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BULLETIN No. 23

MULL AND MOR FORMATION IN RELATION TO FOREST SOILS

By

W. R. C. HANDLEY, Ph.D.

IMPERIAL FORESTRY INSTITUTE

OXFORD

LONDON: HER MAJESTY'S STATIONERY OFFICE

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FOREWORD

This bulletin presents the results of researches carried out at the Imperial Forestry Institute, Oxford, between 1948 and 1953. It deals with the processes that go on when organic material decays in the soil, and it is believed that its findings will interest foresters and soil scientists in all parts of the world.

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CONTENTS

CONTENTS

INTRODUCTION

SOIL is formed when rock undergoes changes known as weathering, becomes colonised by plants and animals, and the dead organic residues from plants and animals are subjected to the action of chemical, physical and biological agencies ; that is to say, soil formation may be regarded as consisting of a series of physical, chemical and biological processes. For a long time it has been observed that the soils resulting from such systems do not always have the same properties and appearances even though they may have developed from the same parent mineral material and in the same climate. These differences may be very striking, especially in the case of forest soils, and have naturally led to attempts at classification.

The characteristics used in the various classifications have depended to some extent on the purpose it was intended the classification should serve. In some cases, especially those intended for more or less specific purposes of practical application, use is made of soil properties such as colour, texture, organic matter content, pH, exchangeable cations, etc., either singly or in combination. Soil maps showing the areal distribution of soils having particular properties have been made, and these may be of considerable value ; e.g. for agricultural purposes, but such an approach can give little information regarding the fundamental causes underlying the formation of specific soils. Soil classifications based on soil properties have led to an indiscriminate terminology which has resulted in confusion, particularly among workers using different languages. Such classifications have also led to differentiation into specific types of soils, which, differing only in minor characteristics because of slightly varying conditions of formation, may possess the same fundamental characteristics.

Other systems of soil classification have made use of the concept of soil maturity ; i.e. the variation of soil properties or characteristics with time. In one case a soil has been deemed mature if the profile features are well developed, i.e. a strictly morphological basis for differentiation; and in another case a soil in equilibrium with its environment has been considered mature. The latter alternative is dynamic in outlook and places emphasis on soil forming processes, rather than profile morphology, without, however, defining the processes involved. On account of the relatively slow speed of many soil reactions, and the consequent difficulty in checking the validity of assessments of soil maturity, any arrangement of soils as a maturity series must be largely hypothetical and speculative. An isolated observation for the purpose of assessment of maturity may indicate that a soil has the characteristics which are said to be indicative of maturity ; e.g. a podzol which has well developed profile features and is apparently in equilibrium with its environment ; there are, however, a number of examples of this apparently mature (by definition) soil type undergoing reversible change to an apparently fundamentally different type under the influence of various factors, especially the activities of man. In this connection Tamm (1932) found the mosaic of soil types observed whilst mapping the soils of an experimental forest to be almost incomprehensible if soil forming processes only take place in one direction, as appears to be implied by the maturity concept, whereas it could easily be understood by the assumption of opposing processes. Therefore, unless one is dealing with soils of regions of primeval vegetation (and even here there are probably exceptions) of which extremely few remain, or under conditions of constancy of climate, organisms, parent material and topography, the concept of maturity of a soil has little meaning apart from its implication that a dynamic system of processes is involved in soil formation.

Hesselman (1925) was of the opinion that the most important task for the soil scientist lies in endeavouring to elucidate processes which go on in the soil, and that not until such knowledge has accumulated should a terminology be developed and the various phenomena systematised, otherwise a clearly formulated and permanently fixed terminology may easily be a disadvantage. In recent times, essentially the same opinion has been expressed by Jenny (1941)—that the assumptions on which the selection of criteria for genetic or scientific classification is based are theoretical and will remain so until we achieve a more precise knowledge of the physical, chemical and biological reactions occurring in soils. The absence of information regarding soil processes appears to have been the source of much fruitless discussion concerning the various supposed soil forming factors.

Whereas many of the *inorganic* soil processes proceed very slowly and are therefore not readily subjected to experimental investigation, it is probable that processes concerning the *organic* material

go on sufficiently rapidly for experimental investigations to be possible.

A number of different processes or reactions have been suggested as characteristic of biologically different soils, but these have usually been the results of field observations or proximate analyses of one or more constituents of an undefined system, and their validity has not been examined experimentally. In the few instances where experimental investigation has been carried out, the underlying causes of the differences do not seem to have been discovered.

From field observations Müller (1879) found considerable biological differences associated with remarkable constancy with particular soils, but he was careful to point out that even provided the observations were correct, and the conclusions regarding mutual coincidence in point of time and association are also correct, it does not mean that the items are really attached to one another as cause and effect, since simultaneity and association in any phenomena do not necessarily mean there is any causal relationship between the two. Thus, although beech mull is associated with the presence of earthworms and beech mor is characterised by a surface layer of organic matter bound together by beech roots and fungal mycelium, we have not, therefore, thrown light on what it is that brings these two factors into existence each on its site ; and it is probable that the faunistic and floristic features of the soils should only be interpreted as expressions of a condition encompassing a more varied series of possibilities.

As Müller showed, the processes constituting soil formation form a labile and often reversible system, and therefore any suggestions regarding the basis of fundamental differences between soils which do not allow of this variation are not likely to be valid.

In the investigation of soil processes, as in the investigation of chemical and biochemical processes, it is essential to try and define the system as precisely as possible and determine how various factors influence or condition it.

Since the soil types described by Müller can be differentiated biologically, the processes going on in them are likely to be fundamentally different, and as these soils can also be differentiated morphologically and thereby recognised in the field, these contrasting soil types form a logical starting point for investigations.

In the present work it is intended to examine as far as possible the fundamental basis or causes of the differences between the biologically different soils described by Müller from the point of view outlined above.

PART I. A SURVEY OF PREVIOUS WORK ON MULL AND MOR IN RELATION TO FOREST SOILS

Chapter 1

THE BIOLOGICALLY DIFFERENT SOIL TYPES OF BEECH FORESTS AS DESCRIBED BY P. E. MÜLLER

As a result of his studies on the beech woods, oak forests and heaths of Denmark, P. E. Müller (1879 and 1884) concluded that their soils could be separated into two biologically distinct types. These seemed to be so different in character, origin and practical significance that investigations of the topmost layer of soils could be most conveniently considered in relation to them. At the same time he pointed out that there were also soils which could be regarded as intermediate forms of the extreme types. Müller described the contrasting soil types as they occur in association with beech forests as follows.

The Soil of Beech Forests Growing on Mull

In the beech forest growing on mull the surface of the soil is covered by a more or less thick layer of forest detritus, leaves, small twigs, bud scales, male catkins, fruit scales, etc., all lying loosely on top of each other. Here and there a few leaves can certainly be seen bound together by a dense white mycelium, but the mass as a whole is incoherent and lies loosely on the soil surface. If this leaf covering is removed, the underlying blackish-brown or greyish-brown surface displays a granular and lumpy appearance. The boundary between the leaves and the soil, between the undecomposed and the completely disintegrated and converted organic remains, is usually quite sharp. On digging down into the soil it is found to be as completely friable as the best worked garden or field soil and the foot sinks in at every step. The uppermost 1½—3 inches of soil is darker in colour than the rest, often very blackish-brown ; on drying it is greyish-brown and has a granular or lumpy structure. This layer passes over gradually into the material which characterises the underlying soil usually to a depth of ¾—1¼ feet, frequently to a depth of 2 feet and very often to 3, 4 or 5 feet. The soil as far down as this is quite friable and loose, the colour may vary with locality, but is almost always completely uniform throughout the whole mass in the same locality. Müller included among the biological characteristics of the beech mull a thriving growth of beech with vigorous height growth, abundant leaf formation and smooth light bark. The beech mull contains very many mycelia of the most varied shape and colour ; the transparent easily decomposed hyphae seem however to occur in greater numbers. The ground flora contains characteristic species such as *Asperula odorata*, *Mercurialis perennis*, *Milium effusum*, *Melica uniflora*, *Stellaria nemorum*, *Oxalis acetosella* and *Anemone nemorosa*. He defined the beech mull as a deposit of beech forest detrital material, rich in animal life especially earthworms, converted to a loose and incoherent layer in which the organic remains are intimately mixed with the mineral soil, the topmost soil down to a depth of ½ to 1 inch seeming to consist almost exclusively of earthworm casts.

The Soil of Beech Forests Growing on Mor

In contrast, the soil of the fairly well stocked beech forest on mor is firm and does not give way under foot, the surface is so tough that even on loose sandy soils the rain water may form puddles when the mor has covered the surface of the soil ; if, however, after a long spell of wet weather this cover becomes thoroughly soaked through, it may be as saturated with water as a sponge, whereas the soil immediately underneath it is dry. When a profile is exposed, the topmost layer of the soil is seen to be a tough blackish-brown humus layer—the mor. Below the mor, and more or less distinctly demarcated from it, there usually lies a layer of loose sand which entirely lacks the ochre yellow colour so common in the soil mass of drift formations ; its colour varies from greyish-white to grey or blackish-grey, as a rule lighter the farther it extends from the mor layer. Beneath this there is a darker coloured reddish-brown or brownish soil layer and finally below this, friable clay, sand or intermediate forms of these. Biologically Müller characterised the beech stands on mor sites as slow

growing, the older trees being stagheaded, often overgrown with lichens, and showing other signs of an unhealthy condition. The beech roots occur almost exclusively in the mor humus layers, where there is also an immoderate production of a blackish-brown mycelium, which is only found in small quantities in the mull. The most characteristic ground flora species of beech mor are *Deschampsia flexuosa* and *Trientalis europea*, the proportions of these varying according to the density of the stand. In the inadequately thinned forest there is a rich moss vegetation including *Hypnum triquetrum*, *Polytrichum formosum*, *Dicranum scoparium* and *Leucobryum vulgare ;* quite often these are accompanied by bilberry. The ground is only sparsely covered by this vegetation, twigs and small sticks, with here and there some leaf remains forming the surface between these scattered and unobtrusive plants. Even at first glance, it is evident that there must be a considerable difference between the animal life of the mull and that of the mor, for molehills do not disturb the even surface of the mor. The mole, as well as its prey the earthworm, is absolutely absent from true mor formation. Müller also states that entomologists consider mor ground to be barren.

The mor can therefore be interpreted as a deposit of the detrital mass from the beech forest, extremely poor in animal life, bound together into a compact peat by beech roots and a very durable mycelium.

Müller (1884) also described similar biological differences between the mull found under oak, with its ground flora extraordinarily rich in species, and the sharply contrasting mor associated with the *Calluna* heath whose flora is very poor in species. Müller (1879) was of the opinion that the majority of humus forms deposited on dry land could be referred to one or other of these types, and that the numerous variations and transition forms derive their peculiarities essentially from the locality, its flora and fauna.

The fact that Müller's differentiation of soils into two main types has stood the test of time, and has been accepted internationally, is a strong indication of the importance of his ideas. He concluded that the most remarkable and most significant differences between the humus forms, the dissimilar disintegration of the detrital masses and dissimilar mixing with the mineral soil, must be attributed to differences in the soil organisms of the localities in question ; but, as pointed out in the introduction, he was by no means certain that they had the relationship of cause and effect ; and he observed that here, as everywhere in nature, a phenomenon is extremely seldom a simple result of a single cause.

Chapter 2

SOIL-FORMING FACTORS IN RELATION TO MULL AND MOR

SINCE the outstanding work of Müller, the development of different soil types has been the subject of a considerable amount of work, much of which has been concerned with the so-called soil forming factors. However, as pointed out by Romell (1931), in spite of the correlation between type of profile and humus type, the humus layer has been somewhat negelected in such studies, perhaps on account of the overwhelming influence of the climatic concept, and seems often to be looked on merely as the tool of climate in soil formation. Soil forming factors will only be considered here insofar as they throw light on the processes occurring in the organic matter of the two biologically different uppermost soil layers, mull and mor.

During the evolution of the various systems of soil classification, the geologic or lithological nature of the parent material was considered to be the soil forming factor most important in determining the type of soil formed ; subsequently climate came to be considered the most important soil forming factor ; whilst more recently organisms, especially vegetation, have been increasingly cited as an important factor in soil formation.

As in the case of chemical, physical and biochemical processes which are influenced by a variety of factors, it is unlikely that the processes of soil formation are at all times dominated by any one and the same factor ; i.e. a multivariable system cannot be completely described or classified on the basis of a single variable. A single factor could only become universally dominant provided all other factors were the same for all soils.

The geological or lithological factor

There are many instances (Tamm (1932) Hesselman (1925)) where it has been observed that under conditions of climate and/or vegetation usually

considered predisposed to mor formation, the presence of inorganic bases, especially lime, in the mineral matter of the soil appears to promote the formation of mull. There are also observations (e.g. Griffith, Hartwell and Shaw (1930)) showing that mull and mor may develop side by side on soils originally uniform in character and origin of parent material, thereby indicating that a site which allows of the development of mor cannot necessarily prevent the formation of mull. It can also be demonstrated that even though mor has formed, it is often possible to change the mor to mull, e.g. by a change of vegetation, without the addition of inorganic bases, although this might be expected to expedite the process. (Dimbleby (1952b)).

Although it has been suggested that inorganic bases neutralise the acidic products of decomposition, aid the processes of decomposition of organic material by micro-organisms and, in base-rich soils, give rise to litter containing larger concentrations of mineral material which affect the decomposition of organic constituents of the litter, the nature of the part played by inorganic bases in the soil processes leading to mull formation, does not seem to have been demonstrated. The problem of the nature of the influence of inorganic bases is complicated by the apparent, though relatively infrequent, occurrence of raw humus on limestone. Coombe and White (1951) have described the cyclic formation of peat on calcareous dolomite in western Finnmark. In this region, although there is a long annual period of low temperature, snow cover and often high relative humidity, the annual rainfall is low and the peat develops in the absence of water-logging ; nor does a preliminary leaching of the surface layers of the dolomite appear necessary, although this may occur, before peat formation can begin. Usually the peat forms over soils which are still alkaline and contain free carbonate; the peat itself in such cases is frequently alkaline, having a reaction above pH 7.0, and sometimes has particles of dolomite incorporated in it. Only the lowest few centimetres of peat remain alkaline, and it would seem that the surface is soon built up to a level beyond the influence of alkaline ground water. As the depth of the peat increases and becomes more acid, the character of the vegetation changes, the original calcicoles being replaced by plants more usually associated with an acid peat flora, although a few of the calcicoles persist for a very long time. In spite of the fact that it seems certain that a depth of 10 cm. of peat can accumulate within the life span of an individual plant of *Astragalus frigidus* or *Selaginella*, the whole area is not blanketed with peat, and after a certain stage has been reached erosion of the peat can be observed ; this is associated with the degeneration of acidophilous dominants, and the appearance in abundance of lichens, especially species of *Cladonia*. When the upper acid peat has been eroded away, leaving a few centimetres of alkaline peat (reaction pH 7.82), calcicoles may colonise this remaining peat, but they do not seem to be able to prevent its complete erosion, and may not re-establish themselves until all of the peat has dispersed, when presumably the cycle commences again and peat begins to accumulate once more.

Such phenomena do not appear to be restricted to such relatively extreme climatic regions. Webb (1947) has described a similar occurrence in the more temperate climate of western Ireland. Carrowkeel is a flat-topped hill having an altitude of 1,057 feet, and is composed of upper carboniferous limestone almost horizontally bedded. The hill is grazed to the summit by cattle, horses, sheep and goats, and most of the moorland is fired at intervals. Broadly speaking, grassland occurs below 650 feet; moorland predominates above that level; the transition zone has the form of a mosaic with areas of moorland increasing in size as the hill is ascended. Peat is always found beneath a moorland flora, never beneath the grassland. The first patches of peat appear (as the hill is ascended) at points where the soil is shallow and drainage best; i.e. on the crowns of rocky knolls breaking through the soil. This is the opposite of the usual sequence leading to the formation of calcareous heaths, where calcicoles persist on the thin soil above the protruding basic rocks. The transition to moorland on Carrowkeel is associated with the appearance of a large proportion of mosses among the calcicolous plants, followed by an invasion of *Blechnum* and *Vaccinium myrtillus ;* with the appearance of *Calluna vulgaris*, peat formation is definitely established, and other moorland species soon follow. Patches of limestone pavement, of very pure limestone free from chert, occur at all levels in the moorland up to the summit. Where the pavement has not been invaded by the moorland association, the deeper crevices have a vegetation of woodland herbs and undershrubs similar to that of the pavements of the Irish Burren and north-west Yorkshire.

When the peat, which is often only 5—8 cm. thick, is stripped off, there is usually no perceptible layer of mineral soil between it and the limestone. This can be clearly observed when healthy bushes of *Calluna vulgaris* accompanied by *Carex binervis* and other moorland species are rooted only in peat which fills a solution hollow, 10—20 cm. deep, in the limestone pavement. The peat is easily pulled out intact, revealing the pure limestone surface, in most cases with roots of the vegetation

in intimate contact with it. The layer of peat in contact with the rock is black and crumbling, and has a reaction of pH 6.8—7.3. Peat also forms readily on the smooth and level surfaces of fallen limestone blocks, which support a luxuriant growth of *Calluna vulgaris*. Such occurrences indicate that neither good drainage nor base-rich water is in itself an impediment to peat formation, and Webb understandably concludes that these problems cannot be answered until we know more about the nature of the peat and the manner of its formation.

In a region far distant from those mentioned above, Galloway (1940) describes the occurrence of raw humus on dolomitic limestone outcrops. This occurs on the Door Peninsula bordering Lake Michigan at an altitude of 580—800 feet, the associated vegetation being composed chiefly of *Thuja occidentalis* with some *Abies balsamea*, incidental hardwoods and a ground vegetation limited to sporadic occurrence of raw humus plants, such as *Maianthemum canadense* and *Cornus canadensis*. The soil profile shows a thin litter layer sharply delineated from the lower layer, which varies from 4—10 inches in thickness, the lower part of which grades into a narrow strip of nearly black, highly dispersed organic matter, powdery when dry, sticky when wet, which is incorporated to some extent with weathered particles of limestone and is underlain by unconsolidated rock substratum. It is unfortunate that there is no mention as to whether or not these sites have been subjected to interference by fire, etc.; and there is no indication of any vegetation succession phases similar to those observed in Ireland and Finnmark.

Tansley (1939) also considers the occurrence of calcifuge species and acid humus in close contact with calcareous substrata. He regards the mixtures of calcicolous and calcifuge species occurring on chalk and limestone "heaths" as conditioned by grazing, and observes that whilst acid humus has not been recorded in association with chalk there is widespread formation of acid humus on the much harder and older limestones. Here again the sequence of vegetation phases consists of pioneer lithophilous lichens and mosses, which give rise to a thin layer of black humus which is subsequently colonised and largely dominated by a calcicolous turf in which such species as *Empetrum nigrum* and *Calluna vulgaris* occur. *Calluna* may occur in pure patches on as little as 5 cm. depth of humus, the rootlets being in contact with the limestone rock. In some cases the fissures and surface of the limestone pavement have black soil whose reaction is pH 7 and which carries a rich vegetation of woodland plants. Under these conditions *Fraxinus excelsior* may become dominant.

On the coarse dry limestone of the island of Gotland, Romell (1938) observed that where the park meadows were subjected to heavy grazing a poor type of coniferous forest with sparse vegetation developed, whereas when mowing and grazing ceased an almost impenetrable wood of ash, hazel, hawthorn and oak developed in 50 years.

Whilst calcareous rocks exhibit wide variation in composition and physical properties, and consequently in the speed and manner of weathering, it does not seem possible to correlate any of these variable properties with the formation of chalk or limestone "heaths", or the apparent development of raw humus associated with the growth of calcifuge species on limestone pavements. In many, if not all, of the examples of this phenomenon it seems likely that the calcifuge vegetation is largely induced and maintained by grazing or fire, and that if not interfered with such sites would become covered by a scrub of broadleaved woody species or a modified ashwood, in neither case being associated with raw humus. It may well be that the properties of some limestones, e.g. purity and hardness, may make them more prone to give rise to raw humus formations; but the problem of how raw humus materials remains. It is proposed to examine in a subsequent section the mechanism of such exceptions to the generally observed influence of base-rich mineral material on the type of soil formed.

The Climatic Factor

The influence of climate on soil formation has been one of the most disputed problems of soil science. Many investigations have resulted in the conclusion that climate affects soil formation through the influence of rainfall and temperature on the chemical and biological processes of soil formation, and more particularly in the case of mor, by slowing down the rate of decomposition of vegetable debris. In this connection Hesselman (1925) declared that whilst in central Europe the climate conditions mull formation, in Sweden the climate conditions mor formation by depressing the rate of decomposition of vegetable debris and the rate of nitrogen mobilisation. Romell (1931) quotes Glinka as asserting that an excess of humus can arise by reason of slow decomposition brought about by conditions of temperature and moisture unfavourable for the activities of micro-organisms. Waksman and Gerretsen (1931) carried out laboratory experiments to ascertain the influence of temperature and moisture on the decomposition of plant residue (straw) by micro-organisms. Their results indicate that whilst over a period of 273 days there is considerably greater reduction in amount of organic matter at 27;C (81;F.) than at 7;C. (45;F.) (36.4% of original weight of material

remains compared with 76.4%) the difference in rate of decomposition is largely restricted to the first 16 days, after which the rates are almost identical. More recently Lutz and Chandler (1946) have concluded that although the decomposition of plant debris is influenced by many factors, it appears that climate exerts a primary control. Robinson (1949) considered that the effect of climate as a pedogenic factor is principally through the effect of rainfall and temperature on the decomposition of organic matter.

Jenny (1941) has stated that the essential criteria of a climatically determined soil type are similar anatomical or morphological characteristics which are preserved under a variety of geographical environments and geological strata. No matter what the parent material or topography, all soils of a given climatic region must possess certain definite features which are typical for the selected climatic region. It is clear from field observations, however, that there are many difficulties in the way of acceptance of climate as a factor of over-riding importance in soil formation. Thus, although it is generally thought that the mor humus of the forest soils of northern Scandinavia is climatically conditioned, it is clear from the writings of Scandinavian workers that mull soils are by no means unknown in these regions. Hesselman (1917) has reported the occurrence of brown forest soils in Norrland. Tamm (1932) states that mull soils occur plentifully in mountain regions and advance repeatedly into the podzol climate ; and also cites Ramann's observations on the freely occurring brown earths in the mountains far distant from the climatic region of the brown forest soil. A similar phenomenon appears to have been recorded more recently for the central mountain regions of Norway by Lothe (1950); these brown forest soils are more acid in reaction, pH 4.1, than the normal podzols, pH 4.8.

Although Romell (1935) appears to have supported the thesis that mor formation is climatically predetermined, he does point out that activation of mor can occur in such regions, and also that, as shown by Müller (1884) Hesselman (1925) and Plice (1934), under uniform climatic and edaphic conditions the composition of the stand has often been observed to affect the type of humus layer ; this is in agreement with the previously noted findings of Griffith, Hartwell and Shaw (1930). Beale (1951), commenting on the weaknesses of the zonal classification of soils, points out that a podzol strip is faithfully reproduced from map to map as occurring along the south-east coast of Australia, whereas in reality it is, as with the other so-called soil zones of Australia, a mosaic of a number of soil groups including, in this instance, podzols, brown forest soils, etc.

In a recent exploratory study of Alaskan soils Kellogg and Nygard (1951) investigated the climate, vegetation and soils of this arctic and sub-arctic region, and found discrepancies between the zonal classification of soils and the soils to be found in the field. Although the tundra soils have a tough fibrous brown mat of organic matter on the surface, this is underlain by a few inches of dark-coloured humus-rich soil, which fades to lighter coloured grey or mottled soil beneath, down to permafrost or unaltered parent rock. The characteristics may change abruptly at the permafrost contact, but usually they change gradually, especially in the absence of permafrost. These soils occur in both arctic and sub-arctic climatic regions, with or without permafrost, and carry a vegetation of hundreds of herbaceous flowering plants, including many species of *Compositae*, *Claytonia*, *Linnaea borealis*, *Campanula* sp. *Mertensia* sp. as well as numerous shrubs and prostrate woody plants such as *Salix* sp. and *Alnus* sp. on moist sites whilst *Betula nana*, *Ledum decumbens* and *Vaccinium uliginosum* are very characteristic. *Rubus arcticus* and *Rubus chamaemorus* also occur. They point out that whereas schematic maps of zonal soils, based primarily on maps of soil genetic factors, have indicated large areas of podzols in Alaska, in fact most of the land where podzols are expected is occupied by azonal or intrazonal soils; well developed podzols with prominent genetic horizons occupying only a small part of the total area. The podzol soils encountered in Alaska occurred within the Boreal forest of the interior with perhaps some weakly developed examples in the coastal forest, i.e. where interference by man is likely to have been most pronounced. The vegetation of these Alaskan podzols does not differ greatly from that of podzols in the northern States. The most strongly developed podzols in Alaska are found in plant associations, often secondary, consisting mainly of white birch, quaking aspen and other poplars and white spruce trees, with an undercover of dwarf heath shrubs and a ground cover of mountain cranberry, crowberry, mosses, lichens and *Cornus canadensis*. Kellogg and Nygard describe a new intrazonal soil group which they term the subarctic brown forest soils because of their similarity to the brown forest soils of the temperate regions. These occur within the region of podzol soils in Alaska and merge with the tundra in places. They are well drained soils with brown surface horizons that merge through gradual transitions to the parent material underneath. Permafrost may or may not be coexistent but the lower soil is normally too cold for much growth of roots or micro-organisms. The subarctic brown forest soils have acid organic layers on the surface,

often including a fibrous or peaty mat. Usually they are leached of free carbonates and in most cases the surface horizon is slightly to strongly acid, while lower layers are slightly acid or even neutral. The ashy, light coloured, A^2 horizon characteristic of podzols is lacking, although near the margin of the transition to podzols there is a suggestion of it. These subarctic brown forest soils appear to be reasonably stable in this region, so that given the parent materials and geomorphological processes of the landscapes of which they form a part, it cannot be postulated that they will in time become podzols. They occur in a climate colder than that characteristic of well developed podzols in North America. The vegetation is similar to that of the podzols, mixtures of white birch and white spruce being most common ; willow and alder shrubs are more plentiful than on the podzol but are most numerous on poorly drained soils. Although its peat-forming mosses and dwarf heaths become established on the well-drained sub-arctic brown forest soils, they are more prominent on the well-drained podzols. Weakly developed crumb structure and worm casts may also be found in the subarctic brown forest soils. It seems quite clear therefore that brown forest soils and podzols can exist together under the extremely cold climatic conditions of Alaska. This makes it even less likely that climate is a dominant factor in the differential formation of mull and mor.

The apparent occurrence of raw humus under tropical conditions provides an equal or perhaps more striking exception to the climatic theory of mor formation. Although such phenomena have been mentioned from time to time, in many cases the descriptions seem to be inadequate for a correct assessment of their relationship to the temperate zone raw humus to be made, but in view of its potential importance this aspect of mor formation must be considered as closely as possible.

Tamm (1920) mentions that, in propounding his rain factor theory, Lang demonstrated the existence of raw humus in the tropical regions of heaviest rainfall.

Wilde (1946) states that mor humus is formed spasmodically in tropical rain forests, which may perhaps be taken to indicate that raw humus and other soil types occur side by side in the tropics.

Vageler (1933) describes the occurrence of ortstein in the tropics and states that iron ortstein occurs there principally in the light sandy soils of the tropical forest peats and primeval forests. It is not restricted to high altitudes and may even occur at sea level where the humus has a very acid reaction. The iron ortstein of the tropics, like the humus ortstein of the temperate regions, is always covered by a layer of bleached sand which is often of considerable depth and is in turn overlain by a layer of acid humus. In heavy soils these formations are but poorly developed and may not go further than changing the soil colour. The distribution of bleached sands and iron ortstein in the moist tropics has not yet been accurately ascertained. They are probably almost as widely distributed in the forest belts there, and especially in the extensive swampy areas of the tropics, as is ortstein in temperate climates.

From many of the descriptions it is not at all clear whether this acid forest humus of the tropics is formed in the absence of waterlogging or not. Vageler states that forest peats have a much wider distribution in the tropics and subtropics, both in regions of heavy rainfall and also where the climate is intermittently moist, than the swamps forming in water courses and limited to certain places, since the conditions necessary for the development of swamps are not generally satisfied even in districts of high rainfall. In spite of this he states that forest peats extend to the sea coast as in Sumatra and Borneo, and are to be regarded as the end product of forest swamps which readily form in badly drained depressions. Continuing his description of these forest peats Vageler states that they can be formed from water which does not carry much debris, provided excess water is present sufficiently long to retard decomposition of the dead plant matter so that gradual accumulation of material takes place This process occurs comparatively slowly as the species composing the vegetation, which consists of certain characteristic plants, do not grow particularly tall nor are they luxuriant and produce relatively little organic matter each year. In all regions where they occur these tropical and subtropical peats have one feature in common in that the surface layer of humus is usually shallow, only here and there does it exceed a yard in depth where depressions of the subsoil have permitted an exceptional accumulation of organic matter. As far as is known such accumulations of organic matter are, without exception, very acid. The subsoil below such peats, if permeable, shows a thick layer of bleached sand with iron ortstein or, less frequently, humus ortstein. Where the soil is heavier it is usually discoloured to a considerable depth. These forest peats have developed over large areas in the region of the Upper Congo and Lualaba, in the East Indies, in the north of South America (particularly by the Amazon and Orinoco rivers) as well as in Central America. Reports indicate that a considerable part of New Guinea has forest peat formations.

The tropical forest peats described by Vageler appear to exhibit striking similarities to the raw humus formations of temperate climates ; e.g. they

are associated with comparatively small numbers of plant species which are largely characteristic of the soil type, and an acid layer of organic material overlying a layer of bleached sand and ortstein. Since, however, they appear to develop in association with widespread and long continued waterlogging, it is difficult to decide how far these tropical peats are comparable to the waterlogged peats of temperate regions or to the raw humus formations of temperate regions, especially as Vageler considered them as arising from forest swamps. For a layer of bleached sand to be formed over ortstein it would seem that there must have been considerable passage of freely draining water through the mineral soil in the first place, (Vageler describes them as occurring principally on light sandy soils.) resulting in an impermeable layer of ortstein whereupon waterlogging and swamp conditions develop. Such a process would be very similar to the bleached sand and pan formation found in the podzol of temperate regions. It is likely that the leaching may have been associated with a vegetation giving rise to material similar to mor or raw humus of temperate regions; in this connection Vageler observes that certain tropical grasses such as *Imperata cylindrica* vigorously promote ortstein formation in acid soils. *Imperata cylindrica* is not a swamp plant and rapidly invades land cleared of forest.

Recent work by Jenny (1948) on the soils of tropical rain forests in Columbia indicates that organic matter accumulations, comparable with temperate raw humus and associated with a horizon of leached material, occur under conditions which seem to preclude waterlogging. Jenny described a yellow podzolic soil in the hot, equatorial and humid regions of the Pacific lowlands. This soil, occurring at an elevation of 140 metres on a Tertiary alluvial ridge which emerges above the plain of yellow soils consisting of Pleistocene deposits, has a layer of raw humus 10 cm. in depth which is completely absent from the yellow soils. The estimated mean annual temperature of the region is 25—26;C. (77;F.) and the annual rainfall 200—320 inches. The vegetation is rain forest of broad-leaf trees and palms. The profile description indicates that the soil has resemblances to the podzols of temperate regions. Jenny also describes the profile of a yellow soil occurring in the same locality on a level site at an elevation of 100 feet where the mean annual temperature was 25.6°C. and the rainfall 336 inches in 1946. The vegetation is dense tropical rain forest of broad-leaf trees and palms. The parent material is alluvial terrace (late Pleistocene) and the profile description bears close resemblances to that of a mull soil of temperate regions. A somewhat similar phenomenon appears to have been recorded by Richards (1936) for Sarawak. He describes "Heath Forests" whose distribution coincided with that of sandy soil; patches of loamy or clayey soil carried Mixed Forest. The Heath Forest soils are of much lighter texture, especially in the surface layer, than the mixed forest soils, although they are derived from the same rock. Richards comments that in general these Heath Forest soils seem to resemble European podzols. Climatic conditions are similar for the Heath Forest and the Mixed Forest. The water of the streams of the Heath Forest is orange brown in colour whereas the water of the streams of the Mixed Forest is colourless. The association of black water with Heath Forest and white sand soils appears to have been noticed in several parts of Borneo. It seems possible that the relatively small accumulation of surface organic matter under these conditions may be the result of the high rainfall continually washing organic matter in a fine state of division through the sandy soil into the streams; in temperate regions of comparatively low rainfall this material would tend to accumulate at the bottom of the leached layer. In the vegetation of the Heath Forest the number of species is much smaller than in the Mixed Forest, and some of the species most plentifully represented in the Heath Forest seem to be characteristic of it; e.g. *Agathis* and *Casuarina* spp. The canopy of the Heath Forest is much thinner than in the Mixed Forest, and the undergrowth very dense but containing a relatively small number of species. The herbaceous ground flora of the Heath Forest is also poor in species and *Bryophytes* are much more abundant than in the Mixed Forest. Whilst conifers are common in the Heath Forest they are absent from the Mixed Forest. The leaves of the Heath Forest trees are smaller and also tend to be thicker and harder than those of the Mixed Forest. The Heath Forest association appears to be widespread in Borneo, and Richards is of the opinion that the Wallaba forest of British Guiana is a closely similar type in respect of structure and soil profile differences by comparison with adjacent Mixed Forest. The Wallaba forest is dominated by members of the Leguminosae and does not contain conifers. In many parts of the Heath Forest it is as if every individual plant in the Wallaba Forest had been replaced by one of similar habit and general appearance but of different systematic affinities. Davis and Richards (1934) are of the opinion that there has been much interference in the forests in the Wallaba forest region.

Later Richards (1941 & 1952) discussed the problem of lowland tropical podzols in some detail, giving further descriptions of profiles (having the characteristics of the podzols of temperate regions) and vegetation in several tropical regions.

and he appears to consider these soils as very similar to podzols of temperate regions. These lowland tropical podzols seem to be almost always, if not invariably, associated with an unusual type of vegetation which, in a number of instances at least, appears to be secondary in nature and resulting from biotic interference. Richards considers, in some cases at least, that edaphic factors play a considerable part in determining the occurrence of this type of vegetation. The lowland tropical podzols described occur side by side with tropical red earths or tropical yellow earths.

Donis (1949) has recorded the occurrence of podzolisation in yet another tropical region, the Mayombe district of the Belgian Congo, in soils occurring at an altitude of 300—330 metres (about 1,000 feet). Several profiles are described in which there is a very shallow litter layer lying on a layer of dark coloured sand up to 20 cm. in thickness; then follows a thicker layer of greyish bleached sand, a thick accumulation or B zone and finally the parent material. These soils carry a vegetation of forest or savannah; in the latter case the vegetation contains a number of gramineous species including *Imperata cylindrica*. Bush fires have occurred irregularly, especially in the savannah areas; this may account for the absence of a mor layer on the surface of the soil. The rainfall in these regions is of the order of 40 to 56 inches a year, which is considerably less than the precipitation recorded in other tropical regions where apparently similar soils have been observed.

It seems probable therefore that there are in the tropics, at low altitudes and occurring side by side under conditions of high temperature and rainfall but in the absence of waterlogging, contrasting soil types equivalent to the soil types mull and mor of temperate climates. From such considerations of the suggested importance of climate in soil formation it seems clear that there is insufficient evidence for it to be regarded as an overriding factor governing the processes of soil formation ; therefore an investigation of the processes involved in the formation of mull and mor should not be hampered by undue weight being attached to the possible influence of climate. Thus, although soil formation may be regarded as a system of chemical and biochemical processes and therefore likely to be influenced to a greater or lesser degree by temperature and moisture, mull and mor apparently occur side by side throughout the temperature range; and therefore factors other than temperature seem to have an overriding effect on the processes of soil formation.

The Influence of Topography

Observations made in north Sweden indicate that under some circumstances topography influences the processes of soil formation. Although the mechanism of such effects does not appear to have been investigated the observations may provide useful information when considered along with other aspects of the problem.

Hesselman (1917) described the streamside vegetation, which shows marked differences from the adjacent conifer forest throughout Sweden. These, often extremely narrow, belts alongside streams are characterised by a vegetation of grey alder, birch and willow ; the shrub and moss covering of the conifer forest being replaced by a distinctly nitrophilous herb flora. These herb-rich belts continue along the stream sides, above the limit of the conifer forest, in association with birch and even above the tree limit in the lower alpine regions. He noted that the humus layer of these herb-rich belts is of the mull type, but that the organic matter is not so well mixed with the mineral soil as in the true mull soil ; in addition there were sometimes slight signs of podzolisation below the humus layer. Hesselman thought that the moving stream water was the important factor governing the occurrence of these herb-rich belts.

In his studies on the soils of the north Swedish conifer forests Tamm (1920) describes how, although on the gentle to moderate slopes of morainic material the podzol profile is usually normal and similar to that found on the plateaux, on the steep slopes the profile is often not that of a typical podzol. He describes an example of this occurring at Rokliden in Norrbotten where a fine spruce stand, growing on a good mull soil with no signs of a leached zone in the soil, occurs on the lowest, very steep, bank of a swamped slope having an abundant water supply. He also describes a similar example in the same district at Svarttjärn where a very porous rolled-gravel ridge provides a ground-water outlet for a small lochan. Above the level of the lochan normal podzolisation occurs on the ridge, whereas below the level of the lochan definite horizons are absent from profiles which are uniformly brown and mull-like in appearance. Commenting on these phenomena, Tamm is of the opinion that the absence of development of a typical podzol profile on steep slopes may be partly due to the fact that the water runs off over the surface and does not percolate to the same extent into the ground, and partly to the fact that surface ground-water streams, being relatively rapid and containing, according to Hesselman (1910), an abundance of oxygen, encourage herb-rich forest types in which profiles develop differently. He sums up the effect of topography as an indirect action on podzolisation by reason of its effect on vegetation which in turn acts on the processes of soil formation. Tamm (1932), however, does not consider, although

for what reason is not clear, that such soils properly belong genetically to the true brown forest soil, but he finds that separation of these two types of brown forest soil in northern regions causes great difficulty. He regards both the groundwater brown forest soil and the calcareous variant as aclimatic subtypes of the brown forest soil with mull and a rich herbaceous flora. Tamm is of the opinion that Ramann's observations on the frequently occurring brown earths in the mountains far distant from the climatic region of the brown forest soil were also made on this subtype. He also notes that both the groundwater brown forest soil and the calcareous brown forest soil can be associated with coniferous forest, and in both cases there is some factor which invariably results in a rich ground flora. Such coniferous forests with rich ground flora give rise to the same soil formation as the southern broadleaved forest. It is somewhat difficult therefore, in view of such a statement, to understand why Tamm wishes to separate these brown forest soils from those of more southerly regions.

Romell (1935) comments that mull soils associated with flowing groundwater occur even in regions very poor in lime. The effects of topography on the processes of soil formation appear to be associated with the development of a rich ground vegetation, although the mechanism of such effects is unknown. It seems possible that the groundwater prevents either accidental or designed burning on such sites, thereby preventing the development of the vegetation usually associated with mor formation.

The Influence of Vegetation

P. E. Müller (1884) demonstrated that a change in soil type occurred when a vegetation dominated by *Calluna vulgaris* replaced oak forest. He also stated that he knew of no vegetation whose residues always come to lie on the soil in the form of mor, but that it is unmistakeable that certain kinds of vegetation more easily and more generally give rise to mor formation than others. Müller (1879) also noted that when oak forest was replaced by beech on siliceous, lime-poor soils, mor was produced, whereas on base-rich soils beech gave rise to a mull soil.

In 1923-26 Bornebusch also discussed the distribution of mull and mor in Denmark. He is of the opinion that Denmark can be regarded as a transition zone between the mid-European mild humus region and the Scandinavian podzol region, and that it is therefore to be expected that the two soil types will be found occurring side by side even under the same climatic conditions, this being partly due to the nature of the subsoil and partly to the nature of the vegetation. For the formation of a podzol he regards it as necessary that there shall be a vegetation whose litter will become raw humus, and he mentions that in the Danish climate raw humus will develop under a vegetation of ling, bilberry and bracken and also beneath spruce on the sandy soils of Jutland and North Zealand, but not beneath spruce growing on the richer soils. He thinks that much of the raw humus formed beneath spruce is derived from mosses, and he finds raw humus under beech to be associated with ground unprotected against drought, or a stand whose thinning has been neglected so that it is in consequence very dense. Raw humus does not form in oak woods unless the ground is covered with bilberry.

Tamm (1920) recorded that conifer forest with an understorey of Ericaceous shrubs, mosses and in some cases lichens, is the most characteristic vegetation of the podzol areas of north Sweden. If, however, the ground flora of a conifer forest is rich in herbs then a mull soil is likely to be found. Writing about the brown forest soils of Sweden, which he considers equivalent to Müller's mull humus type, Tamm quotes Ramann as stating that they correspond with the occurrence of deciduous broad leaf forest, and that differences permitting of the separation of these soils into those of warm and cold regions were not discernible.

Hesselman (1925) also commented on the striking effect on the soil exercised by a mixture of birch, aspen, grey alder and similar trees in the Norrland conifer forests. He noted that in spite of the mixture of broad-leaved trees the soil did not become mull in character, the organic matter lying in a distinct layer, which is usually very loose and friable in consistency, on the ground. The reaction of the soil under these circumstances is often considerably displaced in the alkaline direction compared with that of the soil in the pure conifer forests.

In their studies on the types of humus layer in the forests of north-eastern United States, Romell and Heiberg (1931) concluded that in this region, as in Europe, the mull is mainly a hardwood type, but that it can sometimes persist also under white pine and can even be found under pure hemlock in a lime-influenced area. Observations showed that crumb mull seems to be the normal soil type in the redwood flats in the fog belt of California and also in the coastal Douglas Fir region mull was commonly observed. They frequently observed that hardwood stands with mull contained a smaller proportion of beech and more hard maple, etc., than those with duff. Certain more exacting species, such as *Tilia americana* and *Fraxinus americana*, seemed in New York to be strongly correlated with the occurrence of crumb mull.

More recently Braun (1950) has also commented on the distribution of mull and mor in relation to vegetation in the forests of the United States. In the forests of coniferous and deciduous species on the Adirondacks "spruce flats" there is a fibrous mor, but with local increases in the population of *Fagus grandifolia* and *Acer saccharum* the fibrous mor layer gives way to a mull-like humus and herbs of the hardwood forest appear in the ground vegetation. Whilst mull commonly develops under mixed hardwood stands supporting a rich herbaceous vegetation, mor commonly develops under conifers, being particularly characteristic of the northern coniferous or spruce-fir forest and of coniferous communities in the hemlock-white pine-northern hardwoods region. It is also stated that a hardwood type of mor is seen frequently in oak and oak-chestnut forest on a non-calcareous substrata which commonly supports an ericaceous shrub layer and a sparse herbaceous flora of species very different from those of the mull. Shallow podzols with a laminated or fibrous mor layer occur on dry slopes and ridges occupied by oak-chestnut or oak-pine communities.

Locally where very abundant hemlock has caused the formation of a deep mor layer, which almost excludes herbaceous plants, Lunt (1932) observed, in Connecticut, that podzols are more likely to be found under hemlock, and that even in hemlock hardwood mixtures the podzol may be confined to mere patches beneath a few individual trees. This very local occurrence of very small areas of one soil type occurring in a much larger area of the other soil type and associated with a different vegetation has also been observed from much earlier times. Thus P. E. Müller (1884) noted islands of mull beneath very small areas of oak scrub in a sea of *Calluna* mor. Hesselman (1925) describes the soil beneath an uprooted, but still living, grey alder in the Kulbächsliden Forest. The humus covering has altered, and beneath the crown of the windfall it is friable and mull-like, whereas outside the area of the grey alder crown there is the normal raw humus of the conifer forest. The margin between the two forms of humus follows in the main the form of the crown of the uprooted tree. Below the alder crown the reaction of the humus layer is pH 5.0 and outside its influence pH 4.0. The formation of very localised podzols has been recently recorded by Wright (quoted by Robinson (1949)) in the southern hemisphere under isolated specimens of Kauri in New Zealand. Vageler (1933) also reported the local production of a miniature ortstein, from which no typical characteristic is lacking, under almost every tuft of *Imperata cylindrica* growing under tropical conditions.

Such records of extremely localised occurrence of the one soil type within a large area of the other and associated with the occurrence of a different vegetation, indicates that the establishment of a vegetation known to be associated with mull on a mor should result in the mor changing to mull and vice versa. There are, in fact, a number of observations of such instances.

Discussing the soil types of South Sweden, Tamm (1932) is of the opinion that there the brown forest soil is the climatically determined soil type and describes its occurrence on the most different parent deposits if the natural vegetation, i.e. beech or oak forest, is growing there. Where, however, the broadleaved forest has been replaced by conifer forest or *Calluna* heath, as often happens under the influence of man, podzols or brown forest soils developing into podzols are found. Tamm also describes the reverse phenomenon whereby a mor becomes a mull. If beech or conifers which are giving rise to mor are replaced by birch then in time the ground flora becomes rich in herbaceous species, grasses and dwarf shrubs, the reaction becomes markedly less acid and the mor and leached horizon are converted into mull. He also points out that if beech or spruce colonise or are planted under the birch on such soils then mor will be formed again.

Similar phenomena have been observed by Fisher (1928) and Griffiths, Hartwell and Shaw (1930) in New England, where white pine has developed on abandoned fields. After 80 years there is almost no vegetation under the white pine and under the thin layer of dry needles there is a thick layer of raw humus and a strongly podzolised horizon. On an adjacent plot hardwood forest has been developing on a similar white pine plot which had a similar soil profile at the time the white pine was removed ; now there is a true mull profile, all accumulated raw humus has merged with the mineral soil and less than a single year's leaf fall remains on the surface.

More recently Bornebusch (1943) has described profound changes in soil type brought about through the influence of *Quercus borealis* growing on mor produced by *Pinus sylvestris* and Norway spruce on sandy soils in Denmark. In 20 years the horizons of the profile under the spruce raw humus have become obscured ; the mor layer has for the most part disappeared and is covered by the brownish earthworm mull mixed with mineral soil. The oak roots are abundantly spread through the whole top soil and occur here and there in the subsoil down to 48 inches depth. The fauna has also become more characteristic of the mull soil type and contained *Lumbricus rubellus*, mull soil arthropods and large ground beetles.

It seems clear that, as in the consideration of the importance of lithological material and climate as soil-forming factors, rigid conceptions of vegetation as the determining influence in soil formation are inadequate to explain the observed facts. Thus, whilst it cannot be shown that particular changes in vegetation will under all conditions bring about similar changes in soil type, it can be shown that under some conditions when other factors remain constant then a change of vegetation will bring about a change in soil type. As a working generalisation it can be said that in temperate climates coniferous, ericaceous and, in some instances, beech vegetation is likely to be associated with mor, whilst other broad-leaved trees and shrubs and herbaceous ground flora are likely to be associated with mull.

In a number of the examples quoted above of the influence of changes of vegetation on the type of soil formed, the changes in vegeation have been the result of the activities of man. It therefore seems desirable to consider the importance of the activities of man, by his influence on the floristic composition of vegetation, in relation to the geographical distribution of mull and mor. For example, where there are large areas of mor is this the result of segregation of certain vegetation forms by man for economic or other reasons, or is it the result of agencies other than the activities of man ?

Although soil cannot be formed in the absence of vegetation or vegetable debris, the view has been put forward that vegetation cannot be regarded as an independent factor in soil formation since the distribution of plant species is closely governed by geology and climate. Whilst the floristic composition of the vegetation on a particular site may be determined by factors such as climate and geological characteristics, this does not necessarily mean that if vegetation were allowed to develop on a site free from direct or indirect interference by man it would of necessity, in some cases, be composed only of species which give rise to raw humus solely on account of the influence of climatic and geological factors.

It is becoming increasingly apparent that it is extremely difficult to find areas of vegetation which have not been interfered with to any greater extent than by animals in equilibrium with their predators. In addition there is uncertainty regarding the ultimate structure of such vegetation (Jones (1945)) so that it may not yet be possible to recognise areas of vegetation of this nature. Descriptions of remote areas, in temperate regions, which would seem to have been immune from the activities of man, do not usually include accounts of the associated soils. But the vegetation of these areas, except possibly in certain exceptional areas where natural fires or other widespread natural catastrophies are said to result in a vegetation containing only one species of tree, usually a conifer, appears to consist of a mixture of species which elsewhere would give rise to a mull soil. In view of the difficulties and uncertainties of this line of approach it may be useful to consider the problem from other angles.

As a result of his studies on forest soils which had developed from very different kinds of parent mineral material, calcareous, clayey and siliceous material, Duchaufour (1950) is of the opinion that when they reach stable equilibrium with their respective stable forest vegetation associations, the soils are essentially the same, that is, a brown soil slightly leached. The development of these soils is therefore relatively independent of the parent mineral material, the two chief factors influencing the development of the soil being the micro-climate of the forest and the properties of the forest humus. When, however, such forests are destroyed the soil developing as a result of degradation is closely dependent on the type of parent mineral material ; on calcareous material a rendzina develops, on clay a peat with gleying, and on sand a podzolised soil develops.

Looking at the problem from another angle it has been demonstrated in some cases that long established mor has taken the place of mull by a change in vegetation, due to the activities of man. Taking the heathlands of temperate regions as an example, those which occur in association with a podzol in an advanced stage of development have existed since before the earliest historical records; and there now seems little doubt that they arose after man cleared the forest which had developed on these sites after glaciation. Pollen analyses have shown that the vegetation before the establishment of the heathland was of such a composition that the soil was undoubtedly mull in type. (Dimbleby (1952a).)

It is known that burning either by accident or design is one of the chief factors in the maintenance of the heathland. In the absence of such interference it seems doubtful whether mor could maintain itself indefinitely, as it would appear to be a most unstable system. A vegetation which continues to be composed predominantly or solely of species giving rise to mor would, as the depth of mor increases, find it increasingly difficult to perpetuate itself (Hesselman (1925)), until finally there was no growth of autotrophic plants. At this stage erosion of the mor will probably occur similar to that described by Pearsall (1950) for peats. Subsequently, if seed is available, it seems likely that if the site were protected from fire and other activities of man the vegetation arising would be

of such a composition that it would give rise to a mull soil. It will be recalled that when mor which has developed on limestone disintegrates, recolonisation is by calcicoles.

On the other hand the indications are that mor, protected from burning but with no other interference by man, would, if seed were available, very probably become colonised by mull-forming species ; e.g. the invasion of heathlands by birch, and in course of time a mull soil would be formed.

If one concludes that the segregation of species giving rise to mor is the result of the activities of man, the questions of the autecological position of species giving rise to mor, and the occurrence of local areas, often beneath individual trees, of mor in mixed forest, have to be resolved. In addition there is the problem of the apparent continuity of an association containing only a mor-producing conifer as the tree species.

Under the influence of the activities of man, *Calluna vulgaris* may occur as the dominant species over large areas and give rise to mor. It can also occur however as healthy isolated individuals in a herbaceous vegetation and under these conditions the influence of the debris from the herbaceous vegetation will prevent the formation of mor even over a long period of time. The position is probably somewhat similar in the case of apparently stable forest communities with a conifer as the only tree species, for in many instances the forest is open and between the trees there is a herbaceous ground vegetation, though here there may be accumulation of debris and mor formation beneath the crown of each individual during its considerably longer life than in the case of a species such as *Calluna*. Such a system is very similar to that in which local areas of mor are formed, even beneath individual trees, in the mixed forest. In these cases it is probable that on the death of an individual the mor formed beneath it would be colonised by mull-forming herbs or shrubs and in due course a mull soil would be established again. In the meantime the progeny of the mor-forming species would be maintaining the species by colonisation of a recently vacated adjacent mull site and in due course the newly occupied site would come to have a layer of mor on it. We have therefore a system in which mull alternates with mor at more or less irregular intervals depending on which areas of mull are colonised by individuals of the mor-forming species.

To sum up the influence of these soil-forming factors on mull and mor formation it can be said that whatever the influence of climatic factors on soil formation may be, it seems quite clear that the biologically different soil types described by Müller can occur side by side in regions of widely differing climate. The formation of these different soil types side by side seems to be influenced by the lithological nature of the mineral materials of the soil and especially by the nature of the vegetation growing on the soil. It seems quite clear that the debris from mull-forming species can exert considerable influence on the debris from potential mor-forming species in the prevention of mor formation.

Thus, although some indication of the factors concerned with the occurrence of mull and mor has been obtained, these yield little if any information regarding the detailed mechanism of the causes of their occurrence. Since mull and mor types seem to be components of a reversible system, the characterisation of the reversible process or processes involved is necessary for an understanding of these very different uppermost soil layers. In view of the apparent importance of the species from which the vegetable debris is derived in relation to the type of soil being formed, it seems desirable to ascertain first of all whether there are any characteristic qualitative or quantitative differences between the vegetable debris giving rise to the two types of soil.

Chapter 3

THE CHARACTERISTICS OF VEGETABLE DEBRIS IN RELATION TO THE FORMATION OF MULL AND MOR

The Amount of Litter Falling each Year on Unit Area of Mull and Mor Sites

ONE of the most striking differences between mull and mor is the apparent difference in amount and distribution of organic matter. It seems possible that the large amounts of organic matter lying on the surface of the mineral soil in the case of the mor could be related to considerable difference in the amounts of vegetable debris falling on unit area in the two cases. Of the relatively small number of investigations of the amount of litter falling per unit area per annum very few have

been concerned with the type of soil on which the vegetation was growing.

Ebermayer (1876) gave the results of a large number of observations made in the Bavarian State Forests. The forests were of beech, spruce and pine growing in pure stands and in mixture, in some cases along with small numbers of other species, on soils derived from different kinds of parent mineral material and at various altitudes between about 200—1,100 metres above sea-level. Although the soils associated with the stands are described to a certain extent it is difficult to ascertain when they correspond to mull or mor. The results do not seem to point to a correlation between soil type and amount of litter falling on the site. There is also much variation from year to year within one and the same stand and between different stands and different tree species, the range being approximately 2,000—6,000 kilograms of air-dry litter, containing about 14% moisture, per hectare per annum, with most values about the mean of this range independent of soil and species. According to Büsgen and Münch and Thomson (1929) Ney has criticised the methods used by Ebermayer for collection of the material. Ebermayer (1876) apparently used the methods described in *Forstliche Mitteilungen*, but neither this nor Ney's criticism have been available for consultation.

The amount of the annual leaf fall from a number of hardwood and conifer species growing on a number of sites at various altitudes was determined by Ohmasa and Mori (1937). Although there do not appear to be any descriptions of the soils on which the trees were growing, these authors found, as did Ebermayer, that there is considerable variation in the amount of leaf fall for different plots of the same species in the same year and on the same plot from year to year for both conifer and broad-leaved species. Generally the variations in actual amount of leaf fall show close similarities to those obtained by Ebermayer (1876) although a number of the values are far below any of those obtained by Ebermayer ; these values do not seem to be explained by considerations of age or density of the stand.

A number of observations have also been made in North American forests. Alway and Zon (1930) made measurements of litter production in mixed, unthinned stands of jack pine, Norway pine and white pine growing on sandy soil of low productivity. Although the uppermost soil layer is not described it is possibly of the mor type. They found that the average fall of litter in 12 months amounted to 1,738 lbs. per acre on an oven-dry basis, the amount varying as much as 25% from one plot to another in the same year and almost as much on the same plot from one year to the next.

Heyward and Barnette (1936) measured the litter falling from second growth long leaf and slash pine stands growing on what was previously a mull soil developed on fine sands and fine sandy clay deficient in lime and having a reaction of pH 4.0—5.5, the ground vegetation being suppressed by the layer of accumulating pine needles. The annual needlefall was found to vary between 2,400 and 3,500 pounds per acre on an oven-dry basis.

More recent observations on litter production in American forests are those of Chandler (1941 and 1943). In the first paper Chandler deals with litter production in the hardwood forests of central New York. The measurements were made in several hardwood stands on two different soil types. The one group of soils, coarse mull, reaction pH 5.9—6.7, developed on lime containing mineral material and the other group, having matted mor, reaction pH 4.5, on sandstones and shales (Howe (1935)). All the areas had closed stands of mixed second growth hardwoods, the age of the dominant trees varying between 30 and 70 years. 26 samples were examined from the coarse mull sites and 24 from the matted mor sites. The stands on the calcareous soils contained a higher proportion of *Tilia americana* whilst the soils with matted mor had stands containing a large proportion of *Acer saccharum*, *Quercus borealis* var. *maxima* and *Fagus grandifolia* with few *Tilia americana*. The nature of the ground vegetation is not stated. Samples of freshly fallen litter were collected, from within wire frames enclosing a known area, at the conclusion of leaf fall, and dried at about 70°C. and weighed. On the calcareous soils the amount of the current season's litter varied between about 2,600 and 3,000 lbs. per acre and for the more acid soils between about 2,400 and 2,700 lbs. per acre. If mean values are obtained the differences in litter production per acre between the more productive and the less productive soils amounts to 236 lbs. and although this is statistically significant Chandler is of the opinion that large differences in the amount of litter-fall due to differences in site quality should not be expected.

Chandler (1943) has also made observations on litter production by some of the conifers of North-Eastern America. Pure stands of conifers are difficult to locate in this hardwood region and the ages of the stands chosen vary considerably from 24 year-old-plantations to 150-year-old stands. Three plantations near Ithaca were growing on brown forest soils developed on Dunkirk silty clay loam and those in the Adirondacks were growing on an acid podzol. The litter was collected at intervals from particular sections of a series of four burlap cloths placed on the ground under each stand. The samples were dried at 70°C.

The results indicate that a 24-year-old white pine plantation growing on a brown forest soil yielded 2,732 lbs. of litter (dried at 70°C.) per acre in one year whilst a 65-year-old white pine stand growing on a podzol in the Adirondacks yielded 2,730 lbs. of litter (dried at 70°C.) per acre per annum. *Picea rubens*, *Tsuga canadensis*, *Thuja occidentalis* and *Abies balsamea* growing on podzol soils in the Adirondacks gave yields varying from about 1,300—2,570 lbs. of litter (dried at 70°C.) per acre per year. *Pinus resinosa* and *Picea abies* growing on brown forest soils gave yields of about 3,360 lbs. of litter (dried at 70°C.) per acre per annum.

Pearsall (1945) has commented on the apparent lack of data for this country concerning the amount of litter deposited on a natural soil annually. He made observations on the litter falling on the ground in various types of oak wood in Hertfordshire during the period of leaf fall. The results of these observations are given as grammes of dry material per square metre although whether oven or air dry is not stated. The average weight of leaves falling on the ten normal sites, presumably with mull soil, is 9,390 kgms. per hectare varying between 6,620 and 13,400 kgms. per hectare. The average weight of debris when twigs are included is 16,070 kgms. per hectare. Such values are considerably greater than those recorded for soils with mor layers.

Estimates of litter production have also been made in Scandinavia in recent years. Knudsen and Mauritz-Hansson (1939) observed that an almost pure birch stand of average age 35 years and with a rich, largely non-ericaceous shrub and ground vegetation growing on a brown earth in central Sweden yielded 1,865 kgms. of air-dry (at 17°C.) litter per hectare during the period of leaf fall (7/9—21/11) in 1938. The litter was collected after it had fallen into metal containers 50 cms. x 50 cms. x 50 cms. placed on the ground and when air dry it had a moisture content of 10.4 per cent.

In a 90-year-old spruce stand near Stockholm, probably growing on a mull soil, Lindberg and Norming (1943) collected the litter falling between 19/11 1939 and 19/11 1940 in 10 sampling tins each 300 cms. x 70 cms. x 10 cms. and found that approximately 3,364 kgms. of air-dry material (containing 91.86% of dry matter) fell on one hectare.

A little later Andersson and Enander (1948) used collecting tins to ascertain the amount of litter falling on the forest floor beneath a 62-year-old aspen stand near Stockholm, growing on a mull soil having a very rich ground flora. The litter collected between 29/9 and 21/12 amounted to 1,958 ± 174 kgms. of air-dry material per hectare. Collections made in 1941 and 1942 when there was a small admixture of birch and hazel leaves gave values of 2,078 ± 99 and 2,120 ± 133 kgms. of air-dry material, having a dry matter content of 92.18%, per hectare respectively.

By the collection of falling litter in containers 20 cms. in height and 19.5 cms. internal diameter Mork (1942) has made observations on a number of spruce and birch stands growing at varying altitudes above sea-level ; the type of soil on which the various stands are growing is not clearly indicated in all cases. The amount of needle fall per year for a 40-year-old spruce stand, containing a small admixture of birch and growing at Ås on a site 80 metres above sea-level, for the years between 1939 and 1942 was calculated to vary between 2,123 and 2,665 kgms. per hectare. The litter of other species, including birch, falling on the same area during the same period varied between 381 and 634 kgms. per hectare per annum. For a 63-year-old spruce forest growing at Veldre (180 metres above sea-level) and a 50-year-old herb-rich birch forest, probably growing on mull soil, in the same district, Mork obtained values of 1,517 kgms. spruce needles (total litter 1,883 kgms.) per hectare and 1,269 kgms. birch leaves (total litter 1,876 kgms.) per hectare respectively for the litter falling on the ground. He also compared the litter fall in 140-year-old spruce, 100-year-old birch and 200-year-old pine forests at Hirkjølen 800 metres above sea-level. The soil was a brown earth in the case of the *Geranium*-type spruce and herb-rich birch forests and probably had a mor layer in the *Calluna-Cladonia*-type forest. In each case the litter fall was measured over a period of 6 years. The average values obtained for the litter fall per annum were :—

Spruce needles 810 kgms. dry weight per hectare (total litter 1,465 kgms. per hectare)

Birch litter 629 kgms. per hectare (total litter 799 kgms. per hectare)

Pine needles 391 kgms. per hectare (total litter 797 kgms. per hectare)

These various observations show that there can be very wide variations in the amount of litter produced by forests growing in different places and on different soil types. Although comparisons are not easy to make, there are definite indications that there is probably no fundamental difference between the amount of litter falling on a mull soil and that falling on a soil with a mor layer on otherwise similar sites. In other words, there is no reason to believe that the cause of mull and mor formation lies in differential amounts of litter falling on the respective sites.

The Mineral Composition of Litter

The reason for the association of certain plant species with the development of different soil types

has been ascribed by some investigators to differences in the content of inorganic material, especially basic substances, in their litter. These differences have been based on chemical analyses; but it is essential to bear in mind that similar contents of a given substance in biological materials of different origin, as determined by chemical analysis, may not necessarily mean that these materials will be identical in those biological properties which are associated with this particular substance. Assessments of this kind must await the development of suitable bio-assay techniques.

Considering the chemical analyses of litter giving rise to different soil types Plice (1934) has pointed out the difficulties of obtaining suitable comparable samples of litter and that few investigators seem to have taken the necessary precautions.

Observations indicate that litter from different individuals of the same species may have rather widely differing contents of mineral material. Melin (1930) analysed samples of freshly fallen litter from a number of North American trees of both conifer and broad leaf species. In some cases litter from the same species from more than one site, the soil types of which are not indicated, was examined. Values for nitrogen and ash contents were obtained which show wide variations for litter from the same species, *Betula papyrifera*, *Fagus grandifolia* and *Pinus strobus*, growing on different sites. Hesselman's (1925) observations, although here again the soil types on which the trees from which the specimens were collected are not specified, also show variations in some cases of ash and CaO contents of the litter of the same species growing in different localities ; e.g. in the case of litter from *Pinus sylvestris*, *Alnus incana*, *Betula verrucosa*, *Populus tremula* and *Salix caprea ;* on the other hand litter samples from *Picea abies*, *Vaccinium vitis idaea*, *Betula pubescens*, *Fagus sylvatica* and *Quercus robur* showed little or no variation, although the number of samples in each case is only 2 or 3. Hesselman also estimated what he terms assimilable lime, that is lime soluble in ammonium chloride, but such observations would appear to have little if any relation to biological processes.

Wittich (1943) states that the litter from beech growing on ground moraine has approximately twice the calcium content as litter from beech growing on sand. He found the calcium content to vary characteristically between species growing on the same site, thus beech litter contains more calcium than pine litter and ash litter four times as much calcium as larch litter when the trees are growing on the same site. On the other hand the calcium content of the litter varies very much with the site, so that pine litter from trees growing on glacial loam contains more calcium than beech litter from trees growing on poor sand. Wittich (1947) makes the generalisation that the litter of the broad leaf tree species containing the smallest amount of calcium contains more calcium than the litter of the conifer species containing most calcium, when the trees are growing on the same soil. He also points out that grass litter, which decomposes readily, has a very low calcium content, to the extent that the litter of the grass species (*Dactylis glomerata*) containing the highest concentration of calcium contained only half as much calcium as the litter of the broad-leaved tree species, beech growing on sand, containing the smallest concentration of calcium.

Comparatively few observations seem to have been made on the composition of the litter from vegetation growing on mull and mor. Bornebusch (1930) compared the newly fallen leaves from beech growing on mull with those from beech growing on mor. Although the subsequent behaviour of such litter would be quite different in the two cases their initial reaction and total nitrogen content were very nearly the same, the nitrogen content being slightly higher in the case of the litter from the mor site.

The litter, dried over phosphorus pentoxide, from birch trees growing on mull soil in central Sweden was found by Knudsen and Mauritz-Hansson (1939) to contain 5.01% ash, 1.02% total nitrogen, 1.87% CaO, 0.52% K_2O and 0.12% P_2O_5. For aspen growing on mull soil near Stockholm, Andersson and Enander (1948) found the water-free litter to contain 9.53% ash, 3.34% CaO, 0.98% K_2O, 0.129% P_2O_5 and 0.58% N_2.

Mork (1942) collected leaves, at the time of leaf fall, from ten birch trees growing on a brown earth and also from ten birch trees growing on an iron podzol. In each case the leaves from five of the trees were yellow, from two of the trees greenish-yellow and from the remaining three trees green. The leaves were dried at 100° C. and ash for chemical analysis obtained by incinerating at 575° C. The following analytical results, mean values for 10 trees, were obtained as percentages of dry matter and percentages of ash. (Table 1.)

These findings indicate that whilst the mean CaO content of the leaves from the trees growing on the brown earth is considerably greater than that of leaves from trees growing on the iron podzol, the mean nitrogen content is the same in both cases and the P_2O_5 and K_2O contents are considerably higher in the leaves from the trees growing on the iron podzol.

Analyses of oven dried (70° C.), freshly fallen, litter from a number of coniferous species, including

TABLE 1

	Percentage of Dry Matter				Ash percentage	Percentage of Ash		
	CaO	K_2O	P_2O_5	N_2		CaO	K_2O	P_2O_5
Leaves from birch trees growing on brown soil	2.166 ±0.305	0.658 ±0.253	0.197 ±0.077	1.251 ±1.126	5.669 ±0.718	38.23 ±3.179	11.70 ±4.603	3.435 ±1.1094
Leaves from birch trees growing on iron podzol	1.359 ±0.308	0.807 ±0.182	0.517 ±0.100	1.249 ±0.490	4.496 ±0.607	30.07 ±4.791	18.23 ±4.857	11.89 ±2.487

white pine growing on both mull and mor sites, were made by Chandler (1943) and the following results obtained. (Table 2.)

If the moisture content of the different samples dried at 70° C. can be assumed to be the same, then there would appear to be no characteristic differences between the mineral constituents of litter from trees growing on mull sites and those of trees growing on mor sites. It is unfortunate that, in view of the difficulty of locating pure stands, a considerable age spread of the stands had to be accepted. The nitrogen content of the litter of *Pinus strobus* growing on mor is higher than that of litter from *Pinus strobus* growing on mull and thereby resembles the findings of Bornebusch (1930) for beech litter.

Chandler (1941) carried out similar investigations on the litter of hardwood species growing on crumb mull soils and also on what are stated to be mor sites, and obtained the following results for litter dried at 70°C. (Table 3.)

The values for both the mull and mor soil samples represent the average values for three sites.

The average values for N, P, K, and Ca contents are in a number of cases higher for litter samples from trees growing on mor sites than for litter from the same tree species growing on mull soils. The nitrogen content of *Fagus grandifolia* litter from a mor site is greater than for litter from a mull site, which again resembles the findings for *Fagus sylvatica* by Bornebusch (1930). The average values for N, P, K and Ca contents of the litter of all species are either very nearly equal or slightly greater for samples from the mor sites than for samples from the mull sites. In the case of Mg the average value for all species is slightly less for litter samples from the mor sites than for samples from the mull sites.

Plice (1934) ascertained the calcium content of the litter of maple and beech, both growing on both mull and mor sites. In the case of both species the litter from the mor site contained slightly less calcium than the litter from the mull sites but the species difference was maintained.

These various observations taken together seem to indicate that the mineral content of litter can show more or less large variations in material from the same species growing on different sites, but that these variations are not usually so great as the differences between different species growing on the same site. The litter of various species growing on mull and mor sites may show differences in content of mineral elements which are positively or negatively related to each other according to the mineral element concerned ; these differences are not necessarily always in the same direction for any particular mineral element when pairs or groups of species are being considered. Whilst it seems to be the case that the litter of plant species usually associated with mor sites generally has rather low contents of the various mineral elements compared with those of the litter of species usually

TABLE 2

Age of stand	Species	As percentages of oven dry (70° C) weight				
		Ca	Mg	K	P	N
25 yrs.	Pinus resinosa (mull)	0.58	0.18	0.35	0.07	0.69
65 yrs.	Pinus strobus (mor)	0.60	0.16	0.18	0.05	1.14
65 yrs.	Pinus strobus (mull)	0.60	0.21	0.18	0.07	1.00
150 yrs.	Thuja canadensis (mor)	0.68	0.14	0.27	0.07	1.05
150 yrs.	Picea rubens (mor)	0.79	0.20	0.35	0.10	0.89
25 yrs.	Abies balsamea (mor)	1.12	0.16	0.12	0.09	1.25
25 yrs.	Picea abies (mull)	1.96	0.23	0.39	0.09	1.02
65 yrs.	Thuja occidentalis (mor)	2.16	0.15	0.25	0.04	0.60

TABLE 3

Tree species	Nitrogen content %		Phosphorus content %		Potassium content %		Calcium content %		Magnesia content %	
	Mull	Mor	Mull	Mor	Mull	Mor	Mull	Mor	Mull	Mor
Tilia americana	1.04	1.14	0.14	0.17	0.39	0.66	3.24	3.22	0.39	0.28
Liriodendron tulipifera	0.51	—	0.11	—	0.95	—	2.56	—	0.45	—
Hicoria cordiformis	0.68	0.67	0.10	0.13	0.45	0.43	3.41	3.41	0.50	0.60
Populus tremuloides and P. grandidentata	0.70	0.77	0.08	0.12	0.36	0.58	1.85	2.37	0.23	0.23
Ostrya virginiana	1.01	1.04	0.09	0.11	0.35	0.50	2.52	2.15	0.35	0.23
Fraxinus americana	0.59	0.67	0.15	0.16	0.46	0.62	2.28	2.46	0.29	0.25
Prunus serotina	0.55	0.64	0.18	0.18	0.47	0.64	2.58	2.15	0.44	0.41
Ulmus americana....	0.77	—	0.15	—	0.44	—	2.06	—	0.32	—
Prunus avium	0.88	—	0.15	—	0.63	—	2.42	—	0.57	—
Acer saccharum	0.43	0.45	0.12	0.15	0.45	0.58	1.65	1.68	0.28	0.19
Betula lenta	—	0.72	—	0.17	—	0.75	—	1.65	—	0.30
Magnolia acuminata	0.59	0.57	0.28	0.29	0.81	0.71	1.62	1.80	0.30	0.28
Acer rubrum	0.41	0.44	0.11	0.11	0.30	0.49	1.35	1.17	0.32	0.21
Quercus borealis var. maxima	0.67	0.57	0.11	0.11	0.55	0.76	1.49	1.28	0.31	0.21
Quercus alba	0.50	0.58	0.12	0.14	0.52	0.52	1.22	1.51	0.24	0.24
Fagus grandifolia....	0.59	0.69	0.10	0.10	0.65	0.65	1.09	0.99	0.26	0.18
Average value for all species	0.65	0.69	0.13	0.15	0.46	0.60	2.01	2.02	0.33	0.28

associated with mull sites, it also seems to be the case that the litter of a number of species usually associated with mull sites has no greater content of mineral elements than the litter of species associated with mor sites and indeed some have less.

In connection with the above indications, it should be pointed out that it is probably the form, and not the amount, of the mineral which is important and there is no information available on this point. Also, since mull and mor are parts of a dynamic system, it is important to know whether the litter of a particular species growing on a site, and whose litter is being analysed, has actually produced, is maintaining, or even changing the soil type on which it is growing i.e. whether it really is a mull-forming or a mor-forming species on a particular site, e.g. the case of birch growing on a mor site. This is often a difficult matter to decide and information on this point has not been given in investigations of the mineral content of litter ; in many cases only prolonged observation in the future will answer this question, in some cases the history of the site may help.

It can only be concluded therefore that there is no information indicating characteristic quantitative differences between the mineral material in litter from mull sites and the litter from mor sites.

The Reaction of Vegetable Debris

Hydrogen ion concentration has occupied the attention of soil scientists for a considerable time. Generally, though not necessarily, soils having a mor layer seem to have a more acid reaction than mull soils. It therefore seems desirable to see whether any correlations have been observed between the reaction of freshly fallen litter and the soil type formed, especially in view of the possible effects of different reactions on microbial activity. The method of preparation of the material and the method of measurement can have appreciable influences on determinations of the reaction of litter. In a number of investigations information on these points is not given, and whilst the results can only be compared within each investigation, it is possible to compare the values obtained for litter of species which would probably form mull with those for litter of species which would probably form mor.

Hesselman (1925) determined the reaction of water which had been in contact with the litter of a number of tree species. There is no description of the soil types on which the trees, from which the litter was collected, were growing. He gives the following pH values as representing the reactions of the aqueous extracts of the litter of various species :—

	pH
Pinus sylvestris	4.2
,,	4.1
,,	4.0
Picea abies	4.2
,, ...	3.8
Juniperus communis	4.3
Larix decidua	3.9
,, leptolepis	4.5
,, sibirica	3.8
Vaccinium vitis-idaea	3.7
,, ,, ,,	3.8
,, myrtillus	4.5
,, ,, ...	4.0
,, uliginosum	4.7

Empetrum nigrum 4.2
Calluna vulgaris 4.4
Acer platanoides 3.7
Aesculus hippocastanum 6.9
Alnus incana 6.3
" " 6.1
" glutinosa 4.6
Betula alba 5.0
" pubescens 5.9
" " 5.4
" " 5.5
" verrucosa 6.1
" " 5.3
" " 5.6
Corylus avellana 6.6
Fagus sylvatica 6.6
" " 5.3
Fraxinus excelsior 6.4
Populus tremula 6.1
" " 5.3
Quercus robur 4.8
" " 4.9
Salix caprea 6.1
" " 5.6
Sorbus aucuparia 4.8
Ulmus glabra 7.3
Athyrium filix-femina 5.9
Deschampsia flexuosa 5.5
Thelypteris dryopteris 5.5
" phegopteris 6.0
Dryopteris spinulosa 6.3
Geranium sylvaticum 4.1
Maianthemum bifolium 5.6
Melica uniflora 6.0
Mercurialis perennis 7.4
Cicerbita alpina 6.9
Pteridium aquilinum 4.7
Stachys sylvatica 6.8
Trientalis europaea 6.1
Hypnum schreberi 4.6
Hylocomium splendens 4.6

These findings indicate some variation in reaction within the same species on different sites and also that whilst extracts from the litter of species which are frequently associated with mor have reactions varying between pH 3.7 and 4.7 there are some species, whose litter would be expected to give rise to mull, which yield extracts whose reaction falls within this range, or is very slightly less acid; although the reactions of extracts of the litter of the greater number of species associated with mull are less acid than those of extracts of litter of species usually associated with mor.

The reaction of freshly fallen beech leaves from both mull and mor sites was measured by Bornebusch (1930) although the method of measurement is not stated. He found that the reaction was pH 5.9 in both cases.

Melin (1930) also measured the reaction of recently fallen or still attached leaves of a number of species of American trees. Unfortunately there is no mention of the method of measurement nor the soil type on which the trees were growing, but if the results in the following table are compared among themselves we observe, as in the case of Hesselman's values, that whilst the litter of probable mor-forming species are in the more acid part of the reaction range there is considerable overlap with the reactions of the litter of probable mull-forming species.

The reactions of thick suspensions of the finely-ground litter of a number of American conifer and hardwood species were measured by Plice (1934) using the quinhydrone electrode and saturated calomel half cell. Although there is no clear indication of the soil type or types on which the trees, from which the samples were collected, were growing, the same general picture is again apparent i.e. the probable mor-forming species being somewhat crowded at the more acid end of the range, although there is also a considerable scattering among the probable mull-forming species as shown below. (Table 4).

TABLE 4

Species	pH of litter Plice (1934)	pH of litter Melin (1930)
Magnolia acuminata	5.9	—
Ulmus americana	5.5—5.4	—
Acer platanoides	5.4	—
Populus grandidentata	—	5.3
Carya glabra	5.3	—
Fagus grandifolia	4.7—5.4	5.2 : 6.0
Betula populifolia	—	4.9
Fraxinus americana	4.9—5.0	6.0
Betula papyrifera	—	4.8—5.1
Thuja sp.	4.8	—
Betula lutea....	4.7—5.0	—
Hicoria microcarpa	4.6	—
Pinus sylvestris	4.9—4.3	—
Quercus maxima	4.5—4.4	—
Larix decidua	4.4	4.3
Quercus alba	4.3—4.45	—
Castanea dentata	4.1—4.6	—
Pinus strobus	4.4—4.3	4.6—4.7
Acer rubrum	4.3	4.2
Quercus prinus	4.3	—
Pinus rigida....	4.2—4.3	4.2
Larix laricina	4.2	—
Picea canadensis	4.2	—
Picea abies	4.2	—
Abies balsamea	4.2	3.9
Acer saccharum	4.1—4.25	4.0
Pinus resinosa	4.15	—
Picea rubra	—	4.1
Picea mariana	4.1	—
Pinus banksiana	3.8	—
Tsuga canadensis	3.7—3.3	—

Mattson and Koutler-Andersson (1941 and 1944) using suspensions of ground litter in water in the ratio of 1 : 10 or 1 : 20 measured the reaction of litter from various tree species in Sweden using the quinhydrone method and obtained the following values :—

1941 1 : 10 *suspension*

	pH
Pinus sylvestris, Ultuna woods, gravelly soil slightly podzolised in places	4.22
Fagus sylvatica, Hallandsås, brown earth with low base status	5.08
Betula pubescens, Ultuna park, river clay, with high base status	5.99
Fraxinus excelsior, Ultuna park, river clay with high base status	6.24
Alnus incana, Ultuna park, river clay with high base status	4.89
Ulmus glabra, Ultuna park, river clay with high base status	5.91
Quercus robur, Ultuna park, river clay with high base status	4.48
Acer platanoides, Ultuna park, river clay with high base status	4.49

1944 1 : 20 *suspension*

Fagus sylvatica, Ultuna park	4.77
Alnus incana ,, ,,	4.60
Quercus robur ,, ,,	4.43
Betula pubescens ,, ,,	4.55
Fraxinus excelsior ,, ,,	5.50
Ulmus glabra ,, ,,	5.31
Pinus sylvestris ,, ,,	3.98

Although the material used in the two experiments was very largely from the same tree species growing in the same area it is unfortunately not stated whether they are from the same sites; so that the differences in reaction may represent site differences, differences between individuals or differences from year to year.

From the rather inadequate data available it appears that the position regarding the reaction of the litter from various species is probably similar to that in the case of mineral nutrients i.e. the potential mor-forming species tend to be grouped at the more acid end of the scale ; the variation within a species and the degree of overlap of mor-forming species and mull-forming species is such however that these properties do not by themselves allow of the characterisation of mor-forming litter and mull-forming litter.

Acid and Basic Buffering Materials in Litter

Plice (1934) added 5 gms. of ground litter to water containing a known quantity (excess) of hydrogen ions and determined the quantity of hydrogen ions inactivated by the litter. Attempts were made to investigate the buffer capacity towards alkali by the use of sodium hydroxide and a hydrogen electrode but the determinations gave only poorly reproducible values. The values obtained for inactivation of hydrogen ions show most of the conifers as a compact group with low inactivation of hydrogen ions almost independent of the mineral base content (Ca+Mg+K) of the litter and those species with litter having higher base content as inactivating larger amounts of added hydrogen ions. Although most of the results are not related to soil type, Plice does give comparative values for maple and beech growing on both crumb mull and root mor ; there is however nothing to indicate whether the maple or the beech has actually given rise to the mor. (Table 5.)

TABLE 5

	Crumb mull		Root mor	
	Maple	Beech	Maple	Beech
CaO content, as percentage	3.1	1.7	2.6	1.2
Ca+Mg+K (mE)	6.8	4.1	5.8	2.9
H ions inactivated (mE out of 4 added)	3.4	2.7	3.3	2.4
Reaction (pH)	4.2	4.9	4.2	4.7

More recently Mattson and Koutler-Andersson (1941) have carried out a considerable number of investigations on the quantities of various forms of acid and basic material in plant material and soils. The only observations which will be considered here are those in which the acidity was determined by electrometric titration with NaOH to pH 7.0 and the titratable bases determined in the ash from litter. (See Table 6.)

The litter from *Pinus sylvestris* is the only litter of a species growing on gravelly soil showing slight podzolisation in places. The results indicate that whilst the litter of *Pinus sylvestris* has a titratable acidity or titratable base content within the range of the values obtained for the litter of mull-forming species, the ratio of titratable base content to titratable acidity, and also excess of base content over acidity, is lower in this instance than for any of the mull-forming species examined. It is unfortunate that similar information for other mor forming species is not available for comparison and therefore, although the excess of base content over titratable acidity may prove to be characteristic for mull and mor-forming litter, either of these two properties independently is not characteristic.

TABLE 6

Species	Acidity of the litter as determined by electrometric titration with NaOH to pH 7 in M.e./100 gm. dry matter	Titratable bases in ash from litter in M.e./100 gm. dry matter
Pinus sylvestris	33.9	43.2
Fagus sylvatica	26.3	76.2
Betula pubescens	16.0	140.4
Fraxinus excelsior	16.2	264.2
Alnus incana	49.2	90.1
Ulmus glabra	25.0	261.0
Quercus robur	47.2	100.1
Acer platanoides	35.0	136.8
Gramineae	21.7	40.5
Triticum aestivum (straw)	9.8	39.5

The Nitrogen Content of Litter

Since nitrogen is a substance of great importance for the growth of living organisms, it is likely that the nitrogen content of litter and the form in which the nitrogen occurs will be of considerable importance in relation to the growth and metabolism of the micro-organisms growing on and breaking down litter in the processes of soil formation. Later considerations will indicate the importance of the form of the nitrogen in litter from the point of view of soil-forming processes. At present only the total nitrogen content of the litter of various species, as far as possible in relation to soil type, will be considered.

Although the determinations of the nitrogen content of fresh litter of various species made by Hesselman (1925) do not seem to be clearly related to soil type the results are interesting for comparison among themselves.

Species	*Nitrogen content as percentage of dry weight*
Picea abies	1.5
,, ,, ...	0.9
Pinus sylvestris	0.6
,, ,,	0.5
Larix decidua	0.8
,, leptolepis	0.6
,, sibirica	0.5
Acer platanoides	0.6
Aesculus hippocastanum	1.4
Alnus glutinosa	2.1
,, incana....	2.3
Betula alba	0.7
,, pubescens	1.0
,, ,,	0.9
,, verrucosa	1.0
,, ,,	1.3
Corylus avellana	1.5
Fagus sylvatica	1.0
,, ,,	0.7
Fraxinus excelsior	1.1
Populus tremula	1.2
Quercus robur	1.2
,, ,,	1.1
Salix caprea	0.7
Sorbus aucuparia	0.6
Ulmus scabra	1.5
Vaccinium myrtillus	0.9
,, vitis-idaea	1.0
Melica uniflora	1.1
Mercurialis perennis	1.2
Stachys sylvatica	2.4
Pteridium aquilinum	0.6

The duplicate results for the same species are for different sites.

The nitrogen content of the litter of a number of American trees, in some cases for the same species from more than one site, was determined by Melin without reference to the soil type on which the trees were growing. The nitrogen content of the litter is expressed as a percentage of the dry matter.

Acer rubrum....	0.37
,, saccharum	0.80
Betula papyrifera a.	0.81
,, ,, b.	1.49
,, ,, c.	1.94
,, populifolia	1.44
Fagus grandifolia a.	0.97
,, ,, b.	0.67
,, ,, c.	0.60
Fraxinus americana	1.68
Populus grandidentata	1.31
Abies balsamea	0.83
Larix decidua	0.65
Picea rubens	0.84
Pinus rigida	0.41
Pinus strobus a.	0.89

Species	*Nitrogen content as percentage of dry weight*
Pinus strobus b.	0.38
Aralia nudicaulis	1.18
Pteridium aquilinum a.	0.63
,, ,, b.	1.19
Kalmia angustifolia	0.77
Vaccinium pennsylvanicum	0.17

Bornebusch (1930) estimated the total nitrogen content in the litter from beech trees growing on mull and mor sites and found that the dry matter of beech litter from a mor site contained 1.19% nitrogen whilst for a mull site the value was 1.15%.

Chandler (1941 and 1943) determined the nitrogen content of a number of hardwood and conifer species growing on both mull and mor sites :—

Species	*percentage nitrogen in litter dried at 70° C.*	
	Mull site	Mor site
Tilia americana	1.04	1.14
Liriodendron tulipifera	0.51	—
Hicoria cordiformis	0.68	0.67
Populus tremuloides and Populus grandidentata	0.70	0.77
Ostrya virginiana	1.01	1.04
Fraxinus americana	0.59	0.67
Prunus serotina	0.55	0.64
Ulmus americana....	0.77	—
Prunus avium	0.88	—
Acer saccharum	0.43	0.45
Betula lenta	—	0.72
Magnolia acuminata	0.59	0.57
Acer rubrum	0.41	0.44
Quercus borealis var. maxima	0.67	0.57
Quercus alba	0.50	0.58
Fagus grandifolia....	0.59	0.69
Pinus resinosa	0.69	—
Pinus strobus	1.00	1.14
Tsuga canadensis	—	1.05
Picea rubens	—	0.89
Abies balsamea	—	1.25
Picea abies	1.02	—
Thuja occidentalis	—	0.60

If the two site types can be taken as being equivalent to mull and mor as described by Müller then these values for the nitrogen content of litter show clearly that the nitrogen content of a sample of litter does not enable it to be characterised as coming from a mull soil or from as oil with mor; although for the *same species* there is a distinct tendency for litter from a mor site to have a higher nitrogen content than litter from a mull site. The apparent formation of mor by the hardwood species concerned is a little difficult to understand and for this reason it is unfortunate that more information concerning the stands, especially their previous history, is not available so that the source of the mor might be established.

Analyses of the nitrogen content of the litter of a number of species, probably growing on a mull soil in Ultuna Park, were carried out by Mattson (1944) with the following results:—

Species	*Percentage of nitrogen in organic matter*
Fagus sylvatica	1.00
Alnus incana	2.54
Quercus robur	1.21
Betula pubescens	0.74
Fraxinus excelsior	1.47
Ulmus glabra	0.82
Pinus sylvestris	0.49

For aspen litter from ca. 40-year-old trees growing on a mull soil, with a very rich ground flora along with *Corylus avellana* and *Lonicera xylosteum*, near Stockholm, Andersson and Enander (1948) give 0.58% as the nitrogen content. Lindeberg and Norming (1943) give 0.96% as the nitrogen content of spruce litter from 90-year-old trees growing on what is probably a mull soil near Stockholm, whilst for birch litter, from trees ca. 35 years old growing on a brown forest soil in central Sweden, Knudsen and Mauritz-Hansson (1939) found a nitrogen content of 1.02%.

In Norway, Mork (1942) has determined the nitrogen content of spruce, birch and pine litter from trees growing in several localities. (Table 7.)

TABLE 7 Nitrogen Content as Percentage of Oven-dried (100°C) Litter

	Spruce	Birch	Pine
Ås (soil probably a brown earth)	1.05% (40 yrs.)	—	—
Veldre (soil probably a brown earth)	1.03% (60 yrs.)	0.89%	—
Veldre (,, ,, ,, ,,)	1.00%	0.96% (50 yrs.)	—
Hirkjolen (brown earth, average value for 6 years)	—	1.717% (100 yrs.)	—
Hirkjolen (probably mor, average value for 6 years)	—	1.315%	0.543% (200 yrs.)

Taking the abovementioned observations as a whole it seems quite certain that there is unlikely to to be any direct relationship between total nitrogen content of a sample of litter and its propensity to form mull or mor.

In the investigations quoted only total nitrogen content of the litter has been ascertained. There do not seem to have been any attempts to ascertain whether the litter of species giving rise to mull contains nitrogen, at least in part, in a form with different biological potentialities compared with the nitrogen in the litter of a species giving rise to mor even though Melin (1930) found that whereas *within a species* breakdown of litter, as measured by CO_2 production, is proportional to the nitrogen content of the litter, *between species* there was, however, no correlation between nitrogen content of the litter and rate of CO_2 production. This does not seem to be in accord with the opinion of Wittich (1943) that the C/N ratio is the controlling influence in litter decomposition, although he did find the position of *Ulmus procera* to be anomalous. There are difficulties in the way of acceptance of these conclusions in both cases, for whereas Melin found rye straw to decompose at approximately the same rate as oak litter which had a nitrogen content three times as great, the conditions of the experiment were somewhat artificial, In the case of Wittich's experiments it does not seem possible to determine how much of the litter material which disappeared was ingested and removed by animals and therefore not necessarily completely decomposed.

Chapter 4

THE ORGANIC CONSTITUENTS OF LITTER

LITTER falling on the forest soil is an important source of organic material for the metabolism of micro-organisms involved in the processes of soil formation. If the organic matter available to soil micro-organisms differs in composition, either qualitatively or quantitatively, in one case compared with another, then the populations of micro-organisms, the metabolic activities and therefore the end products of metabolism may be different in the two instances. It might well be expected therefore that litter giving rise to mull may exhibit considerable and perhaps characteristic differences in the kind and amount of its constituent organic materials compared with litter giving rise to mor.

The definition and quantitative estimation of the various organic constituents of vegetable debris is extremely difficult and many investigators do not seem to have given sufficient consideration to these difficulties in problems concerned with litter decomposition and soil formation.

Analyses of litter are usually given in terms of one or more of the following constituents:—

Ether soluble substances	Hemicelluloses
Chloroform ,, ,,	Cellulose
Cold water ,, ,,	Lignin
Hot water ,, ,,	Protein
Alcohol ,, ,,	

Little or no attempt appears to have been made to separate the constituents of the material extracted by the various solvents, the amount of extractives for each solvent being given as a percentage of the dry matter. In the case of hemicellulose, cellulose, lignin and protein it would appear from current opinion that the methods used for isolation and estimation are often inadequate and the results obtained probably represent groups or mixtures of substances rather than single substances. Although in some instances the individuals of each group may be somewhat similar to each other physically and chemically, it is possible that they may be very different biologically.

Hemicellulose

The term hemicellulose originally implied dilute alkali soluble polysaccharide present in plant materials. The present meaning of the term is usually restricted to alkali soluble polysaccharides, criteria of purity of which are almost completely lacking, which are hydrolysed by dilute acids giving monosaccharides and in some cases uronic acids. It seems clear that the polysaccharides known as hemicelluloses comprise at least two groups; one group containing uronic acid groups which are largely absent from the other, but it is doubtful whether satisfactory separation has been effected as yet. Although the properties of these two groups appear to be similar in some ways, it is thought that they may be derived from different sites in the tissue. There is reason to believe, therefore, that estimations of hemicellulose in litter may have been estimations of more than one substance possibly coming from different sites in the tissue. Analytical

results showing equal contents of hemicellulose in different samples may therefore conceal marked qualitative differences.

Estimations of hemicellulose in litter have usually involved acid hydrolysis of material extracted by dilute alkali followed by estimation of the reducing power thereby produced. On account of the delicate balance between liberation and destruction of monosaccharides during acid hydrolysis of hemicelluloses such methods of estimation cannot be regarded as entirely satisfactory. In addition, the work of O'Dwyer (1931) seems to indicate that the method of drying the material from which hemicelluloses are to be extracted affects the quantity of some of the hemicellulose fractions.

A number of observations appear to indicate that the various hemicelluloses are combined or associated with constituents of the cell wall e.g. cellulose and lignin. The nature of such associations is unknown but hemicelluloses do not seem to be removed from woody material to any extent without concurrent or prior delignification or at least after some treatment which might dissociate linkages with lignin. When, however, delignification is carried out prior to hemicellulose extraction some of the hemicelluloses are lost in the process. In addition to these uncertainties there are indications that in non-woody tissues the hemicelluloses are either more complex or less homogeneous with the result that their chemistry is relatively more complex.

In the present state of information, the interpretation of estimations of hemicelluloses in various kinds of litter is too uncertain to be of any value.

Cellulose

The cellulose of woody tissue is thought to occur in close association with other polysaccharides and it seems that such drastic treatment is required to remove these cellulose associates, known as cellulosans, that the cellulose itself is altered or degraded. Such considerations must be borne in mind when the cellulose of litter is being investigated. Even though opinion seems generally to favour methods of cellulose estimation whereby lignin is removed by halogenation, most of the estimations of cellulose in litter have made use of the cuprammonium reagent to dissolve the cellulose, which is subsequently precipitated from the solution. Whilst the cuprammonium reagent dissolves isolated cellulose, its capacity for dissolving cellulose from wood varies according to ancillary treatment and the nature of the wood, e.g. whether hardwood or softwood, but even under the most favourable conditions a considerable amount of cellulose remains in the material, and in addition the extracted material, whilst consisting mainly of cellulose, also contains small quantities of pentosans and lignin.

Lignin

Botanists and others interested in the cell walls of plants have for a long time described a process occurring, during life, in the walls of many plant cells as lignification. In many cases this meant that the investigator had observed, by the use of dye solutions, changes in the thickness and staining reactions of the cell walls during the development of the cell; in other cases microchemical reactions, such as that occurring with phloroglucinol, have been used. It is not sound to deduce a specific chemical composition from staining reactions involving the use of dyes. Even in the case of the phloroglucin reaction there is no certainty that the observable changes occurring in a tissue, as demonstrated by the phloroglucin reaction, during the physiological process known as lignification, correspond to the appearance or formation of what the chemist knows as lignin. Thus, whilst it is true that cell walls having a high content of what the chemist understands by lignin give a strong reaction with phloroglucin, it is also true that during delignification woody tissues lose their capacity to react with phloroglucin before they have become entirely free from the chemist's lignin. It therefore seems possible that reactions such as that with phloroglucin indicate substances closely associated with lignin but not the chemist's lignin.

The methods used in the isolation of lignin can be divided into two classes (a) involving the removal of cellulose etc. usually by the use of concentrated acids, leaving lignin as a residue and (b) removal of lignin from the materials with which it is associated, by the use of solvents. None of the methods available appear to allow of the isolation of lignin in an unchanged state, with the possible exception of the method put forward by Brauns (1939) which, however, yields only a fraction of the amount of material obtained by other methods. Lignin, as at present obtained, is an amorphous material to which the usual criteria of purity cannot be applied; therefore it is not possible to determine whether lignin preparations contain small or large amounts of contaminants or not.

Although indications have been obtained concerning the identity of some of the parts of the lignin molecules, the identity and characteristics of lignin remain unknown. Until lignin can be isolated in an unchanged and pure state it will not be possible to know what substance it is that efforts are being made to try and determine; and therefore a rational method of estimation cannot be developed. Hence at present there can be no entirely satisfactory method for the determination of lignin, and only approximate determinations are possible.

It is possible that even though plant material from different species contains similar quantities of lignin,

TABLE 8 As Percentages of Dry Matter

	Acer saccharum	Betula papyrifera (1)	Betula papyrifera (2)	Fagus grandifolia	Populus grandidentata	Abies balsamea	Picea rubens	Pinus rigida	Pinus strobus	Pteridium aquilinum (1)	Pteridium aquilinum (2)
Ether soluble fraction	7.66	7.50	6.71	4.59	6.30	9.52	5.73	7.22	11.37	2.42	1.68
Cold water soluble organic matter	12.12	11.94	4.80	4.18	4.37	7.30	6.30	13.58	4.42	5.07	3.04
Hot water soluble organic matter	9.26	6.08	6.05	4.44	4.95	3.88	5.39	6.22	2.86	3.13	3.25
Alcohol soluble organic matter	5.34	—	2.70	3.45	2.93	3.38	3.02	3.31	12.58	2.16	1.70
Hemicelluloses	13.24	17.21	18.74	18.46	16.00	14.12	16.40	15.38	17.10	15.76	17.13
Celluloses	13.90	10.47	9.59	14.07	9.54	12.41	17.12	16.63	14.79	17.61	14.92
Lignin	15.62	21.24	26.26	24.95	32.69	30.74	28.27	26.06	21.89	26.78	33.50
Crude Protein	4.13	8.21	11.00	3.00	7.50	4.63	4.25	1.99	2.12	3.44	6.99

as estimated by some such method as indicated above, the lignin in the different species may, especially before isolation, have very different biological properties. Such considerations should be borne in mind when analyses of litter are being examined from the point of view of differences between mull and mor-forming material.

Proteins

Since the introduction of chromatographic methods of analysis the detection of protein constituents has been on a much more satisfactory basis. Whilst such methods have been applied to the detection and estimation of the amino acid constituents of the proteins of fresh plant material and more recently of soils, there do not, as yet, seem to have been any such investigations of the protein material present in litter. Estimations of protein in litter have so far been carried out by the empirical method of determining the water-insoluble nitrogen content and then multiplying by the arbitrary factor *b*.25.

Quantitative Analyses of some Organic Constituents of Litter

There do not seem to have been many investigations in which the amounts of various organic materials in litter have been determined even by the relatively uncertain methods available. Even such analyses as have been made do not seem to have been related to the soil type on which the trees were growing.

Melin (1930) investigated various organic matter fractions in the litter of a number of tree species without stating the soil type on which the trees were growing.

Hemicelluloses were estimated from the sugars produced on hydrolysis of an alkali extract of the litter with 2% HCl solution, together with the sugars produced on treatment of the residue from the alkaline extract with 2% H_2SO_4 solution.

Cellulose was estimated by treatment of the residue remaining from the hemicellulose estimations with modified Schweitzer's reagent. Lignin was deter-

TABLE 9 Organic constituents as percentage of organic material on an ash-free basis

	Litter from an old eastern white pine eastern hemlock stand. New Hampshire	Litter from 75 yr. old red spruce stand New Hampshire	Litter from eastern white pine stand
Ether soluble fraction....	6.7	5.15	6.1
Water soluble fraction	5.4	7.17	12.7
Alcohol soluble fraction	6.1	3.72	3.3
Hemicelluloses	13.6	14.0	14.5
Cellulose	13.7	16.3	15.8
Lignin	35.0	38.5	31.8
Protein	6.1	5.47	3.1

TABLE 10 As percentage of dry matter

	Beech litter from Maltesholm, Skåne		Pine needles from Nåntuna Denmark	Beech litter from Uppsala Castle Park	Aspen litter from Fabödarna at Bondkyrka	*Glyceria maxima* straw from Bondkyrka Ultuna
	(1)	(2)				
Lignin	24.6 (mean of 9 values)	23.6 (mean of 6 values)	23.48 (mean of 12 values)	25.67 (mean of 12 values)	21.49 (mean of 12 values)	13.93 (mean of 12 values)
Cellulose	21.7 (mean of 8 values)	19.7 (mean of 5 values)	27.39 (mean of 12 values)	16.63 (mean of 12 values)	13.78 (mean of 12 values)	26.10 (mean of 12 values)

mined from the residue remaining after extraction of the litter material with a mixture containing 10 ml. 18% HCl solution to 50 ml. of 72% H_2SO_4 solution.

Protein insoluble in cold water was estimated by subtracting the water soluble nitrogen from the total nitrogen and multiplying the result by 6.25. The results are shown in Table 8.

Lutz and Chandler (1946) quote Watson as determining some of the organic constituents of the litter from three coniferous sites. The soil types on which the trees were growing and the methods of analysis are not stated, but since L. F. and H. layers are indicated as being present in the soil profiles of two of the sites it seems likely that the soils have mor layers. (See Table 9.)

The lignin and cellulose contents of the litter of various tree species were estimated by Lindeberg (1944 and 1946) the lignin being estimated by extraction with thioglycollic acid and the cellulose by extraction with Schweizer's reagent from the residue remaining after extraction of lignin. The soil type on which the trees were growing is not stated. Results are shown in Table 10.

A number of the organic matter fractions of litter samples from trees growing in Ultuna Park were estimated by Mattson and Koutler Andersson (1944). In previous papers (1941) by these authors this locality has been described as having a soil derived from clay of high base status and it therefore seems likely that the soil will be of the mull type. Lignin was estimated as the residue remaining after treatment of the litter with 72% H_2SO_4 solution. The findings are shown in Table 11.

In the course of investigations on the decomposition of the litter of various tree species, Wittich (1943) estimated a number of organic fractions by methods somewhat different from those used in the investigations cited above. Two of the samples in the accompanying table (Nos. 16 and 17) were from trees growing on sand, the remainder were from trees growing on ground moraine. After extraction of the material with ether, the residue was treated first with cold water then with hot water. The residue was now extracted with 95% HCl solution. One half of the insoluble material remaining is now treated with a mixture of hydrochloric and sulphuric acids by the method of Kalb, Kucher and Tourel for the estimation of lignin, including humified material; the other half is treated with alcoholic nitric acid by Kürschner's method for the estimation of cellulose, which was in addition estimated by calculation by difference from 100. Lignin was separated from so-called humified material by it's differential solubility in acetyl bromide. (See Table 12.)

It is of interest to compare values obtained for lignin and cellulose contents of various kinds of wood by the U.S. Forest Products Laboratory (quoted by Wise (1944)) with the corresponding

TABLE 11 As percentage of organic matter

	Fagus sylvatica	Alnus incana	Quercus robur	Betula pubescens	Fraxinus excelsior	Ulmus glabra	Pinus sylvestris
Water soluble fraction	16.84	23.6	21.5	19.4	27.3	23.1	15.9
Alcohol benzene 1 : 1 soluble fraction....	17.3	24.8	15.9	19.9	17.1	11.4	27.2
Residue after treatment with 72% H_2SO_4 solution	33.7	21.3	22.6	29.6	14.0	14.3	21.8

TABLE 12 — As percentage of water and ash free material

Species	Ether Soluble	Cold Water Soluble	Hot Water Soluble	Alcohol Soluble	Hemi-celluloses	Cellulose	Cellulose (Kürschner)	Lignin + humified substance	Lignin	Humified Substance
1. Ulmus procera	7.92	12.95	5.62	2.43	34.35	16.53	11.67	20.20	3.70	16.50
2. Alnus glutinosa	7.86	17.74	6.11	2.83	31.97	15.50	10.15	17.99	3.10	14.89
3. Fraxinus excelsior	6.68	13.80	5.35	2.18	35.70	20.06	10.70	16.23	2.64	13.59
4. Alnus incana	8.55	6.87	5.29	3.00	32.30	15.40	9.21	28.59	3.49	25.10
5. Robinia pseudoacacia	6.81	10.97	3.53	2.78	32.56	13.30	12.69	30.05	4.50	25.55
6. Carpinus betulus	6.33	16.27	7.92	2.88	32.55	16.74	13.30	17.31	8.08	9.23
7. Acer pseudoplatanus	11.23	19.81	7.69	2.53	27.95	20.07	12.21	10.72	1.15	9.59
8. Tilia cordata	9.01	10.50	7.45	3.61	31.59	14.68	11.82	23.16	4.60	18.56
9. Populus tremula	9.64	16.72	4.92	2.42	30.12	19.02	14.36	17.16	4.44	12.72
10. Quercus maxima	8.32	11.14	6.43	4.78	33.01	15.79	11.46	23.53	1.85	21.68
11. Fagus sylvatica	6.14	8.57	5.15	2.18	33.07	21.61	13.65	23.28	1.45	21.83
12. Carpinus betulus	6.03	24.65	6.50	3.24	30.12	18.73	12.74	10.73	1.47	9.26
13. Quercus sp.	6.42	9.04	7.69	3.12	30.60	20.42	19.65	22.71	6.15	16.56
14. Quercus sp.	7.25	13.28	6.42	2.80	31.95	20.13	21.64	18.17	7.67	10.50
15. Betula sp.	14.48	20.52	9.64	4.05	25.16	10.77	6.71	15.38	5.71	9.67
16. Betula sp.	14.41	9.23	4.71	4.16	28.48	13.44	9.99	25.57	1.70	23.87
17. Fagus sylvatica	6.48	6.00	4.34	3.75	33.30	19.59	6.72	26.54	0.83	25.71
18. Fagus sylvatica	5.88	9.64	6.25	2.47	31.70	20.95	17.89	23.11	0.27	22.84
19. Fagus sylvatica	5.75	11.87	5.48	2.56	24.55	27.26	24.83	22.53	0.64	21.89

values for litter obtained by various investigators. (See Table 13.)

TABLE 13 — Mean values as percentage of samples oven-dry at 105° C.

Species	Cellulose	Lignin
Pinus ponderosa	57.41	26.65
Chamaecyparis nootkatensis	53.86	31.32
Libocedrus decurrens	41.60	37.68
Sequoia sempervirens	48.45	34.21
Pinus monticola	59.71	26.44
Quercus densiflora	58.03	24.85
Prosopis juliflora	45.58	30.47
Ochroma lagopus	54.15	26.50
Hicoria ovata	56.22	23.44
Eucalyptus globulus	57.62	25.07

When estimated by similar methods the values for contents of lignin cover a similar range for both litter and wood, except that in the case of the litter of some species the lignin content is of a considerably lower order than those for wood of the species quoted. The cellulose content of wood appears to be approximately 2 or 3 times that of litter. Lignin is usually associated with secondarily thickened vascular tissue. If this view is correct then in leaves the proportion of such tissue would appear to be relatively small compared with the proportion in wood, yet according to analyses the proportion of cellulose to lignin is much smaller in litter than in wood and analyses also indicate that litter contains lignin in very similar amounts to those contained in wood.

Such considerations tend to throw doubt on whether all the material in litter as determined by certain methods and said to be lignin is really lignin. Although the use of acetyl bromide for the separation of lignin and humified materials is not sanctioned by all, the results obtained by Wittich for lignin content of litter by the use of acetyl bromide would appear to indicate that the material obtained from litter by the usual methods for lignin estimation is a mixture of substances of which lignin forms only a part. Such a view would seem to give a much more reasonable lignin content for litter, compatible with the proportion of vascular tissue it contains, in comparison with the lignin content of wood.

Such a survey of the limited information concerning the organic constituents, as determined by chemical means, of litter falling on the forest floor indicates that, whatever the identity of the organic fractions estimated may be, it is unlikely that it will be possible to characterise mull and mor-forming litter directly by the aid of such estimations. Such lines of approach, at any rate with the methods at present available, do not seem to show signs of being profitable to pursue from the point of view of determining the causal factor(s) of mull and mor formation. Attention must be directed therefore to an examination of mull and mor as a dynamic system and to a consideration of the processes going on in these two components of the system, especially regarding differences in the changes undergone by the various constituents of litter during their integration into a mull or a mor system.

Chapter 5

PROCESSES GOING ON IN MULL AND MOR AS FACTORS IN THEIR FORMATION

IT is possible that the formation of mull and mor may be different by reason of quantitatively and/or qualitatively different processes occurring during the decomposition of litter. If characteristically different processes can be detected it may then be possible to discover the cause of these different processes.

Quantitative Aspects of the Organic Matter in Mull and Mor

Characteristic differences in rate of decomposition might be expected to result in differences in the amount of organic matter in mull and mor developing on similar sites, especially since previously quoted evidence indicates that the amount of litter falling on mull and mor sites can be of a very similar order. Field observations appear to make differences in the amounts of organic matter in mull and mor so obvious that, as Romell (1932) points out, it is generally regarded that there is a fundamental difference between the rates of decomposition of organic matter in mull and mor. In his discussion of the overall quantitative aspect of the organic matter of mull and mor Romell also points out that such opinions have very little factual basis.

The quantitative aspect of soil organic matter can be approached either by ascertaining the amounts of organic matter present in representative samples of the two soil types or by measuring the rate of destruction of organic matter in the two soil types as indicated by CO_2 production.

Romell (1932), who has examined these two questions closely, points out that it is necessary not only to consider organic matter above the mineral soil but also, in addition, that in the mineral soil as contained by the same area in the case of both soil types down to a depth of one metre from the top of the mineral soil; i.e. the organic matter is determined on a volume basis and not on an area basis alone. The depth of one metre would cover most soils but in some cases it may be necessary to sample to even greater depths, if appreciable amounts of organic matter occur below a depth of one metre. Working on such a basis Romell found the organic matter content of the soil of a beechwood growing on crumb mull in the province of Småland (South Sweden) to be 28.7 kgm. per sq. metre down to a depth of one metre. On the same basis the organic matter of a mor soil near Håsjö in Jämtland (Central Sweden) was found to be 27.9 kgm. and in the case of a mor soil at Rokliden, Norrbotten (Northern Sweden) 31.9 kgm. Romell's comment on these observations is—"A type profile of brown forest soil with crumb mull from south Swedish beechwoods shows as high a total humus content as either of two type profiles with pronounced duff from the strongest duff forming and podzolising forest type on normally drained ground in northern Sweden". Romell has also recalculated the results obtained by Morgan and Lunt for two pairs of mull and mor sites in different localities in the U.S.A., so as to be on a comparable basis with his own determinations for Swedish soils, and obtained the following results.

	Total organic matter in kgm. per sq. m.
Thick podzol, Waterville (White Mountains)	62.1
Rich crumb mull, Bethlehem (White Mountains)	57.8
Thin podzol, Connecticut	30.4
Crumb mull, Connecticut	24.9

Although Romell comes to the general conclusion that "there is no indication of a consistent difference between the crumb mull and even pronounced forms of duff in the amount of organic matter stored up either in the humus layer or in the entire profile" he does point out that there probably are mor profiles having an organic matter content greater than those reached in crumb mull profiles, and that in certain cases there may actually be a "practical standstill of decomposition". When Romell says "The greasy duff will not easily burn at any season of the year" it would appear that he is hinting at important considerations but he does not pursue them further. It is well known that where mor occurs the surface organic layer is more or less frequently, accidentally or deliberately, burned; and such occurrences are bound to have an effect, which cannot be estimated, on estimations of organic matter in mor profiles. The organic matter in a mull profile may represent the residue from hundreds or thousands of years of forest growth, whereas the organic matter in a mor profile may represent the organic residue from a relatively short period of production of vegetable debris. Jenny, Gessel and Bingham (1949) have recently considered this problem from a similar angle, and from theoretical considerations have worked out the time required for the organic matter of the forest floor, in various kinds of forest,

TABLE 14 CO_2 production in gm. per sq. metre per hour

	Maximum	Minimum	Mean	No. of observations	Method
Mor type	0.84	0.10	0.36	21	accumulation
Mull type	0.72	0.13	0.33	35	accumulation

to reach equilibrium from the amount of decomposition undergone by the litter of various tree species in a year. It would seem, however, that it has been assumed that the rate of decomposition of say pine litter, although slow, proceeds at a constant rate over a period of about 100 years, when any given needle shoul dhave disappeared completely. Evidence which will be presented later does not support the idea of a constant rate of decomposition. In addition Jenny *et al.* appear to assume that the floor of the pine forest would reach equilibrium in one or two centuries and be able to continue indefinitely under these conditions. There are, however, indications that such a mor system would not be stable and eventually becomes incapable of supporting a mor-producing vegetation.

The comparison of total amounts of organic matter in mull and mor systems, for the purpose of comparing speeds of decomposition, is therefore of doubtful significance.

Carbon Dioxide Production in Mull and Mor

In the same paper Romell (1932) discusses the problem of decomposition of organic matter in the two soil types in relation to soil respiration. He argues that if the amount of litter-fall and root respiration are the same, then once a regime is established, and the total amount of organic matter in the two profiles is the same, the total respiration should be the same in mull and mor. This may not be the case for the reasons mentioned above.

Romell made a detailed survey of the various methods used and the results obtained in previous work on the production of carbon dioxide by forest soils in situ. He points out that the workers making determinations of carbon dioxide production in forest soils were not concerned with a comparison of mull and mor, nor is it always possible to classify the humus types on which they were working from the descriptions given, and therefore data which are satisfactorily comparable for mull and mor are extremely scanty. He quotes Meinicke as determining the CO_2 production per square metre per hour, between June and November, for a number of beech and beech + oak stands of different ages, some growing on mull and some growing on mor. The results obtained are shown in Table 14. Whatever these values may represent, neither the extreme values nor the mean values indicate any significant difference between mull and mor. Romell mentions the results obtained by himself and Porkka for CO_2 production in Swedish and Finnish soils respectively, and points out that as the experiments were probably all on mor sites they do not allow of a comparison between mull and mor sites, but even so the values obtained indicate higher mean and in some cases higher maximum values than those obtained by Meinicke.

Romell concludes from his survey that the determinations of CO_2 production by forest soils indicate considerable variation in this property, with most of the average values lying between 0.2 and 0.7 gm. of CO_2 per sq. metre per hour. This range holds for sites from north Sweden to south Germany at least, and there is no indication of a consistently more intense respiration of mull soils as compared with mor soils.

In view of the lack of data Romell measured the rate of CO_2 production by a mull and a type of mor known as root duff. The accumulation method of

TABLE 15 CO_2 production in gm./sq. metre/hour

	Crumb Mull	Root Duff	Locality
July 1st	0.24	0.41	Enfield, Jacksonville
13	0.24	0.18	,, ,,
22	0.30	0.16	,, ,,
27	0.37	0.12	Camillus, Baldwinsville
29	0.14	0.19	Enfield, Jacksonville
Aug. 1st	0.24	0.12	,, ,,
6	0.24	0.17	,, ,,
14	0.30	0.32	Camillus, Baldwinsville
	Mean 0.26	0.21	

measuring CO_2 production, by means of a metal bell resting on the surface of the soil, was used. The ground vegetation on the sites was sparse and the few small green plants present were left undisturbed. He is of the opinion that the stands produced much the same amount of litter and had the same amount of roots. Organic matter determinations per square metre down to a depth of 40 cm. showed the crumb mull to contain 32.1 kgm. and the root duff, 14.3 kgm. The values obtained for the production of CO_2 in gms. per square metre per hour do not indicate any characteristic difference between the two sites. (See Table 15.)

When referred to unit weight of organic matter in the top decimetre of soil in each case, the rate of CO_2 production is even higher in the root duff than in the mull (32 mgm. per kgm. organic matter per hour compared with 25 mgm*). This investigation does not provide evidence supporting the hypothesis that the surface accumulation of organic matter in mor is the result of a slower rate of loss of organic matter, as measured by CO_2 production, as compared with the rate of loss in mull. It does however point to the possibility that the difference between crumb mull and root duff may lie in the course the decomposition takes rather than its speed. There is however one point which seems to have been overlooked in this work, especially in cases where the mor is due to coniferous species, for most of the measurements have been made over short periods in the summer and autumn months. This means that whereas in the good crumb mull the previous seasons litter has almost, if not completely, disappeared so that substrate for the respiration of microorganisms is likely to be at a minimum, in the conifer mor the old needles will have recently been shed and so provide new supplies of substrate for the micro-organisms. This may to some extent be counteracted by drying out of the mor layer during the summer months, thereby reducing considerably the activity of micro-organisms and consequently CO_2 production. It would therefore seem necessary to make measurements of CO_2 production in both mull and mor over a whole year at least.

Although the work of Melin (1930) was carried out in the artificial environment of the laboratory, his results seem to be of interest in connection with the relative rates of decomposition of organic matter, as measured by CO_2 production, in mull and mor. He collected the dead leaves, freshly fallen or still attached to the twigs, of a number of tree or shrub species and of a fern; in some cases samples were collected separately for the same species growing on more than one site. The soil types of the various sites are not stated. The dry litter was ground and 5 gm. quantities placed in culture flasks along with 100 gm. of pure quartz sand without sterilisation; water equivalent to 15% of the sand and 100% of the leaf material was added. The flasks were then inoculated with an emulsion of beech-maple forest soil and maintained at a temperature of 25°C. Each day the CO_2 accumulating in the flasks was blown out by CO_2 free air and collected. The results of the first experimental series indicate high rates of CO_2 production at the beginning of the experiment, followed by a gradual falling off during the first 27 days. In the second experimental series, using material from the same species as in the first experiment but from different sites and also from additional species, an initial high rate of CO_2 production was, as before, followed by a fall; but in a number of cases there was a subsequent increase; this occurred during the latter part of the first 36 days of the experiment. In both series, measurements of CO_2 production were made over the period 75-77 days from the beginning of the experiment, and in all cases the rate of CO_2 production was then relatively low. Table 16 shows CO_2 production per hour per kilogram of organic matter during the first and second periods, arranged in descending order of magnitude, and loss of organic matter after four months decomposition at 25°C., and has been constructed from Melin's results.

Whilst there is by no means a close parallel between percentage loss of organic matter and mgms. of CO_2 produced per hour per kilogram of original organic matter in the first or second periods during which CO_2 production was measured, there is a tendency for similar values in all these three properties to show parallel grouping.

The rate of CO_2 production in the initial period is generally considerably higher than the values obtained by Romell for soils when expressed on the same basis. In the later stages, however, the values are essentially of the same order as those obtained for soils by Romell. This seems to suggest that any measurements of CO_2 production by mull and mor should be carried out over at least a complete year before adequate comparisons can be made.

These quantitative observations on the organic matter content of, and CO_2 production by, mull and mor sites do not seem to give any support to the hypothesis that mor is the result of a slower rate of decomposition of vegetable debris, which has often been supposed to be the result of unfavourable climatic conditions. The apparent implication from this last concept that litter and its decomposition products are qualitatively similar but quantitatively

* There appears to be miscalculation in the original paper for the value is given as 15 mgm. ; it would seem that it should be 25 mgm.

TABLE 16

Species	Mgms. of CO_2 produced per hour per kg. of original organic matter during first 27 or 36 days	Percentage loss of organic matter after four months incubation at 25° C.	Mgms. CO_2 produced per hour per kg. of original organic matter during 75-77 day period	Species	Mgms. CO_2 produced per hour per kg. of original organic matter during 75-77 day period	Percentage loss of organic matter after four months incubation at 25° C.
Fraxinus americana	131.02	38.8	33.33	Betula papyrifera	67.50	—
Acer rubrum	91.33	—	26.25	Betula populifolia	56.25	44.9
Betula papyrifera	87.25	54.6	55.42	Betula papyrifera	55.42	54.6
Betula papyrifera	85.00	—	67.50	Acer saccharum	45.00	32.2
Pinus strobus	84.51	—	36.25	Betula papyrifera	43.75	—
Aralia nudicaulis	76.69	37.4	30.83	Pinus strobus	36.25	—
Betula papyrifera	68.12	—	43.75	Fraxinus americana	33.33	38.8
Betula populifolia	66.18	44.9	56.52	Vaccinium pennsylvanicum	31.25	—
Larix decidua	63.95	—	15.42	Aralia nudicaulis	30.83	37.4
Fagus grandifolia	52.13	29.7	20.00	Populus grandidentata	30.00	30.4
Acer saccharum	49.38	32.2	45.00	Picea rubens	28.75	30.4
Vaccinium pennsylvanicum	45.14	—	31.25	Acer rubrum	26.25	—
Picea rubens	44.81	30.4	28.75	Pinus strobus	23.33	29.5
Abies balsamea	43.89	26.2	15.00	Kalmia angustifolia	22.92	34.7
Pinus rigida	42.84	30.3	11.67	Pteridium aquilinum	21.25	24.3
Pinus strobus	40.93	29.5	23.33	Fagus grandifolia	20.00	29.7
Populus grandidentata	39.95	30.4	30.00	Larix decidua	15.42	—
Kalmia angustifolia	36.92	34.7	22.92	Abies balsamea	15.00	26.2
Fagus grandifolia	35.74	—	14.17	Fagus grandifolia	14.17	—
Fagus grandifolia	32.53	25.9	14.17	Fagus grandifolia	14.17	25.9
Pteridium aquilinum	28.24	—	8.75	Pinus rigida	11.67	30.3
Pteridium aquilinum	23.56	24.3	21.25	Pteridium aquilinum	8.75	—

different in the two soil types would, as far as present evidence goes, seem to be incorrect.

It may well be, however, that the quantitative observations referred to do not give an accurate picture, since measurements of CO_2 production have not been carried out over at least a whole year nor would they give any idea of the amount of breakdown of litter material which is subsequently followed by resynthesis of the breakdown products by micro-organisms into different complex materials. In laboratory experiments on isolated samples of litter, even when inoculated with soil, there is usually such a high ratio of litter to soil compared with conditions in nature that, unless there is repeated inoculation, it is possible that organisms playing an active part in the later stages may be absent as a result of the antibiotic effects produced by organisms active in the early stages of litter breakdown. This could result in a greater breakdown of litter in nature than under experimental conditions, provided other environmental factors such as temperature and moisture remained the same.

Since a number of observations give indications that the course of breakdown of litter may be more important than the rate of breakdown in determining the formation of mull and mor, some indication as to the possible reasons for the formation of mull and mor may be obtained by consideration of the changes which are going on in the organic constituents of litter, and the nature of the organic matter of these two soil types. Proximate chemical analyses have been used in many of the observations on these problems but, on account of the complexity and intractability of many of the materials involved, many of the observations are empirical in nature and difficult if not impossible to interpret.

Changes in the Cellulose of Litter During the Formation of Mull and Mor

Cellulose is one of the more well-defined substances present in the litter of plants and is generally readily attacked by a number of micro-organisms under suitable conditions. Yet there are reports of its occurrence in organic matter of vegetable origin which has been in contact with the soil for a considerable time. Although there have been a number of investigations on the cellulose contents of peat formed under conditions of water-logging (Waksman (1938)), it is not easy in the case of forest soils to find data allowing of a comparison of the cellulose contents of mull and mor. Waksman (1938) states that in humus which is still rich in partially decomposed plant residues, such as the F. and H. layers of the raw humus of a forest soil profile and highmoor peats, considerable quantities of true cellulose may still be present. Lutz and Chandler (1946) quote Watson's findings for the cellulose content of the various horizons of the soil from an old eastern white pine-eastern hemlock stand and also from a 75-year-old red spruce stand. The values for these soils may be compared with those obtained by Waksman, Tenney and Stevens (1928) for the cellulose content of the F and H layers of the soils of a northern hardwood-spruce forest in which hardwood leaves form the great bulk of the litter, a mixed forest of coniferous and deciduous trees, and a spruce forest with a heavy growth of *Hylocomium parietinum*. It is not possible to ascertain whether similar methods were used for cellulose estimation in both cases. The results of these two investigations are shown in Table 17.

Although the soil types are not specified it is perhaps of some importance that the relative percentages of cellulose remaining in the H layers increase from the northern hardwood-spruce forest to the spruce and eastern white-pine-eastern hemlock forest. In terms of absolute amounts, if the amount of organic matter completely decomposed in the various cases differs widely, there may have been very much greater relative destruction of cellulose in, say, the northern hardwood-spruce soil than in, say, the spruce forest soil than the figures lead one to believe. It would probably be very difficult, if not impossible,

TABLE 17

	Old eastern white pine-eastern hemlock			Red spruce			Northern hardwoods-spruce		Mixed hardwood conifer		Spruce	
	L	F	H	L	F	H	F	H	F	H	F	H
Cellulose as percentage of dry weight				16.3	10.6	4.17	9.44	2.56	7.28	3.84	9.62	5.64
Cellulose as percentage of ash-free material	13.7	8.2	5.2									

Note: The letters L, F, and H, refer to the three main humus layers, L signifying the upper "litter" layer, F the central or "fermentation" layer, and H the lower layer of "static" humus. These layers, however, cannot be precisely defined.

to determine cellulose loss on this basis for field samples.

Waksman (1938) expresses the opinion that as one proceeds along a soil series indicated by the terms podzol to serozem, which includes mull and mor sites, differences in the chemical composition of the organic matter of the various soils are evident; among these is the disappearance of cellulose, which is completely absent from the serozem whereas the humus of podzols is characterised by an abundance of cellulose.

These indications appear to be supported by the indirect experimental observations of Lindeberg (1944) who investigated the disappearance of cellulose from the litter of a number of plant species, under the influence of various species of *Marasmius*, by cultivating pure cultures of fungi in the laboratory on sterilised litter for 6 or 7 months. The cellulose was determined by the use of Schweizer's reagent. The results obtained for destruction of cellulose are shown in Table 18.

TABLE 18

	Marasmius species	Percentage of cellulose destroyed
Pine needles from Nåntuna Denmark	*M. androsaceus*	20.5
	M. chordalis	12.8
	M. fulvobulbillosus	19.3
	M. perforans	34.7
	M. peronatus	16.1
	M. putillus	17.5
	M. rotula	12.4
	M. scorodonius	35.6
Beech litter Uppsala Castle Park	*M. alliaceus*	84.9
	M. foetidus	86.6
	M. putillus	19.7
	M. ramealis	84.9
	M. scorodonius	67.2
Aspen litter from Fäbodarna, Bondkyrka	*M. epiphyllus*	76.6
Straw of *Glyceria maxima*	*M. graminum*	50.6
	M. oreades	91.3

Although the soil types on which the plants, from which the litter was collected, were growing are not stated and the experimental conditions are artificial, it is interesting to note that the pine litter, which quite possibly represents mor-forming material, shows considerably less reduction in cellulose content than the litter from the other species, which probably represents mull-forming material. Lindeberg deduces from this that the cellulose in pine needles seems more resistant to attack.

There is therefore a suggestion that the breakdown of cellulose is either less rapid or not so complete in mor as in mull. As far as is known attempts have not been made to ascertain the origin of such cellulose; i.e. whether it is derived from all the tissues of the litter or only from certain tissues, e.g. the vascular tissue, or whether it comes from roots present in the mor or is a product of microbial synthesis.

Changes in the Organic Nitrogen of Litter During the Formation of Mull and Mor.

Plant tissues falling to the ground as litter contain varying amounts of nitrogen, a considerable proportion of which is in the form of protein. If the litter protein is in an uncombined form it should be rapidly decomposed by the soil micro-organisms as is the case with other proteins added to soil, e.g. Lathrop (1916) who observed that in 240 days 79% of the nitrogen of dried blood added to arable soil was converted to ammonia nitrogen. Although Waksman (1938) states that when proteins are introduced into soil or compost they are attacked by a great number of organisms and changed to peptides, amino acids and finally ammonia, he also states that the proteins of certain plant residues, such as oak leaves and pine needles, seem to be highly resistant to microbial attack by reason either of their specific nature or of the formation of complexes with other plant constituents.

Analyses indicate that up to 98% of the total nitrogen in the soil may be in organic form. It would therefore appear to be important to try and ascertain whether any changes in the form or amount of organic nitrogen have occurred during the transformation of litter to soil organic matter and whether any such changes are characteristically different in mull and mor. Some observers have been concerned with changes in total nitrogen of the organic matter or in water-insoluble nitrogen of the organic matter, others have multiplied values for water insoluble nitrogen content by the arbitrary figure of 6.25 and the results have been deemed to represent protein content.

Bornebusch (1930) gives the results of analyses of the nitrogen content of samples from two mull sites and two raw humus sites. When the values for total nitrogen are recalculated as a percentage of organic matter for various parts of the profile the results shown in Table 19 are obtained.

The analytical methods used are not mentioned nor are the histories of the stands before the present crops. Weis (1932) states that in his work on podzols, analyses showed a uniform increase in the nitrogen content expressed as a percentage of the humus as depth increased; in some cases the nitrogen content was as high as 22% of the humus. His later results also showed an increase in nitrogen content of the humus with depth, but the increase in this case

TABLE 19

Beech Mull Site	Nitrogen Content as Percentage of Organic Matter	Spruce Mull Site	Nitrogen Content as Percentage of Organic Matter
Newly fallen leaves 1-5 cm.	1.47	Needle layer 1 cm.	1.72
Old leaf layer 0-5 cm.	1.70	Mull layer 0-2 cm. depth	2.22
Worm casts	1.91	Upper topsoil at 8 cm. depth	2.50
Upper mull soil 0-5 cm. depth	2.07	Topsoil at 22 cm. depth	2.00
Lower mull soil 5-15 cm. depth	2.14		
Topsoil at 35 cm. depth	2.00		
Beech raw humus site	**Nitrogen Content as Percentage of Organic Matter**	**Spruce raw humus site**	**Nitrogen Content as Percentage of Organic Matter**
Newly fallen leaves 2 cm.	1.45	Moss and needle layer 4 cm.	1.91
Old leaf layer 2 cm.	1.97	Upper raw humus 0-5 cm. depth	1.87
Upper raw humus 0-4 cm. depth	2.21	Middle raw humus 5-8 cm. depth	1.69
Middle raw humus 4-7 cm. depth	2.08	Lower raw humus 8-10 cm. depth	2.16
Lower raw humus 7-9 cm. depth	2.00	Leached sand 20 cm. depth	2.00
Leached sand 15 cm. depth	2.00	Soft pan 30 cm. depth	1.83
Soft pan 30 cm. depth	2.00		

is much smaller. The average values for the nitrogen content of the humus in the various horizons for 10 profiles from the Grinstead Heath Plain are shown in Table 20.

TABLE 20

	Raw Humus	Bleached Sand	Humus Hardpan	Iron Hardpan	Subsoil
Average values for nitrogen content as percentage of humus	1.81%	1.71%	2.52%	2.34%	2.52%

He associated this increase in nitrogen content with depth with the more rapid downward movement of organic colloidal constituents containing highest amounts of nitrogen. The differences between his earlier results and the later ones he put down to differences in the geological characteristics of the mineral soil. The results obtained for the nitrogen content of the organic matter in the various horizons of a Russian podzol by Zacharov are quoted by Waksman (1938) as set out in Table 21.

TABLE 21

	Soil Horizon	Percentage of nitrogen in humus
Strongly podzolised fine sandy soil at Vologda	A 1	5.65
	A 2	8.11
	B 2	8.38
	C 1	8.49

He also makes the generalisation that although the concentration of humus and nitrogen diminishes with depth in the case of podzol soils, the nitrogen content of the humus becomes greater in the lower horizons; and further that although the amount of humus found in chernozem soils is much higher than in the podzols (except for the surface organic layer of the latter) the nitrogen content of the humus is lower; in addition, the humus is more uniform in chemical nature and is distributed to a much greater depth.

A marked increase in the nitrogen content of the organic matter of a mor soil as distance from the surface increases is indicated by the results obtained by Watson (1930) quoted by Lutz and Chandler (1946), and set out in Table 22. The changes are shown in terms of "protein". The samples were obtained from an old eastern white pine—eastern hemlock stand in New Hampshire. These observa-

TABLE 22

Soil Horizon	"Protein" content as a percentage of organic matter
L	6.1
F	10.6
H	11.1
A 1	15.0
A 2	17.5
B 1	36.2

tions are in agreement with the findings of Weis. Nemec and Kvapil (1926) also made a number of estimations of variation in nitrogen content of the organic material with increasing depth in the profiles of soils on which pure and mixed stands of spruce, pine, oak, beech and other hardwoods, which had had various treatments, were growing. Although many of the stands seem to have developed something approaching a mor layer, only two pine stands and one spruce stand, with the possibility of two further spruce stands, are associated with mor and quoted as having leached layers. The history of the stands prior to the present tree crops is not given, but it seems possible that the soils were mull-type soils and therefore in many cases any nitrogen distribution characteristics due to the present mor-forming stands, and those of possibly previous mull conditions, may be masking each other. If the variation in nitrogen content of the organic matter of various horizons of the pine podzols are compared with those of mull soils on which young and mature hardwood stands are growing, although the percentage of nitrogen in the organic matter may be greater in the mull soils of the broadleaved stands, the relative changes in nitrogen content are not necessarily very different and do not seem to allow of characterisation of the two types of soil (see Tables 23-26). It is interesting to note that the organic matter of the lower layers of the soil from beneath a 100-year-old spruce stand, which had been recently clear-cut, contained very high nitrogen contents reminiscent of those obtained by Weis. It is difficult to understand what form an insoluble material containing such a high concentration of nitrogen could take, and it seems probable that the small concentrations of organic matter in the horizons, where the high nitrogen concentrations have been observed, have led to analytical inaccuracies. It is also difficult to understand why the nitrogen distribution in the profile of the spruce stand, examined soon after clear-felling, should show such differences in comparison with the nitrogen distribution in the profile of the mature spruce stand of which it had formed a part and which in turn resembles more closely, in nitrogen distribution in the profile, the soil of the clear-cut area after it had been clear-cut for a period of two years. Some of the findings obtained by Nemec and Kvapil (1926) are given in Tables 23 to 26.

The results obtained by Nemec and Kvapil also indicate high nitrogen contents in the organic matter in the lower horizons of a number of other soils. Thus a young ash stand was growing on a soil which, at a depth of 55-75 cm., contained 0.33% organic matter and had a nitrogen content which is given as 9.33% but which would seem to be 37.58%. A pure beech stand was growing on a soil which at a depth of 6-16 cm. contained 0.19% organic matter, having a nitrogen content given as 12.65% but which would seem to be 32.63%. Similarly, a spruce polewood was growing on a soil, on to which oak leaves had blown, which at a depth of 60-80 cm. contained 0.19% organic matter, having a nitrogen content given as 10.73% but which would seem to be 40.00%. Although it is not stated it seems likely that these last-mentioned soils are mull soils.

From the data available it would seem that in many soils there is a considerable increase in the concentration of nitrogen in the organic matter of the soil with increasing depth in the soil. These increases in nitrogen content of the organic matter with depth do not seem to be especially characteristic of either mull or mor sites, and it seems that the extremely high values are likely to be artifacts in both cases.

The accurate interpretation of such findings requires the compilation of a complete nitrogen balance sheet for the various soils; this would be extremely difficult if not impossible to obtain. For example, it would be necessary to know the amount of original litter and its nitrogen content from which the soil organic matter and its associated nitrogen content arose. In addition it would be necessary to know whether or not, in the case of both mull and mor, much of the original organic nitrogen of the litter has been completely decomposed, in the sense that cellulose can be completely decomposed to carbon dioxide, since there is often a relative increase in the concentration of insoluble organic nitrogen during the decomposition of litter, i.e. is the organic nitrogen of the litter resistant to breakdown in some cases, whilst in other cases a new and resistant microbial nitrogenous material is built up from the decomposition products. Roots may also absorb considerable quantities of nitrogen released during the decompositon of litter. There is little or no evidence by which such questions can be answered. Müller (1879) was of the opinion that the black fungal hyphae developing abundantly in beech mor were almost indestructible; the protein of the protoplasm of such hyphae may have disappeared soon after death leaving a chitinous envelope which, if composed of glucosamine units, may represent nitrogen stable to breakdown under mor conditions and built up from the decomposition products of the organic nitrogen compounds initially present in vegetable debris.

The problem may also be complicated by the microbiological fixation of atmospheric nitrogen during the decomposition of litter. In 1897 Henry gathered the dead, but not yet fallen, leaves from young oak and hornbeam trees and dried them first at room temperature and then at 100°C. The leaves of each species were exposed in the open air, in

Table 23

82-YEAR-OLD PINE STAND WITH SPRUCE UNDERGROWTH

Horizon	Percentage of nitrogen in organic matter	Percentage of organic matter in dry soil
Humus layer 0–6 cm.	2.60*	36.13
Black infiltrated layer 6–20 cm.	2.88	8.05
Grey leached sand 20–40 cm.	1.25	0.08
Hard pan 40–42 cm.	1.42	3.01
Light yellow subsoil 42-75 cm.	3.05	0.36
Sand 75–85 cm.	2.16	0.37

Note: *It would appear that this value should be 2.33.

TABLE 24

100-YEAR-OLD-SPRUCE STAND AFTER CLEAR-CUTTING

Horizon		Percentage of nitrogen in organic matter	Percentage of organic matter in dry soil
Humus layer 0–2 cm.		3.07	24.69
Vegetal layer 2–12 cm.		3.88	3.60
Loamy sand	12–22 cm.	3.59	1.14
	22–37 cm.	11.34	0.52
	37–52 cm.	15.66	0.60
	52–62 cm.	25.71	0.28
	62–72 cm.	31.76	0.17
	72–87 cm.	29.16*	0.12

Note: *It would appear that this value should be 45.83.

TABLE 25

75-YEAR-OLD CLOSED PURE SPRUCE STAND

Horizon		Percentage of nitrogen in organic matter	Percentage of organic matter in dry soil
Humus layer 0–4 cm.		2.58	50.42
Vegetal layer 4–6 cm.		2.10	9.80
Loamy sand	6–16 cm.	3.35	2.83
	16–30 cm.	6.30	0.92
	30–45 cm.	7.39 (1)	0.69
	45–60 cm.	7.28	0.59
	60–75 cm.	8.11 (2)	0.53
	75–90 cm.	10.66 (3)	0.33

Note: It would appear that 1, 2 and 3 should be respectively 7.29, 9.06 and 10.61.

TABLE 26

90–100-YEAR-OLD HEAVILY THINNED BEECH, MAPLE, ASH AND HORNBEAM

Horizon		Percentage of nitrogen in organic matter	Percentage of organic matter in dry soil
Humus 0–4 cm.		4.29	25.65
Vegetal layer 4–15 cm.		5.07	10.45
Mineral soil	15–30 cm.	16.92	1.30
	30–45 cm.	10.11	0.92
	45–60 cm.	5.56	1.04
	60–75 cm.	4.78	1.35
	75–90 cm.	4.43*	0.79

Note: *It would appear that this value should be 10.12.

TABLE 27

	Percentage of nitrogen in leaves at beginning of experiment	Percentage of nitrogen in leaves after 1 year	Percentage loss of organic matter	Percentage of nitrogen in terms of original dry weight	Nett gain in percentage of nitrogen
Oak	1.108	1.923 (limestone)	21.63	1.508	0.400
Hornbeam	0.947	2.246 (sandstone)	23.01	1.727	0.780

	Percentage of nitrogen in leaves after 2 years	Percentage loss of organic matter	Percentage of nitrogen in terms of original dry weight	Nett gain in percentage of nitrogen
Oak	1.73 (sandstone)	29.64	1.22	0.11
Hornbeam	2.15 (limestone)	28.6	1.53	0.58

zinc boxes protected from emanations from the soil and from sources of ammonia, the leaves lying in contact with both limestone and sandstone blocks. Analyses of the litter at the commencement of the experiment showed them to contain 1.108% nitrogen in the case of oak and 0.947% nitrogen in the case of hornbeam. The nitrogen content was measured after one year. After lying for 18 months the material in each of the two remaining boxes was inoculated with 50 gm. of fine forest soil whose water and organic matter content were known and then allowed to lie for a further six months (two years in all) before nitrogen estimations were carried out. The results are given in Table 27.

Both the oak and the hornbeam leaves were black but still perfectly recognisable at the end of the experiment, which seems to demonstrate that comparatively large amounts of atmospheric nitrogen are fixed during the decomposition of forest litter although there are difficulties associated with such experiments which may throw some doubt on the validity of the results. Nemec and Kvapil (1924) demonstrated that in many cases isolated samples of the various horizons of forest soils could, in the presence of nutrient material and under very artificial laboratory conditions, fix considerable quantities of atmospheric nitrogen during a period of 30 days. It has been demonstrated that bacteria can fix atmospheric nitrogen, and it seems reasonably certain that a number of soil fungi can fix atmospheric nitrogen even though in small quantities. Foster (1949) has discussed the present position of this problem. The soil fungi which seem to be most active in fixing atmospheric nitrogen, but which are much less active than the nitrogen fixing bacteria under experimental conditions, are those forming mycorrhizal associations with members of the Ericaceae, although the wood and litter destroying fungi do not seem to have been investigated from this point of view. The use of isotopic nitrogen (N 15) in recent times has made it even more certain that some fungi at any rate are able to fix elementary nitrogen. The biological fixation of atmospheric nitrogen may therefore be of considerable importance in any considerations of nitrogen changes during the decomposition of vegetable debris.

It seems reasonable to suppose that there is a relative increase in the nitrogen content of soil organic matter compared with the nitrogen content of the litter from which it was formed. Depending on the extent, if any, of the fixation of atmospheric nitrogen under natural conditions of litter decomposition, this relative increase in the nitrogen content of soil organic matter may also represent an absolute increase in nitrogen content, or it may indicate that the nitrogenous constituents of litter are more resistant to decomposition than some of the non-nitrogenous constituents; it may also represent nitrogenous material, relatively resistant to decomposition, resynthesised from the decomposition products of the nitrogenous material of litter.

Since a considerable part of the nitrogen added to the soil in litter is in the form of protein it becomes important to know why the nitrogenous compounds of the soil appear to be relatively more resistant to breakdown than some of the non-nitrogenous materials of the litter. If the nitrogenous constituents of litter are resistant to decomposition then it should be possible to detect the presence of considerable amounts of protein in soil organic matter.

It has been deduced from various investigations that proteins are present in soil, but it does not seem to have been possible up to the present to isolate free proteins from soil. The evidence for the occurrence of proteins in soil has been obtained largely from analyses of soil hydrolysates, but whether the protein material whose presence appears to have been demonstrated in soil is a constituent of living

material in the soil, or represents inanimate material in the soil derived more or less directly from vegetable debris, has not been determined. In the first instance amino acids were isolated by chemical methods from the hydrolysates, e.g. by Suzuki (1906-8) and Robinson (1911), Schreiner and Shorey (1910), and Kojima (1947b). In other investigations (Kojima (1947a)) the Van Slyke manometric and ninhydrin methods have been used. In some recent investigations chromatographic methods have been used. By this method Bremner (1950) detected the presence of 20 different amino acids in hydrolysates from each of ten soils studied, but was unable to detect the presence of free amino acids in concentrated cold aqueous extracts of these soils, although in further work on acid peats (Bremner (1952)) very small amounts of free amino acids were detected on chromatograms of aqueous extracts. Dadd, Fowden and Pearsall (1953) also detected small amounts of free amino acids in the fluid expressed, by means of a tincture press, from a number of soils covering a wide range of acidity and humus types. The results obtained suggested that a relationship may exist between the reaction of the soil and the free amino acid content of the expressed fluid. The more acid the soil the greater the total concentration of free amino acids in the expressed fluid. There is also some evidence of seasonal differences in the free amino acids present in the expressed fluid. There does not, however, appear to be a correlation between the amino nitrogen detected as free amino acids and the total nitrogen content of the soils expressed as a percentage of their dry weight. When amino acids, including those observed by Dadd, Fowden and Pearsall in fluids expressed from soils, are added to solutions with which cultivated soils are being perfused (Quastel and Scholefield (1949)) they are rapidly nitrified. It therefore seems probable that only very small amounts, at the most, of the amino acids detected in soil hydrolysates represent amino acids in the free state in the untreated soil. These investigations indicate that about 37% of the nitrogen in the soils examined was liberated as amino nitrogen, but such values are probably minimal on account of destruction of amino acids during hydrolysis. Soil hydrolysates contain large amounts of ammonia and it is thought that some of this is derived from acid amide residues of protein material; so that it seems that at least 50% of the soil nitrogen is in the form of protein. Kojima places the value as high as 75%.

There have not been many investigations of the amounts of the various amino acids yielded by hydrolysates from different kinds of soil, and still fewer in which mull and mor have been compared in this respect. Bremner (1950) found that chromatographic analyses of the acid hydrolysates of ten different soils, which did not, however, seem to include raw humus, indicated that the protein materials of these soils are similar in their amino acid composition. Davidson, Sowden and Atkinson (1951) and Parker, Sowden and Atkinson (1952) extracted organic material from a podzol and from a prarie soil by the use of reagents such as a sodium hydroxide, and compared, by chromatographic methods, the amino acid constituents of the fractions obtained. Although the methods used did not allow of the determination of the identity of the whole of the nitrogen of the soils, the final results obtained, for the nitrogenous material extracted, do not indicate distinctive differences in the amino acid composition, either between the soils or between the various fractions isolated from each soil. Bremner (1952) has also demonstrated the presence of amino acids in considerable amounts in acid hydrolysates (using 6 N hydrochloric acid) of various humic acids, as usually prepared, both before and after purification.

Some time ago Waksman (1938) expressed the opinion that a knowledge of the chemical composition of plant and microbial proteins is highly essential for an understanding of the decomposition of plant residues and the formation of the nitrogenous constituents of humus. This view is emphasized by the isolation and detection of amino acids in soil. As yet, however, there has been no attempt to compare the composition of the protein material present in litter falling on a soil and that of the protein material of soil organic matter, and therefore such comparisons have not been made for mull and mor.

It is therefore evident that very little is known of possible differences between the organic nitrogen of different soil types; but in view of the observations on the relative increase in the nitrogen content of soil organic matter with depth, it would seem to be important to know the nature of this apparent resistance of the nitrogenous material to decomposition, especially since a considerable proportion of it appears to be protein in nature. If the original nitrogenous material of the litter remains unchanged in the soil it would seem that at least the protein fraction must be united to some substance which renders it relatively immune to decomposition. If on the other hand the organic nitrogen of the litter is largely decomposed and subsequently resynthesized into structural or extracellular microbial organic nitrogen, then the extracellular organic nitrogen especially, may have quite different properties compared with the organic nitrogen of the litter. The microbial proteins may be united, especially on the death of the micro-organisms concerned, with materials rendering them resistant to decomposition. Morton (1951) has demonstrated that extracellular "organic" nitrogen compounds are formed in culture

by various soil fungi growing on inorganic sources of nitrogen. The nature of these organic nitrogen compounds is not known, but they seem to increase in amount with time and may reach 10 or 20% of the originally available nitrogen and then remain constant. These substances appear to contain amino nitrogen and to be stable in the presence of the organisms producing them. It seems possible that materials of this kind may form at least part of the nitrogenous materials which are resistant to decomposition in soils. On the other hand Waksman (1938) points out that when plant and animal residues are added to soil they become subject to various chemical and other influences which may result in interaction between amino acids and carbohydrates, tannins and proteins, and lignins and proteins; thus the proteins of certain plant residues such as oak leaves and pine needles seem to be highly resistant to microbial attack either by reason of their specific nature or of their formation of complexes with other plant materials. Bremner (1951) states that up to 30% of soil nitrogen is resistant to acid or alkaline hydrolysis, and this has been taken to indicate that much of it is non-protein in nature and has led to the suggestion that part of the organic nitrogen of soils is in the form of heterocyclic compounds. It is clear that much further work is necessary on this aspect of the problem.

Another aspect of the decomposition of complex organic nitrogenous materials in soil which may indicate differences between mull and mor, concerns the supply of comparatively simple, perhaps largely inorganic, forms of nitrogen for the growth of higher plants. In an attempt to ascertain how the combined nitrogen of litter is set free as mineralised nitrogen in the soil, Hesselman (1925) ground up freshly fallen litter and mixed it in one case with strongly nitrifying mull and in the other with a strongly ammonia-forming mor. In both instances the litter powder and infecting soil were mixed in two proportions: 9 pts. powder to 1 pt. soil and 9 pts. soil to 1 pt. powder. Nitrate and ammonia estimations were made on the mixtures after storing for three months in a moist condition. Analyses were also carried out on samples at the commencement of the experiment. The results showed that there are only relatively small amounts of nitrogen in the fresh litter at the beginning of the experiment which can be extracted in the form of ammonia, and after three months both the extractable ammonia and nitrate are very small in amount.

Hesselman (1917) also investigated the mobilisation of nitrogen in terms of ammonia and nitrate formation, expressed as percentage of total nitrogen of the samples, after storing for three months, in different soil types. He extracted the ammonia with $\frac{N}{10}$ HCl and the nitrate with distilled water. He demonstrated that, in general, in conifer raw humus the process only proceeded as far as ammonia formation, whereas in mull soils nitrates were formed even in decidedly acid soils. Even so it would seem that only a small proportion of the nitrogen is mobilised even under these relatively good conditions.

The influence of various factors on the production of ammonia and nitrate was considered by Hesselman (1925). Concerning reaction in terms of pH units he found that whilst there is a general connection between mobilisation of nitrogen, as measured by NH_3 and NO_3 production, and soil reaction, when soils are grouped in half pH units, with an optimum of pH 4.5—4.9 for ammonia formation and pH 5.5—6.9 for nitrate formation, it should be noted that within one and the same pH group there are great variations in the amount and intensity of nitrogen mobilisation. Thus soils having reactions as acid as pH 3.9 and 4.0 may have the high nitrate coefficients of 2.18 and 3.0 respectively, whereas soils with reactions nearer neutrality may have very low nitrate coefficients. Romell (1931) also considered the relationship between soil reaction and nitrification. He found nitrifying organisms in soils to be active over a very wide pH range, so that inhibiting acidity seems to occur only at such an acid reaction as hardly or just barely occurs in any forest humus type in the region studied; he finds it questionable whether acidity as such can anywhere be the inhibiting factor, for even in the lowest pH class of 3.0 and below there are samples showing definite nitrification. Comparing the relationship between reaction and nitrification for different soil types, his observations showed hardly any distinct correspondence between reaction and nitrification in the case of mull soils, whereas for mor there is a clear correlation between reaction of the sample and intensity of nitrification which is positive over all the range represented. He concludes that it seems unlikely that reaction is the inhibitory factor where nitrification does not occur.

Hesselman (1925) could find no very close relation between lime content of the organic matter and nitrogen mobilisation. He also investigated the effect of added lime, in the form of finely divided calcium carbonate, on nitrogen mobilisation in mor. He observed that in a fully stocked spruce forest with pure moss covering, the addition of lime increased nitrate formation in the decomposition (F) layer, but did not change the total nitrogen mobilised, i.e. merely altered its form. In the humus salt (H) layer the nitrogen could not be mobilised to the same extent by liming and inoculation, so that even though the reaction became quite

neutral the mobilisation of nitrogen remained weak. Mor from old slow-growing spruce forests showed no nitrate formation after liming until inoculated with nitrifying soil; even then nitrification was weak and ammonia formation was scarcely affected by the addition of lime. In these old spruce forests nitrogen mobilisation is very weak and is not affected by liming, even when inoculated. The mor of soils on which a good proportion of birch were growing showed active ammonia formation, and on adding lime active nitrification occurs. Inoculation brings about nitrification but it is greater when lime is added. Hesselman concluded that the reason for feeble nitrogen mobilisation in old mor is not simply due to the acid reaction or deficiency of buffer salts; he suggests that the nitrogen compounds may be difficult of access by nitrogen mobilising organisms. Recent work by Johnston (1953) on H and B horizon material from a podzolised gravel supporting a mature pine stand, supports Hesselman's findings; for he obtained very little demonstrable change in these materials when environmental factors were varied or mineral nutrients were added.

Nitrogen mobilisation in the different organic horizons of the mor was also investigated by Hesselman (1925). He found nitrogen mobilisation (using the storage method) to be livelier in the decomposition layer than in the humus salt layer. He found it difficult to understand why the liveliest nitrogen mobilisation should take place in the decomposition layer, which is formed directly from the litter whose nitrogen is difficult to mobilise. He considers it possible that the mobilisable nitrogen of the decomposition layer is derived from micro-organisms living therein, and that its origin may be traced to nitrogen assimilated directly from the atmosphere. Whilst there are considerable differences between the often considerable ammonia, and possibly also nitrate, liberation in the decomposition layer and the often negligible liberation in the humus salt layer of the mor profile, in a mull profile there are but slight differences between the samples from different depths. Similar differences apply to the response of the various samples to inoculation with nitrate forming soil. Hesselman concludes that the nitrogen remaining in the soil organic matter becomes more closely bound as it becomes older, but much more so in the case of mor than in the case of mull. He also observed mor profiles in which nitrogen mobilisation was the same or higher in the humus salt layer than in the decomposition layer; this occurred either in very young stands or in stands of conifers strongly mixed with broad leaf trees such as birch and aspen. The age of the stand also seemed to affect nitrogen mobilisation, for in old stands with raw humus the rate of nitrogen mobilisation is often very low, both in the layer of decomposition and in the humus salt layer. The very different nitrogen mobilisation in the decomposition layer compared with the humus salt layer cannot be ascribed to differences in reaction.

A number of similar observations were made on nitrogen mobilisation in various American soils by Romell (1931). He pointed out that in all the earlier work perhaps the most consistent difference between mull and mor, which can be ascertained by laboratory tests, has been found in the nitrifying activity. Using the technique used by Hesselman he recorded nitrifying activity in various American soils, and found a considerable percentage of nitrifying samples within every type of humus layer including the most extreme duff types. In all types except the fibrous duff he obtained samples having a very high nitrifying activity, although within the two most pronounced types of duff several samples failed to show any nitrifying activity. He divided the humus into various kinds of duff: root duff, leaf duff, greasy duff and fibrous duff; as the scale is descended there is a decreasing nitrifying activity and also a much smaller nitrifying activity in the H layers than in the F layers, which is in agreement with the earlier findings of Hesselman. Romell observes that his experiments demonstrate a much wider distribution of active nitrifying organisms in the organic layers of forest soils than has been recognised hitherto.

Romell (1935) discusses this problem again and states that as far as he is aware the differences between the nitrogen regimes of mull and mor have not been really accounted for by previous theories. He points out that according to all the indications the difference lies not merely in the presence or absence of nitrification but in the level of available nitrogen, and that the usual lack of nitrification in mor seems to be due less to acidity than to other factors. Weis (1924) observed the strongest nitrification in the soil of Danish beechwoods from a district with very acid mull on an acid soil. Romell considers that the simplest explanation is that nitrification is governed principally by the level of available raw material (ammonia); this is perhaps indicated by the generally greater luxuriance of both forest growth and ground vegetation on mull soils. Following up this argument Romell is of the opinion that excess nitrogen (for ammonia production and nitrification) occurs when the C/N ratio of the decomposing material has reached a critical, though variable, value. This hypothesis does not, however, seem to agree with the observations that the nitrogen content of the soil organic matter increases with depth on both mull and mor sites, yet nitrogen mobilisation is markedly less in the H layer than in the F layer.

Romell (1935) also considers the striking effect of various forestry measures on nitrogen mobilisation

in mor. He links this up with the very high nitrogen mobilisation in stored samples of mor which, except for the most acid samples from the porrest types, is higher than samples from better types of humus layer, including mull. He finds these observations hard to reconcile with the ecological character of the corresponding soil types. Since tests frequently fail to demonstrate the presence of ammonia in fresh mor samples, yet culture experiments indicate the presence of a very good supply of available nitrogen in isolated lots of mor, there must be a real increase in nitrogen mobilisation as compared with the natural state. Romell considers the answer to be in some factor which affects mor samples more than mull samples, and points to the structural differences between mull and mor as clearly indicating one factor; for disruption of natural connections inevitably accompanies the sampling of mor, i.e. oisruption of roots, mycorrhizal or other hyphae. In the incubating sample, and the mor after forestry operations, much of this material will be in a state of necrobiosis, with the natural conditions of competition radically disturbed, so that material formerly unavailable as food for micro-organisms now becomes available. The effect is one of green manuring with material rich in nitrogen, so that the abnormally strong nitrogen mobilisation observed in the isolated sample of mor can be expected. Mull samples, on the other hand, being naturally worked by soil animals and consisting of loose crumbs or grains, would not be expected to be affected to anything like the same extent by sampling. Thus one may deduce that the dead nitrogenous material of mor is considerably more inert and resistant to mobilisation than the nitrogen of mull.

Pearsall (1938 and 1952) has also commented on the apparent differences in the nitrogen regimes in mull and mor. He points out that a striking feature of mor in Britain is the normal absence of nitrates, as demonstrated by field tests, quite apart from their absence from more normal soils in spring when there is rapid utilisation. The absence of nitrate in mor is considered to be due not to oxygen deficiency but to a very low rate of formation, even though nitrate-forming organisms appear to be present in some types of more at least, since when these are disturbed rapid nitrate formation takes place. The negligible amounts of nitrate present in mor under natural conditions is ascribed to an important difference in metabolism, which appears to be associated with a lower rate of conversion of nitrogenous material in mor soils. There are therefore indications that the nature of the organic nitrogen, other than that present in living cells, in mull and mor may be an important source of difference between mull and mor; and that this point requires further investigation.

Changes in the Lignin of Litter Added to Soil

In the section in which litter composition was considered it was pointed out that the term lignin does not yet apply to a substance which can be isolated in a pure and unchanged state and consequently quantitative methods of estimation cannot be developed. Attempts have been made to estimate so-called lignin in litter and soil organic matter without due regard to the fact that the substance obtained by a certain procedure from soil organic matter may be the same as that obtained from litter by the same procedure or it may be different, the material having undergone more or less profound changes during incorporation in the soil or being of entirely different origin. There are no criteria which allow of a decision to be made in this matter.

In spite of these circumstances the opinion is expressed from time to time that lignin is the most resistant constituent of litter and therefore tends to accumulate in the organic matter of the soil. Falck (1930) ascribed mor formation to the activity of the brown rot fungi in bringing about a progessive accumulation of acid humus from the undecomposed lignin of the litter. But Romell (1935) was unable to find the remains of undecomposed lignified tissue from litter even in the heaviest mor. Falck (1930) considered that in the same way as fungi are thought to decompose woody tissue in two different ways, litter can also be decomposed in different ways. He termed one of these "corrosion" where lignin and cellulose are decomposed almost simultaneously, and the other "destruction" where cellulose is decomposed and lignin remains unattacked, and in the case of soil this lignin accumulates; this accumlating lignin-rich material is only attacked with great difficulty by higher fungi. He contends that the two processes can be related to the distribution of fungi in the field—that in deciduous woods basidiomycetes are present which are able to decompose lignin, but only very few cellulose decomposing higher fungi are known to be present; whereas in pure coniferous woods not many lignin-destroying fungi are known to be present so that lignin accumulates and raw humus forms. In addition to Romell's (1935) criticism, the fact that fallen branches and stumps of trees, which should contain heavily lignified tissue, readily rot even when the associated litter forms raw humus, does not agree with Falck's hypothesis. A similar state of affairs is found in the case of the dead woody stems of *Calluna vulgaris* on the raw humus of a *Calluna* heath. For Falck's hypothesis to be valid it would have to be demonstrated that the lignin of raw humus-forming litter has different biological properties compared with the lignin of woody stems and mull-forming litter.

In the present inadequate state of knowledge concerning lignin it would not be wise to put too much

emphasis on the function of lignin in a hypothesis concerning mor formation. Perhaps the most appropriate comment on the position of lignin in this matter is that of Waksman (1938)—"So many formulae have been proposed to explain the chemistry of lignin and of "humic acids" and so much importance has been attached to them that one is surprised to find that they are largely illusory."

The Identity of Soil Organic Matter in Relation to the Formation of Mull and Mor.

Although from the above considerations there are possibly indications of differences in the fate of certain organic constituents of litter, depending on the soil type with which they are associated, it seems desirable to consider what information is available concerning the nature of soil organic matter and the processes by which it is formed, other than those which have already been considered.

In the processes of the transformation of plant debris into soil organic matter it may well be that, apart from the possibility of differences in the composition of the litter of one species as compared with another, some fractions of the debris undergo certain changes under some circumstances and different changes, or perhaps no change at all, under other conditions. The changes likely to occur in the case of the various fractions include complete breakdown to carbon dioxide, or partial breakdown followed by synthesis of breakdown products, by micro-organisms, into substances, perhaps differing according to soil type, quite different from those from which they were derived. Other fractions of the litter may or may not be changed by the activities of micro-organisms or by purely chemical processes which up to the present have not been clearly defined. Some or all of these processes may occur in different types of soil and under different environmental conditions. The products, some of which may be relatively resistant to further change, at any rate under some environmental conditions, of the processes undergone by vegetable debris in any particular soil, are often termed "humus". Since there are a number of different starting materials in plant debris, and there is also the possibility of variation in the transformations these various materials may undergo under different conditions, it seems unlikely that "humus" will be a single substance and probable that the "humus" of different types of soil, and of mull and mor, may exhibit distinguishing characteristics, especially since there are such striking differences in the distribution of organic matter in relation to the mineral soil. Whilst there have been many attempts at the chemical characterisation of "humus", or perhaps more strictly soil organic matter since there has been no differentiation between living and dead material, there have been comparatively few attempts to elucidate the nature of the processes by which "humus" is derived from plant debris.

In the case of soil organic matter, as with litter, chemical characterisation of the constituents presents considerable difficulties. Most investigators have used organic material extracted from soil by the use of alkali. Such methods do not allow of differentiation between material extracted from unchanged and altered plant residue in the soil, or between living and dead organic matter in the soil. In this connection it has been claimed (Gortner (1916)) that material having similar characteristics can be obtained by extraction of unchanged vegetable material with alkali solutions as is obtained by similar treatment of soil. In addition, as Forsyth (1947) has pointed out, a number of plant materials, such as polyuronides, are also soluble in alkali and insoluble in acids and organic solvents, i.e. manipulations used in the extraction of soil organic matter. A number of writers have also hinted that they consider "humus" formation in the soil to be only a continuation of processes already started in the dying leaf.

Insolubility in acetyl bromide has been used for the characterisation of "humus", but there is considerable difference of opinion regarding the significance of such an empiricism. Whilst Waksman (1938) has little use for such a procedure it has been used even in recent times by Wittich (1943) and Mattson and Koutler Andersson (1943) for the detection of "humified" material. Wittich considers that the differentiation of "humified" material in litter from lignin by the use of acetyl bromide gives a more reasonable value for the content of so-called lignin in litter than methods normally used for lignin extraction. Franz (1950) has expressed the opinion that humus consists of the excreta of soil animals containing, as well as food residues which themselves contain material insoluble in acetyl bromide, new substances which are insoluble in acetyl bromide and which have been synthesized in the alimentary canal of soil animals.

The organic matter extracted from soil by the use of alkali has been subjected to various types of manipulation. In some cases the extracted material has been fractionated by various treatments, and the non-crystalline materials, for which there are no precise criteria of purity, thus obtained have been given distinguishing names; some investigators have claimed that they are definite chemical compounds but this has not been conclusively demonstrated. In other cases a number of definite chemical compounds have been isolated from these fractions, e.g. Schreiner and Shorey (1910), Kojima (1947) and Forsyth (1947 and 1950). If these compounds such as amino acids, uronides, etc. were present in the soil in the free state they would almost certainly

undergo changes due to the activity of micro-organisms. It has therefore been suggested that the organic material known as humus consists of one or more complexes which, by various treatments, can be split into a number of simpler compounds of definite chemical composition. It is important to bear in mind that whatever the nature of the materials obtained by such manipulations they only represent a fraction of the total organic matter of the soil and do not completely characterise it; in addition there is no differentiation between materials coming from changed and unchanged plant debris. So far such investigations of soil organic matter do not seem to have provided any indications of fundamental differences between mull and mor.

Uronic Acids in Soil Organic Matter

From time to time the uronic acids of soil organic matter have attracted attention. Attempts have been made to apply directly to the organic matter of soil the fact that uronic acids are quantitatively decarboxylated when boiled with 12% hydrochloric acid. The carbon dioxide evolved from soils as a result of such treatment was considered to be derived from uronic acids, especially as no carboxyl groups other than those of uronic acids were known which would yield more than small amounts of carbon dioxide with similar treatment. From such observations there were indications that in some cases a very large proportion of the soil organic matter was composed of uronic acid complexes. Uronides have only been isolated from soils in small quantities (Forsyth (1947 and 1950)) which in no way correspond with the amounts present as indicated by measurements of carbon dioxide evolved on treatment of soils with 12% hydrochloric acid. The reason for the discrepancy may be indicated by the observations of Tracey (1948) that substances other than polyuronides yield considerable amounts of carbon dioxide when treated with 12% hydrochloric acid, e.g. alloxan, ascorbic acid, hypoxanthine, urea. Bremner (1951) has discussed the non-specificity of this reaction for polyuronides. Should uronic acids constitute a fraction of soil organic matter it is difficult to understand how they resist decomposition unless they occur in the form of complexes.

A number of soil bacteria have been shown to produce mucilaginous polysaccharide materials, some of which contain uronic acids (Walker and Warren (1938), Cooper, Daker and Stacey (1938), Schlüchterer and Stacey (1945)). It is well known that the inorganic particles of soil can become aggregated into water stable structures termed crumbs, and it is thought that at least part of the cementing material consists of polysaccharide material produced by micro-organisms. Geoghegan and Brian (1948) showed aggregation of soil particles to be brought about by bacterial and fungal polysaccharides produced in culture. Martin (1946) observed that a polysaccharide synthesised by an unidentified soil bacterium and containing 14.4% of uronic acids, the highest uronic acid content of the polysaccharides used, was a more efficient aggregating agent than other bacterial polysaccharides of lower uronic acid content. Moreover, he found that these polysaccharides were fairly resistant to microbial decomposition although there were always some organisms capable of utilising them. The maintenance of crumb structure in a soil would seem to require that the cementing materials of the aggregates should not be easily decomposed. Since aggregates of mineral particles cemented by organic material are characterstic of mull and not of mor it would seem possible that there might be a detectable difference in the type or amount of polysaccharides in the organic matter of mull and mor, but there has not yet been sufficient investigation of the subject to demonstrate this. If such differences were demonstrated, and it could be demonstrated whether they arose from dead or living plant material in one case and by bacterial synthesis in another, it would still remain to discover the reason for such differences. Some kinds of vegetable debris may be unsuitable for the production of polysaccharides under some conditions, e.g. mor-forming conditions, or polysaccharides may be produced and rapidly destroyed under such conditions; in any case it is clear that such polysaccharides can only be the symptoms or results of more fundamental differences and not the cause.

Similar comments also apply to a consideration of the considerable amounts of chitin of fungal origin in soils, and presumably produced from constituents of plant debris, especially as it has now been shown that there are soil micro-organisms and animals such as snails which can readily break down chitin. Skinner and Davis (1937) used organisms from soils, none of which seem likely to have been mor sites, and found that the most effective organisms were bacteria, although some fungi showed slight ability to decompose chitin. The authors state that there is evidence that chitin of fungal and animal origin is similar. Stanier (1947) isolated a non-fruiting chitin-decomposing myxobacterium (*Cytophaga johnsonae*) from soils by means of aerobic chitin enrichment cultures. This organism is considered to be a common member of the chitin-decomposing microflora of soil. The properties of two strains of chitinoclastic bacteria, one belonging to the genus *Corynebacterium*, isolated from garden soil have recently been described by Veldkamp (1952). The possible differential influence on mull and mor of the chitin-decomposing activities of such organisms does not seem to have been investigated.

It must therefore be concluded that although it is likely that the changes undergone by plant debris vary according to the nature and origin of the plant debris and the environmental conditions under which the changes occur, there is comparatively little precise information concerning the nature of these transformations in the formation of soil organic matter and their possible causal relationship to soil type.

Changes in the Inorganic Constituents of Litter in Relation to the Formation of Mull and Mor.

Plant debris falling on the soil contains mineral material and it is likely that if the properties of this mineral material, due perhaps to the manner of its combination with organic material in various kinds of vegetable debris, differ from a nutritional point of view, especially in relation to different soil types, then it may exert a marked influence on plant growth and the nature of soil organic matter in mull and mor.

In the case of litter decomposing under the conditions present in a mull soil, the resulting soil organic matter is mixed with mineral material derived from the parent mineral material of the soil, and it is therefore difficult to compare the mineral matter of the soil organic matter with that of the litter from which it is derived. In the case of the surface organic matter of a mor, the organic matter derived from the litter is often relatively uncontaminated by mineral material from the parent mineral material of the soil. In both cases it would be extremely difficult to determine the quantity of litter which results in a given amount of soil organic matter and its contained mineral material. It is therefore almost impossible to ascertain if there are differences in the amounts of mineral nutrients liberated and leached out or absorbed by micro-organisms, or by the roots of plants, during conversion of the litter to soil organic matter in the respective cases of mull and mor. The matter is further complicated by the great difficulty in separating soil organic matter into that derived from litter and that from living roots, etc.

If different kinds of litter are allowed to decompose under artificial conditions of isolation in the laboratory, it is not possible by the empirical chemical methods available to ascertain whether there are differences in the amounts of nutrients *available for plant growth* which are liberated from the organic matter of the litter. Differences might be detecteabl by the use of bioassay methods using various plant species.

Using the term duff as equivalent to Müller's mor, Romell (1931) determined the calcium content of the surface layers of a number of mull soils and also of the F and H layers of a number of duff sites. It is of interest that he records calcium contents, either as percentage of dry soil or as per cent of "humus", for H layer material, which are greater than the calcium contents for mull soils. Whilst as a rule in both mull and duff the calcium content of the lower layer is less than in the upper layer, there are also instances of both mull and duff in which the calcium content of the lower layer (H layer in the case of duff) is *greater* than in the upper layer (F layer in the case of duff). Romell himself points out the difficulty of interpreting such findings in view of the complications involved. It is however possible to say that there appears to be no fundamental difference in the absolute amounts of calcium present in the surface horizons of mull and mor. The amounts of calcium available for biological activity may be very different in the two cases. The function of calcium has often been regarded as chiefly if not entirely concerned with neutralisation of acidic material, but there are indications from recent investigations (Taylor (1951)) that free calcium ions have an important effect on the growth of soil micro-organisms. Stephenson (1949) has discussed the importance of calcium in some of the enzyme activities of bacteria. Calcium may also be important for the life processes of members of the soil fauna e.g. earthworms.

It does not seem possible therefore at present to say whether there are characteristic differences in the quantity or form of the mineral matter of litter when this becomes soil organic matter, in mull and mor respectively.

Reaction (pH) in Mull and Mor

The reaction of a soil as characterised by pH measurements has for a long time been considered an important property of soil. Although the precise interpretation of such measurements, made on systems such as soils i.e. colloidal systems in contrast to pure solutions, is uncertain, they have often been used in investigations of mull and mor.

Determinations of the pH values of the surface layers of mull and mor have shown that they cannot be characterised by this property alone. Romell obtained results showing the distribution of pH values for various types of mull and mor; he found that whilst soils whose reaction approached neutrality were more likely to be of the mull type, and soils whose reaction was markedly acid were more likely to be of the mor type, there was a considerable range of pH values, roughly pH 4—6, in which the soils might be either of the mull or the mor type. Hesselman (1925) has also recorded mull soils having a reaction well within the range recorded for mor sites. He points out that whilst conifer forests having a ground flora of herbs instead of shrubs and mosses are not very common in Sweden, he has observed them in Central Europe, i.e. in the Schwarzwald and in the Böhmerwald. In the case of herb-rich pine

forests at Barenthoren the reaction proved to be pH 4.1—4.2. Similarly the mull of the herb-rich spruce forests of the Schwarzwald had a reaction of pH 4.0, whilst under pure coniferous forest in the Böhmerwald the mor has a reaction of pH 4.7. Hesselman also points out that a thin, loose, easily disintegrated humus covering may have the same or an even more acid reaction than a thick matted humus covering, as based on measurements of reaction in the F and H layers. On the other hand Plice (1934) records a correlation between reaction of the H layer and the depth of the organic layers, the thicker the organic layer the more acid the H layer, for a pure stand of hemlock. It may well be that fire very often upsets correlations between reaction and depth of organic layers. Hesselman (1925) concludes that whilst the reaction may be an important factor it is by no means of decisive significance under all circumstances, and the strongly acid reaction does not by itself necessarily constitute an unfavourable or injurious factor.

Since the reaction of the surface layers of a soil by no means characterise it as mull or mor, it may be of importance to ascertain whether any changes in reaction occur during decomposition of vegetable debris which lead to different acidities in different horizons, and different patterns of change in reaction according to soil type, thereby perhaps indicating different soil processes in the different soil types.

As Hesselman (1925) pointed out, if characteristic features of different soils are to be distinguished, and indications of differences in the finer processes of the soil are to be detected, it is important that the measurements should be made in recognisable horizons and not at purely arbitrary depths e.g. 5 and 10 cm. below the surface whatever the soil. He concluded that in a typical podzol profile with raw humus covering, the humus salt or H layer is most acid, and is overlaid by a somewhat less acid decomposition or F layer, whilst below there is less acid bleached earth; and below this, again, acidity decreases still further. In herb-rich spruce woods the limited number of observations indicate that there is a tendency for the reaction to become more alkaline with depth. Plice (1934) agrees with Hesselman that in mor the region of greatest acidity is the H layer. The observations made by van der Drift (1951) on beech forest soils also support this.

Measurements of variation in reaction according to horizon were made by Bornebusch (1930) for mull soils supporting spruce and beech, and also for mor supporting spruce and beech. Whilst there were similar trends in all profiles for the soil to become more acid with depth, followed at first by decreasing acidity with depth (except for a slight initial increase in reaction from pH 5.9 to 6.1 in the case of the beech mull profile) considerably more acid reactions are reached in the mor profiles than in the mull profiles. In the beech mull profile the newly fallen leaves had a reaction of pH 5.9, whilst the old litter had a reaction of pH 6.1; the corresponding values for beech mor were pH 5.9 and 5.6. The corresponding layers for spruce mull and spruce mor both showed changes in the direction of greater acidity, the values for spruce mull being pH 4.7 and 4.3, and those for spruce mor pH 4.3 and 3.6.

Nemec and Kvapil (1926) also give measurements of the reaction of various horizons in a number of forest soil profiles. Although from the descriptions it is not easy to identify the sites they were dealing with as mull or mor in the majority of cases, there is a gradient of decreasing acidity with depth through the profile, whether the site appears to correspond most closely to mull or mor. Only in the very favourable stands of maple, beech, hornbeam and ash is there a definite tendency for the reaction to become more acid with depth. Even in these cases there is only a slight change and the litter itself has only a slightly acid reaction initially.

The reaction of different horizons of brown earths under beech and spruce, and of mor under beech, spruce and pine has been investigated by Mattson and Koutler Andersson (1941). Although only the initial stages of decomposition can be compared, the results indicate that, in these early stages at least, changes in reactions do not seem to be characteristic of mull and mor.

It can therefore be concluded from the somewhat scanty data available that there are conflicting findings; hence it is not possible to characterise mull or mor with certainty from profile reaction gradients.

Whilst the reaction of a soil does not allow it to be characterised as mull or mor, it may be of importance, from the point of view of the processes in mull and mor, that observations indicate that when, through a change of vegetation, a raw humus becomes a mull the reaction becomes less acid. Thus Dimbleby (1952b) has noted the decrease in acidity as a *Calluna* mor progresses towards mull under the influence of birch. Similar findings have also been recorded by Tamm (1932). Hesselman (1925) quotes a number of instances where the introduction of broadleaved species into conifer stands with raw humus often causes considerable decrease in acidity. Thus he says that the effect which a mixture of birch, aspen, grey alder and similar trees exercise in the Norrland conifer forests is very striking. The humus covering does not take on a mould character but lies, in spite of the mixture of broadleaved trees, as a distinct horizon or layer on the ground, but is usually very loose and friable in consistency. The displacement of the reaction in the alkaline direction is often considerable, and a

richer mixture of broadleaved trees causes a displacement of the index of reaction by a half to sometimes a whole pH unit in the alkaline direction. As a specific example he records that under an uprooted but still living grey alder the humus covering has altered. Below the crown of the windfall it is friable and mould-like, whilst outside this area there is the normal raw humus of the conifer forest. Below the crown of the alder the pH of the humus covering is 5.0 and outside its influence 4.0.

It may be useful to consider here, in conjunction with the above observations, the results obtained from investigations on the changes in reaction of litter decomposing under artificial conditions and isolated from the forest floor. Stepanov (1932) investigated, over a period of 11 months, the leachates due to snow and rain coming from litter of various species of trees and shrubs placed in lysimeters beneath the trees from which the litter was freshly obtained. The leachates were collected after a thaw and after each well marked rain. The lysimeters were not protected from infection by airborne micro-organisms, nor do they seem to have been inoculated with soil. The litter of the various species showed very different rates of change in appearance—*Quercus robur*, *Pinus sylvestris* and *Picea abies* showed little if any signs of change after 11 months. The colours of the leachates also showed considerable variation. In all cases the first washings (in the autumn) were the most acid in reaction. Subsequent washings were always more alkaline and even at the end of the experiment the species normally associated with fairly acid soils still had only very slightly acid reactions, i.e. *Quercus robur* pH 6.92, *Pinus sylvestris* 6.77 and *Picea abies* 7.0.

A similar experiment was carried out by Mattson and Koutler Andersson (1941) but in this case the leaching was carried out with distilled water at room temperature and the litter was inoculated with an extract of garden soil and forest humus. In the case of all the species used, including some species which form raw humus, there is a marked tendency for the leachates and transformed residues to become less acid. At the end of the year the litter of some species was giving more acid leachates again, although in no case were they anything approaching the original reaction.

Recently Coldwell and Delong (1950) have examined leachates from the litter of beech, birch, maple and poplar exposed to weathering on trays. They found that the leachates of each species became progressively more alkaline with time.

This phenomenon of the development of a more alkaline reaction as leaching progresses may be related to the observations of Holden (1948) on a nonenzymatic alkali-producing mechanism in the fresh leaves of herbaceous plants. The species used by Holden gave results which allowed them to be divided into two groups; one group showed an increasingly more alkaline reaction with washing but the other group did not. In the experiments on the leaching of litter described above, however, the material from all species became less acid, and it seems improbable that they should all belong to Holden's alkali-producing group. Holden observed that a distinguishing feature of the species showing the development of a more alkaline reaction is a considerably higher content of calcium and phosphorus in the fibre, which is associated with a greatly increased proportion of the total calcium which is insoluble. The development of a more alkaline reaction is also associated in some way with the pectic materials of the tissue. It should however be remembered that Holden was working with fresh plant material, and conditions may be different in litter, even though the reaction is nonenzymatic. Even so the phenomenon observed by Holden may have some bearing on changes observed during at least the early stages of litter decomposition.

In view of these apparently rather anomalous changes of reaction in the litter of at least some species, undergoing decomposition under experimental conditions, when compared with the acid reactions attained in the process of decomposition of litter in the soil, it seems desirable to consider the nature of this acidity.

Since there is no clearly marked dividing line of reaction, as represented by pH values, by which mull can be distinguished from mor, the possible importance of acidity in the differentiation of mull and mor would appear to lie either in qualitative differences in acidity, such as acidic material of biologically different potentiality (e.g. differing resistance to change by micro-organisms) or in differing soil processes by which mor can apparently reach a lower level of reaction, as indicated by pH measurements, than is the case with mull.

Mattson and Koutler-Andersson (1944) have given the following list of possible sources of active acidity in litter and humus.

1. Soluble acids (including their salts)
   (a) Distillable acids
   (b) Acids decarboxylated by 12% HCl (uronic and other acids).
2. Acidoids (colloidal acids):
   (*a*) Hydrolyzable acidoids such as the polyuronides in hemicelluloses, pectins and gums.
   (b) Non-hydrolyzable acidoids such as those of lignin and ligno-humic acidoids.

Many of such sources of acidity are only differentiated by empirical procedures, the weakness of which has already been discussed in other connections. Comparisons of the acidic materials of the

organic matter of mull and mor do not seem to have been made even on an empirical basis. Although the changes in acidity occurring during the decomposition of isolated vegetable debris, which even in the case of potential mor-forming material have not reached the reaction values normally attained in mor, have been investigated, there does not seem to have been any characterisation of these acidic materials.

The numerous more or less profound changes occurring during the decomposition of the organic constituents of vegetable debris may affect acidic and basic materials originally present in the vegetable debris, e.g. by destruction during the metabolic activities of micro-organisms or change by leaching, and also acidic and basic materials arising from the vegetable debris as a result of microbiological activity, e.g. acidic material formed during the decomposition of cellulose. Present information does not allow of the characterisation of these acidic materials in soils, nor is it possible to characterise their mode of origin, e.g. by biological processes or by leaching, or their biological properties such as resistance to decomposition and possible differences in mull and mor. There are however a number of observations which may help indirectly towards an understanding of this problem.

The effect of changes of vegetation on the reaction of mor indicate, that the acidity, although apparently normally comparatively resistant to change, can nevertheless undergo changes, probably due to biological activity. This would appear to indicate that the change in acidity is the result of a shift in the equilibrium of a reversible system.

Pearsall (1952) has recorded considerable changes in reaction when samples of bog peat are allowed to dry out under laboratory conditions i.e. changes in reaction from pH 5.46 to pH 3.25 and from pH 4.72 to pH 3.27. The increased acidity is essentially an oxidation process, and is not due to increase in the concentration of acids already present due to drying. The acidity thus developed is not extractable in aqueous solution and appears to be associated with humus colloids. Romell (1935) mentions several similar findings. Although it is not known whether they were responsible for the development of a reaction of pH 3.28, the presence of considerable numbers of bacteria, yeasts and *Penicillia*, along with a small number of *Dematium pullulans* and a *Cephalosporium* species, was demonstrated in the organic material of a mor bearing a vegetation of *Pinus sylvestris* and *Betula pubescens*, with a field layer dominated by *Deschampsia flexuosa*, by Boswell and Gover (1946). These organisms therefore appear able to live at this reaction even if they are not responsible for it.

Among the wood-destroying fungi those producing brown rots seem to give rise to considerable acidity during the decomposition of wood and this acidity tends to persist for some time. Birkinshaw, Findlay and Webb (1940) have demonstrated that when the brown rot fungus *Coniophora cerebella* is grown on blocks of *Pinus sylvestris* sapwood, considerable amounts of citric acid along with smaller amounts of formic, acetic and oxalic acids and other unidentified acids of higher molecular weight are obtained in the water extracts of the decomposed wood. Although the loss in weight of the wood was steady throughout the six months incubation period, the titratable acidity reached a maximum in two months and citric acid yield is constant after four months. This was thought to represent a balance between production and decomposition of acid.

In cultures of wood-destroying fungi grown on malt extract, the acidity increases initially; later, especially in the case of white rot fungi, the acidity disappears and may even become less than before inoculation with the fungus. In the decomposition of wood and vegetable debris one may therefore expect an acid reaction to be produced initially; and for this to be followed, especially when the solid substrate has become exhausted, by a tendency for the acidity to decrease, due either to oxidative metabolism of the acids or to the synthesis of materials such as complex polysaccharides. Evans, Smith, Linstead and Elvidge (1951) have obtained evidence for the various steps by which the breakdown of a number of biologically important aromatic substances is brought about by bacteria. So far two pathways, each involving a number of aromatic acids, have been discovered, one ending in catechol and the other ending in protocatechuic acid; ultimately a common path of breakdown is followed. The common path of breakdown involves a number of acids:—B ketoadipic acid, succinic acid, acetic acid, formic acid and finally CO_2 and water.

The breakdown of wood by fungi and of aromatic substances by bacteria seem to have similarities with regard to acid production, and it seems possible that if the action of one or more of the enzymes involved in these chains of events is inhibited or slowed up under certain conditions, then an increased acidity under such conditions might be expected. In this connection Chesters and Robinson (1951) have found that in the absence of adequate amounts of some mineral nutrients much larger quantities of acid are formed from glucose by *Aspergillus niger*. Should such a state of affairs exist in mor, in which a considerable amount of mineral material appears to be unavailable, the decomposition of the potential mor-forming litter may lead to unusual amounts of acid, both aromatic and non-aromatic, being produced, which would be incompletely metabolised, thereby giving rise to a pronounced acid reaction.

Investigations along such lines do not seem to have been carried out so far.

Oxidation Reduction Potential in Relation to Mull and Mor

Changes in the oxidising or reducing potentials of bacterial cultures are influenced by many factors such as the species of bacterium, the quantitative and qualitative composition of the culture media, and the nature of the environment (Hewitt 1950). Some of the effects produced are the reverse of what might be expected. In the case of some bacteria the presence of adequate supplies of oxygen, even to the extent of aeration, are required for the reducing activities of the cells to reach maximum intensity. Many bacteria, when supplied with material for growth, give rise to increasing reducing conditions for a time; but afterwards, when growth and enzymic activity decreases, the potential increases to a more oxidising level. Therefore in any population of micro-organisms with variable or intermittent supplies of metabolites, and variable environmental conditions, over a period of time, the system is dynamic; and this will be reflected in the variations in oxidising and reducing potentials of the system with time.

For this purpose soils may be regarded as complex populations of micro-organisms having variable supplies of metabolites, both in quantity and quality, and subjected to environmental variations in moisture, oxygen and temperature. If in a soil, therefore, a particular organism or group of organisms, or a particular metabolite or environmental condition, brings about a characteristic activity when compared with another soil, then one might expect the two soils to be characterised by different potentials.

The difficulties of measuring and interpreting oxidation-reduction potentials in biological systems has been pointed out by a number of workers, e.g. Hewitt (1950), Burrows and Cordon (1936), Romell (1935) and Pearsall (1938a). Bearing these difficulties in mind we find that Burrows and Cordon obtained indications that very different potentials resulted, depending on whether protein or carbohydrate was added to a soil. Romell (1935) carried out experiments with samples of mull and mor under highly artificial conditions. The samples were buffered at pH 6.6 and maintained at a temperature of 60°C. for four or five days when the potentials were approximately constant. The potential in the case of both mull and mor samples now indicated reducing conditions; the mull considerably more so than the mor. Romell considers that these results indicate that the mor did not contain any strongly reducing substances indicating the poor aeration which has been supposed from time to time to be charateristic of mor. Recently Boswell and Gover (1946) observed a mor which had an E' value of 423 mv. which places it in the lower range of the oxidising group of soils.

Pearsall (1938a and b) has investigated the influence of various procedures on the values obtained for soil potentials, and in the light of these experiments has compared the potentials of different types of soil with the aim of making measurements under such conditions that the values obtained represent as nearly as possible the potentials in nature, which must be the ecologically significant ones. Even with a minimum of disturbance of the soil, i.e. no more than is necessary for the insertion of the electrodes, in the field Pearsall found the potential to be so disturbed that equilibrium was not restored until after 10 to 30 minutes. Measurements of changes of potential with time in samples of the same soil under various conditions in the laboratory indicated that drifts in the potential of a soil are largely controlled by the tightness of packing of the sample and hence possibly by degree of aeration. Since drifts in potential are virtually non-existent after strong heating this suggests that they are associated with the activity of micro-organisms. This was confirmed in experiments in which toluene was used to prevent activity of micro-organisms. Waterlogged soils were normally found to have a low potential but several close-textured soils, both mineral and peat soils, which were not waterlogged had low potentials. Pearsall concludes that the soil microflora and the degree of aeration are important factors controlling soil potentials.

He also measured the potentials of a number of soils of different types chosen on the basis of their vegetation but which can be segregated fairly well into mull and mor sites. The samples were all obtained in July, August or September and precautions taken to avoid as far as possible changes in potential as a result of sampling. Eight soil samples from *Vaccinium myrtillus* communities and six samples from *Dicranum majus* communities, all showed high oxidation intensity. All these samples had a reaction at or below pH 3.8, earthworms were absent, and nitrates could not be detected due to other causes than deficiency of oxygen, and all had an organic matter content of over 85%. The oxidation intensity of these soils is also high even when compared with the potentials observed for good mull soils characterised by *Brachypodium sylvaticum* or *Mercurialis perennis* communities, although the highest potentials observed for these two groups were somewhat higher than the highest potentials observed in the case of mor. There is, as in the case of reaction (pH), a considerable range of overlap of the values for the potentials of the two types of soil.

Pearsall extended these observations on the basis

that theoretically if a soil is thoroughly exposed to air the final potential would probably always tend to approach a constant value, while the rate of change would depend on such factors as the degree of aeration and the number of oxidising organisms present. He collected two soil samples from each of the three main soil types he studied, including mull and mor, with as little disturbance as possible, their temperature being 13—14° C. These samples were transported to the laboratory, the electrodes inserted with a minimum of disturbance, and the drift of potential observed over a period of 48 hours, the temperature being maintained at 22° C. The results obtained indicate that there is no significant difference in either the initial potentials or in the extent of drift towards a higher potential in these samples, as determined by the methods used.

The results obtained by Pearsall are of great interest but it would appear, in the light of experience with bacterial cultures and the fact that soil oxidation reduction potentials seem to be biologically controlled, that further observations might show characteristic differences between the oxidation-reduction systems of mull and mor. Pearsall's observations were made at a time of the year when, on a good mull soil, the previous year's litter is probably almost completely destroyed and presumably the amount of metabolic material available for the soil micro-organisms is at a minimum. Under such conditions, by analogy with bacterial cultures, one might expect the system to have moved back to a more oxidising potential. It would therefore seem to be important to measure the potentials of mull and mor in situ over a whole year at least when, if the mull litter is more readily available to the micro-organisms, a more reducing potential might be expected when rate of destruction of litter is at a maximum; unless the various metabolic processes proceed at equivalent rates, so that there is no accumulation of reducing substances and hence little or no variation in potential. If potential mor litter should be somewhat more resistant to decomposition, then the lowest potential reached in this case may not indicate such strongly reducing conditions as in the case of mull. In addition different oxidation systems may be involved in the two cases, giving rise to quite different potentials. The changes in potential subsequent to the addition of mull and mor litter separately to both mull and mor soils might also throw considerable light on the matter.

Lafond (1949) measured the oxidation-reduction potential of firmly packed mull and mor material which had been allowed to stand overnight in a waterlogged condition. He found that as a rule mull humus has a positive oxidation-reduction potential whereas mor has a very low negative potential. Other samples of mull and mor and intermediate soil types were incubated at 25° C. for a number of days at 50% of their water holding capacity, and their oxidation reduction potentials measured at 24 hour intervals on 50 ml. samples. It was found that the changes in oxidation reduction potential are directly opposite in mull and mor during the first four or five days; transitional soil types followed, in their oxidation-reduction potential changes, the soil type to which they are moving, i.e. towards mull or mor formation. The changes in the oxidation-reduction potentials of the mull soil curves during the last five or six days follow the initial trend of the curves for mor. It is not stated at what time of the year the soil samples were collected and therefore it is not known whether the soils were likely to have had recent additions of vegetable debris, a factor which may have an important bearing on the form of the curves.

The work on oxidation-reduction potentials of mull and mor has been carried out under such a variety of conditions by the different workers that comparisons between their findings is not possible. There are, however, indications that further investigations might prove profitable. It must be remembered, however, that whilst any differences in the oxidation-reduction potentials may help towards an understanding of the mull and mor system, such differences would not indicate the primary cause of the difference between mull and mor, although the cause of any differences in the oxidation-reduction systems might.

Chapter 6

BIOLOGICAL DIFFERENCES BETWEEN MULL AND MOR

SINCE the differentiation of mull and mor was initially largely based on biological differences, it now remains to be considered whether there is any evidence that the function of cause or effect in the formation of mull and mor can be ascribed to biological differences.

The biological differentiation of mull and mor with respect to higher plants has already been discussed.

In this section differences between the microflora and fauna in mull and mor will be considered.

Although Müller (1879 and 1884) distinguished between mull and mor largely on account of observed biological differences, he did not ascribe the reason for the differential development of mull and mor to these biological differences. The reason for the differential formation of mull and mor must lie in the causes of differing interactions between vegetable debris and the biochemical activities associated with the living organisms of these soils. It is therefore important to understand the factors controlling the distribution of the various organisms and their biochemical activities. It would seem a possibility that all vegetable debris is biochemically similar from the point of view of the formation of mull and mor, and that environmental factors bring about differences in the populations and activities of the soil organisms, thereby giving rise to two different soil types. Such control of biological populations and their activities by environmental factors would necessitate the possibility of radical variation in the relative dominance of such factors on a given site, in order that such a system could accommodate the field observations that mull $\rightleftharpoons$ mor is a dynamic system on a given site under the influence of a change in vegetation. In a previous section it has been shown that whilst the lithological nature of the mineral material can exert a strong influence on the formation of mull and mor, they can develop side by side on the same lithological material and therefore also under the same climatic conditions; the microclimates may subsequently differ but initially they are the same, the variables being vegetation and soil organisms. The position of vegetation in the mull $\rightleftharpoons$ mor system has been discussed previously and it now remains to consider the nature of the interactions between vegetation and soil organisms which appear to give rise to mull on the one hand or mor on the other.

Microfloral Populations of Mull and Mor

The quantitative and qualitative evaluation of the microflora of a soil is a problem of the utmost difficulty and with the methods so far available is an impossible task. Thus although micro-organisms may have the same microscopical appearance this does not characterise them, since morphologically identical organisms may have very different properties; while it is probable that methods have not yet been devised for the detection and isolation of many of them. By the use of standardised methods it may perhaps be possible to discover differences in the populations of certain organisms in different soil types, e.g. Warcup (1951) found that among the fungi in five grassland soils two large groups were distinguishable, one group common in acid soils and the other group common in more alkaline soils. We can, however, discover little or nothing concerning the populations of some important groups of organisms, such as the wood and litter-destroying fungi, even though it is known that they are present in a given soil, since methods for the isolation and identification in the vegetative phase have not yet been devised. Even when the presence of micro-organisms, such as fungi known to be able to bring about biochemical changes in various substrates, can be demonstrated by the use of culture media, it does not mean, as demonstrated by Dobbs and Hinson (1953), that such organisms are present in a vigorously metabolising state in the soil; and they may therefore have little or no significance from the point of view of soil processes. There are probably also many organisms in soils of whose presence we are not yet aware. The difficulties of this kind of work are well illustrated in the recent work of Manninger and Vamos (1950), and of Skinner, Jones and Mollison (1952). It seems unlikely therefore that it is possible to say with any degree of certainty that there are major differences in even the large groups of soil organisms, e.g. bacteria and fungi, in different soils.

From time to time it has been claimed that the populations of micro-organisms in mull and mor show qualitative differences and in some cases the differential formation of mull and mor has been attributed to these differences. Müller (1879) noted the presence of numerous blackish-brown hyphae in beech mor, whilst in beech mull he observed only small numbers of the blackish-brown hyphae but in addition large numbers of transparent hyphae as well as mycelia of the most varied shape and colour. He did not, however, put forward any reason to account for these differences.

The formation of mull was considered by Falck (1926 and 1930) to be associated with a white rot type of decomposition, whereas mor formation was considered to be associated with a brown rot type of decomposition accompanied by the development of acid conditions. He does not seem to have indicated what factor or factors could lead to the development of these two different types of decomposition in mull and mor.

Direct microscopical studies on humus layers were carried out by Romell (1935) and he came to the conclusion that in a mull as well as in the H layer of a very pronounced mor the bulk of the humus appears to consist of living and dead organic material built up by the microflora active in decomposition processes. It is stated that both the microflora and the synthesized material are strikingly different when extreme types are compared. Thus in the crumb mull, jelly capsules of bacterial colonies are con-

sidered to form a considerable part of what has previously been termed "flocculated humus colloids". In the greasy mor fragments of coarse brown hyphae are dominant. Romell thinks it likely that the jelly capsules and the wall substance of the brown hyphae differ in chemical nature, not merely in base saturation, and that this points again to the importance of type rather than rate of decomposition. Romell appears to suggest that although the breakdown of lignified tissue by bacteria has been little studied, such a process must occur in mull since microscopical traces of the original plant residues are not to be found in the most pronounced bacterial mull type.

It is, however, well known that many basidiomycetes including litter-destroying species, grow and produce sporophores on mull soils and even in grasslands having a reaction of pH 6.8—7.1 (Wilkins and Patrick (1940)). Similar observations were made by Warcup (1951) who also noted that culture plates inoculated with material from beneath sporophores showed in all cases a restricted flora of microfungi compared with those growing from soil samples outside the mycelial zone associated with the sporophores; i.e. there is a considerable amount of fungal mycelium of diverse types in mull soils even to the extent that sporophores can be produced. Romell (1935) is also of the opinion that a more active fauna in mull is important in the maintenance of mull by keeping down the population of acid-producing fungi. He does not, however, appear to attempt to explain what factor determines that a fauna shall develop and consume the fungal hyphae, thereby giving rise to or maintaining mull conditions; whereas when a mor develops, or is maintained, fungus mycelium develops abundantly and a corresponding fungus-consuming fauna does not develop to the same extent as in mull.

Boswell and Gover (1946) found that the organic layer, having a reaction of pH 3.28, developing over, and sharply divided from, an apparently unleached light brown sandy material containing very little organic matter, associated with a vegetation composed largely of *Pinus sylvestris*, *Betula pubescens* and *Deschampsia flexuosa*, contained appreciable numbers of bacteria. The numbers of bacteria per gm. of soil, containing 64.9% moisture, obtained by counts on glucose peptone agar were of the order of 300×10^6 to 400×10^6. Therefore whatever differences there may be regarding the populations of micro-organisms of mull and mor it seems clear that there is no sharp differentiation such as bacteria restricted to mull and fungi restricted to mor, although the bacteria present in a mor may have similar or quite different metabolic processes compared with those in a mull; there is as yet no information on this matter.

It seems likely that any differences in the microflora populations of different soil types would be very largely governed by differences in composition of the substrate (vegetable debris) available for their metabolism. Differences in the composition of various kinds of vegetable debris which have not so far been detected by analyses may be the cause of very different micro-organism populations and processes; e.g. Lindeberg (1944) observed that the cellulose in pine litter appeared to be more resistant to decomposition than the cellulose of the litter of other species. Biological reactions may therefore point to important differences in vegetable debris not yet discovered by chemical methods.

The Soil Fauna of Mull and Mor

Whilst the methods of determination of soil fauna populations of mull and mor are by no means perfect, largely due to difficulties in the extraction of all the animals from a sample of soil, they appear to be more adequate than those available for the determination of microflora populations.

The nematode populations of a number of different soil types, including mull and mor, have been studied by Overgaard Nielsen (1949). He is of the opinion that the methods of extraction used enable him to isolate at least 90% of the nematodes present in a given sample of soil. He found that a very large proportion of the species are common to all soil types and his results indicate that the nematode populations of mull and mor cannot be characterised by differences in numbers of nematodes or the proportion of those having differing feeding habits, including those feeding on bacteria and those which appear to feed on algae.

Earlier Bornebusch (1930) had studied the earthworm populations of mull and mor and found that there was a strong tendency towards a diminution in numbers of both species and individuals and in total weight of earthworms per unit area as one proceeded from mull to mor, thereby confirming the observations of Müller (1879). His conclusions regarding the remainder of the fauna of mull and mor are that any differences are largely quantitative. Comparing the fauna of the soils as a whole he concluded from his observations that the soil in which decomposition of vegetable debris is most active contains the greatest total weight of animals but the lowest number; where decomposition of vegetable debris is slow and a heavy mor is present a greater number of animals is found, but, being on the average very small, their total weight is less than in the best soils. Thus the good forest soil contains few and large animals, the inferior one many and small animals. As Bornebusch points out, animals are able to move about and so are to a considerable

extent largely independent of the conditions offered to them. That the fauna population cannot be regarded as a determining factor in the formation of mull and mor is clear from the fact that a mull fauna population cannot withstand indefinitely the effects of a change in vegetation which results in a change from mull to mor. It would seem, therefore, that the nature of the vegetable debris can control the fauna so that the fauna does not control the manner of breakdown of vegetable debris in these circumstances.

Whilst the fauna of a soil cannot be regarded as determining the type of the soil, differences in the composition and activities of the fauna populations may give indications of the reasons for the differential development of particular soil types, and also indications of differences concerning vegetable debris which influence soil type but which have not so far been detected by purely chemical analyses.

Considering first the earthworms which, of the fauna, exhibit the most striking differences in distribution between mull and mor. Experiments have been conducted on preferences displayed by various earthworms for the litter or fresh leaves of various species. Darwin (1883) recorded that earthworms will consume fresh leaves but that they consume large numbers of half-decayed leaves which he considered to be their chief article of diet. He did not understand the basis of the apparent preference for the leaves of certain species, it did not seem to be related to strength of flavour or softness of texture. They preferred wild cherry leaves to those of lime or hazel. The parenchyma was gnawed off fresh leaves pinned to the surface of the soil but normally leaves, whether fresh or dry, are pulled into the mouth of the burrow and skeletonised when rotten. The needles of *Pinus nigra* were dragged into the mouth of the burrow but not gnawed. Some pine needles were used to line the walls of the burrow. Worms appear to be able to live for a considerable time in the absence of fresh supplies of vegetable debris and presumably obtain food material from the organic matter of the soil.

The preferences of various species of earthworms for various kinds of litter were compared by Lindquist (1941) by means of feeding experiments. His results indicate that, although various species of worms may react somewhat differently to the same kind of litter, and that even the same species of worm may occasionally react somewhat differently in similar experiments to the same kind of litter, in general the earthworms characteristic of mull soils show a preference for the litter of elm, birch, and dog's mercury, consuming only small amounts of beech and oak litter, and pine and spruce needles not at all. In some cases small quantities of bilberry leaves were ingested.

Franz and Leitenberger (1948) compared the consumption of various kinds of vegetable debris by various taxonomic groups of soil animals and found considerable differences. The order of preference for various kinds of vegetable debris can only be compared for two animals, *Glomeris connexa* and the worm *Lumbricus rubellus*. The comparison is made in terms of mgm. of organic matter, in the form of freshly fallen litter, consumed per animal per day, as shown in Table 28.

The order of preference is the same for both animals, i.e. hazel, beech, cocksfoot grass. It must be remembered, however, as pointed out by van der Drift (1951) that fresh litter may not and probably is not the natural food of soil animals, which generally seem to feed on vegetable debris which has been on the ground for a longer time.

The disintegration and disappearance of the litter of various species when placed on the same mull soil was observed by Wittich (1943). Although it must be assumed that the effects are due largely to animals the results show very similar tendencies to those obtained by Lindquist, i.e. oak and beech litter disappear and are disintegrated much more slowly than the litter of other broad leaf species. Wittich found that the needles of conifers were slowly mixed with the soil by the soil animals; of the various kinds of litter utilised larch seemed to be the most unpalatable.

The apparent relative unpalatability of certain kinds of litter may be associated with noxious materials in the litter or to their indigestibility as far as the animals, or micro-organisms in the soil on which the animals feed, are concerned. In this connection Bornebusch (1930) indicated the great

TABLE 28

Glomeris connexa		Lumbricus rubellus	
Litter	Mgm. litter consumed per animal per day	Litter	Mgm. litter consumed per animal per day
Hazel	1.44	Hazel	20.39
Beech	0.64	Beech	6.41
Cocksfoot grass	0.28	Cocksfoot grass	2.28

disparity in the total weights of soil fauna present per unit area in mull and mor respectively; the difference being largely associated with the earthworm population. The differences in the weights of animals in each soil type imply that a much larger amount of nitrogenous material is required for the production of animal tissue in mull than in mor. It is perhaps significant therefore that, as pointed out previously, the nitrogen of mor appears to be less readily available than that of mull, and it is especially the potential mor-forming litter which appears to be generally less acceptable to earthworms, both in feeding experiments and as indicated by the distribution of earthworms in relation to vegetation. This is also supported by the observation that, whatever the adverse factor in mor-forming litter may be as far as earthworms are concerned, even those species of animals which can live in mor apparently do not reach such high levels of productivity, as determined by weight of animal tissue produced, as in mull populations. An important factor in the production of animal tissue is availability of easily digestible organic nitrogenous material, whether of plant or animal origin.

There has been comparatively little work on the details of the digestive processes of the soil fauna. Darwin (1883) was of the opinion that his observations indicated that earthworms exude a fluid which has a digestive action especially on the contents of the cells of fresh leaves. Müller (1879) observed plant remains of very varied size and with well-preserved structure in worm casts; this led him to wonder whether the fungal mycelium associated with the vegetable debris on the forest floor forms the food of earthworms rather than the actual dead plant remains. He considered that earthworms cannot grind up plant remains and that plant remains are only weakly attacked by the digestive system of worms. The findings of van der Drift (1951) confirm Müller's observation; an examination of the excreta of soil fauna showed it to consist largely of very small pieces of litter, of surface area about 0.01 square millimetres, which retained their reactions for cellulose and lignin.

Franz and Leitenberger (1948) carried out laboratory experiments in which various members of the soil fauna were fed on the fresh litter of various plant species and the absolute changes in the content of acetyl bromide insoluble material on passage through the gut determined. The changes in acetyl bromide insoluble material were found to be dependent on the species of animal as well as on the kind of food material eaten. In most of the cases examined by Franz and Leitenberger there was an absolute gain in acetyl bromide insoluble material, in some cases quite a large gain, on passage through the gut; in a very few cases there had been little change. In the case of fresh cocksfoot grass or fresh red clover, grasshoppers brought about a marked reduction in acetyl bromide insoluble material. These authors consider acetyl bromide insolubility to be a characteristic of humus. Since the percentage of carbon remains unaltered during digestion this is taken to indicate that only the early stages of humus formation take place during digestion. In these experiments there has been little consideration of the relative parts played by the microflora of the alimentary canal and the tissues of the alimentary canal itself in the changes undergone by vegetable debris ingested by the animals.

The digestion of oak litter by *Glomeris marginata* has been studied by van der Drift (1951). A sufficiently large number of the animals, 264 individuals weighing 30.5 gms., was used so that sufficient excreta for analysis could be collected in a few days in order to minimise the effects of the action of micro-organisms on the excreta. 20 gms. of oak litter from the F_1 horizon were used, and by difference between the amount of litter which disappeared and the amount of excreta produced the amount of material metabolised was determined. Although there is little difference between the percentage composition of the litter and that of the excreta, there is evidence that a small percentage of nitrogen has been lost from the litter. It is perhaps unfortunate that oak litter was chosen as substrate in view of its apparent relative unpalatability and indigestibility, and more striking results might have been obtained with more readily assimilable litter. Van der Drift concludes that the main result of the activities of *Glomeris marginata* and indeed of the majority of the members of the soil fauna is that of mechanical breakdown, thereby promoting decomposition by favouring the activities of micro-organisms.

Recently Tracey (1951) dissected out the alimentary canal of the worm *Lumbricus terrestris*, and after washing free from gut contents observed the effects of various regions of the alimentary canal on carboxymethyl cellulose, chitosan hydrochloride and finely divided chitin. Evidence was obtained for the presence of cellulase and chitinase in the worm tissues. The enzymes are apparently very largely localised in the wall of the forehalf of the intestine. The amount of enzyme present, on a fresh weight basis, is said to be of the order found in the snails *Helix aspersa* and *Helix pomatia*, which are perhaps the richest sources of animal chitinase and cellulase so far discovered. From these observations it would appear that the worm *Lumbricus terrestris* possesses enzymes of its own and is not completely dependent on the microflora of its alimentary canal for utilisation of cellulose, etc.

Evidence has been obtained by Day (1950) that the gram-negative, non-spore forming bacterium

Serratia marcescens is killed out completely by the pharyngeal secretions of *Lumbricus terrestris*, whilst other species of bacteria appeared to be unaffected by passage through the alimentary canal of this animal. Whether bacteria or other micro-organisms killed in this way serve as a source of nutrient material for the animal appears to be unknown.

The evidence at present available therefore points to the conclusion that either the litter of mor-forming plant species is nutritionally unsuitable or inadequate for earthworms, or, if the intervention of micro-organisms is essential, then for some unknown reason these micro-organisms must be absent or ineffective under conditions which allow of the formation of mor.

Wittich (1939 and 1943) has pointed out that conifer needles placed on the surface of a mull soil are dragged down into the burrows of worms and there undergo very rapid decomposition, whereas those remaining on the surface of the soil were remarkably well preserved and loss in weight took place relatively slowly. Indeed the decomposition of needles which are not within the soil is no further developed than in needles which are of the same age in a thin raw humus layer. In contrast the leaves of broadleaved species decompose even on the surface of the soil. Although the conifer needles may have become unrecognisable in the worm burrows, does this mean that they are completely decomposed, perhaps due to more favourable environmental conditions in the worm burrows for the activity of micro-organisms, or have they merely become unrecognisable amorphous material? The work of Mork (1937-1939) and Johnston (1953) indicates that environmental conditions have a considerable effect on various aspects of the decomposition of litter and soil organic matter under laboratory conditions. Whatever the explanation may be, repeated application of conifer needles alone would, in course of time, lead to the disappearance of the earthworms. Once such a process begins, even the environmental conditions for decomposition are likely to deteriorate, for the increasing tendency for the roots to be restricted to the surface layers will lead to drying out of the organic layers, especially in summer, which might very well slow up decomposition processes which are perhaps already hindered by other factors.

To summarise, such evidence as we have seems to point to some attribute or attributes of particular kinds of vegetable debris as being responsible for determining the differential formation of mull and mor. A corollary of this is that the activities of soil micro-organisms and soil fauna are governed by one or more properties of the vegetable debris. In addition even the mor-forming properties of a mor-forming litter can be modified by the presence of other types of litter and perhaps mineral material rich in bases. Any mechanism which is put forward as the basis of the differential formation of mull and mor will have to satisfy all these conditions and be capable of considerable modification under the influence of various factors.

PART II. THE PRESENT INVESTIGATIONS

Chapter 7

INTRODUCTION TO THE PRESENT INVESTIGATIONS ON THE REASONS FOR THE DIFFERENTIAL FORMATION OF MULL AND MOR

In spite of the amount of work which has been carried out on soil properties and soil genesis there are many aspects of mull and mor on which information is scanty or lacking, and it does not seem possible to point to a generally satisfactory explanation of the cause of the differential development of these types of soil organic matter. In view of its biological importance the problem appears to require re-examination.

If the reasons for the differences between mull and mor are to be found, an understanding of the processes involved in the decomposition of plant and animal residues and the manner of their integration into the organic matter of the soil is essential. From a chemical point of view most of the materials involved in the processes are complex and difficult to manipulate; this probably accounts, to a large extent, for the lack of progress in finding a solution to the problem by chemical analyses alone. Consideration of the results of previous investigations on the processes and characteristics of mull and mor indicates a number of differences which, although they appear to represent effects rather than causes, seem to be possible starting points for further investigations. Even when taken together the detectable differences seem comparatively few, and uncertain to account for the striking differences in the appearance of two contrasting profiles.

Differences in the Characteristics of Mull and Mor

(a) There seems reason to believe that whilst considerable quantities of cellulose are present in mor this material is present in much smaller quantities in mull.

(b) The nitrogen in mor appears to exhibit a greater degree of resistance to change by biological agencies than the nitrogen of mull.

(c) There is a tendency for the production of extremely acid conditions when mor is formed.

(d) The fauna populations of mull and mor exhibit marked differences especially with regard to earthworms.

(e) The differences between mull and mor appear to concern differences in type or course of decomposition and resynthesis, rather than different rates of decomposition.

(f) Whatever the mechanisms responsible for the differential formation of mull and mor may be, a particular condition represents a phase in a dynamic system which is reversible under the influence of the changing intensity of various factors.

P.E. Müller stated in 1884 that there was a need for a foundation of elementary investigations into the nature of the natural humus forms, which must give the essential preliminary information for more intensive and difficult researches; the first steps of the process of conversion of vegetable debris by the action of organisms must be understood, the rougher outlines being first drawn in by the aid of the lens and microscope, before the investigation moves on to the almost invisible. It seems that this opinion can still form the basis of a sound and logical approach to the problems of mor formation. It forms the basis of the present investigations.

Chapter 8

THE MICROSCOPIC AND MACROSCOPIC CHARACTERISTICS OF MULL AND MOR

Müller (1879) himself made microscopical observations on mull and mor. He described how in beech mull the top soil is interwoven with microscopically fine mycelial threads of the most varied shape and colour, so that not even the smallest fragment of soil can be placed under the microscope without showing these structures; the transparent easily destructible threads seem, however, to occur

in greatest numbers. He considered the whole of the topmost soil layer to consist almost exclusively of earthworm castings which, when examined microscopically, were seen to consist mainly of a nondescript detritus of an organic nature, along with inorganic soil constituents, but they are often formed of plant remains of very varied dimensions and having a well-preserved structure. In beech mor Müller observed that it was difficult to separate the fine roots from the dead leaves. Microscopical examination showed the reason for this to be the thick net of fine blackish-brown filaments of fungus mycelium which appear to be tough and hard and hold the roots and dead leaves as a firmly coherent mass. He considered this blackish-brown mycelium to be almost imperishable and a very important constituent of mor. In addition he observed in mor a large number of minor constituents such as plant remains and chitinous parts of insects. Müller also described his microscopical examinations of ling (*Calluna vulgaris*) mor which led him to conclude that its origin is absolutely analagous with that of beech mor, there being an abundance of dark brown mycelium closely related to that of the beech mor but certainly belonging to another species of fungus. Since the work of Müller there have been few microscopical examinations of either soil or decomposing litter which can be related to the differentiation of mull and mor.

Ramann (1911) observed that although macroscopic plant remains were not present in "*Moder*", microscopically detectable plant remains were present. The precise identity of "*Moder*" seems uncertain but it is probably a form of mor. Ramann distinguished two layers in the unincorporated soil organic matter; an upper layer having plant remains which more or less retain their structure and a lower layer in which there are few recognisable plant remains.

The unincorporated organic matter on the surface of the soil beneath a spruce plantation was described by Stepanov (1929). The layer below the litter consisted of half decomposed debris in which small pieces of twig are only hollow shells of bark, whilst cone scales and needles are in less advanced stages of decomposition. The lowest layer consisted of material in which it is very difficult if not impossible to recognise plant remains.

Pearsall (1950) has pointed out that in peats such as those of the Southern Pennines, although the presence of former mor-forming species such as *Calluna vulgaris* is indicated by an abundance of their pollen, other recognisable structures are not present, and even the stems of *Calluna vulgaris* are comparatively quickly decomposed. This decomposition of woody tissue of *Calluna vulgaris* occurs under conditions of mor formation as in the case of Stepanov's spruce twigs.

A microscopical examination of pronounced forms of mor and mull was carried out by Romell (1935). Although in both cases the bulk of the humus appeared to consist of living and dead organic matter built up by the microflora active in decomposition, this synthesised material and its associated microflora is strikingly different when extreme types of mull and mor are compared. He found that in crumb mull the gelatinous capsules of bacterial colonies apparently form a considerable part of what has earlier been regarded as "flocculated humus colloids". In "greasy mor" fragments of coarse brown hyphae were as dominant as the bacteria in mull. The remains of litter are rare in the well-decomposed organic matter and even the heaviest (greasy) mor examined seemed to be built up of dead fragments of brown hyphae and not from undecomposed lignified remains from the litter. Microscopical examinations of the most pronounced bacterial type of crumb mull indicated practically complete decomposition of the original plant residues.

In his microscopical investigations of soils Kubiëna (1943) found much evidence of the activities of the soil fauna. He considers that microscopical examination shows a mull soil to consist almost entirely of the remains of casts of earthworms, all plant structure having disappeared and the finely divided organic matter being intimately mixed with mineral matter. Although this is in agreement with Romell's observations on the absence of plant tissue in mull it does not seem to be in accord with Darwin's (1883) and van der Drift's (1951) findings of small particles of vegetable debris in the casts of earthworms. Kubiëna found a sample of "*Buchenmoder*" to contain a large number of mite droppings and considers these to be the source of durable humus. He also examined material from the B horizon of a podzol and considers the organic material in this situation to have been formed elsewhere and secondarily deposited in this horizon, which appears black to the naked eye. The organic matter of the B horizon is in the form of a thin film around the mineral grains, which are almost entirely quartz. This organic material is also characterised by the absence of any signs of life in it.

The microscopical appearances of mull and mor have been compared by Hartmann (1944). He found mull to consist of the casts of earthworms and amorphous humus material mixed with fine particles of mineral material, but he does not mention whether plant remains are detectable. Mor contained the droppings of small soil animals along with plant remains which still showed morphology and cell structure, the whole being more or less bound together in some instances by fungal hyphae.

Generally few recognisable plant remains appear to have been found in mull soils whereas in the case of mor recognizable remains seem to occur in some cases but not in others. In view of the findings that cellulose is present in mor it would seem to be desirable to know how far this is associated with the presence of recognisable plant remains or originates from some other cource, e.g. synthesis by micro-organisms from other organic materials. Since cellulose is more or less readily decomposed by a variety of micro-organisms it seems unlikely that it would normally remain long in the free state in the soil, although experiments (e.g. Rayner and Neilson Jones 1944) indicate that the rate of breakdown of cellulose buried in soil varies according to the type of soil, being very largely prevented in mor soils.

Chapter 9

THE PRESENCE OF MASKED CELLULOSE IN MOR

On the assumption that the colour reaction resulting on treatment of cellulose with iodine and zinc chloride solutions is reasonably characteristic for cellulose, it seemed that if free cellulose were present in a soil its detection by microscopical examination should be possible after treatment with these reagents. Samples of a mor layer formed from the debris of *Calluna vulgaris* were therefore examined from this point of view.

Although there were slight variations according to the source of the sample, in general the surface layers of the mor contain numerous remains of *Calluna vulgaris* litter which are recognisable by eye; such remains decrease in number with increasing depth until in the lower layers of the surface organic material it is difficult to detect plant residues by the unaided eye. Some of this mor material, after removal of obvious plant remains, was examined microscopically and found to consist largely of dark brown amorphous particles along with fragments of very thin roots and fungal mycelium. Among these constituents were a small number of squarish or rectangular cells, either free or attached to what appeared to be root fragments and therefore presumably root cortical cells of *Calluna vulgaris*, and occasional fragments of larger cells which gave the blue reaction of cellulose; in addition a small number of the fungal hyphae had a dirty bluish-grey colour. The small amount of material giving the cellulose reaction did not seem sufficient to account for the amounts of cellulose which have been recorded as being isolated from mor by chemical means.

In estimations of cellulose in plant material by the use of Schweitzer's reagent it has been found desirable to remove encrusting material first. Therefore, in view of the strongly held opinions that there are considerable amounts of so called lignin in mor, samples of mor similar to those previously examined were treated with a 1% solution of sodium hypochlorite for about one hour at room temperature before being treated with iodine and zinc chloride solutions. Similar methods have been used by Barghoorn (1948) for the identification of plant remains. When this mor material, which had undergone treatment with sodium hypochlorite, was examined microscopically it was observed that the fungal hyphae previously visible had disappeared and the cortical cells and other tissues of the *Calluna vulgaris* root fragments gave a strong reaction for cellulose. In addition the dark brown amorphous masses had lost their dark brown amorphous character and were seen to consist of a matrix of light yellowish-brown material in which fragments of material giving the cellulose reaction were embedded. Further examination of this material from the original dark amorphous masses and now giving the cellulose reaction showed that it was apparently not derived from vascular tissue but from parenchymatous cells having simple pits.

Further samples of *Calluna vulgaris* mor from various parts of the country, i.e. Wareham Heath, Dorset; Allerston Forest, Yorkshire; Clocaenog Forest, North Wales; Brendon Forest, Somerset; and Dartmoor Forest, Devon, were examined and in all cases the previous findings were confirmed. In the case of some of the samples, fragments of *Calluna* debris recognisable by eye were restricted to the extreme surface layer. In those cases where the samples were taken from mor of greater depth the lower parts of the surface organic material was very different in appearance, being almost black and of a much finer and almost greasy texture, compared with the upper part which had a similar appearance to that of the shallow samples of mor. In addition the lower parts of the deeper samples seemed to contained little if any material giving a reaction for cellulose even after treatment with sodium hypochlorite solution. There is reason to believe that this

material may have arisen from vegetation other than *Culluna vulgaris*, which had subsequently been replaced by *Calluna vulgaris*. But on the other hand one gets the impression that the amount of material giving a reaction for cellulose, and not arising from roots, tends to decrease with depth even in the samples from shallow mor formed, almost certainly, entirely from *Calluna vulgaris* debris. In this connection a sample of material from the B horizon of a podzol carrying and probably formed by a vegetation of *Calluna vulgaris* at Caesar's Camp (Berkshire) was examined microscopically. Before treatment with sodium hypochlorite solution the material was observed to consist of mineral material mixed with a large proportion of dark brown amorphous material, along with very fine roots of *Calluna vulgaris* and occasional fungal hyphae, but material giving the reaction for cellulose was not detected. After treatment with sodium hypochlorite solution the proportion of mineral material was seen to have considerably increased. The brown amorphous material had disappeared leaving a residue of yellowish material which may be an endodermal residue from the roots of *Calluna vulgaris* growing in this zone of the soil, since they have a very similar appearance to the inner parts of the fragments of the roots of *Calluna vulgaris*. Except for the root cortical cells there are only very occasional small fragments of material giving the reaction for cellulose. This would seem to agree with the suggestion put forward by Kubiëna (1943) that the brown amorphous material of the B horizon is formed elsewhere (e.g. the surface of the mineral soil) and subsequently transported to the B horizon. It would seem that this is only likely to occur where the mineral material is composed of relatively large particles. The samples of deeper mor mentioned above were formed on very fine textured mineral soils (clay) which would perhaps not allow of the movement of amorphous organic material to the B horizon; and also might frequently give rise to conditions approaching waterlogging, which in the first place may well have been associated with a vegetation other than *Calluna vulgaris*.

Samples of mor formed from the debris of various other plant species have also been examined for the presence of cellulose, as set out below:

1. Sample of mor from Allerston Forest, Yorkshire formed, as far as can be ascertained, very largely from the debris from *Vaccinium myrtillus*. The surface organic layer is much more spongy in texture and much deeper than is the case in most mors associated with *Calluna vulgaris*. It consists of a mass of amorphous purplish-brown material with *Vaccinium* roots and rhizomes of various sizes ramifying in it. The uppermost five centimetres of the mor below the loose surface litter contains *Vaccinium myrtillus* leaves in all stages of disintegration; many of the leaves are skeletonised due apparently to the intervenous tissue becoming dark brown and brittle and falling from between the veins. There are also many fragments of *Vaccinium myrtillus* twigs which though outwardly appearing sound are brittle and collapse on squeezing. Samples were removed at intervals from the top to the bottom of the mor; the amorphous material was examined microscopically after treatment with iodine and zinc chloride solution, both with and without previous treatment with sodium hypochlorite solution. In the absence of treatment with sodium hypochlorite solution the amorphous material is seen to consist of dark brownish irregular masses, round the edges of which appear to be parts of plant cells, along with fairly numerous fungal hyphae and highly refractile brownish-yellow particles, but there are extremely few particles giving the reaction for cellulose. Where the material has been treated with sodium hypochlorite solution most of the dark brown amorphous material and most of the fungal hyphae have disappeared, leaving many cell walls of parenchymatous cells and fragments of cell wall giving the reaction for cellulose. The observations were essentially the same for the different samples taken at various depths below the surface of the mor.

2. A sample of mor was collected from beneath the crown of an old *Taxus baccata* tree growing on a flinty soil on the top of Box Hill, Surrey. On calcareous material near the bottom of the hill *Taxus baccata* is associated with mull. The crown cast a dense shade and as far as could be determined there was no ground vegetation. The mor consisted of a shallow layer of very dark blackish-brown amorphous material 1–2 cm. in thickness lying below a layer of recognisable litter of *Taxus baccata* of the same thickness. There was no visible evidence of leaching in the mineral soil, and beneath an old birch tree (*Betula sp.*) nearby there was a mull soil with no signs of mor formation.

Microscopical examination of the dark amorphous material after treatment with iodine and zinc chloride showed the presence of many round particles which may be the faecal pellets of the soil fauna. Fungal hyphae were fairly numerous but extremely few particles giving the reaction for cellulose were observed. When the material has received treatment with sodium hypochlorite solution before treatment with iodine and zinc chloride solutions, it is seen to contain a considerable amount of material, much of which seems to consist of parenchymatous cells or fragments of them, giving the reaction for cellulose. There is also a considerable quantity of refractile yellowish material which may be cuticular in nature.

3. An almost pure stand of *Fagus sylvatica*,

TABLE 29

	Surface Sample	Sample from a depth of 10 cm.
Before treatment with sodium hypochlorite solution	Brown amorphous granules, fairly numerous fungal hyphae, occasional fragments of yellow refractive material, root vascular tissue but no material giving the reaction for cellulose.	Very similar appearance to that of the surface sample, except that there are very few fragments of tissue. Material giving the reaction for cellulose was not observed.
After treatment with sodium hypochlorite solution	Most of the amorphous brown material has disappeared leaving small particles of refractive orange yellow material and sheets of refractive pale yellow material. Occasional fragments of tissue give the reaction for cellulose but there is nothing corresponding to the material giving the reaction for cellulose as found in mor.	Much amorphous material remains but it has become much lighter in colour. Occasional bundles of vascular tissue giving the reaction for cellulose and probably arising from recently dead roots. There is nothing corresponding to the material giving the reaction for cellulose as found in mor.

having only occasional individuals of *Quercus* sp. in it, growing on gravel in the Chilterns, was found to have a mor layer developed beneath it. As far as could be ascertained there is no ground flora except for very occasional tussocks of moss. The mor was sharply demarcated from the mineral soil, which had only a very thin visibly leached layer at the surface, and consisted of a very dark brown amorphous material on which lay many layers of leaves in all stages of disintegration and containing many roots. The whole of the surface organic layer was about 10 cm. in thickness.

Before treatment with sodium hypochlorite solution the dark brown amorphous material was observed to contain numerous fragments of fungal hyphae and particles of refractive yellow material, but material giving the reaction for cellulose was not observed. After treatment with sodium hypochlorite solution much of the brown amorphous material was seen to have disappeared, leaving many parenchymatous cell walls and fragments of cell walls giving the reaction for cellulose; there were also occasional groups of cells of vascular tissue giving the reaction for cellulose.

For comparison with the mor soils, samples from an alkaline fen peat, at present supporting a vegetation of *Phragmites communis*, were examined. Samples were taken at the surface and from 10 cm. below the surface. The microscopical appearance of the samples with and without treatment with sodium hypochlorite solution is indicated in Table 29.

The microscopical appearances of these highly organic soils were then compared with those of a number of mull soils examined from the same point of view, as stated below:

1. Samples of mull were taken from the calcareous soil on which a mature, pure stand of *Fagus sylvatica* was growing in the Chilterns not far from the previously mentioned stand of *Fagus sylvatica* associated with mor. After removing ¼ to ½ inch of the surface layer, samples of the crumb mull containing numerous earthworms were taken in October, before leaf fall and when all the litter of the previous year had completely disappeared from the soil surface. There appears to be no ground flora, not even traces of a spring ground flora, over large areas of this site.

Samples examined before treatment with sodium hypochlorite solution appeared to consist very largely of mineral material, and of any organic material present none gave the reaction for cellulose. Attempts were made to concentrate any particles of organic matter by centrifuging an aqueous suspension of the soil in the presence of carbon tetrachloride. Even then the lighter material separating out contained only occasional small fragments giving the reaction for cellulose. Treatment with sodium hypochlorite solution made very little detectable change and certainly none in the amount of material giving the reaction for cellulose.

2. A crumb mull soil formed on calcareous material (Coral Rag) and supporting a vegetation of *Fraxinus excelsior* and *Corylus avellana* with a rich herbaceous ground flora was sampled at depths of 1 and 5 and 10 cm. in February 1949. There were only a few isolated pieces of litter on the soil surface. The top 2 or 3 cm. of the soil were dark brownish-black in colour, below this the soil gradually became yellow in colour. Microscopical examination of the samples gave the following results.

Surface Sample (from a depth of 1 cm.):—

Not treated with sodium hypochlorite solution:—

Fairly numerous amorphous granular masses and frequent fungal hyphae were observed but no material giving the reaction for cellulose.

After treatment with sodium hypochlorite solution:—

The granular masses which remain appear to be aggregates of very fine mineral particles.

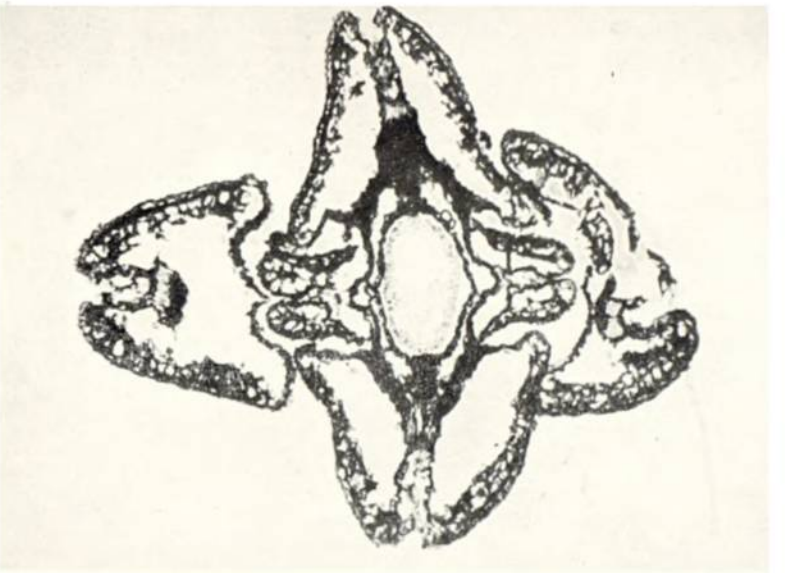


FIG. 1. Transverse section of *Calluna vulgaris* litter taken from surface layer of *Calluna* mor ; vascular tissue beginning to disappear, mesophyll tissue persistent. × 50. (See page 62.)

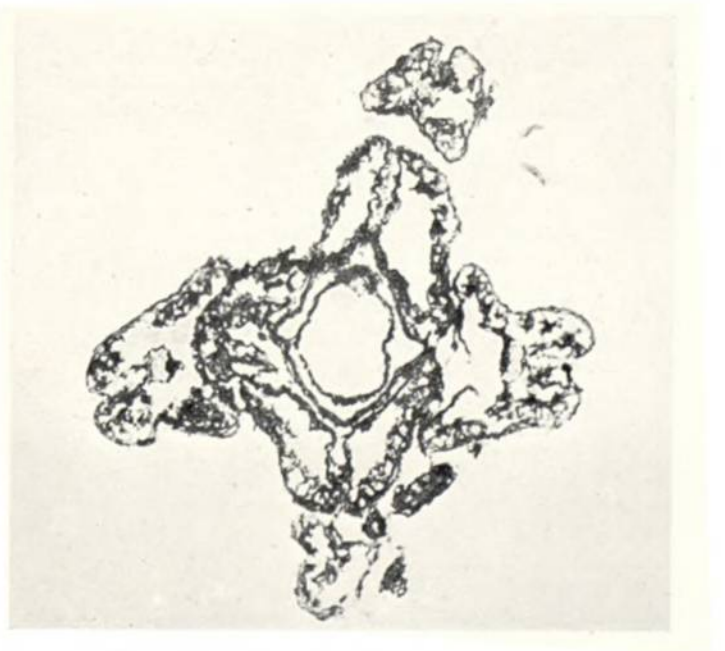


FIG. 2. Transverse section of *Calluna vulgaris* litter taken from a position about 1 cm. below the surface of *Calluna* mor ; further disappearance of vascular tissue compared with Fig. 1, mesophyll tissue still persistent. × 50. (See page 62.)

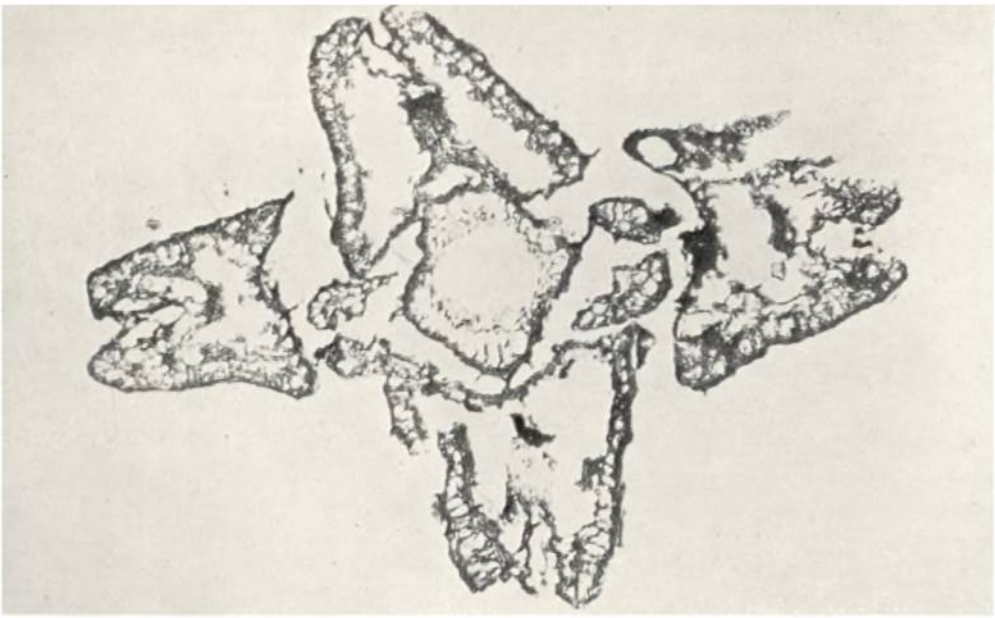


FIG. 3. Transverse section of *Calluna vulgaris* litter from *Calluna* mor; vascular tissue almost completely disappeared although mesophyll tissue still persists. × 100. (See page 62.)

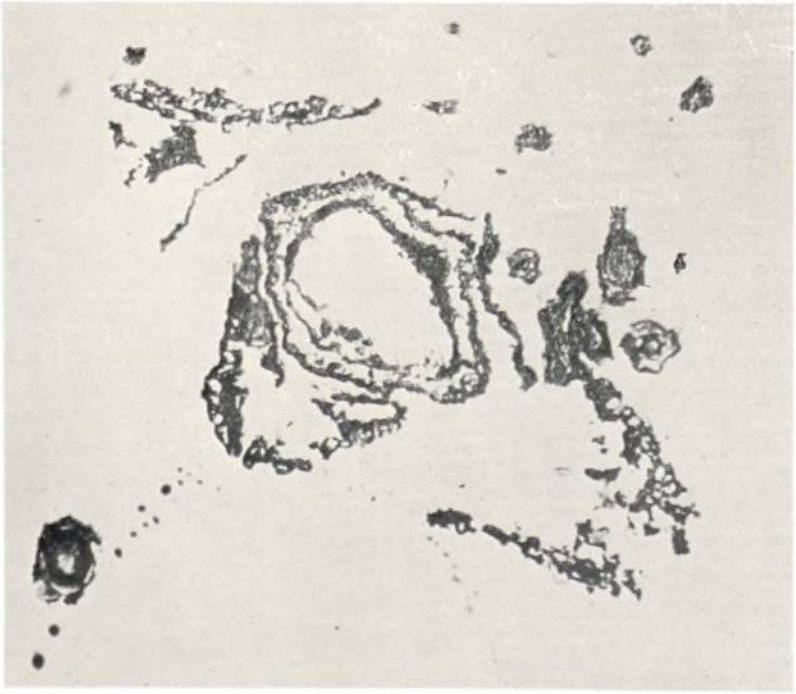


FIG. 4. Transverse section of *Calluna vulgaris* litter taken from a position 2 cm. below the surface of *Calluna* mor. Extremely fragile material with vascular and epidermal tissues disappeared and the residual mesophyll tissue becoming amorphous material. 100. (See page 62.)

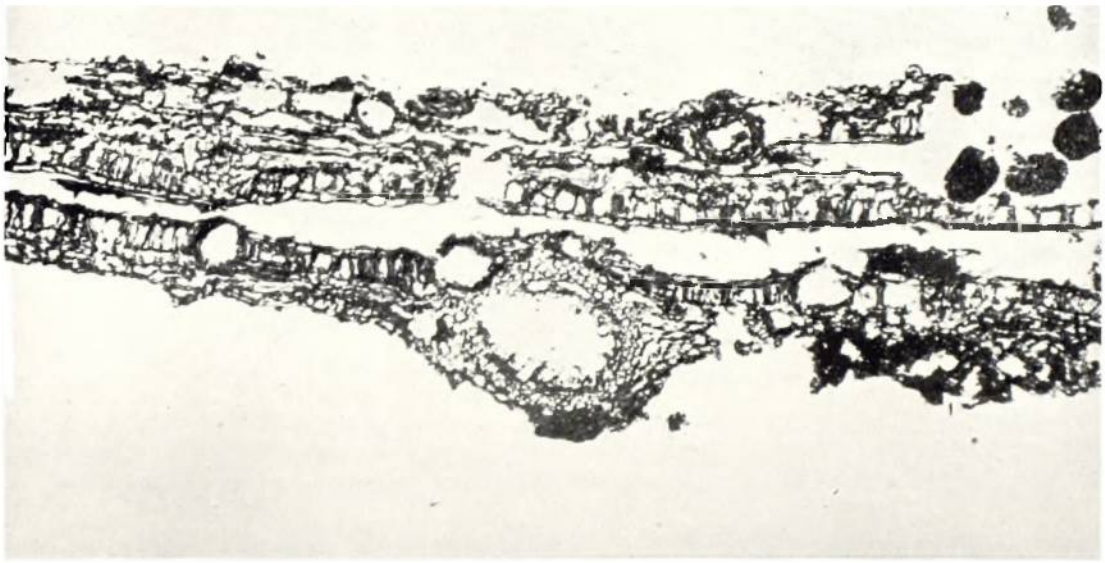


FIG. 5. Transverse section of the most fragile and brittle recognisable litter remains from a Chiltern beech mor. Vascular tissue disappearing, mesophyll tissue persistent, faecal pellets of litter-eating fauna. × 100. (See page 63.)

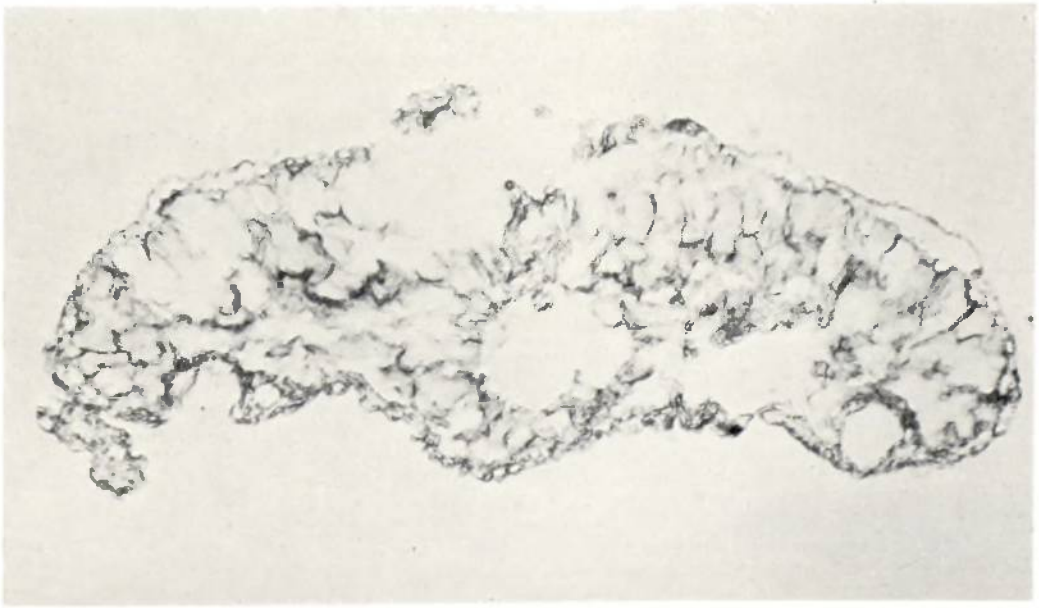


FIG. 6. Transverse section of litter taken from bottom of litter layer beneath *Picea abies*; vascular tissue very largely disappeared, mesophyll tissue persistent. × 90. (See page 63.)

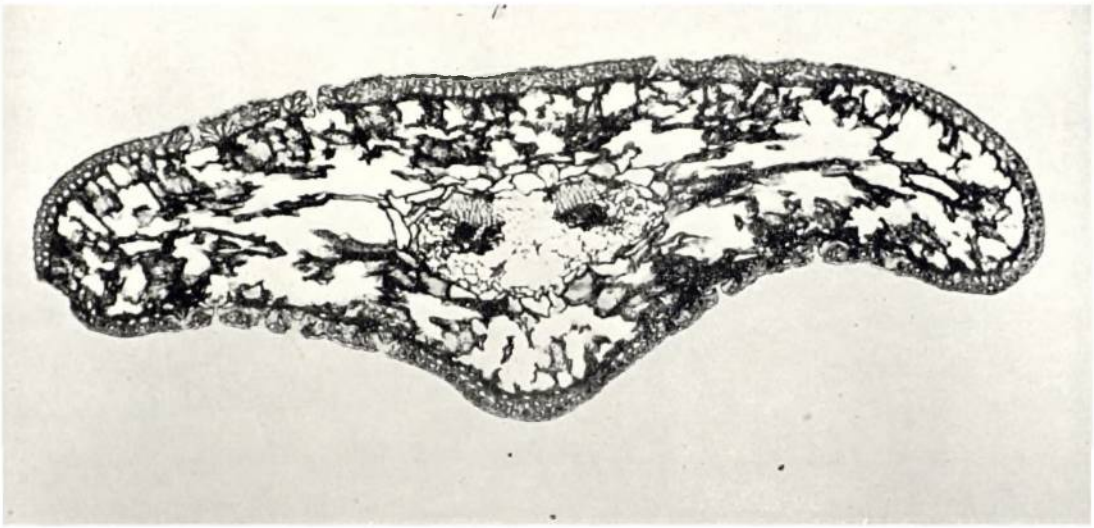


FIG. 7. Transverse section of one-year-old living leaf of *Abies pinsapo*. × 40.
(See page 63.)

FIG. 8. Transverse section of litter taken from lowest layer of litter beneath a specimen of *Abies pinsapo*. Vascular tissue disappearing, mesophyll tissue persistent and containing faecal pellets of the litter-eating fauna. × 30.
(See page 63.)

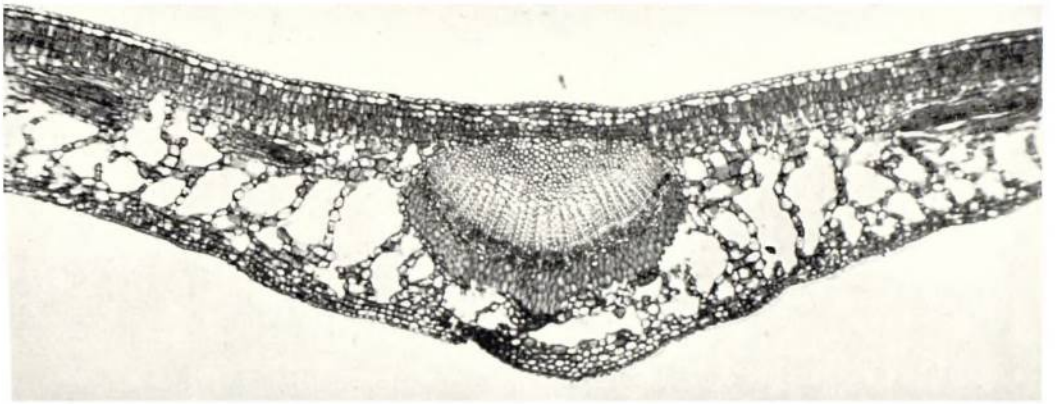


FIG. 9. Transverse section of living leaf of *Ilex aquifolium*. × 30. (See page 63.)

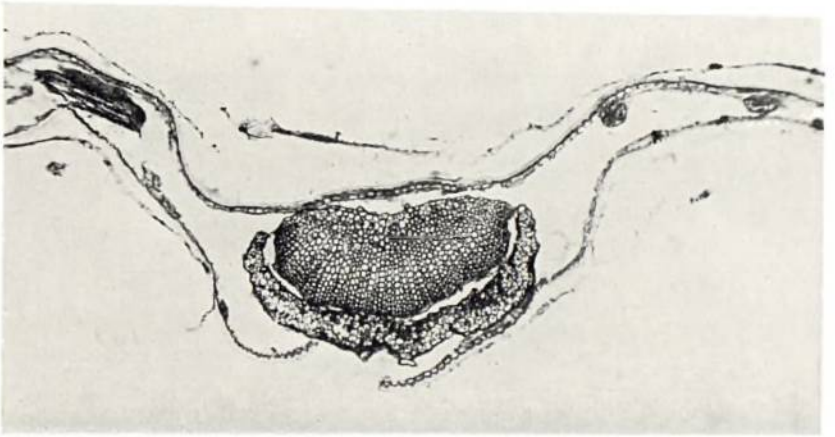


FIG. 10. Transverse section of litter of *Ilex aquifolium* which has undergone considerable decomposition; much of the mesophyll tissue has disappeared whilst the vascular tissue remains apparently unaltered. × 30. (See page 63.)

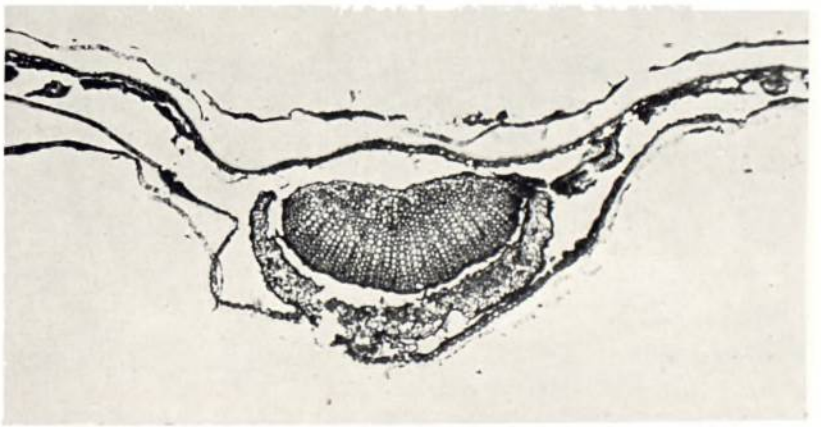


FIG. 11. Transverse section of same material as in Fig. 10. Section treated with sodium hypochlorite solution. × 30. (See page 63.)

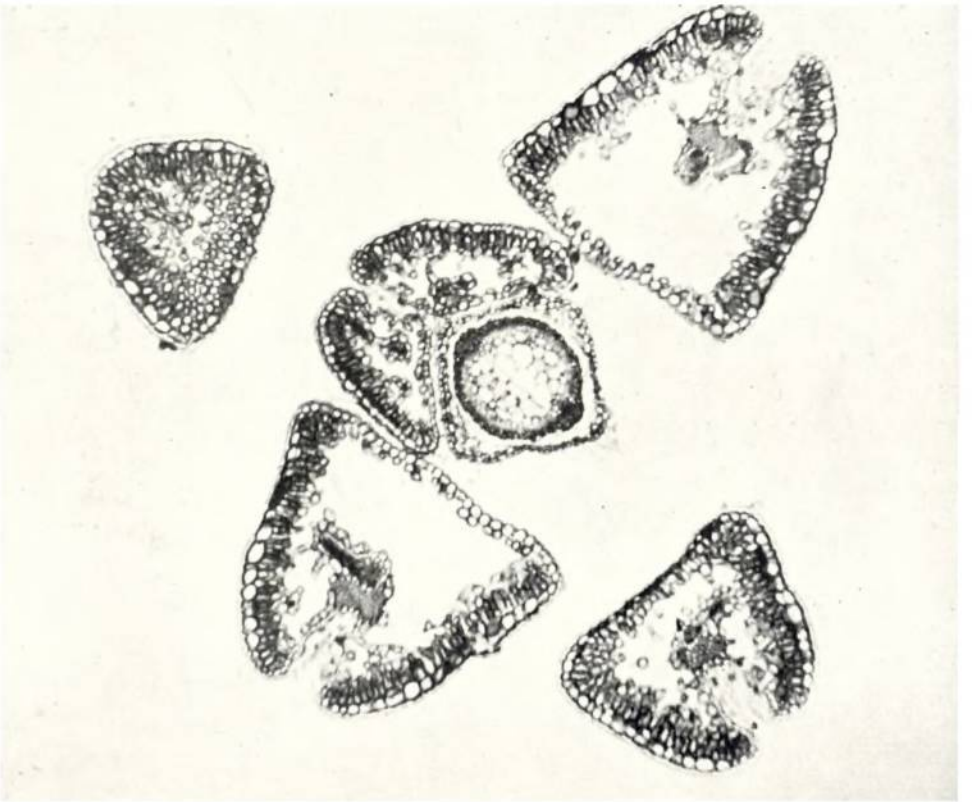


FIG. 12. Transverse section of shoot of *Calluna vulgaris* having both living and dead leaves. Section from the region where all the leaves are alive. × 50. (See page 65.)

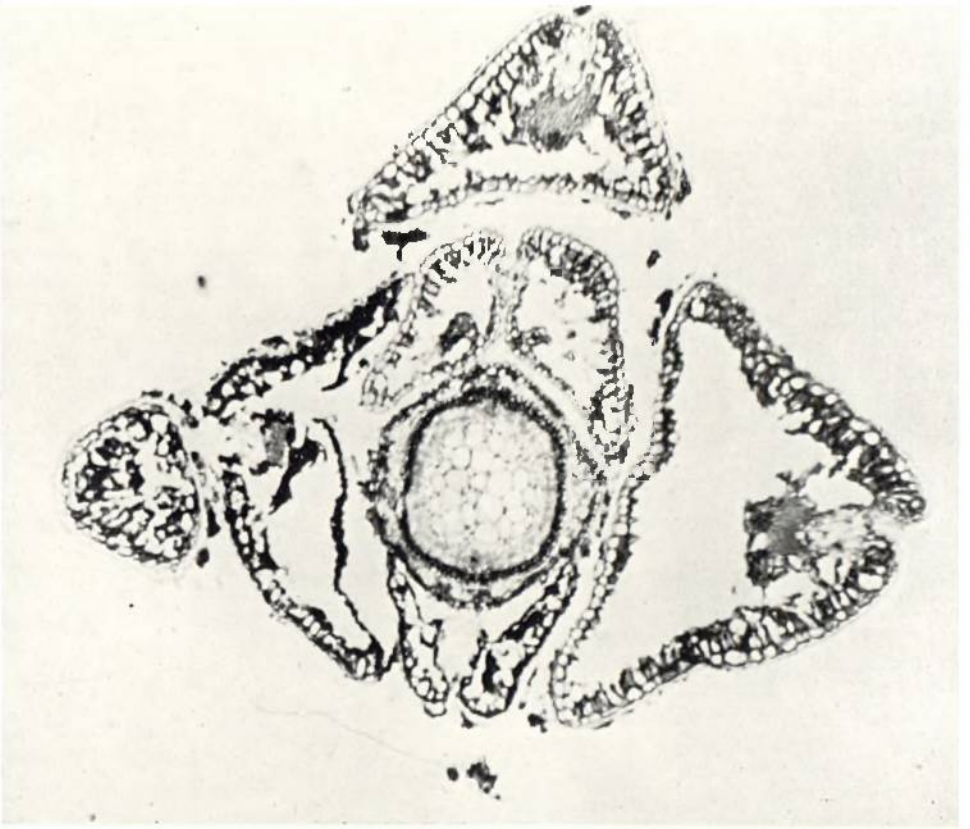


FIG. 13. Transverse section of shoot of *Calluna vulgaris* having both living and dead leaves. Section from the region where some of the leaves are dead and some are alive. × 50. (See page 65.)

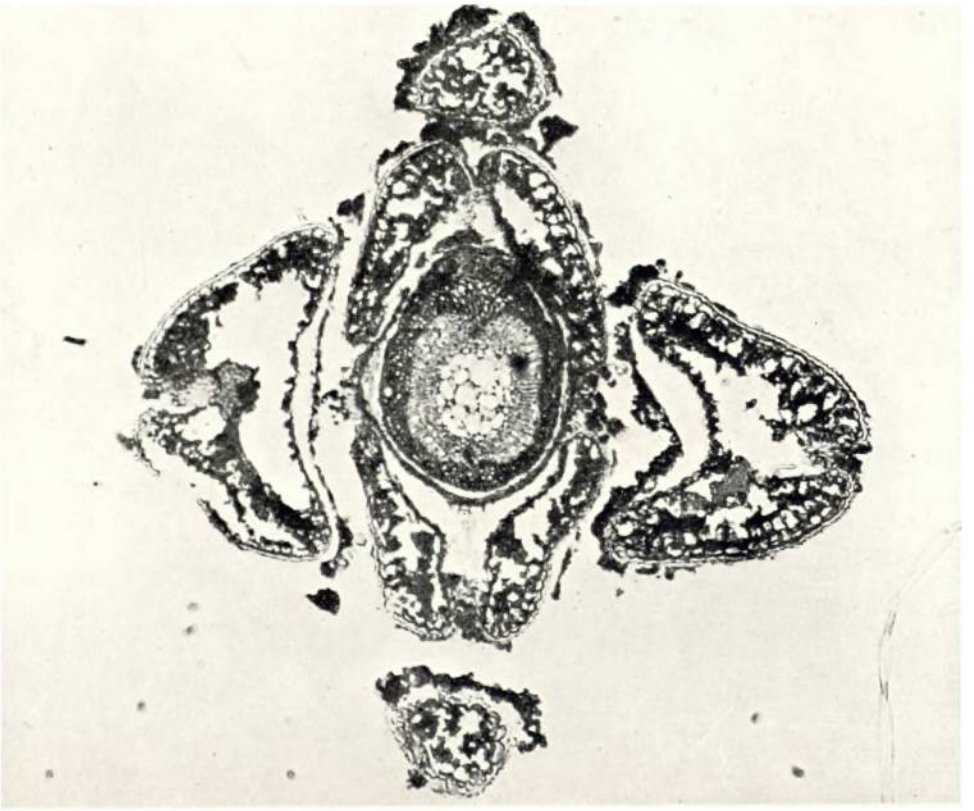


FIG. 14. Transverse section of shoot of *Calluna vulgaris* having both living and dead leaves. Section from the region where all the leaves are dead. × 50. (See page 65.)

There are a few yellow refractive fragments and occasional small fragments giving the reaction for cellulose.

Sample from a depth of 5 *cm.*

Observations as for material from 1 cm. depth but even less material giving the reaction for cellulose.

Sample from a depth of 10 *cm.*

This material proved to be very similar to that from 1 and 5 cm. depths. There are, however, fewer fungal hyphae, and fragments giving the reaction for cellulose were not observed.

3. Samples from young oak stands (40–50 years) growing on more acid mull soils formed from Kimmeridge clay with varying proportions of admixed plateau gravel.

(a) The first site has an oak stand of Spessart provenance and has a ground flora of very sparse grass and weak blackberry shoots. A thin layer of loose litter remains but there is no evidence of the accumulation of a layer of organic material on the surface. The surface layer of the soil had a reaction of pH 3.9, as measured by means of the glass electrode, when moistened with water, and is dark brown in colour quickly changing to a lighter brown as depth increases. Earthworms are present.

Sample from dark surface layer:—

Not treated with sodium hypochlorite solution:—

Consists of a large amount of mineral material and numerous dark brown amorphous particles of all sizes; there are also fragments of plant tissue and fairly numerous fragments of fungal hyphae but only very occasional particles of material giving the reaction for cellulose.

After treatment with sodium hypochlorite solution:—

The only changes in microscopical appearance are that the dark brown amorphous particles have become a little lighter in colour and the particles of material giving the reaction for cellulose are a little more numerous.

Sample from lighter brown layer 10 *cm. below the surface:—*

Not treated with sodium hypochlorite solution:—

This material has a very similar appearance to that of the surface layer except that there are even fewer fragments of plant tissue and cell fragments giving the reaction for cellulose.

After treatment with sodium hypochlorite solution:—

The dark brown amorphous masses have become much lighter in colour and there are a number of the fragments of plant tissue, as distinct from fragments of plant cells, giving the reaction for cellulose.

(b) Samples from the mull soils under two further young oak stands, one naturally regenerated, have also been examined. These stands are growing on mineral material containing a higher proportion of clay than in the case of the Spessart oak, and have reactions of pH 4.1 (natural regeneration plot) and pH 5.0 respectively. In each case the ground flora is sparse:—blackberry, grasses and *Endymion nonscripta* (natural regeneration plot) and blackberry and *Lonicera periclymenum*. Earthworms are present in each case.

Microscopical examination of samples taken at 2 and 10 cm. depth from these soils showed them to be very similar to those from the Spessart oak stand. If anything they contain even less material giving the reaction for cellulose. The small amount of material giving the reaction for cellulose in these soils seems to consist of fragments of plant tissues, and has probably been carried down into the soil by the soil fauna and is not yet decomposed. Samples are lighter in colour after treatment with sodium hypochlorite solution and the brown amorphous particles seen in the untreated soil seem to consist of small aggregates of fine mineral particles cemented together in some way.

Advantage has been taken of an opportunity to examine certain tropical soils from the same point of view:—

1. An air dried sample from the surface layers of the soil of the previously described (page 9) Wallaba forest of British Guiana. The soil consists very largely of coarse sand from which the small content of organic matter is readily separated by flotation. On treatment with iodine and zinc chloride solutions this organic material was seen to consist largely of dark brown amorphous particles, pale brown to almost colourless fungal hyphae, fragments of roots and other plant tissues but only, very occasionally was material giving the reaction for cellulose observed. After treatment with sodium hypochlorite solution the dark brown amorphous particles became pale yellowish-brown and there was now a large amount of material giving the reaction for cellulose. The cellulose material consisted for the most part of fragments of cell walls or almost isodiametric cells; occasionally spirally thickened xylem elements not giving the reaction for cellulose were observed. Appreciable amounts of epidermal tissues, some of which give the reaction for cellulose, are also present. The fragments of cell wall giving the reaction for cellulose now occur free and also embedded, apparently in a random manner, in the amorphous masses. The fungal hyphae have

disappeared as a result of treatment with the sodium hypochlorite solution. The microscopical character-istics of this tropical soil seem to have many similarities to those of a mor of temperate climates.

2. An air dried sample from the surface layer of a silty forest soil adjacent to the Wallaba forest and carrying a different vegetation. After treatment with iodine and zinc chloride solutions microscopical examination showed the soil to consist largely of various kinds of mineral particles with medium brown amorphous particles, a few brownish fungal hyphae, fragments of root material and extremely occasional particles among the mineral particles, giving the reaction for cellulose. Prior treatment with sodium hypochlorite solution does not produce any change in the amount of material giving the cellulose reaction; but the medium brown amorphous particles have become light yellowish brown particles composed of aggregates of small mineral particles.

3. A sample from the top 3 inches of a red forest loam from Nigeria was also subjected to microscopical examination; the findings were essentially the same as those for the silt forest soil from British Guiana.

These findings indicate that tropical soils having similar properties to the mull and mor of temperate climates also have similar microscopical characteristics. Whilst the amorphous organic material of mor appears to contain a considerable amount of masked cellulose, which tends to decrease in amount with depth, there are at most in mull soils only very small quantities of material which might be suspected to be cellulose.

Chapter 10

THE ORIGIN OF THE MASKED CELLULOSE IN MOR

In view of the very small amounts of free cellulose, as compared with masked cellulose, observed in mor samples, it seems possible that the masking material may be hindering the decomposition of the masked cellulose. The origin of both the cellulose and masking material in mor, and the reason for their apparent absence in mull, would seem to be important in understanding the difference between mull and mor.

Microscopical examination of mor showed that the masked cellulose probably originated not from lignified vascular tissue but from parenchymatous tissue. In attempts to determine the source of this parenchymatous tissue mor profiles were examined, and recognisable fragments of litter, situated at various depths in the profile, were removed from a number of mor profiles formed from the debris from different plant species, and sectioned by microtome. It was found that this difficult material could be satisfactorily sectioned by the use of tertiary butyl alcohol and paraffin oil in dehydration and embedding, as advocated by Johansen (1940).

Serial sections were prepared from fragments of litter from various depths in the surface layer of mor formed from *Calluna vulgaris* litter. Although the surface organic layer was 5 cms. in thickness, only the top $1\frac{1}{2}$ cm. contained recognisable litter fragments. After cementing on slides, the sections were dewaxed and treated with iodine and zinc chloride solutions. Microscopical examination of these preparations (see Figs. 1–4) showed the stem vascular tissue and leaf traces in various stages of decomposition, from complete disappearance of vascular tissue through xylem giving the reaction for cellulose, to unchanged vascular tissue. The leaf vascular tissue appears to persist for a longer time than stem vascular tissue. Even in those cases where the stem vascular tissue has completely disappeared the greater part of the palisade parenchyma of the leaves remains, although much of it has a ragged appearance by this time and it does not give the reaction for cellulose until the sections have been treated with sodium hypochlorite solution. It is perhaps important to note here that when sections of litter, in which mesophyll tissue which does not give the reaction for cellulose, and lignified tissue, are both present, are treated with sodium hypochlorite then, under the conditions of treatment used, the mesophyll cell walls subsequently give the reaction for cellulose, whilst the lignified vascular tissue does not give the reaction for cellulose. There would therefore appear to be differences between the material (lignin) which prevents the vascular tissue from giving the reaction for cellulose, and the material which prevents the mesophyll cell walls from giving the reaction for cellulose. In what appear to be the most advanced phases of decomposition, the epidermal cells are also disintegrating, and occasional fragments of fungal hyphae can be observed among the tissues; it looks as though the next stage is the collapse of the litter to give an unrecognisable mixture of remains of palisade parenchyma and cuticular material. These observa-

tions would appear to account for the failure to detect vascular elements in the amorphous *Calluna vulgaris* mor material, apart from those associated with fragments of *Calluna vulgaris* roots, and also Romell's (1935) observation that even the heaviest mor was not built up from undecomposed lignified remains derived from litter.

Although these findings are not in accord with the idea that mor results from the accumulation of lignified tissue, they do agree with the findings that twigs and branches which have fallen on mor, tree stumps in mor, and dead stems of *Calluna vulgaris* on mor, all decompose readily in the mor environment.

Essentially the same picture was observed on examination of material from samples of mor in process of formation from the litter of other species, e.g. *Pseudotsuga taxifolia*, *Picea abies*, *Larix decidua*, *Fagus sylvatica* and *Abies pinsapo* (Figs. 5–8.)

In a number of instances the mesophyll of the decomposing, mor forming, litter contained more or less large cavities, whilst the vascular tissue still remained intact. The cavities contained oval bodies fairly regular in size and shape which are almost certainly the faecal pellets of some members of the soil fauna. Before treatment with sodium hypochlorite solution the pellets are very dense and almost black; but on treatment they retain their shape and are seen to consist of fragments of cell walls, presumably from the mesophyll, giving the reaction for cellulose. In his description of structural changes occurring during the decomposition of larch needles lying on the surface of a mull soil Wittich (1943) observed that after five years on the surface of the ground only slight changes could be seen in the epidermal layer. The cell walls are weakened but the shape of the needle is still maintained. Compared with the condition after three years the parenchymatous tissue of the larch needle was largely destroyed; at this stage the decomposition can be compared to that of needles in the middle of a normal raw humus layer which have also undergone decomposition for several years. This disappearance of parenchymatous tissue before the vascular tissue in the larch litter might also be associated with the activities of members of the soil fauna which ingest the tissue but do not necessarily digest more than a small fraction of it. Wittich also observed that whereas needles lying on the surface of a mull soil did not undergo decomposition any more rapidly than needles of the same age in a raw humus layer, when these needles were dragged into the mull soil by earthworms they appeared to decompose very rapidly. This may represent the influence of the mull soil in bringing about a mull type of decomposition of the larch needles; but it also seems possible that the effect may have been one of increased rate of decomposition of epidermal and vascular tissue under the more favourable environment of the earthworm burrow, leaving the mesophyll tissue as an unrecognisable amorphous material.

For comparison with the observations on the changes taking place during the decomposition of mor-forming litter, sections were prepared of decomposing litter associated with species growing on mull soils. *Ilex aquifolium* and *Buxus sempervirens* were chosen as species having tough leaves but not forming a raw humus. Here the picture is exactly the reverse of that found in the case of mor forming litter, for the mesophyll tissue rapidly disappears leaving the cuticle supported by the network of vascular tissue. (Figs. 9–11.)

Observations which have been made from time to time on the differential breakdown of various tissues in wood may be related to the above findings on the differential decomposition of tissues in litter. Büsgen, Münch and Thomson (1929) point out that many medullary rays are more resistant than the rest of the wood; they cite the example of oak in which the medullary rays may still remain as solid bands when the rest of the wood has become a brown crumbly mass.

In the present investigations the reddish-brown residue from a rotten stump of *Picea abies* was examined microscopically and found to consist of reddish-brown apparently amorphous particles, similar in many respects except colour to the amorphous material observed in *Calluna* mor. Only very occasional fragments of material giving the reaction for cellulose were observed; tracheids were not observed. This material left a creamy-white residue on treatment with sodium hypochlorite solution, which consisted almost entirely of fragments of cell wall, apparently from medullary rays, giving a strong reaction for cellulose with iodine and zinc chloride solutions.

In contrast with these observations Szuleta (1947) states that three wood-destroying fungi, growing on sterilised blocks of *Pinus sylvestris* wood, chiefly attacked the ray cells, resin ducts and tracheids of the early wood.

Boyce (1938) mentions that in oaks attacked by *Polyporus berkeleyi* and *Polyporus frondosus* the rays are unaffected, although the other woody tissues are decomposed by these white rots. On the other hand in *Robinia pseudoacacia* attacked by *Fomes rimosus* the decay extends outward from the centre of the heartwood in a series of radial lines along the larger rays.

Varossieau (1949) examined piles which had been buried below ground water level at Rotterdam for periods varying between 30 and 600 years. The timbers used were *Picea abies*, *Pinus sylvestris* and *Abies alba*. Varossieau concluded that taking the

annual ring as a unit of structure the tissues are not all equally attacked. The ray cells are involved first, followed by the tracheids near the boundary of the annual ring and adjacent to the rays. On the other hand Barghoorn (1949) examined a fresh water marsh peat of considerable, though undetermined, age and found, after treatment with sodium chlorite, innumerable delicate plant fragments such as root hairs and root cap cells. In spite of the fact that these delicate root cells have retained their minute anatomical features the thick walled fibrous or conductive tissues of the roots are absent. Barghoorn considers this to indicate that unlignified primary cell walls are more resistant under conditions of anaerobic degradation than heavily lignified secondary cell walls, and that there are fundamental chemical differences in the cellulose of successively formed lamellae of the cell wall.

A tertiary woody lignite was examined by Barghoorn and Spackman (1950) who found it to be composed almost entirely of the remains of dicotyledonous plants. Material from the root wood of *Cyrilla* sp. was sectioned and it was observed that the aggregates of ray cells were far less altered physically than the longitudinally oriented elements of the wood. The findings were similar for the wood of a *Persea* sp.

There therefore seems to be evidence that, in some cases at least, the medullary ray cells show greater resistance to decomposition when compared with other cellular components of wood. The fact that, like the mesophyll cells of leaves, the medullary ray cells remain alive longer than the cells of associated tissues which are more readily decomposed makes it important to know whether there are any known properties of medullary ray cells which might render them resistant to decomposition. In 1928 Harlow and Wise determined the so-called lignin content of medullary rays and compared it with that of the total wood for two species of oak. In both cases the percentage of lignin was found to be considerably higher in the ray tissues than in the whole wood. Later, whilst examining the changes occurring during the transformation of sapwood into heartwood, Pew (1949) observed that amorphous deposits may accumulate in the cell cavities, principally in the medullary rays. Within a cell a film of the material connects the larger aggregates and completely lines the interior of the ray cells, extending also into the connecting pits of the fibres. In the case of Douglas fir this material is pale orange in colour under the microscope, reddish-brown in the mass, and is chiefly responsible for the orange hue of the heartwood of this species. Other species, both coniferous and dicotyledonous, were found to have similar material in the medullary ray cells; but it varies considerably in amount from species to species, e.g. Blackjack oak heartwood contained considerable quantities. These membrane-like deposits were not observed in the vertical tracheids of any species. In no instance have these membranous materials been observed in green sapwood, nor in green sapwood after rapid drying in air, but in the case of western hemlock seasoned for a long time with the bark on, fragmentary membranes did appear in the sapwood. The formation of membranes in medullary ray cells is evidently associated with the death of these cells.

These membranous deposits first swell and then dissolve when boiled in dilute aqueous sodium hydroxide; they are not extractable by inert solvents and are extremely resistant to the action of acid reagents. This latter property has been made use of in the isolation of the material by treatment of wood with formalin and 72% sulphuric acid; the material remains apparently unchanged by this process even after standing in the reagent for one month. Such preparations, however, no longer swelled when treated with hot dilute sodium hyrdroxide.

There would seem to be the probability of a connection between these membranous materials of medullary ray cells and the increased resistance of some ray cells to decomposition on the one hand, and also between the masking material and the apparent resistance to decomposition of leaf mesophyll cells of mor-forming litter.

Chapter 11

THE ORIGIN AND NATURE OF THE MATERIAL MASKING THE CELLULOSE OF THE MESOPHYLL CELLS OF MOR-FORMING LITTER

THE masking material might be a product of microbial metabolism; but this seems rather unlikely since the tissues would, even in the case of mor-forming litter, presumably be vulnerable to attack as soon as they fell from the tree as in the case of *Ilex aquifolium* and *Buxus sempervirens*, yet in fact they do not seem to be. It is also not easy to account, along these lines, for the apparent localisation of the

masking material in the mesophyll cells. It seems more likely that the masking material is an integral part of the plant material, and if this is the case it might be possible to observe it at, or very soon after, the death of the leaf. Sections have therefore been prepared of living leaves and leaves which were dead but had not fallen from the plant. In the case of shoots of *Calluna valgaris* with dying leaves it is possible to have living and dead leaves next to each other which appear in the same section of a stem with leaves attached. In the dead leaf the tissue has undergone changes so that the mesophyll tissue already has the appearance which it retains until the time when the epidermis disintegrates and the mesophyll becomes an amorphous mass. (Figs. 12–14.) The walls of the mesophyll cells of the dead *Calluna vulgaris* leaf do not give the reaction for cellulose until they have been treated with sodium hypochlorite solution. If on the other hand we go to the other extreme and sections are prepared from living and recently dead leaves of *Bambusa* sp., which gives rise to mull, differences between the living and dead leaves, similar to those observed in the case of *Calluna vulgaris*, are not detectable. Further observations on other species, however, indicate that it will probably not be possible to differentiate between potential mor-forming litter and mull-forming litter from the microscopic difference in appearance between the living and recently dead leaves. Since it seems reasonable to suppose that the changes in the mesophyll taking place at the death of the leaf result in the masking of the cellulose walls of this tissue, the nature of this change was investigated. The invisible stage of Müller's scheme of investigation is now being approached.

It would appear that the material most likely to be present in the cell in sufficient amount and capable of undergoing profound and rapid change on the death of the mesophyll cell is the cytoplasm, the predominant constituent of which is protein. From the observations on the recently dead litter of different species there are indications that if the cytoplasmic proteins are the source of masking material then they undergo changes which are visibly different in different species. Since all mesophyll cells contain cytoplasmic protein it would seem that if cytoplasmic proteins are the source of masking material then the cellulose walls of all recently dead cells, i.e. before microorganisms have normally had an opportunity to bring about changes, might be expected to be masked. In this connection, however, Priestley and Scott (1938) observed that the walls of the meristematic cells of the shoot apex readily gave the reaction for cellulose with iodine and sulphuric acid or zinc chloride, whereas the meristematic cells of the root apex did not react with these reagents unless the tissue was previously treated by prolonged boiling with alkali or warming with alkaline oxidising agents such as Eau de Javelle. From these observations they concluded that the walls of the meristematic cells of the root apex are more impregnated with protein, possibly still with the living protoplasm of the cell which laid them down.

It seems extremely unlikely however that the drying of the cytoplasmic proteins on the death of the mesophyll cell would protect either the proteins or the cellulose walls from decomposition when it is placed under moist conditions on the ground, since protein material is rapidly attacked by micro-organisms under such circumstances. It seems likely therefore that in those cases where the mesophyll cells are resistant to breakdown the proteins are in some way protected from decomposition. Is such a protective agent likely to be a difference in properties of the protein in the case of mor-forming litter, or the result of union of the protein with some other cell constituent which is not present in the cells of mull-forming litter? Although the meaning of staining reactions is often doubtful it is noticeable that sections of the fresh leaves of various species when stained with Safranin and counterstained with Fast Green do show differences. Whereas sections of the leaves of many mull-forming species show the mesophyll cells stained with the Fast Green, sections of the leaves of mor-forming species show the palisade cells and their contents stained to a greater or less extent with Safranin; this also applies to some mull-forming species such as *Chamaenerion angustifolium*.

The Composition and Properties of Leaf Proteins

It is therefore desirable to consider the information available concerning the leaf proteins of different species. The isolation of leaf proteins presents a number of difficult problems, but the use of high speed centrifugation has considerably assisted in the separation of leaf proteins which have been differentiated largely on the grounds of their distribution in the cell, i.e. chloroplastic and cytoplasmic proteins. By comparison with seed and animal proteins, leaf proteins have been studied very little.

Formerly only a very small proportion of the leaf proteins could be extracted by the methods used and, even though much better yields are often obtained by present methods, there is still (Chibnall 1939) often failure to extract proteins in a reasonable degree of purity from the leaves of many important groups of plants; in the case of a number of species there is entire failure to obtain cytoplasmic protein (Vickery 1945). The reason for this may be indicated by the observations of Waygood and Clendenning (1951) that a natural flocculating agent, believed to be a tannin, is present in the cell sap of the leaves

TABLE 30

	Dactylis glomerata		Lolium perenne	Lolium italicum	Poa trivialis		Cynosurus cristatus	Festuca rubra var fallax (Hack)		Festuca trachyphylla	Phleum pratense	Medicago sativa	Trifolium repens	Trifolium pratense
	29.9.36	7.6.37	6.11.33	26.6.33	27.6.33	4.10.32	12.6.33	29.6.33	26.9.32	29.9.32	18.10.32	29.6.33	18.10.32	23.10.33
Yield as a percentage of total leaf protein nitrogen	—	—	21.8	20.5	13.7	13.6	—	—	17.8	16.1	9.7	12.6	28.0	24.5
Total nitrogen (ash free) percentage	14.0	13.1	13.0	14.1	14.0	13.6	14.1	14.4	14.2	15.0	13.8	14.4	13.2	12.8
	Percentages of Total Protein Nitrogen													
Amide nitrogen	5.3	5.0	5.0	4.7	5.1	4.8	4.7	5.1	4.6	4.8	4.8	5.2	5.7	5.4
Arginine nitrogen	13.9	15.5	14.5	13.4	16.4	15.3	15.1	14.3	14.6	14.0	14.8	15.1	15.4	14.9
Histidine nitrogen	2.3	2.3	2.4	2.4	2.3	2.1	2.6	1.9	2.0	2.4	2.4	2.3	1.6	2.9
Lysine nitrogen	6.2	6.0	6.2	5.9	5.8	5.4	5.8	5.2	5.7	5.8	4.9	7.0	6.6	6.5
Tyrosine nitrogen	2.3	2.3	2.2	2.3	2.3	2.3	2.2	2.4	2.3	—	—	2.8	2.5	2.5
Tryptophan nitrogen	1.8	1.8	1.8	1.8	1.7	1.7	1.8	1.9	1.9	—	—	1.9	1.9	1.7
Cystine nitrogen	1.5	1.4	1.3	1.3	1.4	—	1.5	1.3	—	—	—	1.3	1.0	1.2
Methionine nitrogen	1.3	1.2	1.2	1.4	1.5	—	1.4	1.4	—	—	—	1.3	1.2	1.2
Glutamic acid nitrogen	—	7.8	7.2	—	6.8	—	—	6.6	—	—	—	6.4	6.6	—
Aspartic acid nitrogen	—	4.9	5.4	—	5.2	—	—	4.9	—	—	—	5.4	4.7	—

(e.g. *Sambucus canadensis* and *S. racemosa*) which brings about flocculation of non-plastid cytoplasmic protein and then association of this flocculated material with chloroplast material during their attempted isolation by centrifugation.

Wildman and Bonner (1947) have estimated that in spinach leaves the protoplasm constitutes 76.2% of the dry weight of the leaves. This is made up of chloroplasts (26.8% of the dry weight and 37.9% of the total nitrogen of the leaves), cytoplasmic proteins (15.8% of the dry weight and 39.3% of the total nitrogen of the leaves) and water soluble low molecular weight substances (23.6% of dry weight and 22.8% of total nitrogen in the leaves).

Preparations of leaf protein have been investigated chemically to determine the amounts of the various amino acids they contain. Chibnall (1939) gives the results shown in Table 30 for the mixed protoplasmic proteins, for a number of species in terms of percentages of total protein nitrogen.

Results for cytoplasmic and chloroplastic proteins are given in Table 31.

Although the composition of approximately 20% of the protein remained undetermined, the amino acid composition of the rest, as determined by the methods used, indicates a surprising uniformity of composition of the cytoplasmic protein preparations from widely varied species of plants. It is of interest that leaf proteins apparently contain considerable amounts of basic amino acids. So far as can be ascertained there have been no attempts to investigate the leaf proteins of mor-forming species in a similar manner.

Bonner (1950) concluded that a large part of the cytoplasmic protein of the leaf consists of a single, electrophoretically homogeneous, component, the remainder consisting of a variety of minor constituents, which include a considerable number of recognisable enzymes. Wildman and Jagendorf (1952) reached a similar conclusion but consider there are indications that the leaf proteins of at least some monocotyledons show marked differences with regard to electrophoretic and ultracentrifugal properties compared with dicotyledonous leaf proteins.

The protein of chloroplasts presents more difficulties in its isolation than cytoplasmic protein, and the processes involved appear to result in a certain amount of degradation. Such analyses as have been made indicate that its amino acid composition is similar to that observed for cytoplasmic protein.

Lugg (1949) has given an account of the properties of leaf proteins. It has been shown that, for many species of plants, leaf proteins have minimum solubility when the reaction of the solution is within the pH range 4–5. They are more freely soluble on the alkaline than on the acid side of this range. The presence of ethyl alcohol depresses the solubilities still further; the solubility relationships of chloroplastic and cytoplasmic proteins are similar. The solubility of isolated leaf proteins is low and after drying even at fairly low temperatures they are almost insoluble in water or mildly alkaline buffer solutions. The labile nature of leaf proteins is also demonstrated by the fact that acid flocculated leaf protein is readily denatured if heated above 50°C; surface denaturation occurs readily at a reaction of pH 5–6. Extracted leaf proteins may therefore have considerably different characteristics from leaf proteins in situ. Lugg concludes that the bulk of proteins of leaves do not fit into a group of the well-established classes of proteins and should be placed in a separate group; also that variations in composition among plant families are probably slight, but may be definite in some cases, such as the relatively low cystine and methionine values for the Leguminosae compared with those for other plant families.

According to Lloyd and Shore (1938) the most characteristic property of leaf cytoplasmic proteins is their sensitivity, particularly in acid solutions, to traces of salts; they quote the observation of Chibnall

TABLE 31 Yield as percentage of total protein Nitrogen.

	Zea mais	Ricinus communis	Phaseolus multiflorus	Spinacea oleracea cytoplasmic protein	Spinacea oleracea chloroplastic protein
Amide Nitrogen	5.4	5.1	5.4	5.6	5.1
Arginine	14.4	12.9	14.9	14.1	13.9
Histidine	2.1	2.2	2.6	2.2	3.3
Lysine	6.1	6.5	6.1	6.2	4.7
Tyrosine	2.3	2.6	2.5	2.7	2.6
Tryptophane	1.6	1.7	1.6	1.7	1.7
Cystine	1.1	1.5	1.1	1.4	1.2
Methionine	1.3	1.45	1.1	1.3	1.3
Aspartic acid	—	5.6	5.2	5.5	5.8
Glutamic acid	—	6.7	6.7	6.5	6.5

and Nolan that the protein was immediately and completely precipitated from 50 ml. of a 4% solution of alfalfa protein by the addition of 4 drops of a saturated solution of ammonium sulphate. In this respect, with the possible exception of the α—glutelins, the cytoplasmic proteins of green leaves differ from all other known proteins.

Although the leaf proteins of the various herbaceous species examined appear to contain very similar proportions of the various amino acids, this does not necessarily mean that the proteins themselves are identical in properties, for they may be structurally quite different. The only indication of of such differences so far would appear to be the previously noted observation that using the same extraction methods there was complete failure to obtain cytoplasmic protein in the case of a number of species. The reason for this may lie, however, not in the structure of the proteins but in some non-protein constituent of the leaves, such as the protein flocculating agent observed by Waygood and Clendenning (1951) or acidity of the vacuolar sap as suggested by Wildman and Jagendorf (1952).

The investigations of leaf protein have been carried out largely if not entirely on material from fresh vigorous leaves in which the proteins may be in a different state compared with those in the moribund or dead leaf which is the material concerned in the formation of mull and mor. The processes occurring in the dying leaf were described in very general terms by Büsgen, Münch and Thomson (1929) as a loss of nitrogen just before leaf-fall resulting in the impoverishment of the protoplasm whose remains, including the nucleus, are otherwise still present in the faded leaves.

A number of studies have been made on the changes occurring in the constituents, including proteins, of isolated leaves. Most of the work has been carried out on fresh active leaves. The general opinion seems to be that under these starvation conditions protein hydrolysis proceeds at about the same rate in the case of both the chloroplastic proteins and the cytoplasmic proteins, giving rise at first to an increase in free amino acid content which subsequently declines. That such changes may not be the same as those occurring in the dying leaf seems to be indicated by the work of Michael (1935) on the leaves of *Tropaeolum majus*. He observed a difference between the changes occurring when green active leaves were isolated, and the changes when old leaves were isolated. In young leaves isolation retarded the onset of yellowing, especially in the absence of the petiole, but it did not retard yellowing in old leaves which he found to contain very little nitrogen.

It must be concluded that the characteristics of any proteins or their products which may remain in the dead leaf do not seem to be known, nor does there seem to be any information regarding differences between the proteins of potential mor litter and mull litter, nor concerning possible degrees of specificity of the proteolytic enzymes of soil micro-organisms for proteins present in the litter of different plant species. The activity of a particular proteolytic enzyme may vary according to the nature of the protein, so that with different proteins different products of hydrolysis may be produced and different rates of hydrolysis may be observed. Native proteins, whatever their origin, do not seem to exhibit an absolute resistance to hydrolysis by all proteolytic enzymes. Differences in the action of the same enzyme on different proteins are probably related to unknown details of the architecture of the protein molecules. It is possible that the structure of the proteins of potential mor litter and mull litter differs in such a way that whilst the proteins of the mull litter are broken down by proteolytic enzymes those of potential mor litter are not attacked by the same proteolytic enzymes under certain similar environmental conditons, since for example mull and mor can both develop over at least part of the same pH range. It is perhaps more likely however that soon after the death of the leaf the proteins remaining in the cells are changed in some way by other constituents of the leaf so that, they become resistant to breakdown, at least under some conditions. It is perhaps of interest to note that Waksman (1938) makes the statement that the proteins of certain plant residues such as oak leaves and pine needles seem to be highly resistant to microbial attack, by reason either of their specific nature or of their formation of complexes with other plant constituents.

The artificial process of tanning hides renders a putrescible animal protein resistant to change by microbiological and hydrothermic agencies. Since many of the materials used in the tanning of hides are of vegetable origin the possibility of leaf proteins, particularly in mor-forming species, being rendered resistant to microbiological degradation on the death of the leaf, by a process similar to tanning, has been examined.

The Precipitation of Protein by Aqueous Extracts of Fresh Leaves

The detection of protein precipitating material in leaves would appear to be dependent on the presence of an excess of this material in a given leaf over that necessary for precipitation of the proteins in that leaf. Vegetable tanning materials are soluble in water, and therefore extracts of the leaves of a number of plant species were prepared by extracting fresh leaves; with distilled water in the proportion of 150 gm. of leaves to 300 ml. of water, in airtight

jars in the presence of a few ml. of chloroform to prevent the growth of micro-organisms, in one or two cases litter was also used. After one or two weeks the fluid was extracted by means of a press and stored in the presence of a little chloroform. In the detection of protein precipitating factor one drop of an aqueous 1% solution of gelatin was added to one ml. of the leaf extract and the resulting reaction noted immediately, and also after standing overnight. In view of the possibility that tannins or other phenolic compounds might be involved, each leaf extract was examined for material giving a colour reaction with aqueous ferric chloride solution, by adding two drops of 5% ferric chloride solution to 1 ml. of leaf extract.

Preliminary tests showed that extremely active protein precipitating factors could be detected in leaf extracts; visual examination indicated a greater concentration of protein precipitating factor in leaf extracts than in comparable litter extracts; this may well be due to the excess protein precipitating factor being washed out by rain while the litter is lying on the ground. The results obtained for leaf extracts of a number of species and for litter extracts of a few species are given in Appendix I (page 103).

In any interpretation of these results it must be remembered that any precipitation of gelatin is brought about by an excess of precipitating factor over any that may be removed by the proteins of the leaf from which it was extracted. This is perhaps indicated by the fact that, as judged by the visual amount of gelatin precipitates formed, extracts from the leaves of different individuals of the same species yield different amounts of precipitate.

At least three types of reaction seem to be indicated by the results obtained above:—

a. The formation of a precipitate with gelatin by extracts from the leaves of all the species investigated which are known to be associated with the formation of mor.

b. The formation of a precipitate with gelatin by extracts from the leaves of species which are not known to give rise to mor.

c. The absence of formation of a precipitate with gelatin by extracts from the leaves of species which are not known to give rise to mor—these are very largely herbaceous species.

The different colours of the gelatin precipitates indicate that there are probably a number of different precipitating factors in the various species.

Although there is usually an association between the production of a colour reaction, though not always the same colour, when ferric chloride solution is added to the leaf extracts, and the formation of a precipitate with gelatin, there are a number of exceptions. Thus the extracts of the leaves of a number of species give a colour reaction with ferric chloride solution but no precipitate with gelatin. In addition extracts from the leaves of a few species have been observed in which although there is a reaction with gelatin, there is no immediate colour reaction with ferric chloride solution, e.g. *Pinus sylvestris* (from Painswick), *Pinus griffithii*, *Pinus ponderosa* (litter) and *Robinia pseudoacacia*.

There does not seem to be any direct relationship between the reaction, as indicated by pH measurements using the glass electrode, of the leaf extract, and the presence of gelatin precipitating factor, except that the less acid the extract the greater the tendency for absence of gelatin precipitating factor.

Naturally Occurring Protein Complexes, Resistant to Decomposition, in Animals

It is perhaps of importance in this connection that naturally occurring protein precipitation and tanning has been observed in the animal kingdom. These materials seem to be of comparatively widespread occurrence and although they are all similar in showing considerable chemical stability the system involved in their formation may not always be the same. This lends support to the hypothesis that on the death of some plant tissues the proteins undergo reaction with other constituents of the plant tissue, thereby rendering the proteins resistant to attack by micro-organisms, and in this way the cytoplasm becomes a protective film over, and perhaps in, the cellulose walls of such tissues.

A consideration of the methods of formation and properties of these naturally occurring resistant protein complexes in animals may be of help in understanding what may be rather similar materials in vegetable debris formed by the interaction of leaf proteins and protein precipitating factors, and also the part such protein complexes may play in mull and mor formation.

Pryor (1940 a and b) has described how the material for the formation of the ootheca in the cockroach (*Blatta orientalis*) is secreted by two colleterial glands, one gland secreting a water soluble protein, the other a clear watery fluid containing a phenolic substance. The interaction of the protein and the phenolic substance in the presence of an oxidase system causes the gradual darkening and hardening of the ootheca. It was later shown by Pryor, Russell and Todd (1946) that the phenolic substance concerned is protocatechuic acid (3:4 dihydroxybenzoic acid).

Brown (1950) has investigated the tanned protein of the byssus and periostrachum of *Mytilus edule* and the egg cases of *Fasciola hepatica* and found that the precursor of the quinone in these cases was not an alcohol soluble substance such as protocatechuic acid, but an alcohol insoluble material whose properties suggest that it may be an aromatic

amino acid or protein. Brown points out that if this is correct and the side chain is split off before oxidation to the quinone occurs, then the final method of tanning would not be significantly different from that in insects.

The formation of eggshells in trematodes and cestodes has been investigated histologically by Smyth (1951). The shell material produced in the so-called "vitelline" glands of trematodes has been shown to be an orthodihydroxyphenol-protein complex. Smyth found that this protein complex gave a characteristic and specific reaction with methyl green and malachite green which enables shell material to be readily identified in sections. In this way the origin of the shell material in the cells of the "vitelline" glands as spherical globules was demonstrated. Not all the cells produce shell at the same time but in each acini inactive cells free from shell occur. The "vitelline" cells loaded with shell globules may easily be traced along the "vitelline" ducts and into the lower uterus where, on meeting the ova, they release the contained globules which fuse to become moulded into typical shaped eggs. The pseudophyllidean cestodes apparently have the same system and in both cases when the worms are exposed to the air the "vitelline" glands become brown, also when eggs are exposed to the air they rapidly darken. In vitro culture, experiments on the pseudophyllidean cestode *Schistocephalus solidus* showed that under aerobic conditions the "vitelline" glands rapidly darken as maturation approaches and only a few shell-less or very abnormal eggs are produced; this is thought to be due to the shell material becoming prematurely tanned, on account of the high oxygen tension, so that the shell globules are subsequently unable to fuse together to form normal shaped egg shells. The "vitelline" cells of the cyclophyllidean cestodes, whose eggs lack an external eggshell, did not give any reaction for shell material with methyl green, which supports the hypothesis.

These observations on naturally occurring tanned proteins in animals seem to indicate that the tanning agents do not react with protein until exposed to the atmosphere, or perhaps an oxidising system of a particular potential; this may explain how it is possible for a cell to synthesise and excrete a protein tanning agent without putting the protoplasm of the cell out of action. Such a state of affairs may also apply to the cells of leaves containing protein precipitating materials.

The studies on the formation of stable protein complexes of animal origin, although yielding information on the manner of their origin, do not seem to have included consideration of their resistance to breakdown by micro-organisms. This would seem to be essential if large accumulations of this material are not to occur. It would be interesting to know whether the resistance to breakdown by micro-organisms varies according to the species of animal in which the complex originates, i.e. whether the constituents of the complexes vary, or according to the environmental conditions under which a particular complex is subjected to attack by micro-organisms.

The Breakdown of Protein Complexes, Formed by the Interaction of Gelatin and Aqueous Leaf Extracts, by Micro-organisms

The observations on the detection of gelatin precipitating factors in extracts of fresh leaves indicate that the extracts of the leaves of many species (e.g. *Chamaenerion angustifolium*) whose litter is not known to form mor may contain substances which behave as gelatin precipitating agents. Since masked cellulose has not been observed to occur in mull soils as it does in mor soils, it would seem that if resistant protein complexes do protect the cellulose walls of the mesophyll cells from decomposition in mor, then any protein complexes produced in the dying leaves of mull-forming species would seem to have to be different, perhaps in chemical constitution, and less resistant to decomposition. The microbiological properties of model protein complexes obtained by the use of gelatin as the protein and extracts of the fresh leaves of mull-forming species (e.g. *Chamaenerion angustifolium*) and mor-forming species e.g. *Calluna vulgaris*) have therefore been compared by subjecting them to the action of a number of micro-organisms under various conditions.

Aqueous extracts of the fresh leaves of *Chamaenerion angustifolium* and *Calluna vulgaris* were prepared by extracting the leaves with twice their weight of distilled water in the presence of a little chloroform in airtight jars. After two weeks the extracts were separated by means of a press.

The precipitated gelatin complexes were obtained by adding an 0.5% solution of gelatin in distilled water to the aqueous extracts, prepared as described above, with vigorous stirring. The resultant flocculant precipitates were allowed to settle out and then washed twice with distilled water, being separated by centrifugation between each washing. The washed precipitates were spread out in a thin layer, dried in the refrigerator and then powdered. These powders were distributed in 20 mgm. quantities in 50 ml. conical flasks. The flasks containing the powdered *Chamaenerion angustifolium*-gelatin complex and those containing the powdered *Calluna vulgaris*-gelatin complex were then each divided into three groups:

5 ml. of distilled water were added to each flask in the first group.

5 ml. of aqueous extract of *Fraxinus excelsior* litter were added to each flask in the second group.
5 ml. of aqueous extract of *Calluna vulgaris* litter were added to each flask in the third group.

Aqueous extracts of fresh litter (*Fraxinus excelsior* representing a mull-forming species and *Calluna vulgaris* a mor-forming species) were added as indicated above, since it seemed possible that the fungi may require soluble materials from litter in order to be able to grow and break down more complex materials; this is perhaps indicated by the observations that when mull-forming litter is mixed with mor, material changes in the direction of mull formation take place. The litter extracts were prepared by autoclaving milled air dry litter (freshly fallen in the case of *Fraxinus excelsior* and shaken from the bush in the case of *Calluna vulgaris*) with distilled water in the proportion of 1 gm. of milled litter to 4 ml. of distilled water, followed by extraction in a press. The fluids thus obtained were added to the powdered complex in the flasks and the whole sterilised by autoclaving at 10 lbs. per sq. inch pressure for 15 minutes. Of the five flasks in each group four were inoculated with strains of the wood-destroying fungi, *Lenzites betulina*, *Polystictus abietinus*, *Stereum hirsutum* and *Polystictus versicolor*, the other flask remaining uninoculated as control.

Since the particles of the complex become entangled in mycelium and it is not yet possible to estimate it by chemical means, any changes in the complex and the amounts of fungal growth have to be judged by visual examination. The reaction of the fluid in each flask was measured by means of the glass electrode at the end of the experiment. The flasks were inoculated on 12.7.49, incubated at 20°C, and the following observations recorded on 30.11.49. On 4.10.49 and again, 23.11.49, 5 ml. of sterile distilled water were added to each flask to make good losses due to evaporation. Results are shown in Table 32.

TABLE 32

	Polystictus abietinus	Lenzites betulinus	Stereum hirsutum	Polystictus versicolor	Control
0.02 gm. of *Chamaenerion*-gelatin complex. 5 ml. of *Fraxinus* litter extract.	Extremely good growth. Complex unchanged. pH 4.30	Extremely good growth. Complex unchanged. pH 4.35	Extremely good growth. Complex unchanged. pH 4.25	Extremely good growth. Complex unchanged. pH 4.62	No growth. Complex unchanged. pH 4.86
0.02 gm. of *Chamaenerion*-gelatin complex. 5 ml. of *Calluna* litter extract.	No growth. Complex unchanged. pH 3.42	No growth. Complex unchanged. pH 3.39	Very good growth. Complex unchanged. pH 4.31	Very good growth. Complex unchanged. pH 4.69	No growth. Complex unchanged. pH 3.47
0.02 gm. of *Chamaenerion*-gelatin complex. 5 ml. of distilled water.	Fairly small growth. Complex unchanged. pH 5.02	Fairly small growth. Fluid has changed from brown to orange-yellow. Complex completely disappeared. pH 4.60	Fairly small growth. Complex unchanged. pH 4.45	Fairly small growth. Fluid has become deep orange-yellow; complex completely disappeared. pH 4.76	No growth. Complex unchanged. pH 3.98
0.02 gm. *Calluna*-gelatin complex. 5 ml. *Fraxinus* litter extract.	Extremely good growth. Complex unchanged. pH 4.35	Extremely good growth. Complex unchanged pH 4.29	Extremely good growth. Complex unchanged. pH 4.34	Extremely good growth. Complex unchanged. pH 4.64	No growth. Complex unchanged. pH 4.53
0.02 gm. *Calluna*-gelatin complex. 5 ml. *Calluna* litter extract.	No growth. Complex unchanged. pH 3.50	Very good growth. Complex unchanged. pH 5.17	Very good growth. Complex unchanged. pH 4.80	Very good growth. Complex unchanged. pH 4.84	No growth. Complex unchanged. pH 3.41
0.02 gm. *Calluna*-gelatin complex. 5 ml. of distilled water.	Very small growth. Complex unchanged. pH 4.74	Very small growth. Complex unchanged. pH 4.65	Very small growth. Complex unchanged. pH 5.13	Very small growth. Complex unchanged. pH 4.61	No growth. Complex unchanged. pH 4.11

These observations indicate that under the conditions specified the *Chamaenerion*-gelatin complex is less resistant to decomposition than the *Calluna*-gelatin complex. There does not appear to be any connection between the amount of growth of the organism, or the reaction of the fluid at the end of the experiment, and the disappearance of the gelatin complexes. Whereas the presence of litter extract is associated in most cases with much greater growth of the organisms, disappearance of the gelatin complex did not take place when litter extracts were present.

The same complexes were also used in an experiment in which sterilisation was effected by the use of chloroform in order to avoid changes due to autoclaving. Milled filter paper was also added to the flasks, for if similar protein complexes are present in litter they will be associated with cellulose, which may provide a substrate necessary for the long-term growth and metabolism of organisms active in the decomposition of such complexes. The flasks were prepared as described in the previous experiment, except that 0.05 gm. of milled filter paper was added to half the flasks in each case and the flasks were sterilised by the use of chloroform instead of autoclaving. 0.02 ml. of chloroform was added to each flask and the flasks placed in large desiccators containing a few ml. of chloroform, where they remained from 28.7.49 to 9.8.49, when they were heated in a waterbath at 65°C. for 30 minutes to drive off any chloroform remaining inside the flasks. Inocula of the following organisms and material were placed in separate flasks of each group on 10.8.49:—*Polystictus versicolor*, *Stereum hirsutum*, *Marasmius dryophilus*, *Calluna vulgaris* mor, *Chamaenerion* mull, with an uninoculated flask serving as control in each series. On account of evaporation sterile distilled water had to be added to the flasks at intervals during incubation at 20°C. At a late stage in the incubation period the control flasks became contaminated with *Penicillia*, but the growth of these organisms did not produce any visible change in the complexes. Only a very small number of the inoculated flasks showed visible evidence of contamination. The appearance of the flasks was finally recorded on 4.7.50 and the reaction of the fluid in each flask was determined by means of the glass electrode at the conclusion of the experiment. Results are set out in Table 33.

TABLE 33

	Polystictus versicolor	Stereum hirsutum	Marasmius dryophilus	Calluna mor	Chamaenerion mull	Control
0.02 gm. *Chamaenerion*-gelatin complex. 5 ml. *Calluna* litter extract.	Very good growth. Fluid very pale yellow. Complex unchanged. pH 5.21	Good growth. Fluid light brown. Complex unchanged. pH 3.76	Overgrown by Penicillium. Fluid light brown. Complex unchanged. pH 4.29	Small growth. Fluid light brown. Complex unchanged. pH 4.81	Good growth. Fluid almost colourless. Complex unchanged. pH 4.61	Insufficient material
Ditto + 0.05 gm. cellulose	Very good growth. Fluid almost colourless Complex unchanged. pH 5.14	Very good growth. Fluid light brown. Complex unchanged. pH 5.34	Contaminated with Penicillium. Fluid pale brown. Complex unchanged. pH 4.48	Good growth. Fluid light brown. Complex unchanged. pH 4.71	Fairly good growth. Fluid light brown. Complex unchanged. pH 4.62	Insufficient material
0.02 gm. *Chamaenerion*-gelatin complex. 5 ml. *Fraxinus* litter extract	Extremely good growth. Slightly contaminated with *Penicillium*. Fluid dark brown. Complex unchanged. pH 5.52	Extremely good growth Fluid dark brown. Complex unchanged. pH 5.25	Extremely good growth Fluid dark brown. Complex unchanged. pH 7.17	Very good growth. Fluid dark brown. Complex unchanged. pH 8.45	Very good growth. Fluid dark brown. Complex unchanged. pH 8.61	Contaminated. Fluid dark brown. Complex unchanged. pH 7.24

TABLE 33—*continued.*

	Polystictus versicolor	Stereum hirsutum	Marasmius dryophilus	Calluna mor	Chamaenerion mull	Control
Ditto + 0.05 gm. cellulose	Extremely good growth. Fluid dark brown. Complex unchanged. pH 5.78	Extremely good growth. Fluid medium-brown. Complex unchanged. pH 5.27	Extremely good growth. Fluid bright golden colour. Complex disappeared except for 1 or 2 particles. pH 4.94	Good growth. Fluid dark brown. Complex unchanged. pH 7.94	Extremely good growth. Fluid dark brown. Complex unchanged. pH 8.47	Contaminated. Fluid dark brown. Complex unchanged. pH 8.52
0.02 gm. *Calluna*-gelatin complex 5 ml. *Calluna* litter extract	Extremely good growth. Fluid very pale brown. Complex unchanged. pH 5.80	Extremely good growth. Fluid very pale brown. Complex unchanged. pH 5.93	Fairly good growth. Contaminated with *Penicillium*. Fluid light brown. Complex unchanged. pH 5.05	Fairly good growth. Fluid very pale brown. Complex unchanged. pH 5.40	Fairly good growth. Fluid very pale brown. Complex unchanged. pH 5.03	Contaminated. Fluid pale brown. Complex unchanged. pH 4.48
Ditto + 0.05 gm. cellulose	Extremely good growth. Fluid pale yellow. Complex unchanged. pH 6.12	Extremely good growth. Fluid pale yellow. Complex unchanged. pH 6.73	Small growth Fluid light brown. Complex unchanged. pH 4.66	Fairly good growth. Fluid almost colourless. Complex unchanged. pH 5.26	Fairly good growth. Fluid very pale brown. Complex unchanged. pH 5.13	Contaminated. Fluid pale reddish-brown. Complex unchanged. pH 4.48
0.02 gm. *Calluna*-gelatin complex. 5 ml. *Fraxinus* litter extract	Extremely good growth. Slight contamination. Fluid dark brown. Complex unchanged. pH 5.55	Extremely good growth. Slight contamination. Fluid medium-brown. Complex unchanged. pH 5.31	Extremely good growth. Fluid bright golden colour. Complex unchanged. pH 5.35	Very good growth. Fluid very dark brown. Complex unchanged. pH 8.09	Very good growth. Fluid very dark brown. Complex unchanged. pH 8.70	Contaminated. Fluid very dark brown. Complex unchanged. pH 6.33
Ditto + 0.05 gm. cellulose	Extremely good growth. Fluid dark brown. Complex unchanged. pH 8.53	Extremely good growth. Fluid dark brown. Complex unchanged. pH 8.58	Extremely good growth. Fluid medium brown. Complex unchanged. pH 8.21	Extremely good growth. Fluid dark brown. Complex unchanged. pH 8.52	Extremely good growth. Fluid dark brown. Complex unchanged. pH 8.61	Contaminated. Fluid very dark brown. Complex unchanged. pH 7.29

The following points of interest arise from this experiment:—

1. The dark brown colour of the *Fraxinus* litter extract becomes lighter in colour under the influence of *Polystictus versicolor* and *Stereum hirsutum*, and a bright golden colour under the influence of *Marasmius dryophilus*.

2. Only *Marasmius dryophilus* brought about disappearance of the *Chamaenerion*-gelatin complex, even though the growth of organisms in other flasks appeared to be at least equal in amount.

3. Although the *Chamaenerion*-gelatin complex disappeared in the presence of *Fraxinus* litter extract under the influence of *Marasmius dryophilus*, this only occurred in the presence of cellulose. Under similar conditions the presence of *Calluna* litter extract did not lead to the disappearance of the *Chamaenerion*-gelatin complex.

TABLE 34

Species	Reaction with saturated phenol solution	Reaction with 1% gelatin solution	Reaction with ferric chloride solution
Rhododendron sp.	No reaction	Bulky flocculent pale reddish-brown precipitate.	Deep purplish black colour.
Ilex aquifolium	Marked opalescence	No visible change.	No colour change.
Hedera helix	Marked turbidity.	No visible change.	No colour change.

4. Although the dark brown colour of the *Fraxinus* litter extract became much lighter in the presence of *Marasmius dryophilus* and *Calluna*-gelatin complex, the latter showed no signs of disappearing even in the presence of cellulose.

5. The disappearance of the *Chamaenerion*-gelatin complex is not associated with the development of an abnormal reaction.

6. In no case could a gelatin precipitating factor be detected in the fluid at the end of the experiment.

7. The soil inocula did not bring about disappearance of the complexes; this may be caused by the prevention of microbiological ecological succession under these conditions.

The most important result from this experiment is that the complex obtained by the use of material from *Calluna vulgaris* leaves, a potential mor-forming material, is less readily broken down under these conditions than the complex obtained by the use of material from *Chamaenerion angustifolium*, a mull-forming species.

The Breakdown of Protein Complexes, Formed by the Interaction of Leaf Protein and Aqueous Leaf Extracts, by Micro-organisms

In the above experiments gelatin was used as the protein for the formation of complexes as it is an easily accessible protein and relatively easy to manipulate, although it is obvious that leaf protein would be a more appropriate material. The previously noted lack of information concerning leaf protein does not allow of a systematic approach to their extraction in specific cases. General points of guidance previously noted are: that for many plant species minimum solubility of leaf protein is at a reaction of pH 4–5; that drying, even at fairly low temperatures, brings about changes leading to their insolubility in water or mildly alkaline buffer solutions; and that in some cases there has been complete failure to obtain cytoplasmic protein. No attempt was made to isolate the leaf proteins in a pure state and observations have so far been restricted to a species (*Sambucus nigra*) the aqueous extract of whose leaves did not give a precipitate with gelatin solution nor a colour reaction with ferric chloride solution. The method finally adopted for obtaining leaf protein consisted of placing 30 gm. of fresh leaves of *Sambucus nigra* in 200 ccs. of a buffer solution containing 50 ml. of $\frac{M}{5}$ KH_2PO_4 and $\frac{M}{5}$ NaPH and having a reaction of pH 7.8 after the addition of 10 gm. of sodium chloride. The leaves in the solution were now placed in a blender for five minutes, after which coarse particles were removed from the fluid, whose reaction was readjusted to pH 7.8, and a fruther 30 gm. of fresh *Sambucus nigra* leaves added followed by blending again for five minutes. The resulting suspension was freed from coarse particles and centrifgued for 45 minutes at 5,500 r.p.m. when an opalescent very dark greenish-brown solution was obtained; from this solution a flocculent greyish-green precipitate settled out on standing overnight. The resulting clear supernatant produced a very marked turbidity when added to a saturated solution of phenol or to a solution of trichloracetic acid. The turbidity was more marked for a solution prepared in this way than by any of the other methods tried, in addition this solution appeared to give bulkier precipitates with various aqueous leaf extracts.

In an attempt to locate possible sources of leaf protein for experimentation during the winter months the fresh leaves of three evergreen species were also subjected to the same treatment as the leaves of *Sambucus nigra*. The preparations obtained gave the reactions shown in Table 34.

Immediately after blending, the material from the *Rhododendron* leaves formed a flocculent coagulum and the suspension settled out extremely rapidly on standing, leaving a clear supernatant. Preparations made by the use of distilled water instead of buffered sodium chloride solutions showed the same characteristics. It would therefore seem to be impossible to obtain *Rhododendron* leaf proteins in solution by such methods, presumably due to the protein precipitating factor present in the living leaf. This may be the explanation of the failure to obtain leaf proteins for analysis in certain cases, as previously noted.

Precipitates have been obtained as a result of the interaction between preparations containing *Sambucus* leaf protein in solution and aqueous extracts of the fresh leaves of various species. The species used were chosen as being representatives of mor-forming species and of species which, although not giving rise to mor, yield leaf extracts which precipitate gelatin from solution. They were *Pinus contorta*, *Rhododendron* sp., *Taxus baccata*, *Acer pseudoplatanus* and *Sorbus aucuparia*. The resistance of these complexes to decomposition by a number of wood and litter fungi has been investigated. The resistance to decomposition of *Sambucus* leaf protein precipitated from solution by adjustment of the reaction to pH 4.6 with N hydrochloric acid was also examined for comparison. The precipitates were prepared by adding *Sambucus* leaf protein solution to the aqueous extracts of fresh leaves in the proportion of 1 ml. to 5 ml. These quantities gave sufficient precipitate for each 50 ml. flask, except in the case of *Taxus baccata* and *Acer pseudoplatanus* when approximately the same bulk of precipitate was obtained by using twice these volumes. In all cases the reaction of the mixture of leaf protein solution and leaf extract was more acid than pH 5.8. The precipitates were washed with three successive quantities of distilled water, each of the same volume as the fresh leaf extract used in the production of the precipitate. All the precipitates produced in this way were dirty greenish-grey in colour and therefore different to the chocolate-brown, foxy red and other clear coloured precipitates produced by the use of *Sambucus* leaf protein solutions obtained by blending in distilled water.

To facilitate distribution and perhaps also the action of micro-organisms, the precipitates which had been prepared in bulk were suspended, without drying, in distilled water or aqueous extract of *Fraxinus* litter and distributed in 5 ml. quantities in 50 ml. conical flasks, half of which contained 0.05 gm. milled cellulose. Whereas in previous experiments the litter extracts had been prepared by autoclaving, in this case they were prepared by extracting 75 gm. of air dried milled *Fraxinus* litter with 300 ml. of distilled water for three weeks in the presence of chloroform. After separation from the solid material the extract was diluted in the proportion of 1 ml. of extract to 4 ml. of distilled water for use in the experiment. The flasks containing the precipitates were then sterilised, by the use of chloroform as previously described, before inoculation on 20.9.50 with strains of the following fungi: *Marasmius dryophilus*, *Mycena pura*, *Collybia butyracea*, *Lentinus lepideus*, *Polystictus abietinus*, *Lenzites betulina* and *Polystictus versicolor*. One flask in each series was left uninoculated as a control. The results given in Appendix II recorded on 8.5.52 show little variation from those recorded on 5.11.51. (See page 108.)

These results do not indicate any sharp differentiation between complexes formed by the use of extracts of the leaves of mor-forming species, and those formed by the use of extracts of the leaves of mull-forming species. This may be partly due to the fact that the precipitates never became air dry in the way litter almost certainly does before decomposition. It is also possible that it would have been more satisfactory to use *Sambucus* leaf protein obtained by blending the leaves with distilled water, even though such a preparation contains a considerably lower concentration of protein than that obtained by the use of buffered sodium chloride solution. None of the uninoculated controls showed any signs of disappearance of the complexes. In many cases the presence of cellulose appears to assist in the disappearance of the complex in the presence of both distilled water and *Fraxinus* litter extract. In a number of cases the organisms have grown well in the presence of the protein complex, cellulose and distilled water, without any visible difference appearing in the protein complex, which may indicate that a slow change is being produced in the complex or that the organisms are assimilating atmospheric nitrogen. The *Sambucus* leaf protein precipitated by acidification does not show any marked differences compared with the *Sambucus* leaf protein preparations in which precipitation was brought about by fresh leaf extracts; except that whereas *Mycena pura* failed to bring about noticeable disappearance of any of the protein-leaf extract complexes, it caused complete disappearance of the acid precipitated *Sambucus* leaf protein, but only in the absence of *Fraxinus* litter extract. On the other hand some of the organisms which caused complete disappearance of the protein-leaf extract complexes, produced no visible effect on the acid precipitated *Sambucus* leaf protein which might be expected to be less resistant to breakdown.

In the previously described experiments indicating a greater disappearance of *Chamaenerion*-gelatin complex than *Calluna*-gelatin complex, the complexes had been air dried before being subjected to the action of fungi. In view of this, and the previously noted observations on the decreased solubility of extracted leaf proteins after drying, an experiment in which the effect of fungi on air dried complexes was compared with their effect on complexes which were not dried at any time during preparation, has been carried out.

The complexes were prepared from gelatin and the leaf proteins of *Sambucus nigra* in buffered sodium chloride solution, and extracts of the fresh leaves of *Quercus* sp., *Chamaenerion angustifolium* and *Calluna vulgaris* as sources of protein precipita-

ting factors. In all cases the leaf extracts were made slightly more acid by the addition of small amounts of hydrochloric acid before precipitation of the protein, as this appears to assist in the precipitation of the protein, especially in the case of the buffered solution of *Sambucus* protein. Whereas the complexes formed from *Sambucus* leaf protein tend to remain in the form of discrete particles, the floccules of the gelatin complexes tend to coalesce after a time. The complexes were washed three times with distilled water, being allowed to settle out between each washing. Half of each complex was then kept moist in the presence of chloroform, and the other half dried in a thin layer at room temperature and subsequently reduced to a fine powder. The moist and dry complexes were then placed under various conditions. In each case both moist and dry complexes were placed in the presence of aqueous *Calluna* litter extract and aqueous *Fraxinus* litter extract, both prepared in the same way as the *Fraxinus* litter extract for the previous experiment, in the presence and absence of cellulose. The same amounts of litter extracts and cellulose were used as in the previous experiment. The dry powdered complexes were distributed in 50 ml. conical flasks in the following quantities per flask:—

Quercus-gelatin complex .... 0.01 gm.
Chamaenerion-gelatin complex .... 0.01 gm.
Calluna-gelatin complex .... 0.007 gm.
Quercus-Sambucus protein complex 0.007 gm.
Chamaenerion-Sambucus protein complex .... 0.004 gm.
Calluna-Sambucus protein complex 0.0045 gm.

The moist complexes, which should contain the same total amount of solid material in each case as the dry complexes, were then distributed among the same numbers of flasks so that each flask containing dry complex should contain the same amount of solid material as the corresponding flask containing moist complex.

After sterilisation by the use of chloroform, each series of flasks was inoculated with strains of the following organisms on 27.8.51: *Collybia butyracea*, *Polystictus abietinus*, *Lenzites betulina*, and *Polystictus versicolor*, with an uninoculated control in each case. The results for this experiment are given in Appendix III (p. 111). The appearance of the flasks on 7.5.52 allows of the following comments :—

The air-dried complexes show very much less tendency to disappear under the influence of the fungi than the complexes which have been kept moist; there is no marked difference between the gelatin complexes and the *Sambucus* leaf protein complexes in this respect. There is a strong suggestion that the presence of cellulose assists in the disappearance of the complexes.

Neither the kind of protein used nor the species from which the protein precipitating factor was obtained seem to have any influence on the disappearance of the complexes in the presence of *Calluna* litter extract, whether in the presence or absence of cellulose. All are similarly highly resistant to the action of the fungi, and the *Calluna* litter extract appears to have a marked inert or even inhibitory action on the disappearance of the complexes.

Although the disappearances of the air-dried *Sambucus* leaf protein—*Chamaenerion*, *Quercus* and *Calluna* leaf extract complexes shows no difference in the absence of cellulose and in the presence of *Fraxinus* litter extract, the *Chamaenerion* complex appears to disappear much more easily than *Quercus* and *Calluna* complexes when in the presence of cellulose. In the case of the air-dried gelatin—leaf extract complexes, in the presence of *Fraxinus* litter extract the disappearance of both the *Chamaenerion* and *Calluna* complexes appears to be very considerably assisted by the presence of cellulose, whilst the *Quercus* complex appears to be almost unaffected.

These experiments give sufficiently strong indication that the biological properties of these protein complexes exhibit sufficiently marked differences, which are in accordance with what might be expected from other laboratory and field observations on the differences between mull and mor, as to warrant further investigation of the systems involved. The findings would also appear to be in agreement with the conclusions reached by Melin (1929) that certain internal factors, which were characteristic for a species within a certain amplitude of variation, of the leaves, resulted in the nitrogenous compounds being decomposed with more difficulty in some cases than in others.

Chapter 12

PROTEIN PRECIPITATING FACTORS IN AQUEOUS EXTRACTS OF FRESH LEAVES

PRELIMINARY attempts have been made to discover some of the characteristics of the protein precipitating factors which are extractable with water from the fresh leaves of many species.

The stabilisation of proteins, similar to that which seems to occur in certain leaves when the leaf dies, can be brought about by their irreversible combination with tanning agents. By such a process the putrescible proteins of hide are rendered microbiologically and hydrothermically resistant. The tanning of proteins can be brought about by a variety of substances such as vegetable tannins, unsaturated oils, aldehydes and basic chromium salts. Since vegetable tannins occur in many kinds of leaves it is tempting to suppose that these may be the materials which unite with leaf proteins to give a product which appears to be concerned in the formation of mor. It is likely that if tannins are not involved in this process, the substances which are involved may undergo reactions with proteins similar to those occurring between tannins and proteins; therefore consideration of tannins and their reactions with proteins is desirable.

Vegetable Tannins

The tannins appear to be a numerous and very incompletely understood group of substances known largely from empirical reactions and the substances produced when the molecules are broken down, which indicate that they are, or contain, polyphenols of complex structure. In an early classification the tannins were divided into "iron blueing tannins" and "iron greening tannins" on the basis of their differing reactions with iron salts. The reactions between tannins and iron salts do not appear to be specific, since phenolic substances give similar reactions. The tannins have later been classified as (1) hydrolysable tannins which are hydrolysed by acids and enzymes, being esters of hexoses and phenolcarbonic acids and (2) condensed tannins of the catechin type, with hydrolysable tannins containing ellagic acid sometimes considered as a third type. Such a group of substances would seem to allow of reaction of the various members with proteins, resulting in products having variable, including biological, properties.

As with so many other complex materials of biological origin, isolation in the pure state is difficult. It seems hopeful, however, that the application of chromatographic methods of analysis along the lines indicated by the work of Clark and Levy (1950) may lead to the separation and purification of tannins so that their structure may be determined.

Although a large number of the aqueous extracts of fresh leaves which are able to precipitate gelatin give colour reactions with solutions of ferric chloride, there are, as previously noted, a number of exceptions; which may indicate that the protein precipitating substances and the substances giving colour reactions with ferric chloride are perhaps not necessarily one and the same material. If the colour reactions with ferric chloride solution do arise from the substances responsible for the precipitation of protein, it is clear that there is variation from species to species. Whether the protein precipitating factors in leaf extracts are the substances giving colour reactions with ferric chloride solutions, and hence possibly tannin in nature, or not, it is essential that they should be isolated in a pure state if their relationship to raw humus formation is to be studied precisely. Although it has not been possible to isolate the protein precipitating materials from leaves in a pure state some of their properties have been observed.

Precipitation of Protein Precipitating Factor from Aqueous Extracts of Fresh Leaves by Ethyl Alcohol

It has been found that protein precipitating materials can be precipitated from aqueous leaf extracts by ethyl alcohol. The most convenient method seems to be to concentrate the fresh leaf extract from 500 ml. to 25 ml,. when dark brown syrupy fluids are obtained from which solid material is removed by centrifuging. The syrupy fluid is now added slowly with vigorous stirring to 400–500 ml. of absolute ethyl alcohol. A very bulky, flocculent, light pinkish-brown precipitate is produced in the case of *Calluna* leaf extract, and a similar pale yellow precipitate in the case of *Chamaenerion* leaf extract. These precipitates were washed with absolute alcohol and dried over calcium chloride, and very pale reddish-brown and very pale yellow powders were obtained. The dry powders dissolve to a large extent in distilled water, completely in the case of the *Chamaenerion* powder when the solution is acidified from a reaction of pH 6.2 to a reaction of pH of 4.8 (the pH of the *Chamaenerion* leaf extract) by addition of hydrochloric acid, but not completely in the case of the *Calluna* powder even when the solution was acidified from a reaction of pH 5.0 to that of the *Calluna* leaf extract, i.e. pH 4.2. The protein precipitating factors insoluble in alcohol only seem to be precipitated when the alcohol is absolute or very near in strength. There are also indications that successive solution in water and precipitation by alcohol results in a decreased solubility in water of the alcohol insoluble material. This may be associated with the development of a less acid reaction in successive solutions, and in turn may be associated with oxidation of these protein precipitating materials.

There is evidence that the precipitation of protein is facilitated in some cases by a more acid reaction. In the case of the solution of the alcohol precipitated material from *Chamaenerion* leaf extract, there was little if any visible reaction with gelatin until the reaction was made more acid by the addition of

TABLE 35

	Solution of alcohol precipitated material from *Calluna* leaf extract	Solution of alcohol precipitated material from *Chamaenerion* leaf extract	
	Reaction pH 5.0	Reaction pH 6.2	Reaction adjusted to pH 4.8
Reaction with gelatin	Very bulky light purplish-brown precipitate	Very slight turbidity	A yellowish - brown flocculent precipitate, adherent to the sides of the tube, separates out.
Reaction with ferric chloride solution	Dark greyish-green colour and turbidity	Very deep blue-black colour	Very deep blue-black colour

hydrochloric acid. Details appear in Table 35.

As a result of these observations leaf extracts not previously observed to give precipitates with gelatin, and having a reaction about neutrality, were acidified with the effects on gelatin precipitation set out in Table 36.

This may indicate that the protein precipitating factor is in some cases more active at more acid reactions. It is very unlikely that the precipitates obtained by the use of absolute ethyl alcohol represent pure materials, and probably they contain polysaccharides in addition; this is indicated by the fact that there seems to be no relation between the reaction of a leaf extract with gelatin and the amount of alcohol precipitated material obtained from it.

Leaf extracts were prepared in the usual way from *Pseudotsuga taxifolia* growing on two different sites, and from *Pinus sylvestris* on four different sites. In each case 25 ml. of leaf extract were evaporated almost to dryness on a waterbath and then taken up in 2 ml. of distilled water and the solution centrifuged. The clear supernatants were added drop by drop to 100 ml. of absolute alcohol, and the resulting precipitates washed with absolute alcohol and dried to constant weight over calcium chloride. Results are given in Table 37.

TABLE 36

	Reaction with gelatin before acidification	Reaction with gelatin after acidification
Bambusa sp. pH 6.06	No turbidity or precipitate	Very marked turbidity.
Moss sp. pH 6.74	No turbidity or precipitate	No change.
Moss sp. pH 6.66	No turbidity or precipitate	No change.
Ulmus procera pH 5.90	Slight opalescence but no precipitate	Flocculent reddish - brown precipitate.
Urtica dioica pH 7.49	No turbidity or precipitate	No change.

The reactions of the leaf extracts (as indicated by pH measurements) are sufficiently close to each other for it to be unlikely that this factor is the cause of the different reactions with gelatin. It must be remembered however that proteins other than gelatin may react differently towards these substances, being either more readily or less readily precipitated by them.

The protein precipitating materials appear to be considerably more soluble in water than in organic solvents such as ether, benzene, chloroform and butyl alcohol, whether acidified or not. Although ether was used as an extractive for protocatechuic acid, the protein coagulant in the case of the ootheca of *Blatta orientalis*, the protein precipitating materials in leaf extracts do not seem to be extractable to any appreciable extent in this solvent. Clark and Levy (1950) found that a sample of Italian chestnut tannin was largely soluble in water, pyridine, ethylene glycol and cellosolve, but was only slightly soluble in ether, acetone, benzene, chloroform, carbon tetrachloride, butanol and collidine. They also found that when increasing amounts of sodium chloride were added to an aqueous solution of the tannin, increasing weights of tannin were thrown out of solution.

Precipitation of Protein Precipitating Factor from Aqueous Extracts of Fresh Leaves by Sodium Chloride

Observations have shown that part of the protein precipitating material is precipitated by saturation of leaf extracts with sodium chloride. For a series of leaf extracts the great majority show a close parallel between gelatin precipitation and the production of a precipitate on saturation with sodium chloride.

TABLE 37

Reaction of leaf extract	Leaf extract	Reaction of leaf extract with gelatin solution	Reaction of leaf extract with ferric chloride solution	Amount of absolute alcohol precipitate from 25 ml. leaf extract
pH 4.40	Pseudotsuga taxifolia (1)	Marked opalescence but no precipitate	Intense reddish-purple colour	0.14 gm.
pH 4.29	Pseudotsuga toxifolia (2)	Bulky flocculent light yellowish-brown precipitate	Intense reddish-brown colour	0.31 gm.
pH 4.57	Pinus sylvestris (1)	Slight opalescence. No precipitate	Very slight green colour	0.13 gm.
pH 4.78	Pinus sylvestris (2)	Slight opalescence. No precipitate	Very slight green colour	0.18 gm.
pH 4.46	Pinus sylvestris (3)	Slight opalescence. No precipitate	Very slight green colour	0.21 gm.
pH 4.31	Pinus sylvestris (4)	Bulky flocculent light yellowish-brown precipitate	Dark greenish-grey colour	0.22 gm.

The Breakdown of Protein Complexes, formed by the Interaction of Gelatin and Protein Precipitating Factor Precipitated from Aqueous Extracts of Fresh Leaves by Sodium Chloride, by Micro-organisms

The material precipitated from leaf extracts by sodium chloride is soluble in water and yields precipitates with gelatin. Such gelatin precipitates have been prepared and subjected to the action of various fungi. In the preparation of these complexes leaf extracts of *Chamaenerion angustifolium* and *Calluna vulgaris* were saturated with sodium chloride and the resulting precipitate, after washing several times with a saturated solution of sodium chloride, dissolved in distilled water, and gelatin added to the resulting solutions. The precipitated complexes were washed with distilled water and then distributed, in the form of a suspension without being allowed to dry, among 50 ml. conical flasks; and placed under the following conditions: (a) in distilled water; (b) in extract of *Calluna* litter; (c) in extract of *Fraxinus* litter, all in the presence and absence of cellulose. The additions were carried out in such a way that there was the same amount of suspension of complex in all flasks. Before inoculation with strains of *Marasmius dryophilus*, *Polystictus versicolor* and *Lenzites betulina*, with an uninoculated flask as control, the flasks were sterilised with chloroform. Results were recorded after incubation of the flasks from 5.4.51 to 11.9.51 at 20°C, and are shown in Table 38.

The general trend of these results is very similar to that for the gelatin complexes prepared by the use of fresh leaf extracts i.e. the *Chamaenerion*-gelatin complex disappears under a wider variety of conditions and under the influence of more of the organisms than is the case with the *Calluna*-gelatin complex. It should be borne in mind that the complexes used in the above experiment have not been air dried.

It is therefore probable that the material present in aqueous extracts of fresh leaves which brings about the precipitation of proteins is itself precipitated by ethyl alcohol and sodium chloride; but the identity and properties of the protein precipitating material, especially in different plant species, require chemical and biochemical investigation.

TABLE 38

	Control	Marasmius dryophilus	Polystictus versicolor	Lenzites betulina
Calluna-gelatin complex in distilled water + cellulose	No growth Complex unchanged	Very small growth. Complex unchanged	Fairly good growth. Complex unchanged	Fairly good growth. Complex unchanged
Ditto without cellulose	No growth Complex unchanged	Very small growth. Complex unchanged	Small growth Complex unchanged	Small growth Complex unchanged
Calluna-gelatin complex in *Calluna* litter extract + cellulose	No growth Complex unchanged	Small growth Complex almost completely disappeared	Good growth Complex unchanged	Good growth Complex unchanged
Ditto without cellulose	No growth Complex unchanged	Very small growth. Complex unchanged	Good growth Complex unchanged	Good growth Complex unchanged
Calluna-gelatin complex in *Fraxinus* litter extract + cellulose	No growth Complex unchanged	Good growth Complex unchanged	Very good growth. Complex diminished in amount	Very good growth. Considerable amount of complex disappeared
Ditto without cellulose	No growth Complex unchanged	Good growth Complex unchanged	Good growth Complex unchanged	Good growth Complex unchanged
Chamaenerion-gelatin complex in distilled water + cellulose	No growth Complex unchanged	Very small growth. Complex completely disappeared	Small growth Complex completely disappeared	Small growth Complex diminished in amount
Ditto without cellulose	No growth Complex unchanged	Very small growth Complex unchanged	Small growth Complex unchanged	Very small growth Complex unchanged
Chamaenerion-gelatin complex in *Calluna* litter extract + cellulose	No growth Complex unchanged	Extremely small growth. Complex completely disappeared	Good growth Some disappearances of complex	Good growth Considerable disappearance of Complex.
Ditto without cellulose	No growth Complex unchanged	Small growth Complex unchanged	Fairly good growth. Complex unchanged	Small growth Complex unchanged
Chamaenerion-gelatin complex in *Fraxinus* litter extract + cellulose	No growth Complex unchanged	Good growth Complex unchanged	Good growth Considerable disappearance of Complex	Good growth Complex completely disappeared
Ditto without cellulose	No growth Complex unchanged	Fairly good growth. Complex unchanged	Good growth Complex unchanged	Good growth Considerable disappearance of complex

Chapter 13

OBSERVATIONS ON THE NATURE OF THE REACTION BETWEEN PROTEIN PRECIPITATING MATERIALS IN LEAF EXTRACTS AND PROTEINS

It has already been pointed out that proteins of different origin, e.g. gelatin and *Sambucus nigra* leaf protein appear to exhibit differences in their reactions with the protein precipitating factors of leaf extracts, and it is therefore desirable to enquire into the nature of the reaction between proteins and protein precipitating factors.

The Influence of Reaction (pH) and Origin of the Protein on the Formation of Complexes Between Proteins and Aqueous Extracts of Fresh Leaves

The first possibility to be considered is that the precipitation of proteins by leaf extracts is an isoelectric phenomenon brought about by the reaction of the leaf extracts. The isoelectric point or range for leaf proteins is given by Chibnall (1939) as being within the range pH 4.0—pH 5.0; the isoelectric point for gelatin also falls within the same range (Seifriz (1936)). Observations indicate that it is very unlikely that the protein precipitation is an isoelectric phenomenon. Freshly prepared 1% gelatin solution had a reaction of pH 5.42, when the reaction was adjusted to pH 4.6 there was no detectable precipitation.

Solutions containing *Sambucus nigra* leaf proteins were prepared by blending fresh leaves with distilled water and also with a phosphate buffer having an initial reaction of pH 7.9. The final reactions of the two solutions containing *Sambucus* leaf protein were pH 6.0 in the case of distilled water and pH 7.37 in the case of the phosphate buffer. Both these solutions were tested for precipitation reactions with *Rhododendron* leaf extract having a reaction of pH 5.34, and also with *Rhododendron* leaf extract the reaction of which had been adjusted to pH 7.4. In all cases a bulky flocculent precipitate was immediately produced when three drops of *Sambucus* leaf protein solution were added to 1 ml. of *Rhododendron* leaf extract.

Globin prepared from sheep haemoglobin has also been examined from this point of view as an example of a protein having an isoelectric range on the alkaline side of neutrality i.e. pH 7.3 to 8.4. The globin was dissolved in distilled water to give solutions containing various concentrations from 0.02% to 2.0%. These solutions and a 1% solution of gelatin were tested against aqueous extracts of fresh leaves of *Urtica dioica* and *Rhododendron* sp. As, prepared without adjustment, the extract of *Urtica* leaves had a reaction of pH 7.6, the reaction of a portion of this extract was adjusted to pH 4.6. Similarly the reaction of the *Rhododendron* extract as prepared was pH 5.4, and the reaction of a portion of this extract was adjusted to pH 7.5, the precipitate thrown down during adjustment of the reaction being removed by centrifuging before the tests were carried out. The results of these tests are shown in Table 39; in each case two drops of protein solution were added to 1 ml. of leaf extract, except in the case of 2% globin and 1% gelatin solutions, where only one drop was used.

Besides indicating that the precipitation of proteins by leaf extracts is not an isoelectric phenomenon, these findings provide further evidence that the precipitation reaction varies according to the nature of the protein involved.

The Production of Complexes by the Interaction of Amino Acids with Aqueous Extracts of Fresh Leaves

The proportions of the various constituent amino acids vary in different proteins; in addition variations in the linkages between the constituent amino acids may result in proteins with different molecular architecture and different properties.

Observations have been made on precipitation reactions between a number of leaf extracts, giving various types of reaction with 1% gelatin solution and ferric chloride solution, and a number of amino acids and related compounds. The tests were carried out by "layering" two drops of the amino acid solution (100 mgm. in 5 ml. of distilled water, or sturated if the solubility is less than this concentration) on the surface of 1 ml. of aqueous extracts of the fresh leaves of the species concerned, which were chosen on the following basis:

Urtica dioica, *Sphagnum* sp., *Ulmus glabra* } No precipitation reaction with gelatin, no reaction with ferric chloride.

Acer pseudoplatanus, *Fraxinus excelsior* } No precipitation reaction with gelatin, colour reaction with ferric chloride.

TABLE 39

Protein solution	Urtica dioica leaf extract. pH 7.6	Urtica dioica leaf extract. pH 4.6	Rhododendron sp. leaf extract. pH 7.5	Rhododendron sp. leaf extract. pH 5.4
0.02% globin	No visible reaction	No visible reaction	No visible reaction	Small amount of flocculent precipitate
0.04% globin	Small amount of granular precipitate	Small amount of granular precipitate and slight turbidity	Slight turbidity	Small amount of flocculent precipitate
0.08% globin	Small amount of granular precipitate	Small amount of granular precipitate and slight turbidity	Slight turbidity	Moderate amount of flocculent precipitate
0.2% globin	Moderate amount of granular precipitate	Considerable flocculent precipitate which settles out rapidly	Moderate amount of flocculent precipitate	Fairly bulky flocculent precipitate
2.0% globin	Very bulky fibrous precipitate with clear supernatant	Bulky flocculent precipitate and turbidity	Bulky flocculent precipitate	Bulky flocculent precipitate
1.0% gelatin	No visible reaction	No visible reaction	Bulky flocculent precipitate	Bulky flocculent precipitate

Chamaenerion angustifolium, *Aesculus hippocastanum* — Precipitation with gelatin and colour reaction ferric chloride.

Pseudotsuga taxifolia (Site 12) No precipitation reaction with gelatin, colour reaction with ferric chloride.

ditto (Site 21) Precipitation with gelatin, colour reaction with ferric chloride.

Pinus sylvestris (Site 23) No precipitation reaction with gelatin, colour reaction with ferric chloride.

ditto (Site 24) Precipitation with gelatin, colour reaction with ferric chloride.

Fagus sylvatica (from mull site), *Larix decidua*, *Libocedrus decurrens*, *Cedrus deodara*, *Pinus griffithii* — Species which probably give rise to mor at least under some circumstances. Extracts give little or no precipitate with gelatin, but a colour reaction with ferric chloride.

Rhododendron sp., *Calluna vulgaris*, *Pinus contorta*, *Thuja plicata*, *Taxus baccata* — Species giving rise to mor, precipitation with gelatin and colour reaction with ferric chloride.

The amino acids and related compounds used were β-alanine, valine, nor-leucine, serine, methionine, sodium glutamate, lysine, histidine, arginine, glycine, α-alanine, asparagine, phenylalanine, aspartic acid, leucine, isoleucine, citrulline, ornithine, glycylglycine, creatinine, urea, Roche peptone and edestin. Since edestin is only soluble in the presence of sodium chloride, a saturated solution in 10% sodium chloride being used, and some of the leaf extracts tend to give precipitates with sodium chloride when this is added in the same amount as is present in the edestin solution used (as shown in the accompanying table), the leaf extracts of additional species were used in order to include more species whose extracts give little or no precipitate when saturated with sodium chloride, i.e. *Larix leptolepis*, *Bambusa* sp., *Ilex aquifolium*, *Mercurialis perennis*, *Salix* sp. These additional species were also tested against Roche peptone at a concentration of 2%.

None of the leaf extracts examined gave any kind of precipitation reaction with β-alanine, valine, nor-leucine, serine, methionine, sodium glutamate, glycine, α-alanine, asparagine, phenylalanine, aspartic acid, leucine, isoleucine, citrulline, glycylglycine and urea. The results with the remaining substances are given in table 40.

These observations seem to indicate that the formation of precipitates by the interaction of proteins and leaf extracts is concerned with those amino acids having amino groups other than α-amino

TABLE 40

Leaf extracts	Lysine	Histidine	Arginine	Creatinine	Ornithine	Roche peptone	*Edestin + 0.1 gm. sodium chloride	0.1 gm. sodium chloride	Gelatin
Urtica dioica	No reaction	No reaction	Very small precipitate greenish-yellow	Not tested	No reaction	No reaction	No reaction	No precipitate	No reaction
Sphagnum sp.	ditto	ditto	No reaction	Not tested	ditto	ditto	Bulky white precipitate	No precipitate	Very slight opalescence
Ulmus glabra	ditto	ditto	Bulky precipitate brownish-yellow	No reaction	Small precipitate	ditto	Moderate flocculent yellow precipitate	No precipitate	No reaction
Acer pseudoplatanus	ditto	ditto	Bulky precipitate greenish-yellow	ditto	No reaction	Moderate precipitate	Very bulky white precipitate	Moderate precititate	Abundant finely flocculent precipitate
Fraxinus excelsior	ditto	ditto	Bulky precipitate brownish-yellow	ditto	ditto	Small precipitate	Very bulky pale yellow precipitate	Moderate precipitate	Turbidity only
Chamaenerion angustifolium	ditto	Bulky precipitate	Bulky precipitate greenish-yellow	Moderate precipitate	Very small precipitate	Very bulky precipitate	Very bulky pale yellow precipitate	Moderate precipitate	Bulky precipitate
Aesculus hippocastanum	ditto	No reaction	Bulky precipitate yellow	No reaction	No reaction	Small precipitate	Bulky cream coloured precipitate	Slight precipitate	Bulky precipitate
Pseudotsuga taxifolia (12)	ditto	No reaction	Small precipitate greenish-yellow	Very small precipitate	Very small precipitate	Small precipitate	Bulky white precipitate	Slight precipitate	Turbidity only
ditto (21)	ditto	Small precipitate	Bulky precipitate yellow	Moderate precipitate	Very small precipitate	Bulky precipitate	Bulky white precipitate	Moderate precipitate	Bulky precipitate
Pinus sylvestris (23)	ditto	No reaction	Small precipitate white	No reaction	No reaction	Small precipitate	Bulky white precipitate	Slight precipitate	Trace of precipitate

continues

TABLE 40—*cont.*

Leaf extracts	Lysine	Histidine	Arginine	Creatinine	Ornithine	Roche pepton	*Edestin + 0.1 gm. sodium chloride	0.1 gm. sodium chloride	Gelatin
Pinus sylvestris (24)	No reaction	No reaction	Bulky precipitate pale brownish-white	No reaction	No reaction	Bulky precipitate	Very bulky white precipitate	Slight precipitate	Bulky precipitate
Fagus sylvatica (mull)	ditto	Very small precipitate	Moderate precipitate pale brown	No reaction	No reaction	Small precipitate	Bulky pinkish white precipitate	Slight precipitate	Small precipitate
Larix decidua	Very slight precipitate	Small precipitate	Bulky precipitate light brown	Small precipitate	No reaction reaction	Bulky precipitate	Bulky white precipitate	Moderate precipitate	Moderate precipitate
Libodcedrus decurrens	Very slight precipitate	Very small precipitate	Small precipitate yellow	No reaction	Very small precipitate	Very small precipitate	Bulky white precipitate	Slight precipitate	Turbidity only
Cedrus deodara	No reaction	No reaction	Very small precipitate white	Small precipitate	No reaction	Small precipitate	Bulky white precipitate	Turbidity	Turbidity
Pinus griffithii	No reaction	No reaction	Very small precipitate white	Very small precipitate	Very small precipitate	Very small precipitate	Bulky white precipitate	Very slight precipitate	Turbidity only
Rhododendron sp.	Bulky precipitate	Bulky precipitate	Very bulky precipitate orange	Very bulky precipitate	Bulky precipitate	Very bulky precipitate	Bulky pale cream precipitate	Moderate precipitate	Very bulky precipitate
Calluna vulgaris	Small precipitate	Small precipitate	Bulky precipitate foxy red	Bulky precipitate	Small precipitate	Moderate precipitate	Bulky very pale brown precipitate	Moderate precipitate	Bulky precipitate
Pinus contorta	Bulky precipitate	Bulky precipitate	Bulky precipitate very pale brown	Bulky precipitate	Moderate precipitate	Bulky precipitate	Bulky white precipitate	Moderate precipitate	Bulky precipitate
Thuja plicata	Bulky precipitate	Bulky precipitate	Very bulky precipitate pale brown	Very bulky precipitate	Bulky precipitate	Bulky precipitate	Bulky pale brown precipitate	Moderate precipitate	Bulky precipitate

continues

TABLE 40—*cont.*

Leaf extracts	Lysine	Histidine	Arginine	Creatinine	Ornithine	Roche peptone	*Edestin + 0.1 gm. sodium chloride	0.1 gm. sodium chloride	Gelatin
Taxus baccata	No reaction	Very small precipitate	Bulky precipitate foxy red	Moderate precipitate	Small precipitate	Bulky precipitate	Very bulky orange-yellow precipitate	Moderate precipitate	Moderate precipitate
Larix leptolepis						White opalescence	Moderate flocculent precipitate white	No precipitate	Moderate precipitate
Bambusa sp.						No reaction	Moderate flocculent precipitate yellow	ditto	No reaction
Sambucus nigra						ditto	Moderate flocculent precipitate yellow	ditto	No reaction
Ilex aquifolium						ditto	Moderate flocculent precipitate	ditto	Extremely slight turbidity
Mercurialis perennis						ditto	Moderate flocculent precipitate very pale brown	ditto	No reaction
Salix sp.						Marked pale brown opalescence	Moderate flocculent precipitate very pale brown	ditto	Abundant finely flocculent precipitate
Distilled water						No reaction	Extremely slight opalescence	ditto	No reaction

*In the absence of added sodium chloride the edestin solution gave a precipitate even with distilled water.

Note: Numbers in brackets refer to the sites from which samples were obtained.

groups; the differences between the reactions of norleucine and lysine are particularly interesting in this respect. Many of the leaf extracts giving little or no precipitate with lysine, histidine and ornithine give bulky precipitates with arginine. There is a marked tendency for the leaf extracts of species which give rise to mor to give strong reactions with lysine, histidine and ornithine as well as with arginine and gelatin.

In view of the different precipitation results obtained by use of the same leaf extract with different diamino acids, experiments were carried out to ascertain whether, after exhaustive precipitation of a protein or amino acid by a leaf extract, the leaf extract still retains the capacity to precipitate other proteins or amino acids.

To 10 ml. quantities of extracts of the fresh leaves of *Chamaenerion angustifolium* and *Thuja plicata*, 1 ml. of 10% gelatin solution was added, the extracts being continuously stirred during the additions. The bulky precipitates were removed by centrifuging. When further drops of gelatin were added to the clear supernatants further precipitation was not observed, and the supernatants were submitted to various tests as shown in Table 41.

The two leaf extracts seem to behave quite differently. This is also reflected in their changes in appearance on exhaustive precipitation with gelatin, for whereas the *Thuja* leaf extract is a much deeper colour than the *Chamaenerion* leaf extract before treatment, after treatment it is much lighter in colour than the *Chamaenerion* leaf extract. The results indicate that exhaustive precipitation of gelatin has removed the material precipitated by saturation with sodium chloride, and also the material which precipitates histidine and to some extent arginine in the untreated extracts. The treatment also removes the factor which precipitates ornithine and lysine in the case of *Thuja plicata* extract, but appears to have little or no influence on the reaction of *Chamaenerion* extract towards these substances.

The converse of the above experiment was carried out, i.e. exhaustive precipitation of leaf extracts by basic amino acids, and the clear supernatants obtained after centrifuging subjected to the tests shown in Table 42. *Rhododendron* leaf extract was used in place of *Thuja plicata* extract.

Although the reaction of the *Chamaenerion* leaf extract with ferric chloride solution is altered in colour after exhaustive precipitation by arginine, it is still a strong reaction, whereas there is little if any reaction of the *Rhododendron* leaf extract with ferric chloride solution after exhaustive precipitation with arginine. In the case of both leaf extracts exhaustive precipitation by arginine is the only

TABLE 41

	Untreated Chamaenerion extract	Chamaenerion extract after exhaustive precipitation with gelatin	Untreated Thuja extract	Thuja extract after exhaustive precipitation with gelatin	1% gelatin solution
Saturation with Sodium chloride	Bulky precipitate	No precipitate	Bulky precipitate	No precipitate	
2 drops of 2% ornithine solution to 1 ml. of leaf extract	Very slight precipitate	Very slight precipitate	Considerable amount of precipitate	Extremely slight precipitate	No precipitate
2 drops of 0.5% lysine solution to 1 ml. of leaf extract	Very faint trace of precipitate	Faint trace of precipitate	Considerable amount of precipitate	Extremely slight precipitate	No precipitate
2 drops of approx. 2% histidine solution to 1 ml. leaf extract	Considerable amount of precipitate	No precipitate	Very considerable amount of precipitate	No precipitate	No precipitate
2 drops of 2% arginine solution to 1 ml. of leaf extract	Bulky precipitate	Considerable amount of precipitate	Bulky precipitate	Considerable amount of precipitate	No precipitate
1 drop of 5% ferric chloride solution to 1 ml. of leaf extract	Very bulky blue-black precipitate	Bulky blue-black precipitate—much less than in untreated leaf extract	Bulky greyish-green precipitate	Little if any reaction	

TABLE 42

Chamaenerion leaf extract	1 drop of 1% gelatin solution added to 1 ml. of leaf extract	2 drops of 5% ferric chloride solution added to 1 ml. of leaf extract	1 ml. of leaf extract saturated with sodium chloride
After exhaustive pre-precipitation by histidine	Abundant flocculent precipitate	Abundant blue-black precipitate	Abundant precipitate
After exhaustive precipitation by arginine	No visible reaction	Dark greenish-grey precipitate	No visible reaction
After exhaustive precipitation by ornithine	Abundant flocculent precipitate	Abundant blue-black precipitate	Abundant precipitate
After exhaustive precipitation by lysine	Abundant flocculent precipitate	Abundant blue-black precipitate	Abundant precipitate
Rhododendron leaf extract	(See text.)	—	—
After exhaustive precipitation by histidine	Abundant flocculent precipitate	Dark green precipitate	Abundant precipitate
After exhaustive precipitation by arginine	No visible reaction	Very little if any reaction	Very small amount of precipitate
After exhaustive precipitation by ornithine	Abundant flocculent precipitate	Dark greenish-grey precipitate	Abundant precipitate
After exhaustive precipitate by lysine	Abundant flocculent precipitate	Dark greenish-grey precipitate	Abundant precipitate

treatment which results in the subsequent absence of formation of precipitate with gelatin or on saturation with sodium chloride. Although there is a complicating factor in that arginine was the only substance used in the form of free base, and consequently may have been more reactive than the others which were added in the form of mono or dihydrochloride, the above observations give some indication of the complexity and variation in the systems involved.

The Breakdown of Complexes, formed by the Interaction of Arginine with Aqueous Extracts of Fresh Leaves, by Micro-organisms

Experiments were carried out to ascertain whether complexes formed by the interaction of arginine and leaf extracts possessed similar properties with regard to microbiological decomposition as the protein complexes. In addition if arginine should prove suitable for such experiments it should be a much more convenient substance to work with than proteins themselves.

Some difficulty was experienced in selecting species giving suitable leaf extracts. Of the species whose litter decomposes rapidly and whose extracts give little or no reaction with gelatin solution, *Mercurialis perennis*, *Bambusa* sp., *Sambucus nigra* and *Urtica dioica* all give leaf extracts yielding little or no precipitate with arginine, but an extract of the leaves of *Ulmus procera* gave a bulky brown precipitate with arginine. It was found that the arginine precipitates formed from leaf extracts of species from the group whose litter does not give rise to mor, but whose leaf extracts give bulky precipitates with gelatin, dissolved during washing with distilled water, and therefore this group was not represented. As representatives of the group whose litter gives rise to mor, the leaf extracts of *Calluna vulgaris*, *Pinus sylvestris* and *Vaccinium myrtillus* gave precipitates with arginine which did not dissolve in distilled water. The arginine precipitates were prepared by adding 10 to 18 ml. of 2% arginine solution to 35 to 50 ml. of leaf extract, according to species, so as to give approximately the same bulk of precipitate in each case. The resulting precipitates

were washed several times with distilled water and finally suspended without drying in distilled water. Each experimental flask contained 4 ml. of suspension. The activity of strains of the fungi *Marasmius dryophilus*, *Polystictus versicolor* and *Lenzites betulina*, active in the decomposition of gelatin leaf extract complexes, in the breakdown of the arginine-leaf extract complexes, was tested under various conditions:—

(a) in distilled water alone, i.e. 1 ml. of distilled water added to 4 ml. of suspension.
(b) in *Fraxinus* litter extract, i.e. 1 ml. of *Fraxinus* litter extract added to 4 ml. of suspension;
(c) in *Calluna* litter extract, i.e. 1 ml. of *Calluna* litter extract added to 4 ml. of suspension.

In all three cases the effect of the presence and absence of cellulose was examined. After sterilising with chloroform the flasks were inoculated on 4.12.50 and incubated at 20°C. The results (Appendix IV) on 11.9.51 indicate similar trends for the decomposition of the arginine complexes as were previously obtained for protein complexes. The *Ulmus* complexes disappeared completely under all conditions, except in the case of one flask where there was almost complete disappearance. In the case of the remaining arginine complexes, formed with the leaf extracts of the other species, visual inspection indicated partial disappearance of the complexes in many of the flasks and complete disappearance in a number of cases. There again seems to be evidence that the presence of cellulose assists the disappearance of the precipitates. It is possible that the results would have been more sharply differentiated, as in the case of the protein complexes, if the arginine complexes had been air dried before use instead of being kept moist the whole time. (See also page 114).

The fact that edestin yields precipitates with leaf extracts which do not form precipitates with gelatin (table on pp. 161–163) is a further indication of the influence of the nature of the protein on precipitation. Quantitative evidence for this is provided by an experiment in which the amounts of precipitate produced, when gelatin and *Sambucus nigra* leaf protein react with the same leaf extracts, were compared. The *Sambucus* leaf protein solution contained 103.2 mgm. of nitrogen per 100 ml., and the strength of the gelatin solution was adjusted to give a similar concentration of nitrogen. In all cases excess of leaf extract was used, so that after precipitation of the protein the supernatant was still able to precipitate protein. In each case 10 ml. of the protein solution was added to the leaf extract, and the precipitates formed washed three times with 50 ml. of distilled water. The precipitates were then dried at 100°C. and the results shown in Table 43 obtained.

TABLE 43

Protein solution	*Calluna* leaf extract	*Chamaenerion* leaf extract	*Quercus* leaf extract
Gelatin	0.078 gm.	0.094 gm.	0.082 gm.
Sambucus leaf protein	0.035 gm.	0.027 gm.	0.017 gm.

It has been assumed that the nitrogen content of the *Sambucus* leaf protein solution represented only protein nitrogen, and it is unlikely that more than a very small percentage was non-protein nitrogen. The results indicate a marked difference in the weight of precipitate produced when proteins of different origin are used under similar experimental conditions.

It also seems possible that either the concentration or nature of the protein precipitating substances in extracts from the leaves of species giving rise to mor allows them to form precipitates with proteins such as gelatin, whereas extracts of the leaves of many species giving rise to mull are unable to form precipitates with gelatin. There is thus the possibility that variation in the composition and structure of the protein precipitating factors in the leaves of different species will give protein complexes having a range of biological properties. Such a system would allow of the formation of a large number of different complexes in different plant species, so that the litter from these plants may vary in biological properties only slightly or very markedly from species to species. Should environmental factors such as climate or lithological nature of the soil influence the amount or chemical composition of the protein precipitating factor, then the biological properties of the litter of a species may vary considerably with the environment. Hoffmann (1949) observed that in the case of some plant species the content of certain constituents such as ethereal oils, alkaloids and tannins varies according to the amounts of various inorganic constituents in the soil on which they are growing. There is also the likelihood that differences in environmental factors such as climate, lithological nature of the mineral material of the soil, and presence of the litter of other plant species, may influence the action of soil micro-organisms on such protein complexes. The differing behaviour of beech litter on different sites may be an example of either or both these possibilities.

The Action of Micro-organisms on Vegetable Tanned Hide

Allusion has already been made to the possible parallel between the process of protein precipitation

described above and leather formation, and it may therefore be useful to consider at this stage the processes of leather formation and its decomposition by micro-organisms.

The effect of micro-organisms on leather has attracted attention for obvious reasons. So far investigations seem to have shown that the profuse growth of mould of various kinds on leather, under favourable conditions of moisture and temperature, is at the expense of fats, water soluble materials and tannins and it has not been possible to demonstrate that the growth of the moulds is a direct cause of loss of tensile strength of leather (Kanagy, Seebold, Charles and Cassel (1949)). Nor does prolonged and prolific mould growth appear to cause significant change in the properties of vegetable tanned leather, the tanned collagen fibres being unattacked (Hyde, Mitton and Musgrave (1951)). Similarly it has not been possible to demonstrate, by histological examination, any deterioration of the collagen matrix of vegetable tanned hide as a result of mould growth (Barghoorn 1950).

The results of attempts to use leather as a fertiliser are also interesting. Unless leather is extensively detanned it does not seem to have any manurial value for crops such as swedes, corn, spinach, beet, etc., but after treatment with alkali, expecially sodium hydroxide, a product can be obtained which is equal in fertilising value to hoof and horn meals. These observations were carried out on crop plants of relatively rapid growth, and experiments indicate that even without chemical treatment chamoix and formaldehyde tanned leathers, which are much more easily broken down than vegetable tanned leathers, may be completely broken down in soil within twelve months (Turner 1950). This raises the point as to whether the vegetable tanned protein fibres of leather, although unattacked by moulds such as *Penicillium* and *Aspergillus niger*, may be broken down, even though comparatively slowly, by soil organisms such as wood- and litter-destroying fungi which are able to decompose other complex and relatively resistant material. In this connection Colin—Russ (1940) mentions an isolated experience of profound mycological action on the collagen tannate complex. In this instance all but the soles of a pair of willow calf shoes, lying near a slightly damp wall, were either eaten away or made tender, during a period of three to four months, by a process he likens to dry rot in timber; there is however no indication of the identity of the fungus involved.

The Nature of the Tanning Process in the Tanning of Hides by Vegetable Tannins

Vegetable tanned leather and the protein complexes formed by the interaction of proteins and leaf extracts appear to have somewhat similar properties and therefore information regarding the processes involved in the tanning of hides may be useful in further investigation of the protein complexes formed from leaf extracts.

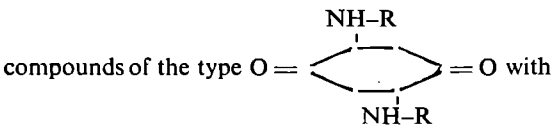
Lack of precise knowledge of the molecular architecture of proteins and tannins has resulted in much discussion and speculation regarding the nature of the tanning process. Gustavson (1949) has reviewed the current ideas concerning the nature of vegetable tanning and points out that although the various investigators have emphasized one or other of two types of reaction, it is probable that the final answer will have to recognise both types of reaction, with preference for a particular reaction in certain circumstances. The two types of reaction are (1) electrovalent reactions in which the tannin is attached to the basic groups of the protein and (2) co-ordination reactions where the tannin reacts with the peptide groups. He is of the opinion that the same large tannin molecule may interact with collagen by means of both types of valency, thereby giving rise to multipoint fixation.

Although there are considerable differences of opinion regarding the precise nature of tanning processes there are a number of points on which there is a considerable measure of agreement. Thus, whatever the function of peptide groups may be in the irreversible attachment of tannins to collagen, it seems clear that the basic amino groups play a considerable part in the fixation of vegetable tannin. When the basic groups of a protein are inactivated there is a decrease in the amount of tannin fixed by the protein. The hydrothermal stability imparted to the fibres by the action of tannins is found to be associated mainly with the fraction of tannins attached to the basic groups. Vegetable tanning also imparts to collagen increased resistance towards the action of trypsin. Equilibrium is rapidly attained in the reaction of the basic groups with tannins, whilst in the co-ordination reactions equilibrium is only reached after some time. This may however be restricted to insoluble fibrous proteins, for, with such material, swelling of the micellar structure of the hide in order to make the reactive groups accessible to the tannins seems to be an important factor which chiefly affects the co-ordinate groups. The accessibility factor also seems to be concerned in the observed effect of acidity and alkalinity on the degree of swelling of the protein. Such considerations may perhaps not be so important in the case of soluble proteins. The factors controlling the reactivity of the basic groups of the basic amino acids in proteins are the subject of considerable discussion, but seem to be associated with the molecular architecture of the proteins, so that when this undergoes change, as for example when

denaturation takes place, there are likely to be changes in reactivity. (Bowes and Moss (1951) Bailey (1951) and Porter (1950)).

Gustavson (1949) has discussed the probable importance of spatial relationships and molecular architecture in problems of tanning, and the formation of other complexes between proteins and smaller molecules. The reactions are likely to be influenced by the size and shape of the introduced molecule, the distance between the reactive groups of the protein, and the distance between these and the reactive groups of the tanning agent; there is, however, little information concerning such matters as yet. Gustavson therefore pictures the tanning process as the incorporation in the protein of substances possessing affinity for various protein groups, resulting in the immobilisation of the tanning agent in the protein lattice. Such more or less stable combinations would also lead to stabilisation of protein structure, perhaps by the formation of bridges, by the tanning agent, between reactive groups of adjacent chains of the protein molecules, the degree of stabilisation being governed not only by the strength of the valency bonds between the tanning agent and the protein, but also by the spatial architecture of the reacting molecules.

The problem of quinone tanning, which seems to be of considerable importance in the animal kingdom, is also discussed by Gustavson (1949). At first it was considered that the C=O groups were those actively concerned in the tanning reaction, whereas now it is considered that the formation of



compounds of the type with

the amino groups of proteins seems more likely; this latter idea being supported by the fact that tetrachloroquinone is devoid of tanning power.

In view of the complexity of the molecules of leaf proteins, and possible variations in the composition and molecular structures of substances in leaf extracts which unite with proteins, apparently in a similar manner to the reaction between tannins and hide, there would appear to be the potentiality for widely varying degrees of stability in complexes which may be formed in leaves between the leaf proteins and protein precipitating materials. Such variation in stability may be the basis for the experimentally observed differences between the complexes formed by the reaction of proteins with extracts of the leaves of different species, and also for the resistance to decomposition under some conditions of a fraction of the litter of species which give rise to mor.

Although the protein precipitating substances observed in leaf extracts may not be tannins, their reactions with proteins seem to be sufficiently similar to those of tannins with hide as to warrant a consideration of the nature and occurrence of vegetable tannins.

The Occurrence of Vegetable Tannins

As previously mentioned the vegetable tannins are complex substances concerning which there is little precise information. Skene (in Nierenstein (1934)) discusses the botanical position of tannins and emphasises that only where microchemical, methods are used to study the distribution or behaviour of tannins, in plants known to contain tannins, can conclusions be drawn if caution be used; even then the conclusions are based on probability rather than rigorous proof. The tannins only seem to be of widespread occurrence in the higher plants, and the type of metabolism resulting in tannin formation runs in families. Tannins may occur in every part of the plant, and bark often contains the highest concentrations, although Clarke, Rogers, Sievers and Hopp (1949) found that the leaves, leaflets and flowers of sumac (*Rhus*) had a high tannin content whilst other parts of the plant had a low tannin content.

Skene (1934) states that there appears to be no diffusion of tannin through the plasma in the living cell, and that on plasmolysis of tannin-containing cells the withdrawal of water may result in the separation of the tannin as a gelatinous mass. Further, he considers that the tannins of the vacuole are not by any means certainly in contact with the proteins of the plasma, the vacuolar membrane probably being lipoid in nature. Very high concentrations of tannin are usually found only in moribund cells and tissues. The protein precipitating materials found in leaves, even if they are not tannin in nature, may therefore be present only in the vacuole during the life of the cell, and only they unite with the cell protein on the death of the cell. In the case of the previously mentioned tanning agent protocatechuic acid, however, which is secreted by cells of *Blatta orientalis*, it would appear that the tanning agent must pass through the protoplast, but here oxidation appears to be an essential part of the tanning process. It seems likely in the case of both animal and plant tanning materials that whilst their synthesis probably involves contact with protein material, the tanning of proteins is essentially a process concerning proteins which are not part of a living system; the properties and activity of living protoplasm preventing interaction between cell tannins and the proteins of protoplasm.

The Decomposition of Vegetable Tannins by Micro-organisms

Whilst the growth and metabolism of many

micro-organisms are inhibited by tannins, there are also many yeasts and bacteria which grow actively in vegetable tan liquors, and moulds may also occur. This aspect of tannins has been recently reviewed by Musgrave (1948) and evidence is put forward that moulds such as *Penicillium* sp. and *Aspergillus* sp. can bring about a decrease in tannin concentration. Thus *Penicillium glaucum* and *Aspergillus niger* caused a decrease in tan concentration in a 2% Philippine cutch liquor. Some vegetable tannins support mould growth better than others, e.g. moulds grow freely on gambier and sumac but sparingly on unsulphited quebracho, and, in general, the catechol tans seem less able to support growth than the pyrogallol tans. Musgrave also cites evidence indicating that the tannases are specific adaptive enzymes.

The Inactivation of Protein Precipitating Factor in Aqueous Extracts of Fresh Leaves by Micro-organisms

The parallel between leather and the protein complexes formed by leaf extracts now becomes even closer for, as in the case of leather which is resistant to decomposition by micro-organisms whereas its separate constituents collagen and vegetable tannins are not, proteins precipitated by some leaf extracts become resistant to decomposition whereas the protein alone (gelatin) is not resistant to decomposition. Experiments have shown that the substances in leaf extracts which bring about protein precipitation are either destroyed or rendered unable to precipitate protein as a result of the activity of micro-organisms.

In the first instance the effect of the growth of a number of wood- and litter-destroying fungi on the protein precipitating materials in aqueous extracts of the fresh leaves of *Rhododendron* sp. and *Chamaenerion angustifolium*, in the presence or absence of cellulose was investigated. The fresh leaf extracts, prepared as previously described, were distributed in 5 ml. quantities among 50 ml. conical flasks, to half of which 0.05 gm. of milled filter paper was added. The flasks were sterilised by chloroform inoculated on 3.8.50, and incubated at 20°C. until 15.11.50; then the fluid in each flask was examined for gelatin precipitating capacity, reaction (pH), reaction with ferric chloride solution, and changes on saturation with sodium chloride. The organisms used were strains of *Lenzites betulina*, *Lentinus lepideus*, *Polystictus versicolor*, *Stereum hirsutum*, *Trametes gibbosa*, *Polystictus sanguineus*, *Polystictus abietinus*, *Marasmius dryophilus*, *Marasmius peronatus*, *Marasmius oreades*, *Marasmius hariolorum*, *Mycena pura*, *Collybia butyracea*, and *Clitocybe nebularis*. The results may be summarised:—

1. There is a much greater tendency for there to be a diminution in detectable gelatin precipitating factor in the leaf extracts after the growth of the wood-destroying fungi, than after the growth of the litter-destroying fungi.

2. In a number of cases the changes in gelatin precipitation, reaction with ferric chloride solution, and production of precipitate on saturation with sodium chloride, do not run parallel; this may indicate that the substances involved in these reactions are not identical.

3. The presence of cellulose does not seem to have played any considerable part in the changes which have occurred.

4. A number of the test organisms, but especially the litter-destroying fungi, seemed able to grow only feebly or not at all on the leaf extracts. This is particularly interesting in the case of *Collybia butyracea* which, although not growing vigorously, was able to bring about the disappearance of the gelatin-*Chamaenerion* leaf extract complexes, especially in the presence of *Fraxinus* litter extract and cellulose; whereas in the present experiment it has not brought about any change in the protein precipitating factor.

5. In a small number of the flasks a *Penicillium* sp. developed as a contaminating organism. This was usually accompanied by a considerable increase in acidity, the reaction becoming as acid as pH 3.1, but in no case was there a detectable change in gelatin precipitating capacity or reaction with ferric chloride solution.

6. In a considerable number of the flasks a jelly-like material was produced, often in comparatively large quantities, by a number of the organisms but especially by the wood-destroying fungi.

In a further experiment the effect of the growth of three organisms on the gelatin precipitating properties, and reactions with ferric chloride solution, of the leaf extracts of a number of species was examined. The organisms used were strains of *Lenzites betulina* and *Polystictus versicolor*, organisms which in the previous experiment brought about complete disappearance of the gelatin precipitating capacity of the leaf extracts; and *Marasmius dryophilus* which, although not causing any change in the gelatin precipitating capacity of the leaf extracts, was able to grows satisfactorily on the leaf extracts.

Leaf extracts of the following species, prepared by the usual method, were distributed in 5 ml. quantities in 50 ml. flasks and sterilised by chloroform; cellulose being added to the leaf extracts in an equal number of flasks. The plant species from which the leaf extracts were prepared were *Abies procera*, *Abies grandis*, *Araucaria imbricata*, *Juniperus virginiana*, *Pinus contorta*, *Sequoia gigantea*, *Pseudotsuga*

taxifolia, *Taxus baccata*, *Taxodium distichum*, *Calluna vulgaris*, *Cornus sanguinea*, *Corylus avellana*, *Fagus sylvatica*, *Quercus robur*, *Sorbus aucuparia*, *Eriophorum* sp., and *Vaccinium myrtillus*. The flasks were inoculated on 22.12.50 and observations recorded on 27.4.51. The results may be summarised:—

1. *Lenzites betulina* and *Polystictus versicolor* seem better able to grow on the leaf extracts than did the litter-destroying organism *Marasmius dryophilus*.

2. Of the various leaf extracts used only two gave results in which there was not complete disappearance of gelatin precipitating capacity caused by at least one organism; even in these cases, i.e. extracts of *Vaccinium myrtillus* and *Sequoia* leaves, there was evidence of some reduction in gelatin precipitating capacity.

3. The changes observed may be divided into two types:

(a) both gelatin precipitating capacity and the reaction with ferric chloride solution disappear;

(b) gelatin precipitating capacity remains and the reaction with ferric chloride solution disappears.

Cases of the gelatin precipitating capacity disappearing and the reaction with ferric chloride solution remaining have not been observed.

4. In this experiment, whilst cellulose again appears to have little influence on the changes in gelatin precipitating capacity and reaction with ferric chloride solution, there appears to be a considerably greater development of jelly-like material when cellulose is present. Jelly formation in comparatively large quantities seems to occur more frequently with extracts of leaves of Dicotyledonous species than with extracts of leaves of Coniferous species. The amount of jelly formed does not seem to be related to the amount of growth of the organism.

CONCLUSIONS

MANY conclusions have already been indicated at appropriate points in the text, and only the general conclusions necessary to outline a working hypothesis concerning the differential formation of mull and mor will be considered here.

In Part I a consideration of previous work did not indicate any acceptable mechanism which might be considered responsible for the differential development of mull and mor. Of the factors considered to have an important influence on soil formation, it would appear that no single factor can be shown to have an overriding influence in determining the differential formation of mull and mor.

It is clear, however, that under some conditions at least the species composition of the vegetation growing on the site, acting through the properties of the vegetable debris produced, determines whether mull or mor will be formed. Consideration of the known quantitative and qualitative characteristics of the vegetable debris from which mull and mor arise does not give any indication of the factor or factors responsible for the differential formation of mull and mor.

From a consideration of the relatively small amount of information available concerning soil processes it is concluded that one or more hitherto unobserved attributes of the various kinds of vegetable debris may be responsible for determining the differential formation of mull and mor.

It seems probable that the activities of soil micro-organisms and the soil fauna are controlled by one or more properties of the various kinds of vegetable debris.

Even the mor-forming properties of a mor-forming litter appear to be capable of modification by the presence of other types of vegetable debris and perhaps also by mineral material rich in bases.

Observations which have been made on the decomposition of vegetable debris, with the above conclusions in mind, are described in Part II. These lead to the hypothesis that stabilised leaf proteins are an important factor in the processes of mor formation, and this hypothesis is also in agreement with the following characteristics which seem to differentiate mull and mor:—

(a) The apparently larger amounts of cellulose present in mor than in mull.

(b) The greater resistance to breakdown which seems characteristic of nitrogenous material in mor.

(c) The differences in the faunal populations of the two soil types which may well be a reflection of a relative unavailability of nitrogen in mor.

Whilst a connection between stabilised leaf proteins and the unusual degree of acidity which occurs in many examples of mor has not been demonstrated as yet, there is reason to think that stabilisation of leaf proteins, by immobilisation of mineral nutrients (in this connection Rennie (1952) has found concentrations of Ca, K, Mg and P in the mor formed from *Calluna* debris ten to fifteen times as great as in the leached and parent mineral layers below, as determined by base exchange methods) or by other means, may well upset stages of intermediate metabolism of micro-organisms, resulting in the accumulation of organic acids which are relatively resistant to decomposition under such conditions.

It is now possible, as a result of these investigations, to formulate a variable biological system of soil processes as the basis of the dynamic system of which mull and mor represent the extremes.

The presence in vegetable debris, especially in potential mor-forming debris, of proteins stabilised in the dying leaf by materials bearing resemblances to tannins forms an essential part of the system. The stabilised proteins of potential mor-forming vegetable debris occur in the mesophyll tissues and are, under certain conditions, so resistant to decomposition that the various parts of the debris in which they do not seem to occur, especially the vascular tissue, decompose, and leave, as a layer lying on the surface of the mineral soil, an amorphous residue of leaf mesophyll cell walls protected from decomposition by the resistant stabilised protein. It is possible that, in addition to resisting decomposition itself, the stabilised protein, by withholding adequate supplies of available nitrogen and possibly other materials, considerably delays the decomposition of unprotected tissues such as the vascular tissue, and may also considerably modify the metabolic pathways, thereby giving rise to abnormal acidity.

Whilst stabilised proteins may also occur in mull-forming vegetable debris, there is reason to believe that these are by no means so resistant to decomposition, probably on account of differences in molecular composition and structure, compared with those of the potential mor-forming debris. Vegetable debris from mull-forming species in which stabilised proteins may not occur, decomposes readily, probably on account of adequate supplies of more readily available nitrogen, and in some cases at least the mesophyll tissue decomposes in advance of the vascular tissue.

The greater ease of transformation of mull-forming vegetable debris, as compared with the resistance to decomposition of certain parts of potential mor-forming debris under mor-forming conditions, is probably the reason for the lack of recognisable plant debris in mull soils; the quite considerable amounts of organic matter present in mull soils being in part at least the more or less stable products of synthesis by micro-organisms, perhaps similar to those formed in experiments on the decomposition of protein precipitating factors in leaf extracts, from the products of decomposition of the vegetable debris. If a considerable amount of resynthesis goes on in mull soils, then the proportion of the organic matter of the litter which is completely broken down to carbon dioxide may not be very different in the case of mull-forming litter and mor-forming litter. These synthesised materials appear to be in part polysaccharide in nature, and it seems possible that the more or less stable nitrogenous material present in mull soils, which has been shown to contain amino acids, consists of microbial protein or extracellular organic nitrogen compounds, such as those described by Morton (1951), stabilised to some degree by other metabolic products of micro-organisms, such as hydroxy aromatic substances, e.g. similar to homoprotocatechuic acid and 2.4.5 trihydroxyphenylglyoxylic acid obtained from the metabolic liquor of *Polyporus tumulosus* by Ralph and Robertson (1950), or the orthodiphenol obtained from cultures of soil organisms by Lemoigne (1928).

The transition to mull which can be produced in mor by the repeated addition of mull-forming litter seems to be well explained by postulating that some materials in the mull-forming litter allow of the decomposition of the stabilised proteins in mor. There is an indication of this in the experiments with protein complexes. The effect may well be similar to that observed by Lohnis (1926) that addition of leguminous plant debris to agricultural soils results, especially when the soil is rich in organic matter, in the liberation of more nitrogen than that contained in the plant material added.

If the problem of the formation of mor on limestone be reconsidered on the basis of the above hypothesis, it seems that the phenomenon can be explained along the following lines. At first the limestone is colonised by calcicolous species which would normally give rise to a mull soil, but the mull-type of soil organic matter produced cannot be mixed with mineral particles as it lies directly on the limestone surface and may therefore be expected to accumulate as an organic layer on the surface of the limestone. Perhaps aided by such factors as grazing the calcicolous flora is replaced by calcifuge species such as *Calluna vulgaris* which will, some time after it achieves dominance, produce mor lying on the layer of mull organic matter.

Such a hypothesis of the formation of mull and mor allows of the possibility of a gradation of intermediate soil types between mull and mor, and also leads to the conclusion that mull is the more satisfactory soil type biologically, for by allowing optimum rates of turnover of nutrients it permits optimum biological activity on a particular site, as a long term consideration.

The precise composition of the protein complexes, and the nature of the microbiological populations and enzyme systems bringing about their decomposition, together with the influence of variation in environmental factors on the activities of these micro-organisms and their enzyme systems, should be the subjects of further investigation.

SUMMARY

In Part I previous observations relating to the differential formation of mull and mor are considered.

Of the factors influencing the processes of soil formation, there are indications that the characteristics of the parent mineral materials of the soil and the vegetation are important in relation to the occurrence of mull and mor. The distribution of mull and mor yields little if any information regarding the detailed mechanism of the causes of their occurrence. Mull and mor seem to be components of a reversible system. For an understanding of this system it is necessary to characterise the reversible processes involved, and to define as clearly as possible the qualitative and qantitative characteristics of the materials concerned in the processes.

It has not been possible to demonstrate, from information available, that there is any reason to believe that the cause of mull and mor formation lies in disproportionate amounts of litter falling on the respective sites. Similarly there are no indications that the mineral constituents of litter, its reaction in terms of pH units, its content of basic and acidic buffering materials, or its nitrogen content, allow of the differentiation of vegetable debris which will give rise to mull from that which will form mor. Qualitative and quantitative aspects of the organic constituents of litter which have been investigated also do not appear to allow of the characterisation of mull- and mor-forming litter.

The possible differentiation of mull- and mor-forming vegetable debris by changes undergone by the litter and its various constituents during integration into a mull or a mor system has been considered, since the detection of characteristically different processes during mull and mor formation could lead to the cause of these different processes and thence to the reasons for the differential development of mull and mor.

Overall changes in the organic matter of mull and mor, from such observations as have been made on comparisons of total amounts of organic matter in mull and mor systems and of carbon dioxide production by the two systems, do not lend support to the hypothesis that mor is the result of a slower rate of decomposition of vegetable debris. The evidence available on this matter cannot however be considered to be conclusive one way or the other.

Detection and interpretation of changes in individual organic constituents, during the decomposition of litter and the formation of mull and mor, by proximate chemical analyses, are rendered difficult by the complexity and intractability of many of the materials involved. In this connection there are indications that cellulose tends to be present in greater quantities in the organic matter of mor than in the organic matter of mull.

In many soils there appears to be an increase in concentration of nitrogen in the organic matter of the soil with increasing depth, but from the evidence available this does not seem to be especially characteristic of either mull or mor sites. Since much of the nitrogen added to soil would seem to be in the form of protein in litter, the apparently greater resistance to decomposition of the nitrogenous compared with the non-nitrogenous constituents of litter would appear to be important; especially considering the observations indicating that the complex nitrogen of mor is usually more resistant to mobilisation than the complex nitrogen of mull, and also in view of the demonstrated presence of considerable amounts of amino acids in soil hydrolysates. There are therefore indications that the nature of the organic nitrogen in mull and mor may be an important source of differences between them.

Although much importance is sometimes attached to the function of lignin in mor formation, Waksman's (1938) comment "So many formulae have been proposed to explain the chemistry of lignin and of 'humic acids', and so much importance has been attached to them, that one is surprised to find that they are largely illusory" still seems most appropriate, especially since certain heavily lignified materials readily decompose even on mor sites.

Other investigations on the nature of soil organic matter do not seem to have provided any indications of fundamental differences between mull and mor.

It does not seem possible to point to any qualitative or quantitative differences in the inorganic constituents of litter which might be characteristic of litter giving rise to mull or mor.

In the case of reaction, as represented by pH values, there is no clearly marked dividing line by which mull can be distinguished from mor, although mor does tend to have a more acid reaction than is found in mull. The cause or nature of these more acid reactions does not seem to have been ascertained.

The difficulty of investigating and interpreting oxidation reduction potentials in biological systems such as mull and mor is probably the reason why little useful information is available regarding the oxidation-reduction potentials of mull and mor. There is no information at present indicating

characteristic differences between the oxidation-reduction systems of mull and those of mor.

Methods for investigating the identity and activities of populations of micro-organisms in soil are at present inadequate to make possible a useful comparative study of mull and mor in this respect. The behaviour of micro-organisms may indicate differences in the composition and properties of litter from different plant species, even though such differences have not been demonstrated by chemical methods.

There seem to be fairly well marked differences between the populations of the soil fauna of mull and mor, which may indicate differences concerning the properties of litter, so far not detected by chemical methods, influencing the differential formation of mull and mor. There are indications that some undetermined properties of the vegetable debris control the soil fauna, so that the soil fauna cannot be regarded as a primary determining factor in the formation of mull and mor.

The available evidence seems to point to some attribute or attributes of certain kinds of vegetable debris as being responsible for determining the differential formation of mull and mor. It appears that the properties of a mor-forming litter can be modified by the presence of other kinds of litter, and probably also by mineral material rich in bases.

Any mechanism which is put forward as the basis of the differential formation of mull and mor will have to be capable of considerable modification under the influence of various factors.

In Part II investigations on the reasons for the differential formation of mull and mor are based on the following differences in the characteristics of mull and mor, derived from Part I.

(*a*) The apparently greater quantities of cellulose in the organic matter of mor compared with the organic matter of mull.

(*b*) The nitrogen of mor appears to exhibit a greater degree of resistance to mobilisation by biological agencies than the nitrogen of mull.

(*c*) There is a tendency for the production of extremely acid conditions when mor is formed.

(*d*) The soil fauna populations of mull and mor seem to exhibit characteristic differences.

(*e*) The differences between mull and mor appear to concern differences in type or course of decomposition and resynthesis, rather than different rates of decomposition of litter.

(*f*) Whatever the mechanisms responsible for the differential formation of mull and mor may be, a particular condition represents a phase in a dynamic system which is reversible under the influence of the changing intensity of various factors.

Samples of mull and mor have been examined microscopically. Cellulose can be detected in mor in considerable amounts only after treatment with a 1% solution of sodium hypochlorite. This cellulose material appears to be derived not from vascular tissue but from parenchymatous cells. The amount of this cellulose material seems to decrease with depth. Cellulose material similar in kind and amount has not been observed in mull samples.

Recognisable fragments of litter from increasing depths in mor formed from the litter of *Calluna vulgaris* were embedded in wax. Serial sections of this material indicated that vascular tissue disappears first, leaving a residue of leaf mesophyll tissue which ultimately becomes an amorphous mass containing mesophyll cell walls apparently coated with some protective material. Similar findings were obtained for material from samples of mor formed, for example from beech litter, Norway spruce litter and *Abies pinsapo* litter. Similar examination of mull-forming litter indicated that here the mesophyll tissue disappears first, leaving the more resistant vascular tissues.

From serial sections of shoots of *Calluna vulgaris*, having both dead and living leaves, changes have been observed in the mesophyll tissue when the leaf dies, whilst still on the living plant, which give the mesophyll tissue the appearance it retains until it becomes amorphous material in the mor. This change, occurring whilst the leaf is still on the plant, would not appear to be due to a product of microbial metobolism. The material appearing as a result of changes in the mesophyll tissue when the leaf dies, would seem to be that which protects the cellulose walls of the mesophyll from decomposition when the litter falls on to the surface of the mor.

Experimental evidence is put forward to show that the essential part of the change occurring when the leaf dies is a precipitation or stabilisation of cytoplasmic protein by a process which can be likened to that occurring when hide is tanned. Active protein precipitation agents have been found in aqueous extracts of the fresh leaves of a number of plant species, including some commonly associated with the formation of mull.

By the action of wood- and litter-destroying fungi on model substances prepared by the use of gelatin, representing the leaf cytoplasmic protein, and aqueous extracts of fresh leaves, it has been observed that model substances prepared from aqueous extracts of the fresh leaves of species giving rise to litter forming mull on the one hand, and mor on the other, show a differential resistance to breakdown; the models from material from mor-forming species

were the more resistant. Model substances which have been air dried seem to be more resistant to breakdown than the same materials which are not allowed to dry before inoculation with wood-destroying and litter-destroying fungi. The presence of aqueous extracts of litter, especially of species such as *Fraxinus excelsior*, seems to aid the breakdown of the model substances, especially in the presence of cellulose.

The protein precipitating materials present in aqueous extracts of fresh leaves can be precipitated by saturation of the extracts with sodium chloride, or the use of ethyl alcohol; but it is unlikely that the materials so obtained represent only substances concerned with protein precipitation.

Fresh leaf extracts containing protein precipitating material usually give a colour reaction with ferric chloride solution.

It is unlikely that the precipitation of proteins by leaf extracts is an isoelectric phenomenon.

Evidence has been obtained which indicates that the basic amino groups of diamino acids are actively concerned in the formation of precipitates by interaction between proteins and extracts of fresh leaves. Such systems would seem to allow of the formation of a large number of more or less different complexes, with the possibility of a considerable range of resistance to breakdown by biological agencies.

The similarity of these protein precipitating materials in extracts of fresh leaves to vegetable tannins is indicated, and it has been shown that, as in the case of vegetable tannins, the protein precipitating materials are readily rendered inactive by micro-organisms when they are not united to protein, whereas the complexes formed with protein are resistant to breakdown.

It is concluded that leaf proteins stabilised by protein precipitating materials on the death of the leaf are an important factor in the processes leading to mor formation. It is suggested that these stabilised proteins are, in certain species, those whose litter gives rise to mor, so resistant to decomposition, under some conditions, that much of the mesophyll tissues of the litter comes to lie as a layer of organic material on the surface of the soil. Although similar materials may be formed in the mesophyll tissue of the dying leaves of species whose litter gives rise to mull, in these cases the materials are probably much less resistant to decomposition; with the most important consequence for biological processes that whereas available nitrogen is very scarce in mor, it is more plentiful in mull.

There are indications that the presence of constituents from the litter of some species associated with the development of mull aids the breakdown of the resistant nitrogenous material derived from species associated with the formation of mor, thereby accounting for the reversibility of the mull $\rightleftarrows$ mor system.

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APPENDIX I

PROPERTIES OF THE AQUEOUS EXTRACTS OF FRESH LEAVES AND FRESHLY FALLEN LITTER OF VARIOUS SPECIES

(See page 69)

A. FRESH LEAVES

Conifers

Species	Date of Collection	Reaction of Extract (pH) (Glass Electrode)	Reaction with Gelatin Solution	Reaction with Ferric Chloride Solution
Abies alba	21/2/50	4.2	Very bulky flocculent light brown precipitate	Bulky blue black precipitate
Abies firma	7/3/50	4.1	Extremely bulky flocculent cream-coloured precipitate	Bulky blue black precipitate
Abies grandis	21/2/50	3.6	Extremely bulky flocculent cream-coloured precipitate	Dark blue grey precipitate
Abies procera	7/3/50	3.8	Extremely bulky flocculent cream-coloured precipitate	Extremely bulky blue black precipitate
Abies pinsapo	7/3/50	4.1	Extremely bulky flocculent cream-coloured precipitate	Dark greenish grey precipitate
Araucaria araucana	7/3/50	4.9	Flocculent light brown precipitate	Pale greenish brown colour
Cedrus deodara	21/2/50	3.9	Marked light brown turbidity but no rapid flocculation	Slight greenish colour
Cryptomeria japonica	7/3/50	4.4	Flocculent light purplish-brown precipitate	Deep green colour
Chamaecyparis lawsoniana	21/2/50	5.1	Extremely bulky flocculent orange-yellow precipitate	Greenish grey precipitate
Cupressus macrocarpa	7/3/50	4.6	Bulky flocculent pale brown precipitate	Deep green colour
Juniperus virginiana	7/3/50	5.0	Bulky flocculent brown precipitate	Grey brown colour
Larix laricina	30/8/49	3.4	Bulky flocculent light brown precipitate	Pale brown colour
Larix decidua	30/8/49	3.9	Extremely bulky light brown precipitate	Dark grey brown colour
Larix leptolepis	23/5/50	4.0	Marked cream-coloured turbidity with some flocculation	Grey brown colour
Libocedrus decurrens	21/2/50	5.0	Slight turbidity but no flocculation	Pale greenish brown colour
Picea abies (27)	2/8/49	4.5	Bulky flocculent precipitate	Green colour
Picea abies (5)	21/2/50	4.4.	Very bulky flocculent dirty.white precipitate	Grey colour
Picea abies (17) (calcareous mull)	7/2/50	4.0	Extremely bulky flocculent pale brown precipitate	Green colour

Note: Numbers in brackets refer to the sites from which samples were obtained.

Species	Date of Collection	Reaction of Extract (pH) (Glass Electrode)	Reaction with Gelatin Solution	Reaction with Ferric Chloride Solution
Picea abies (18) Kimmeridge clay mull	7/3/50	4.4	Marked pale brown turbidity but no rapid flocculation	Pale green colour
Picea abies (19) Kimmeridge clay mull	7/3/50	4.2	Extremely bulky flocculent pale brown precipitate	Dark green colour
Picea abies (20) Kimmeridge clay mull	7/3/50	3.9	Extremely bulky flocculent pale brown precipitate	Dark greenish grey colour
Picea glehni	7/3/50	4.4	Extremely bulky flocculent brown precipitate	Dark brownish grey precipitate
Picea orientalis	7/3/50	3.8	Bulky flocculent cream-coloured precipitate	Bulky blue grey precipitate
Picea sitchensis	21/2/50	4.4	Bulky flocculent very pale brown precipitate	Greenish grey colour
Pinus contorta	2/8/49	4.0	Extremely bulky flocculent pale brown precipitate	Dark grey green colour
Pinus griffithii	7/3/50	4.3	Cream coloured turbidity but no rapid flocculation	No visible change
Pinus peuce	7/3/50	4.6	Very bulky flocculent cream-coloured precipitate	Very finely divided dark grey precipitate
Pinus ponderosa	21/2/50	4.0	Bulky flocculent dirty-white precipitate	No visible change
Pinus radiata	21/2/50	3.7	Very bulk flocculent dirty-white precipitate	Bulky dark blue grey precipitate
Pinus thunbergii	7/3/50	4.2	Marked cream-coloured turbidity but no rapid flocculation	Very pale green colour
Pinus sylvestris	21/2/50	4.6	Light brown turbidity but no rapid flocculation	Pale greenish brown colour
Pinus sylvestris (calcareous mull)	14/1/50	4.8	Marked light brown turbidity but no rapid flocculation	Pale greenish brown colour
Pinus sylvestris (calcareous mull)	26/2/50	4.5	Marked pale brown turbidity but no rapid flocculation	No visible change
Pinus sylvestris (calcareous mull)	26/2/50	4.3	Bulky flocculent dirty-white precipitate	Dark greenish grey colour
Pseudotsuga taxifolia (12) Fraser River Form	21/2/50	4.4	Marked light brown turbidity but no rapid flocculation	Intense red colour
Pseudotsuga taxifolia (21) var. glauca	14/2/50	4.3	Extremely bulky flocculent pale brown precipitate	Intense red colour
Pseudotsuga taxifolia (29) Fraser River Form	2/8/49	4.4	Bulky flocculent pale yellow precipitate	Intense red colour
Pseudotsuga taxifolia (30)	2/8/49	4.1	Bulky flocculent cream-coloured precipitate	Dark brown colour
Pseudotsuga taxifolia (31)	2/8/49	4.0	Bulky flocculent pale yellow precipitate	Dark brown colour

Note: Numbers in brackets refer to the sites from which samples were obtained.

Species	Date of Collection	Reaction of Extract (pH) (Glass Electrode)	Reaction with Gelatin Solution	Reaction with Ferric Chloride Solution
Sciadopitys verticillata	7/2/50	4.8	Marked cream coloured turbidity but no rapid flocculation	Pale greenish brown colour
Sequoia wellingtonia	7/3/50	3.7	Extremely bulky flocculent cream-coloured precipitate	Bulky dark greenish grey precipitate
Sequoia sempervirens	21/2/50	4.0	Bulky flocculent very pale brown precipitate	Greenish grey colour
Thuja plicata	21/2/50	5.1	Extremely bulky flocculent orange precipitate	Bulky brownish grey precipitate
Taxodium distichum	2/8/49	3.3	Bulky white precipitate which coalesces to a fibrous mass	Green colour
Taxus baccata	21/2/50	4.9	Extremely bulky flocculent orange precipitate	Dark green colour
Thuja plicata	21/2/50	5.1	Extremely bulky flocculent orange precipitate	Bulky brownish grey precipitate
Tsuga heterophylla	7/3/50	3.9	Extremely bulky flocculent cream-coloured precipitate	Bulky dark greenish grey precipitate

Broadleaved Trees

Acer campestre	3/11/49	4.4	Bulky flocculent light brown precipitate	Almost black precipitate
Acer pseudoplatanus	2/8/49	4.4	Bulky flocculent brown precipitate	Very dark green colour
Aesculus hippocastanum	17/10/49	5.2	Bulky flocculent light brownish yellow precipitate	Greenish brown colour
Ailanthus glandulosa	17/10/49	5.3	Marked greyish brown turbidity	Dark brown colour
Betula sp.	12/10/49	4.8	Bulky flocculent light brown precipitate	Dark greenish brown colour
Castanea sativa	12/10/49	4.6	Bulky flocculent greyish brown precipitate	Blue black precipitate
Fagus sylvatica (14) (calcareous mull)	–/9/49	5.3	Bulky flocculent light reddish purple precipitate	Green colour
Fagus sylvatica (15)	–/9/49	5.1	Bulky flocculent reddish purple precipitate	Greenish brown colour
Fagus sylvatica (64) (mor)	20/6/51	4.6	Bulky flocculent pale pinkish brown precipitate	Greenish brown colour
Fagus sylvatica (65) (calcareous mull, sun leaves)	20/6/51	4.7	Bulky flocculent pinkish brown precipitate	Dark brown colour
Fagus sylvatica (66) (mor, sun leaves)	20/6/51	4.7	Bulky flocculent pale pinkish brown precipitate	Greenish brown colour
Fagus sylvatica (67) (calcareous mull, sun leaves)	20/6/51	4.8	Bulky flocculent dark pinkish brown precipitate	Dark greenish brown colour

Note: Numbers in brackets refer to the sites from which samples were obtained.

Species	Date of Collection	Reaction of Extract (pH) (Glass Electrode)	Reaction with Gelatin Solution	Reaction with Ferric Chloride Solution
Fagus sylvatica (68) (calcareous mull, shade leaves)	20/6/51	4.3	Bulky flocculent very dark purplish brown precipitate	Dark greenish brown colour
Fraxinus excelsior	12/10/49	4.9	Marked brownish turbidity	Dark green colour
Platanus orientalis	17/10/49	5.1	Bulky flocculent light brown precipitate	Greenish brown colour
Populus sp.	16/10/49	4.9	Marked brownish opalescence	Greenish brown colour
Quercus borealis	17/7/51	4.2	Bulky flocculent pale pinkish brown precipitate	Dark blue precipitate
Quercus sp. (calcareous mull)	3/11/49	4.7	Bulky flocculent light brown precipitate	Black precipitate
Quercus sp. (non-calcareous mull)	3/11/49	4.6	Bulky flocculent light brown precipitate	Black precipitate
Robinia pseudoacacia	17/10/49	5.4	Pale brown opalescence	No visible change
Tilia vulgaris	16/10/49	5.4	Bulky flocculent light brown precipitate	Brown colour
Ulmus procera	17/10/49	5.9	Brownish opalesence	Brown colour
Ulmus glabra	12/10/49	5.8	No visible change	Light brown turbidity

Shrubs and Small Trees

Aucuba japonica (current leaves)	20/6/51	4.3	No visible reaction	Green colour
Aucuba japonica (2-year leaves)	20/6/51	4.8	No visible reaction	Green colour
Calluna vulgaris	Spring 1949	4.2	Bulky flocculent reddish brown precipitate	Dark greenish brown colour
Calluna vulgaris	–/9/50	4.2	Very bulky flocculent pinkish brown precipitate	Bulky greyish green precipitate
Cornus sanguinea	12/10/49	4.9	Bulky flocculent cream-coloured precipitate	Dark purplish black precipitate
Corylus avellana	12/10/49	5.2	Marked light brown turbidity	Brown colour
Crataegus monogyna	12/10/49	5.2	Bulky flocculent light brown precipitate	Greenish brown colour
Erica cinerea	6/10/48	4.5	Flocculent pale yellowish brown precipitate	Pale greenish brown colour
Erica cinerea	–/9/50	5.0	Bulky flocculent pale brown precipitate	Slight greyish green turbidity
Ilex aquifolium	2/8/48	5.0	Very slight brownish turbidity	Pale greenish brown precipitate
Ligustrum vulgare	16/11/49	4.9	Slight brownish opalescence	Bulky greenish brown precipitate

Note: Numbers in brackets refer to the sites from which samples were obtained.

Species	Date of Collection	Reaction of Extract (pH) (Glass Electrode)	Reaction with Gelatin Solution	Reaction with Ferric Chloride Solution
Prunus laurocerasus (current leaves)	20/6/51	4.4	No visible reaction	Intense bright green colour
Prunus laurocerasus (2-year-old leaves)	20/6/51	4.9	Bulky flocculent pale yellow pre-precipitate	Intense bright green colour
Rhododendron sp.	23/5/50	5.3	Bulky flocculent greyish precipitate	Bulky greenish black precipitate
Salix sp.	16/10/49	5.2	Bulky flocculent brown precipitate	Greenish brown colour
Sambucus nigra	12/10/49	5.0	No visible change	Dark green precipitate
Sorbus aria	17/10/49	5.2	Bulky flocculent brown precipitate	Dark brown colour
Sorbus aucuparia	12/10/49	5.0	Bulky flocculent light reddish brown precipitate	Dark brown colour
Vaccinium myrtillus (leaves)	–/9/50	4.2	Very bulky flocculent pale brown precipitate	Bulky greyish green precipitate
Vaccinium myrtillus (young stems)	–/9/50	4.8	Bulky flocculent pale brown precipitate	Bulky greyish green precipitate
Viburnum lantana	12/10/49	4.7	Bulky flocculent light reddish brown precipitate	Bulky dark greenish brown precipitate

Grasses, Ferns and Herbs

Bambusa sp.	12/10/49	6.1	No visible reaction	Brown turbidity
Brachypodium sylvaticum	19/10/51	5.5	No visible reaction	Pale brown precipitate
Circaea lutetiana	28/8/51	4.2	Bulky flocculent cream-coloured precipitate	Blue black precipitate
Dactylis glomerata	1/10/51	6.0	No visible reaction	Bulky dark grey/green precipitate
Deschampsia caespitosa	30/9/51	5.7	No visible reaction	Bulky cream-coloured precipitate
Deschampsia flexuosa	–/9/50	5.1	No visible reaction	Bulky greyish green precipitate
Chamaenerion angustifolium	14/9/49	4.8	Very bulky flocculent cream-coloured precipitate	Blue black precipitate
Eriophorum angustifolium	–/9/50	5.2	Bulky flocculent reddish brown precipitate	Dark green colour
Mercurialis perennis	–/9/48	5.0	No visible reaction	No visible reaction
Molinia caerulea	–/9/50	5.4	No visible reaction	Bulky brownish precipitate
Plantago major	8/8/51	4.8	No visible reaction	Green colour
Pteridium aquilinum	–/9/48	5.1	Very slight opalescence	No visible reaction

Note: Numbers in brackets refer to the sites from which samples were obtained.

Species	Date of Collection	Reaction of Extract (pH) (Glass Electrode)	Reaction with Gelatin Solution	Reaction with Ferric Chloride Solution
Ranunculus acris	8/8/51	5.2	No visible reaction	Dark brown colour
Rumex sp.	23/9/51	5.2	No visible reaction	Greenish blue colour
Taraxacum officinale	23/9/51	6.0	No visible reaction	Bulky dark greenish brown precipitate
Trifolium repens	8/8/51	5.3	No visible reaction	Geenish brown colour
Urtica dioica	16/9/48	7.5	No visible reaction	Pale brown turbidity
Vicia faba	20/7/51	5.3	No visible reaction	Greenish brown colour

Note: Numbers in brackets refer to the sites from which samples were obtained.

B. FRESHLY FALLEN LITTER

Calluna vulgaris	10/11/47	3.4	Marked pale brown opalescence but no precipitate	Very pale green colour
Fraxinus excelsior	2/11/50	5.7	No visible reaction	Brown colour
Pinus contorta	23/5/50	4.0	Orange turbidity	Bulky brown precipitate
Pinus ponderosa	23/5/50	3.9	Bulky pale brown flocculent precipitate	No visible change

APPENDIX II

THE DECOMPOSITION OF COMPLEXES, FORMED BY THE INTERACTION OF SAMBUCUS LEAF PROTEIN AND AQUEOUS EXTRACTS OF THE LEAVES OF VARIOUS SPECIES, BY STRAINS OF VARIOUS WOOD-DECOMPOSING AND LITTER-DECOMPOSING FUNGI.

Amount of fungal growth is indicated by + signs on the left ; — = no growth ; +++++ = extremely good growth. + signs on the right indicate degree of disappearance of complexes; — =no disappearance, + =considerable though incomplete disappearance, ++ =complete disappearance. Incubation period 20/9/50—8/5/52. (See page 75.)

A. SAMBUCUS-TAXUS COMPLEX

Organism	Sambucus-Taxus complex in distilled water		Sambucus-Taxus complex in distilled water + cellulose		Sambucus-Taxus complex in Fraxinus litter extract		Sambucus-Taxus complex in Fraxinus litter extract + cellulose	
Marasmius dryophilus	±	+	+	+	++++	+	++++	+
Mycena pura	±	—	±	—	++++	—	++	—
Collybia butyracea	±	—	++	+	+++	++	++++	+
Lentinus lepideus	±	—	+++	—	++++	—	++++	—
Polystictus abietinus	±	—	+++	—	++++	—	++++	++
Lenzites betulina	±	—	+++	—	++++	+	++++	++
Polystictus versicolor	+	—	+++	+	+++	+	++++	+
Control	—	—	—	—	Slight contamination	—	—	—

B. SAMBUCUS-PINUS COMPLEX

Organism	Sambucus-Pinus complex in distilled water	Sambucus-Pinus complex in distilled water + cellulose	Sambucus-Pinus complex in Fraxinus litter extract	Sambucus-Pinus complex in Fraxinus litter extract + cellulose
Marasmius dryophilus	± —	+ +	++++ —	++++ +
Mycena pura	ditto	+ —	++++ —	++++ —
Collybia butyracea	ditto	+ ++	++ ++	++++ ++
Lentinus lepideus	ditto	+ —	++++ —	++++ —
Polystictus abietinus	ditto	+ +	++++ ++	+++ ++
Lenzites betulina	ditto	+ —	++++ +	+++++ ++
Polystictus versicolor	ditto	++++ —	++++ +	++++ +
Control	— —	— —	— —	— —

C. SAMBUCUS-RHODODENDRON COMPLEX

Organism	Sambucus-Rhododendron complex in distilled water	Sambucus-Rhododendron complex in distilled water + cellulose	Sambucus-Rhododendron complex in Fraxinus litter extract	Sambucus-Rhododendron complex in Fraxinus litter extract + cellulose
Marasmius dryophilus	± ++	+++ +	+++ +	++++ —
Mycena pura	± —	+ —	+++ —	+ —
Collybia butyracea	± +	+ ++	+++ ++	+++ ++
Lentinus lepideus	± —	+ —	++++ —	++++ —
Polystictus abietinus	± —	+++ +	++++ ++	+++ ++
Lenzites betulina	± —	++++ —	++++ ++	+++++ ++
Polystictus versicolor	± —	++++ —	++++ +	+++++ +
Control	— —	— —	— —	Slight contamination —

D. SAMBUCUS-ACER COMPLEX

Organism	Sambucus-Acer complex in distilled water	Sambucus-Acer complex in distilled water + cellulose	Sambucus-Acer complex in Fraxinus litter extract	Sambucus-Acer complex in Fraxinus litter extract + cellulose
Marasmius dryophilus	± —	+++ ++	+++ —	+++++ —
Mycena pura	+ ++	± —	+++ —	+ —
Collybia butyracea	± ++	— —	+++ ++	++++ ++
Lentinus lepideus	± ++	— —	++++ —	++++ —
Polystictus abietinus	— —	++ ++	++++ +	++++ ++
Lenzites betulina	± —	++ —	++++ +	++++ +
Polystictus versicolor	± ++	+++ ++	++++ +	+++++ ++
Control	— —	Very slight contamination —	Slight contamination —	Very slight contamination —

E. SAMBUCUS-SORBUS COMPLEX

Organism	Sambucus-Sorbus complex in distilled water		Sambucus-Sorbus complex in distilled water + cellulose		Sambucus-Sorbus complex in Fraxinus litter extract		Sambucus-Sorbus complex in Fraxinus litter extract + cellulose	
Marasmius dryophilus	+	+	+	—	+++	—	++++	+
Mycena pura	+	—	+	—	+++	—	++++	—
Collybia butyracea	+	—	+	—	++	++	+++	++
Lentinus lepideus	+	—	+	—	++++	—	++++	—
Polystictus abietinus	—	—	+	++	++++	++	+++	++
Lenzites betulina	+	—	+++	+	++++	++	++++	++
Polystictus versicolor	+	—	+++	+	++++	++	++++	++
Control	—	—	—	—	—	—		—

F. ACID PRECIPITATED SAMBUCUS PROTEIN

Organism	Acid precipitated Sambucus protein in distilled water		Acid precipitated Sambucus protein in distilled water + cellulose		Acid precipitated Sambucus protein in Fraxinus litter extract		Acid precipitated Sambucus protein in Fraxinus litter extract + cellulose	
Marasmius dryophilus	±	—	++	—	++++	—	++++	—
Mycena pura	+	++	+	++	++++	—	+	—
Collybia butyracea	±	—	±	—	+++	++	++++	++
Lentinus lepideus	±	—	+++	+	++++	—	++++	—
Polystictus abietinus	+	—	+	+	++++	+	+++	++
Lenzites betulina	+	—	+	—	++++	++	+++++	++
Polystictus versicolor	+	++	+	++	++++	+	+++++	++
Control	—	—	—	—	Slight contamination	—	—	—

APPENDIX III

THE INFLUENCE OF DRYING ON THE RESISTANCE OF PROTEIN COMPLEXES TO ATTACK BY WOOD-DESTROYING AND LITTER-DESTROYING FUNGI

Amount of fungal growth is indicated by + signs on the left; — =no growth, +++++ =extremely good growth; + signs on the right indicate degree of disappearance of complexes; — =no disappearance, + =considerable though incomplete disappearance, ++ =complete disappearance. Incubation period 27/8/51—7/5/52 (See page 76.)

A. QUERCUS-GELATIN COMPLEX

	Undried Quercus-gelatin complex + Fraxinus litter extract	Dried Quercus-gelatin complex + Fraxinus litter extract	Undried Quercus-gelatin complex + Fraxinus litter extract + cellulose	Dried Quercus-gelatin complex + Fraxinus litter extract + cellulose	Undried Quercus-gelatin complex + Calluna litter extract	Dried Quercus-gelatin complex + Calluna litter extract	Undried Quercus-gelatin complex + Calluna litter extract + cellulose	Dried Quercus-gelatin complex + Calluna litter extract + cellulose
Collybia butyracea	++ ++	++ —	++++ +	+++ —	± +	± —	± ++	+ —
Polystictus abietinus	+++ +	+++ —	++++ ++	++++ +	— —	— —	+++ +	+ —
Lenzites betulina	++++ +	++++ —	++++ ++	++++ —	+ —	+ —	+++ —	++ —
Polystictus versicolor	++++ —	++++ —	+++++ +	++++ —	+ —	+ —	+++ —	+++ —
Control	Slight contamination —	— —	Slight contamination —	— —	— —	— —	— —	Slight contamination —

B. CHAMAENERION-GELATIN COMPLEX

	Undried Chamaenerion-gelatin complex + Fraxinus litter extract	Dried Chamaenerion-gelatin complex + Fraxinus litter extract	Undried Chamaenerion-gelatin complex + Fraxinus litter extract + cellulose	Dried Chamaenerion-gelatin complex + Fraxinus litter extract + cellulose	Undried Chamaenerion-gelatin complex + Calluna litter extract	Dried Chamaenerion-gelatin complex + Calluna litter extract	Undried Chamaenerion-gelatin complex + Calluna litter extract + cellulose	Dried Chamaenerion-gelatin complex + Calluna litter extract + cellulose
Collybia butyracea	++ +	+++ ++	+ +	+++ ++	± —	+ +	± +	± +
Polystictus abietinus	+++ +	+++ —	++++ ++	++++ ++	— —	± —	+++ +	++ —
Lenzites betulina	++++ +	++++ —	++++ ++	++++ +	± —	± —	++++ —	+ —
Polystictus versicolor	+++ +	+++++ —	+++++ +	+++++ +	++ —	± —	++++ —	+ —
Control	Slight contamination —	Slight contamination —	Contaminated —	Contaminated —	— —	Slight contamination —	— —	Slight contamination —

C. CALLUNA-GELATIN COMPLEX

	Undried Calluna-gelatin complex + Fraxinus litter extract		Dried Calluna-gelatin complex + Fraxinus litter extract		Undried Calluna-gelatin complex + Fraxinus litter extract + cellulose		Dried Calluna-gelatin complex + Fraxinus litter extract + cellulose		Undried Calluna-gelatin complex + Calluna litter extract		Dried Calluna-gelatin complex + Calluna litter extract		Undried Calluna-gelatin complex + Calluna litter extract + cellulose		Dried Calluna-gelatin complex + Calluna litter extract + cellulose	
Collybia butyracea	++	+	++	+	++++	++	+++	++	+	++	+	+	++	++	+++	+
Polystictus abietinus	++++	+	++++	—	++++	++	++++	+	+	—	+	—	+++	++	+++	—
Lenzites betulina	++++	—	++++	—	+++++	++	+++++	+	+	—	++	—	+++	—	+++	—
Polystictus versicolor	++++	—	++++	—	+++++	—	+++++	—	+	—	++	—	+++	—	+++	—
Control	Slight contamination	—	Slight contamination	—	Slight contamination	—	Slight contamination	—	Slight contamination	—	—	—	—	—	—	—

D. QUERCUS-SAMBUCUS COMPLEX

	Undried Quercus-Sambucus complex + Fraxinus litter extract		Dried Quercus-Sambucus complex + Fraxinus litter extract		Undried Quercus-Sambucus complex + Fraxinus litter extract + cellulose		Dried Quercus-Sambucus complex + Fraxinus litter extract + cellulose		Undried Quercus-Sambucus complex + Calluna litter extract		Dried Quercus-Sambucus complex + Calluna litter extract		Undried Quercus-Sambucus complex + Calluna litter extract + cellulose		Dried Quercus-Sambucus complex + Calluna litter extract + cellulose	
Collybia butyracea	++	++	+	—	++++	++	++	—	±	++	—	—	+	++	+	—
Polystictus abietinus	++++	++	++++	—	++++	++	+++	+	±	—	±	—	+	++	+	—
Lenzites betulina	+++++	++	+++++	—	++++	++	++++	—	±	—	±	—	++++	++	+++	—
Polystictus versicolor	+++++	++	+++++	—	++++	++	+++++	—	+	—	±	—	++++	—	++	—
Control	Slight contamination	—	Slight contamination	—	—	—	—	—	—	—	—	—	—	—	—	—

E. CHAMAENERION-SAMBUCUS COMPLEX

	Undried Chamaenerion-Sambucus complex+Fraxinus litter extract		Dried Chamaenerion-Sambucus complex+Fraxinus litter extract		Undried Chamaenerion-Sambucus complex+Fraxinus litter extract + cellulose		Dried Chamaenerion-Sambucus complex+Fraxinus litter extract + cellulose		Undried Chamaenerion-Sambucus complex+Calluna litter extract		Dried Chamaenerion-Sambucus complex+Calluna litter extract		Undried Chamaenerion-Sambucus complex+Calluna litter extract + cellulose		Dried Chamaenerion-Sambucus complex+Calluna litter extract + cellulose	
Collybia butyracea	+	++	+	—	++	++	++	—	±	++	±	—	±	++	±	—
Polystictus abietinus	++++	++	++++	—	++++	++	+++	++	—	—	±	—	±	+	±	—
Lenzites betulina	++++	++	++++	—	+++++	++	++++	+	±	+	±	—	+++	++	±	—
Polystictus versicolor	++++	++	++++	—	+++++	++	+++++	+	±	—	±	—	+	—	±	—
Control	—	—	Accidentally destroyed		Slight contamination	—	Slight contamination	—	—	—	Accidentally destroyed		Slight contamination	—	—	—

F. CALLUNA-SAMBUCUS COMPLEX

	Undried Calluna-Sambucus complex + Fraxinus litter extract		Dried Calluna-Sambucus complex + Fraxinus litter extract		Undried Calluna-Sambucus complex + Fraxinus litter extract + cellulose		Dried Calluna-Sambucus complex + Fraxinus litter extract + cellulose		Undried Calluna-Sambucus complex + Calluna litter extract		Dried Calluna-Sambucus complex + Calluna litter extract		Undried Calluna-Sambucus complex + Calluna litter extract + cellulose		Dried Calluna-Sambucus complex + Calluna litter extract + cellulose	
Collybia butyracea	+++	++	+++	—	+++	++	+++	—	+	—	±	—	+	++	±	—
Polystictus abietinus	++++	++	++++	—	++++	++	++++	+	+	—	+	—	+	++	+	—
Lenzites betulina	++++	+	++++	—	++++	++	+++++	—	+	—	+	—	+++	+	+++	—
Polystictus versicolor	++++	+	++++	—	+++++	++	+++++	—	+	—	+	—	+++	—	+++	—
Control	Slight contamination	—	Slight contamination	—	Slight contamination	—	Slight contamination	—	Slight contamination	—	—	—	Slight contamination	—	—	—

APPENDIX IV

THE DECOMPOSITION OF COMPLEXES, FORMED BY INTERACTION BETWEEN ARGININE AND AQUEOUS EXTRACTS OF THE LEAVES OF VARIOUS SPECIES BY STRAINS OF VARIOUS WOOD-DECOMPOSING AND LITTER-DECOMPOSING FUNGI

Amount of fungal growth is indicated by + signs on the left; — =no growth, +++++ =extremely good growth. + signs on the right indicate degree of disappearance of the complex; — =no disappearance, + =considerable though incomplete disappearance, ++ =complete disappearance. Incubation period 4/12/50—11/9/51. (See page 88.)

A. ARGININE-PINUS SYLVESTRIS COMPLEX

	Arginine-Pinus sylvestris complex + distilled water + cellulose		Arginine-Pinus sylvestris complex + distilled water		Arginine-Pinus sylvestris complex + Fraxinus litter extract + cellulose		Arginine-Pinus sylvestris complex + Fraxinus litter extract		Arginine-Pinus sylvestris complex + Calluna litter extract + cellulose		Arginine-Pinus sylvestris complex + Calluna litter extract	
Marasmius dryophilus	±	++	±	+	++++	+	+++	—	±	+	±	+
Polystictus versicolor	++	+	++	—	++++	++	++++	—	+	+	+++	—
Lenzites betulina	+++	+	++	—	++++	++	++++	++	++++	+	+++	—
Control	—	—	—	—	—	—	—	—	—	—	—	—

B. ARGININE-CALLUNA VULGARIS COMPLEX

	Arginine-Calluna complex + distilled water + cellulose		Arginine-Calluna complex + distilled water		Arginine-Calluna complex + Fraxinus litter extract + cellulose		Arginine-Calluna complex + Fraxinus litter extract		Arginine-Calluna complex + Calluna litter extract + cellulose		Arginine-Calluna complex + Calluna litter extract	
Marasmius dryophilus	±	+	±	—	++++	+	+	+	±	++	±	++
Polystictus versicolor	±	+	±	—	++++	++	++++	+	++++	+	+	+
Lenzites betulina	±	—	±	—	++++	+	++++	+	++++	+	+	—
Control	—	—	—	—	—	—	—	—	—	—	—	—

C. ARGININE-VACCINIUM MYRTILLUS COMPLEX

	Arginine-Vaccinium myrtillus complex + distilled water + cellulose		Arginine-Vaccinium myrtillus complex + distilled water		Arginine-Vaccinium myrtillus complex + Fraxinus litter extract + cellulose		Arginine-Vaccinium myrtillus complex + Fraxinus litter extract		Arginine-Vaccinium myrtillus complex + Calluna litter extract + cellulose		Arginine-Vaccinium myrtillus complex + Calluna litter extract	
Marasmius dryophilus	±	+	±	++	+++	+	+++	+	+	+	±	+
Polystictus versicolor	++++	+	+	—	++++	+	+++	+	++++	+	++	+
Lenzites betulina	+	+	±	—	+++++	++	++++	++	++++	+	+	+
Control	—	—	—	—	—	—	—	—	—	—	—	—

D. ARGININE-ULMUS PROCERA COMPLEX

			Arginine-Ulmus procera complex + Fraxinus litter extract + cellulose		Arginine-Ulmus procera complex + Fraxinus litter extract		Arginine-Ulmus procera complex + Calluna litter extract + cellulose		Arginine-Ulmus procera complex + Calluna litter extract	
Marasmius dryophilus			+++	++	++++	+	+	++	+	++
Lenzites betulina			++++	++	++++	++	+++	++	+	++
Control			—	—	—	—	—	—	—	—

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