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Botaniska Notiser

En kort historik

AV TYCHO NORLINDH

Botaniska Notiser, som grundades 1839, är den äldsta botaniska tidskriften i Norden och en bland de äldsta i världen. Den har utgivits omväxlande i Lund, Stockholm och Uppsala.

I regel ha samtliga häften tillhörande en årgång av Botaniska Notiser utkommit under det år, som angives på titelbladet. Vid citering av arbeten publicerade i denna tidskrift har det sålunda räckt med att angiva årgången, t.ex. Fries, E.: Öfver Parthenogenesi hos växterna. — Bot. Notiser 1857, pp. 105, 125. Uppsala 1857. Undantag utgöra dock årgångarna 1855, 1856, 1858 och 1867, där det sista häftet utkommit i början av påföljande år.

Från åtskilliga botanister har det under senare år framställts en önskan om att redaktionen skulle införa volymbeteckning på årgångarna av Botaniska Notiser. Redaktionen har nu ansett sig böra tillmötesgå detta önskemål.

När tidskriften firade sitt 100-årsjubileum 1939 med att utgiva ett generalregister för perioden 1839—1938 hade 91 årgångar utgivits. Sedan dess har Botaniska Notiser utkommit regelbundet varje år. Botaniska Notiser för år 1955 skall sålunda bära volymbeteckningen 108.

I detta sammanhang har jag ansett det vara lämpligt att lämna en översikt över de hitintills utgivna årgångarna av Botaniska Notiser, deras utgivare, redaktörer och tryckorter.

Under åren *1839—1846* utgavs Botaniska Notiser av Al. Ed. Lindblom. Dessa 8 årgångar (motsvarande vol. 1—8) trycktes i Lund.

Sedan blev det ett avbrott i tidskriftens utgivande under åren 1847—1848.

Årgångarna *1849—1851* (motsv. vol. 9—11) utgavs av N. J. Andersson under namnet »Nya Botaniska Notiser» med Stockholm som tryckort.

Årgångarna *1852—1856* (motsv. vol. 12—16) utgavs av K. F. The-

denius. Även dessa trycktes i Stockholm under namnet »Nya Botaniska Notiser».

De båda följande årgångarna *1857—1858* (motsv. vol. 17—18) utgavs av Th. M. Fries med Uppsala som tryckort. Han återupptog det ursprungliga namnet Botaniska Notiser.

Sedan blev det ett avbrott i utgivandet av tidskriften under åren 1859—1862.

N. J. Andersson återinträdde som redaktör *1863*, men utgav endast en årgång (motsv. vol. 19), vilken trycktes i Stockholm.

Följande år utkom ej tidskriften.

Th. M. Fries återupptog redaktörskapet under åren *1865—1868*. Dessa 4 årgångar (motsv. vol. 20—23) trycktes i Uppsala.

Under de båda följande åren utkom ingen årgång av tidskriften.

År 1871 utgavs Botaniska Notiser ånyo i Lund och har sedan dess årligen utkommit där.

Årgångarna *1871—1921* (motsv. vol. 24—74) ha utgivits av C. F. O. Nordstedt. Under inte mindre än 51 år utövade sålunda prof. Nordstedt sin gärning som redaktör för Botaniska Notiser. Fram till 1903, då »Arkiv för botanik» grundades av Kungl. Svenska Vetenskaps-Akademien, var Botaniska Notiser den enda, rent botaniska tidskriften i Sverige. Vid Nordstedts avgång som redaktör 1921 övertogs tidskriften av Lunds Botaniska Förening. Hitintills hade Botaniska Notiser varit en av redaktörerna ägd tidskrift.

Under perioden *1922—1928* var Harald Kylin redaktör för Botaniska Notiser. De 7 årgångar, som då utkom, motsvara volymerna 75—81.

Under perioden *1929—1937* var N. Sylvén redaktör för tidskriften. Hans 9 årgångar motsvara sålunda vol. 82—90.

Med årgång 1938, då Henning Weimarck inträdde som redaktör, avslutades den första 100-årsserien av Botaniska Notiser och tillika tidskriften i dess mindre format med en satsyta av omkring 10×16 cm. För att tillmötesgå författarnas önskemål om större sidyta för bilder och tabeller beslöt styrelsen, att Botaniska Notiser fr.o.m. 1939 skulle utgivas i ett större format med en satsyta av omkring 12×18 cm.

Under jubileumsåret 1939 utgavs ett utförligt generalregister till Botaniska Notiser för 100-årsperioden 1839—1938. Detta register, som omfattar X+1108 sidor, utkom den 1 maj just på hundraårsdagen av tidskriftens grundande. 1939 var även ur andra synpunkter ett märkesår för Botaniska Notiser, ty då utkom två festskrifter. Häfte 1 tillägnades professor Harald Kylin på hans 60-årsdag och häfte 4 professor Svante

Murbeck på hans 80-årsdag. Denna årgång, som omfattar 856 sidor, är den största hitintills i serien av Botaniska Notiser.

Årgångarna *1938—1949* (motsv. vol. 91—102) redigerades av Henning Weimarck. Denna period kännetecknas av en stark uppgång i föreningens och därmed prenumeranternas medlemsantal.

Under perioden *1950—1953* var Hakon Hjelmqvist redaktör. De 4 årgångar som då utkom, motsvara vol. 103—106. Sistnämnda år uppgick föreningens medlemsantal till 672.

Fr.o.m. 1954 redigeras Botaniska Notiser av Tycho Norlindh. Årgång 1954 motsvarar vol. 107.

Botaniska Notiser, som under 1800-talet huvudsakligen behandlade nordisk botanik, har fått en alltmera internationell prägel, särskilt under de sista decennierna. Tidskriften står öppen för alla grenar inom botaniken och bidrag mottagas från botanister från alla länder. En förutsättning är dock att bidragsgivaren, eller den institution till vilken han är knuten, är medlem av Lunds Botaniska Förening.

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Botaniska Notiser

A historical Survey

By TYCHO NORLINDH

Botaniska Notiser, founded in 1839, is the oldest botanical journal in Scandinavia and one of the oldest in the world. It has been edited alternately in Lund, Stockholm, and Uppsala.

As a rule all the fascicles belonging to a volume of Botaniska Notiser have appeared in the year given on the title-page. When quoting treatises published in the periodical, one has accordingly only had to state the year, e.g. Fries, E.: Synopsis Caricum distigmaticarum, spicis sexu distinctis in Scandinavia lectarum. — Bot. Notiser 1843, pp. 97—109. Lund 1843. However, the volumes of 1855, 1856, 1858 and 1867 form exceptions, the last fascicle of each having appeared in the beginning of the following year.

Of late years several botanists have expressed the desire that the volumes should be numbered. The editorial board has now decided to comply with this wish.

When the journal celebrated its centenary in 1939, by publishing a General Index for the period 1839—1938, 91 volumes had appeared. Since then Botaniska Notiser has appeared regularly every year. Thus the volume of 1955 will be numbered 108.

In this connection it is appropriate to give the following survey of the volumes published up to now, their publishers, editors, and their place of printing.

1839—1846 (Vol. 1—8), edited by Al. Ed. Lindblom, printed in Lund. (1847—1848 the journal did not appear.)

1849—1851 (Vol. 9—11), edited by N. J. Andersson under the name "Nya Botaniska Notiser", printed in Stockholm.

1852—1856 (Vol. 12—16), edited by K. F. Thedenius under the name "Nya Botaniska Notiser", printed in Stockholm.

1857—1858 (Vol. 17—18), edited by Th. M. Fries, printed in Uppsala.

(1859—1869 the journal did not appear.)

1863 (Vol. 19), edited by N. J. Andersson, printed in Stockholm.

(1864 the journal did not appear.)

1865—1868 (Vol. 20—23), edited by Th. M. Fries, printed in Uppsala.

(1869—1870 the journal did not appear.)

1871—1921 (Vol. 24—74), edited by C. F. O. Nordstedt, printed in Lund.

Thus Professor Nordstedt served as editor of Botaniska Notiser for no less than fiftyone years. Up to 1903, when "Arkiv för botanik" was founded by K. Svenska Vetenskaps-Akademien, Botaniska Notiser had been the only, exclusively botanical journal in Sweden. When Professor Nordstedt retired, the publication of the journal was taken over by the Botanical Society of Lund, which is the oldest botanical society in Sweden, founded in 1858. Before 1922 Botaniska Notiser was owned by the editors.

1922—1928 (Vol. 75—81), editor Harald Kylin, printed in Lund.

1929—1937 (Vol. 82—90), editor N. Sylvén, printed in Lund.

1938—1949 (Vol. 91—102), editor H. Weimarck, printed in Lund.

The volume of 1938 terminates the first 100-year period of Botaniska Notiser. From 1939 the page-size of the journal was changed from about 10×16 cm to about 12×18 cm.

In the jubilee year 1939 a detailed General Index to Botaniska Notiser for the first hundred years 1839—1938 was published. This index, comprising X+1108 pages, appeared on May 1st, the very day on which the journal was founded. The year 1939 was a landmark in the existence of Botaniska Notiser in other respects, too, for in that year two dedicated publications appeared. Fascicle 1 was dedicated to Professor Harald Kylin on his 60th birthday, and fascicle 4 to Professor Svante Murbeck on his 80th birthday. This volume, comprising 856 pages, is up to now the biggest in the series of Botaniska Notiser.

1950—1953 (Vol. 103—106), edited by H. Hjelmqvist, printed in Lund.

1954—1955 (Vol. 107—108), edited by Tycho Norlindh, printed in Lund.

Botaniska Notiser, which in the 19th century treated mainly Scandinavian botany, has acquired an increasing international character, especially during the last decades. The journal is open to all branches of botany and contributions are welcome from botanists in all countries. The only condition is that the contributor or the institute with which he is associated is a member of the Botanical Society of Lund.

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Endosperm Formation in Myrica Gale L.

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Most angiosperms of the taxon *Amentiferae* have so called endosperm-less seeds, endosperm had certainly been formed after fertilization but had disappeared during seed development. Such seeds also occur in *Casuarinales* which some authors have included in *Amentiferae* but more often is considered a separate order showing relationship with this taxon. *Amentiferae*-orders lacking endosperm are *Betulales, Myricales, Juglandales, Fagales, Salicales* (compare Hjelmquist 1948, concerning the range of *Amentiferae*; he unites *Myricales* and *Juglandales* into one order, *Juglandales*). The orders *Leitneriales* and *Balanopsidales* have, however, seeds with endosperm; in *Leitneria floridana* which also has perisperm the endosperm is large (Pfeiffer 1912); in the order *Balanopsidales* the endosperm is small (compare Hutchinson 1926). The embryology of *Garrya* which has a large endosperm, but often is considered related to *Amentiferae,* shows, however, a more advanced position of the order *Garryales* near *Umbelliflorae* (Hallock 1930). The embryology of *Balanopsidales* has as it seems not been investigated, while the embryological investigation of *Leitneria floridana* has given inconclusive evidence of the relations of *Leitneriales* (Pfeiffer l.c.).

Embryological investigation of endosperm formation has been somewhat neglected in most *Amentiferae*, but surely the degree of development and mode of formation of the evanescent endosperm has some morphological and probably phylogenetical interest. A development after the so called Nuclear type is, however, established in all families. Our knowledge of *Myricales* is very fragmentary. I had good opportunity to collect material to study endosperm formation in the bog myrtle, *Myrica gale,* in Holmsjö, northern Blekinge. The fixations were

made last summer, from 18/6 to 2/8. The chromosome number of the investigated population I have determined many years ago, it is n=24 (unpublished), the same number one finds in chromosome lists. Other known chromosome numbers of the family *Myricaceae* are n=8 in *Myrica rubra, M. carolinensis, M. cerifera* and *M. pumila,* n=16 in *Comptonia peregrina.*

Myricaceae has been divided into three genera *Myrica, Comptonia* and *Gale,* the bog myrtle being called *Gale palustris.* Floral organization is in *Gale* at most derived, while *Comptonia* is separating from *Myrica* in an other direction than *Gale* (Hjelmquist l.c. p. 29). The chromosome numbers seem to support such views.

Earlier investigations have shown (Treub 1891, mostly on *M. lobbii,* Kerschaw 1909) that the nucellus has only one embryosac mother cell, that the embryosac develops after the Normal type, that the pollen tube uses the micropyle (absence of chalazogamy), that endosperm formation is nuclear. I had not intended to study development of the embryosac, some observations made confirm earlier results (Fig. 1—3).

It is well known that the gynoeceum of the *Myricaceae* is interesting. The ovary is unilocular and has one basal, orthotropous ovule. Hagerup (1934) has stressed that there is an open passage in the top of the ovary, the carpels not being completely closed until after the pollen tube has passed (similarity to *Reseda*). He considers that the ovule is apical, formed of the stemtip, and that the integument is a leaf. The more common view is, however, that the ovule belongs to the anterior of two median carpels. A fine figure of the ovule has been published by Kerschaw (1909 a). The embryosac occupies only the upper part of a rather long nucellus, between the embryosac and the chalaza is a string of narrow, elongated cells in the centre of the basal part of the nucellus, probably here nutrients pass to the embryosac. Kerschaw also describes vascular branches in the chalaza and their passage into the integument.

The material collected 18/6 showed no fertilized embryosac, 22/6 the pollen tube could be observed above the nucellus, 24/6 many ovules contained endosperm nuclei. The shedding of pollen in the bog myrtle usually occurs at the end of April and clearly fertilization is much delayed. This year spring was late (compare p. 12), but there must pass four or five weeks between pollination and fertilization. Slow growth of the pollen tube has been observed in many true *Amentiferae.* Delayed fertilization seems already known in *Myrica rubra* (Yen 1950).

It is sometimes considered a primitive character; its occurrence in *Orchidaceae* has, however, been considered an argument against primitiveness. Be this as it may, its general occurrence in *Amentiferae,* as well as in *Coniferales,* is a fact. In view of the often enormous amount of pollen, produced in these cases, it is perhaps of importance that the tree finds time to produce nutrients to a high seed production.

In the collection made 18/6 the micropyle had still not been formed in many ovules. The one integument is rather narrow, 3—5 cell layers. Thus it has not been formed through fusion of two separate integuments. Here the unitegmous ovule must be a primitive character, or else result from the abortion of one integument of a bitegmous ovule. In certain cases the growth of the integument was arrested, there was no micropyle, the top of the nucellus remained naked. This absence of a micropylar canal does not, as it seems, prevent fertilization of the embryosac, since endosperm nuclei have been observed in an ovule lacking micropyle (Fig. 5); the upper part of the ovary being narrow, the pollen tube cannot fail to attain the top of the nucellus, which on all sides is close to the ovary wall.

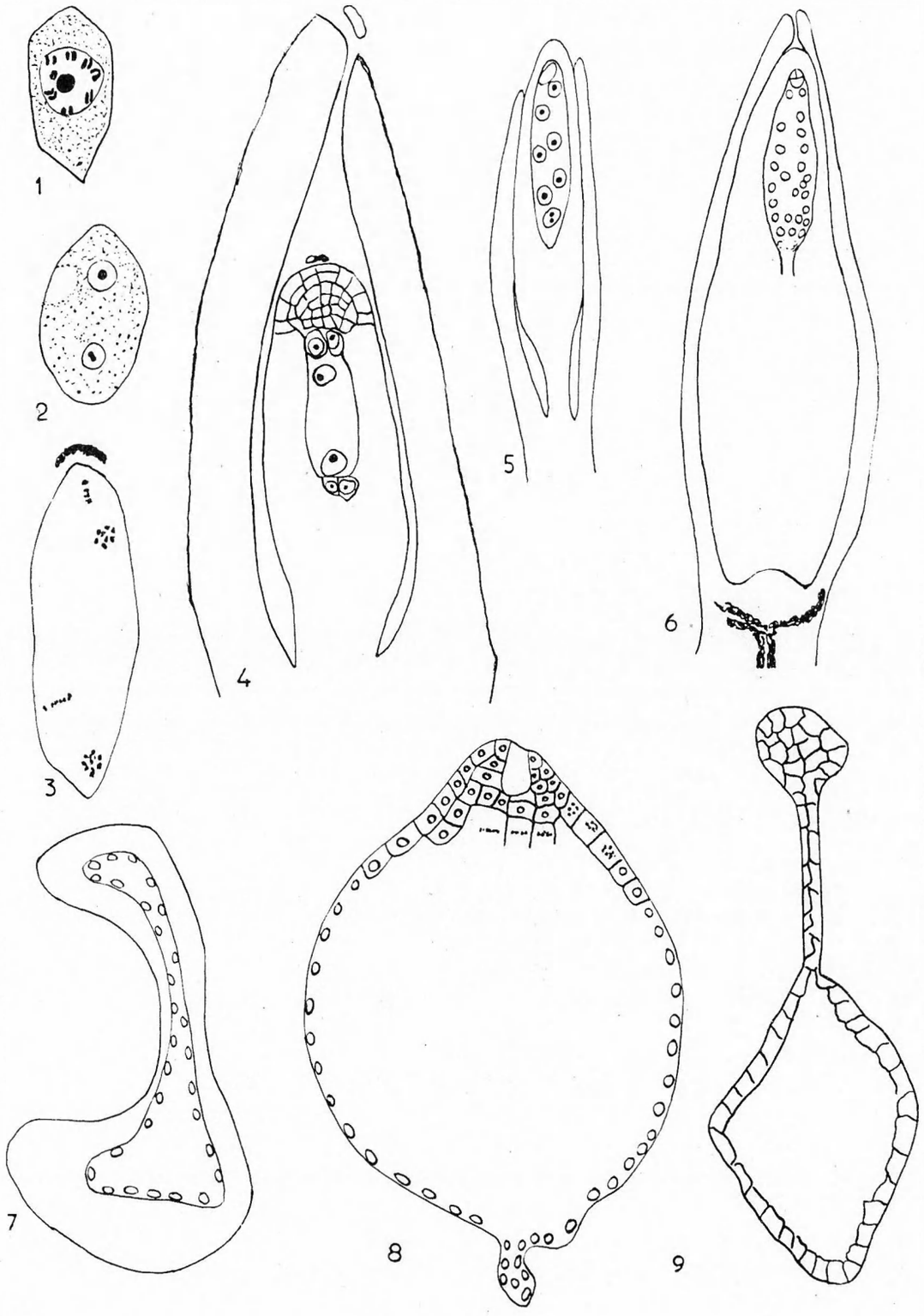
The organized embryosac is separated from nucellusepidermis through four to six parietal layers. Sometimes also some epidermis cells have been divided through a periclinal wall (Fig. 4). In the earliest fixation the embryosac was sometimes at the two- or four-nucleate stage. The organized embryosac had at first one polar nucleus at each end, later a central nucleus. The antipodal cells were often indistinct, presumably they are rather ephemeral. In one instance the nucellus contained two embryosacs lying side by side; this ovule had perhaps had two embryosac mother cells.

The occurrence of as many as 32 endosperm nuclei already on 24/6 shows that the development of the endosperm proceeds rapidly. As a rule the ovules contained an undivided zygote, but in rare cases embryo formation had been initiated (compare Fig. 6). Thus the division of the fertilized egg cell is delayed, though not much. The ovule and embryosac increase in size after fertilization, at first rather slowly, then more rapidly. After one week 2/7 the aspect of the endosperm is changed, because it has grown down into the basal part of nucellus (Fig. 7), the embryo may now comprise 60 cells at the highest.

The assemblage of endosperm nuclei at the antipodal end of the embryosac seen in Fig. 6 is probably a prelude to the growth of the endosperm into the nucellus base. The basal part of the ovule widens considerably (Fig. 10); at the same time a large hole is formed in the

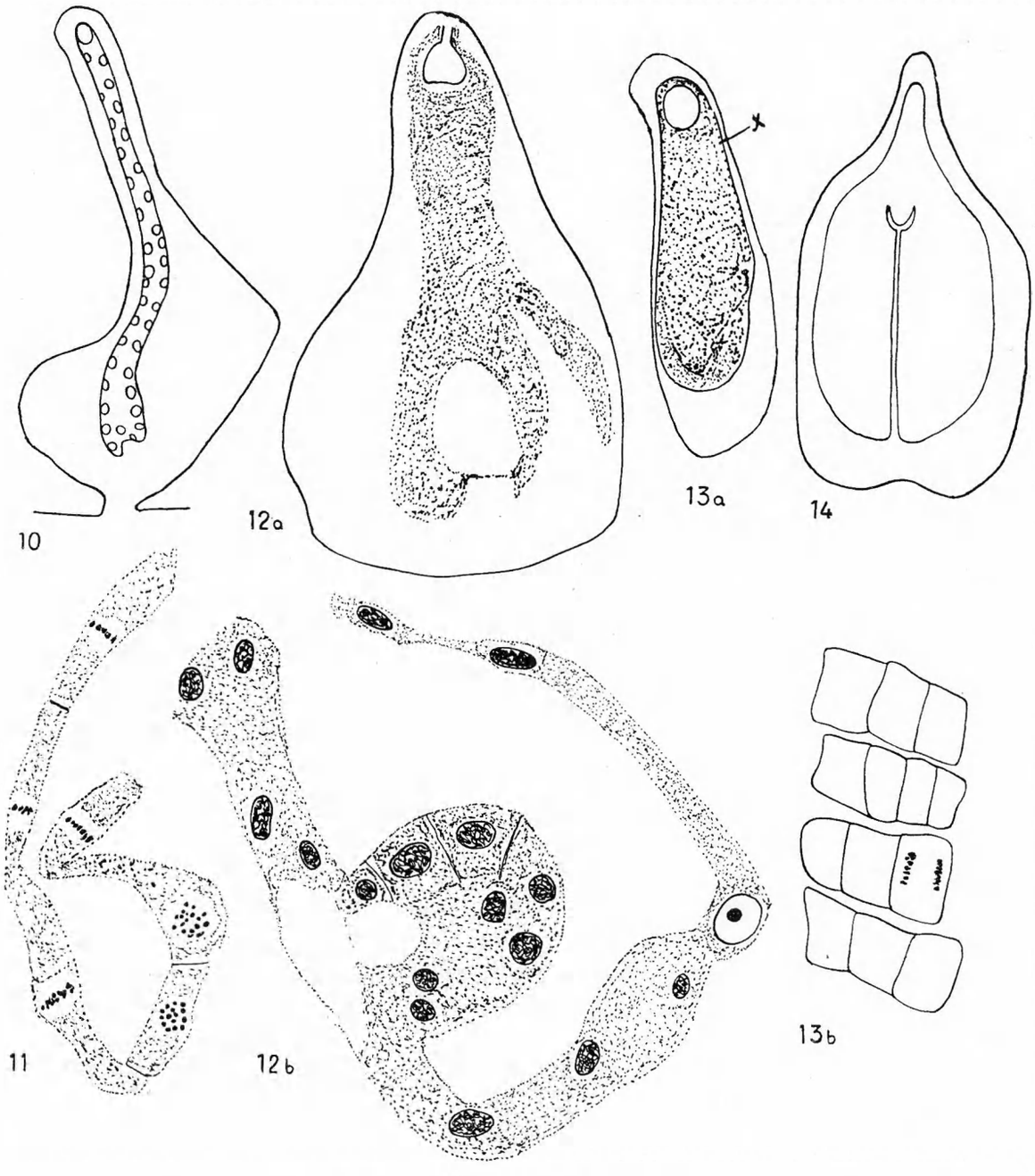
nucellus centre along the strip of elongated cells. This hole thus is formed passively, not through the activity of the endosperm. It is also seen in ovules with aborted young endosperm. Later nucellus tissue is destroyed by the growing endosperm. The antipodals degenerate early and form no barrier to endosperm growth.

The volume of the young endosperm is very variable. There is a central vacuole which may be large, as seen in Fig. 8. Cell formation has occurred in the upper part of the endosperm and proceeds towards the chalazal end. This is common in angiosperms, as also the fact that walls are formed when there is one layer of free nuclei. The direction of the mitotic spindles shows that the growth of the tissue is proceeding towards the centre of the vacuole, while the one-layered endosperm still grows only through anticlines. Cell formation was sometimes initiated on the 2/7 and seeds with a small central vacuole soon have a compact tissue. The period of seed development with predominant endosperm formation and slow growth of the embryo seemed to be approaching its end 11/7. At this date the formation of the cotyledones may be initiated (Fig. 12 a). The more narrow upper part of the endosperm was cellular but in the broad basal part the central vacuole may not be filled (Fig. 9). Fig. 11 shows simultaneous mitosis in the basal endosperm, the largest part of this seed had a compact endosperm tissue. Fig. 12 a shows a larger ovule in which the remainder of the vacuole is small. Fig. 12 b shows endosperm from the basal part of this ovule. Cell formation is very restricted here and most nuclei are shrunk and seem incapable of further division. The endosperm has formed a protrusion similar to an embryo. Such usually spherical endosperm formations have been observed in several angiosperms (compare Maheshwary, 1950, p. 222). They occurred in *Salix cinerea* (Håkansson, 1954). Fig. 13 a shows an ovule with compact endosperm tissue, the central vacuole has been completely filled here. This endosperm was still in active development as mitotic figures showed. Fig. 13 b shows periclinal divisions in the "epidermis" layer of the endosperm. In this way the endosperm tissue grows thicker and expands in centrifugal directions. During endosperm development the nucellus tissue is destroyed, the cap above the embryo is more resistant. The divisions often cause the formation of rows of endosperm cells, but in the interior of endosperm the divisions have more variable directions, the tissue formed is more irregular. The interior is filled in different ways, through centripetal growth from peripheral endosperm tissue and through wall formation between scattered endosperm nuclei.



Figs. 1—9

1: The e.m.c. of *Myrica gale*. ×900. — 2: 2-nucleate e.s. ×900. — 3: The last division during e.s. development. ×900. — 4: Nucellus with organized e.s. and integument. Remains of the pollen tube are seen. ×200. — 5: Ovule with arrested integument growth; the e.s. contains free endosperm nuclei. ×65. — 6: Ovule with e.s. containing an increased number of endosperm nuclei and a few-celled embryo. At



Figs. 10—14

10: Ovule with nuclear endosperm. × 50. — 11: chalazal part of the endosperm from an ovule largely filled of endosperm tissue. × 700. — 12 a: ovule with cellular endosperm (the latter dotted). ×50. — 12 b: chalazal endosperm from this ovule. ×700. — 13 a: ovule with solid endosperm tissue (dotted). ×50. — 13 b: the endosperm cells marked in 13 a. ×700. 14: The embryo fills the seed; no endosperm is left.

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the base of the ovule are vascular bundles. × 65. — 7: The endosperm has grown into the basal part of the ovule. ×65. — 8: Endosperm with a very large central vacuole. The micropylar part of the endosperm is now cellular. ×125. — 9: Cellular endosperm, the vacuole is here in the basal part of the endosperm. ×125.

In many ovules the endosperm formed is rather small. Clearly its development is checked in many seeds, in other it may be very slow. The embryo in such ovules is small and shows no divisions. Probably the embryo aborts in seeds with imperfect endosperm. Sometimes no embryo was observed. In many cases an endosperm approaching normal size had been formed but the tissue had a rather sickly appearance indicating its degeneration before the embryo had grown down into the endosperm.

The material collected 2/8 showed instances of the last period of seed development with rapid growth of the embryo and disappearance of the endosperm. In the largest fruits an embryo with thick cotyledones filled the seed (Fig. 14). There was storage substances in the cotyledons and outer cell layers of the upper part of the hypocotyl (not in epidermis).

In the seeds of *Myrica gale* an often rather large endosperm thus is formed which is completely cellular and has no central vacuole. It shows, however, no special differentiations in the form of haustoria or regions with dense cytoplasm or enlarged nuclei. There is never storage in the endosperm which is ephemeral. Its functions are probably production of growth substances inducing the enlargement of the ovule, and, later, nutrition of the embryo.

Through the courtesy of Dr. Hakon Hjelmqvist I have been able to investigate a number of his slides showing seed development in bog myrtle in 1953, a year with rather early development of the vegetation. As expected, development that year was more advanced, surely the result of a more early pollination. The collection made 20/6 showed that cytoplasm had grown into the basal part of nucellus and had a different number of free endosperm nuclei; embryos with 16 or 20 cells were found in the most advanced ovules. In the material collected 30/6 the endosperm was more or less cellular but had apparently not attained its full size. The embryo was undifferentiated, showing no evidence of cotyledons.

Conclusions. — The endosperm of *Myrica gale* is perhaps typical of *Amentiferae*: a continuous, rather weak tissue, rapidly replaced by the embryo. As already indicated most families have evanescent endosperm, namely *Myricaceae, Juglandaceae, Fagaceae, Betulaceae, Corylaceae* and *Salicaceae*. In the isolated family *Salicaceae,* particularly in *Salix,* the endosperm is most reduced: cell formation is very restricted and the central vacuole is surrounded of free nuclei (Håkansson, 1954).

Here endosperm formation begins only slightly before the division of the eggcell and the embryo soon contains more nuclei than the endosperm, in these respects the other families are different.

On the embryology of *Juglandaceae* a number of papers has been published. This family seems related to *Myricaceae,* Hjelmqvist regards it "as merely a further development of the *Myricaceae* type". The seeds of this family are large. Here the endosperm becomes cellular, but the large central vacuole is never filled, being lined by several layers of endosperm cells. Later the embryo rapidly fills the vacuole. This is clear after the investigations of Langdon (1934) on *Carya glabra* and *Juglans mandschurica,* of Nast (1941) on *Juglans regia,* and of McKay (1947) on *Carya illinoensis.* Endosperm development is very slow. McKay states that it begins two weeks after fertilization and then continues during two months; the stage of free nuclei lasts long, cell formation beginning at the chalazal end. On the last point doubts may be expressed, in *Juglans regia* it begins at the micropylar end, a more common mode in angiosperms. Thus the endosperm of *Juglandaceae* is large and cellular when the rapid embryo growth begins but the persistance of a large central vacuole may indicate a derived condition in comparision with *Myricaceae.* The division of the fertilized eggcell seems more delayed in *Carya illinoensis* to nearly 40 days after fertilization.

There are three families in which the degree of endosperm formation before rapid embryo growth sets in is less investigated. In *Betulaceae* a large tissue is formed in *Alnus rugosa,* judging from the figures in a paper on polyembryony and parthenogenesis in this species (Woodworth 1930). Concerning *Fagaceae* we have the statement of Conrad (1900) on early and copious development of endosperm in *Quercus.* Here cellformation is very late. Further investigations are desirable.

The endosperm of *Casuarina* is now known (Swamy 1948). It forms a tissue rather similar to *Myrica* endosperm, but in *Casuarina* the endosperm tissue in the micropylar region of the seed shows secondary growth not observed in *Myrica.*

Certain embryological characters of *Amentiferae* other than endosperm formation may be discussed briefly.

1) The nucellus of the ovule is large, several layers of parietal cells separate the embryosac from the epidermis. In *Salicaceae* the nucellus is relatively small, the mature embryosac occupies its whole length from chalaza to epidermis. In all other families the nucellus has a large basal part. The description of Hjelmqvist (1954) of the embryo-

logy of *Quercus robur* indicates, however, that the nucellus in *Fagaceae* shows reductional traits. In *Quercus robur* formation of parietal cells sometimes fails; later the upper part of nucellus is destroyed, a large part of the mature embryosac is limited by integument tissue. — Important ovular characters are variable in *Amentiferae* as for instance the number of integuments (*Fagaceae* and certain species of *Populus* have two integuments) and the archesporium (there is one embryosac mother cell in *Myricaceae, Juglandaceae, Salicaceae* while *Fagaceae, Corylaceae* and *Betulaceae*(?) have a multicellular archesporium). The form of the ovule also varies, antropous in most families, it is orthotropous in *Myricaceae* and *Juglandaceae*. Vascular bundles in the integument are described in several families, they are absent in *Salicaceae*.

2) Chalazogamy, detected in *Casuarina* 1891, has been observed in *Juglandaceae, Betulaceae, Corylaceae*. In *Salicaceae, Myricaceae* and *Fagaceae,* however, the pollen tube uses the micropyle. Chalazogamy was once considered a primitive trait but this seems doubtful.

3) Delayed fertilization is very common in *Amentiferae*. Fertilization occurs often three weeks or more after pollination. Such delay is common in conifers and is often considered a primitive character in angiosperms. Delayed fertilization does not occur in *Salicaceae* and more recent evidence makes its occurrence in *Juglandaceae* seem doubtful. Nast (1935) states that in *Juglans regia* fertilization occurs 2—5 days after pollination; McKay (1947) observed fertilization of polar nuclei 4 days after pollination in *Carya illinoensis*; in *Hicoria pecan* Woodroof and Woodroof (1927) found 4—7 weeks, but Shuhart (1932) only two weeks between pollination and fertilization. Delayed fertilization is often connected with early pollination, many wind pollinating deciduous trees shed their pollen before the leaves are fully expanded. Wind-pollination is, for instance in conifers, connected with enormous pollen production. Surely it is advantageous, that embryo formation is delayed here, two great strains on nutrient supply from the tree being separated through a period of production and accumulation of new substance. In all *Amentiferae* except in the families just mentioned delay is generally though it is more or less pronounced.

4) Caecum formation from the embryosac has been found in *Fagaceae* and *Corylaceae*. An outgrowth which grows in the direction of the nucellus base, is formed near the antipodals immediately before or at fertilization. The primary or first endosperm nuclti move into this caecum where the endosperm is largely formed. Treub (1891) described

caecum formation in *Casuarina,* but already from the one- or two-nucleate embryosac (see also Swamy 1948). In *Amentiferae* its function seems to be to bring the young endosperm into the large basal part of nucellus. Persistance of antipodals and resistance of their surrounding may prevent direct downward growth.

Two families, *Leitneriaceae* and *Balanopsidaceae,* have not been considered here, because they have endosperm in their mature seeds. The family *Salicaceae* is "non-amentiferous" in the four points discussed. Its small seeds, adapted to wind-dispersal, develop and germinate rapidly. The fruit is a capsule with several or many seeds. The gynoeceum of *Myricaceae* and *Juglandaceae* shows great similarity. The ovary is unilocular with only one, basal ovule. The latter is orthotropous, and has one integument. The nucellus is large but has only one embryosac mother cell, which is separated from nucellus epidermis through several parietal layers. Some embryological differences exist however, as pointed out above. *Juglandaceae* is chalazagamous, shows little or no delay of fertilization, the endosperm vacuole is never filled. Chalazogamy is here the most important difference.

Summary

Myrica gale forms a solid, though ephemeral, endosperm tissue. The embryosac is eight-nucleate, fertilization is delayed, occurring several weeks after pollination, endosperm development precedes the embryo formation during about four weeks.

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Some Interesting Lichens from the West of Scotland (Argyll)

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During the summer of 1954 I had for some weeks an opportunity of making lichenological excursions in the West of Scotland. All districts visited were in Argyll and my headquarters were Oban, Ballachulish and Salen on the Island of Mull. In the environs of these places I made several excursions and in this paper I intend to present the more rare or in other ways interesting species. Most of them belong to that group of oceanic lichens which Degelius treats in his work 1935. All the localities visited were earlier lichenologically unknown.

During this century Argyll has been the object for only a few lichenological investigations. W. Watson visited Argyll a short time in 1929 and collected on Ben Doran and by Loch Trulle in the north-eastern part of the county. In 1940 I. M. Lamb visited the north-west shore of Loch Awe and also made an excursion to Ben Cruachan. His paper 1942 has been of special interest to me as Loch Awe is situated only 11—12 miles east of Oban. In 1949 G. Degelius visited especially the south-west of Scotland and collected also in Argyll.

Dr. Degelius has been kind enough to give me some localities from his journey in 1949 concerned with the species mentioned, which I also include in this paper.

All my material is preserved in the Herbarium of the Institute of Systematic Botany in Uppsala (Dr. Degelius' collection in Herb. Degelius, Institute of Plant Ecology, Uppsala).

1. *Lecidea (Biatora) gothoburgensis* H. Magn.

Glencoe, Chailleach, about 560 m s.m., on overhanging rocky walls. Sterile (n. 83).

Described by Magnusson in 1925 and found in several localities especially in the West of Sweden (Bohuslän, Västergötland and Hal-

land). My specimen belongs to f. *maculosa* H. Magn. and this form seems, like the type, to have a wide distribution though overlooked on account of its sterility. (Magnusson 1929 p. 120, 1935 p. 4). — *New for the British Isles.*

2. ***Leptogium burgessii*** (L.) Mont.

Island of Mull, Killichronan, Kellan Wood, on a big mossy *Quercus*. Fertile (n. 121)).

Coll. G. Degelius: Loch Awe, Cladich, on *Corylus* and *Fraxinus* on a slope with decid. wood by the road, about 50 m s.m. — Glen Aray, above Inverary Castle, on *Quercus* by the road, about 40 m s.m. — Loch Fyne, between Strachur and St. Catherines, on *Fraxinus* by the road. — Loch Fyne, Strachur, on *Fraxinus* in the park.

Extremely oceanic distribution (southerly euoceanic) and known from Portugal, the British Isles and western Norway (Rogaland) (Degelius 1935 p. 192, 1936 p. 114). Watson 1953 mentions it also from Argyll.

3. ***Lobaria amplissima*** (Scop.) Forss.

Oban, Dunollie Woods on *Acer pseudoplatanus* (n. 3). — Loch Etive (north side)), Kennacraig, on *Quercus* by the road. Abundant (n. 31). — Loch Etive (south side)), about 1 km east of Dailnamac, on solitary *Quercus* by the main road. Abundant (n. 35 a). — Kerrera, Ardchoric, on old *Quercus*. Abundant (n. 49). — Island of Mull, Salen, near Chapel (in ruins), about 1 km east of the bridge of River Forsa, on steep rocks by the shore. Rather sparse (n. 106 a).

In all localities with *Dendriscocaulon,* n. 49 fertile, all others sterile.

Coll. G. Degelius: Loch Fyne, between Strachur and St. Catherines, on *Quercus* by the road (near the shore). — Loch Awe, Cladich, on *Corylus* in a slope with decid. wood by the road, about 50 m s.m. — Glen Aray, near upper Kennachregan, on *Quercus* in decid. wood, about 90 m s.m. In all localities sterile, with *Dendriscocaulon.*

Wider distribution (omnivagously suboceanic) than the former. (Degelius 1935 p. 204, map p. 72). On the British Isles distributed chiefly in the western areas especially in western Scotland and northern England. In Argyll earlier known from Loch Long near Roseneath, Inverary and Barcaldine (Smith 1918 p. 114). The other suboceanic species *L. laetevirens* is rather common and often locally very abundant in the investigated districts.

4. ***Pannaria sampaiana*** C. Tav.

Loch Feochan (south of Kilbride), about 1 km east of Knipoch, on big mossy boulders by the road. Rather abundant. Sterile (n. 41 a).

Coll. G. Degelius: Loch Fyne, between Strachur and St. Catherines, on *Fraxinus* by the road (near the shore). Abundant.

Described by Tavares 1950 from Serra do Gerês in Portugal. Its most closely related species is *P. leucosticta* from which it differs primarily

in possessing soredia occurring on the central lobes of the thallus. Tavares does not mention apothecia. (Tavares 1950 p. 76).

In the Uppsala Herbarium I have seen a fertile specimen from Ternowa not far from Idria, north-east of Trieste, collected 1873 by J. Glowacki. Apothecia lecanorine, 0.25—0.7 mm in diam., discus mahogny red (Ridgway 1912). Margo thallinus unregulary crenate. Spores, asci and paraphyses sought in vain. The apothecia seem not to be well developed or are only young — interesting in this case as the species in question generally does not have apothecia. — *New for the British Isles.*

5. *Parmeliella atlantica* Degel.

Oban, Dunollie Woods, on *Fraxinus*. Rather abundant (n. 9 b). — Oban, Dungallan Parks, on a big mossy *Acer pseudoplatanus*. Abundant (n. 18 a). — Dun Uabairtich (about 3 km south-west of Oban), on mossy boulders on the slopes of the hills above the ferry. Rather abundant (n. 23 c). — Kerrera, near Ardchoric, on mossy trunks of *Alnus glutinosa* (n. 53 a). — Loch Feochan, on the slopes of the hill "1032", about 1 km east of Knipoch, on big mossy boulders. Rather abundant (n. 41 b). — Kilmore, Cleigh, on a big *Fraxinus* by the road. Abundant (n. 29 b). — Ballachulish, on the trunk of a big solitary *Fraxinus*. Abundant (n. 86 c). — Island of Mull, by the Salen-Drumlang road, near Feith Bhan, on a mossy trunk of *Salix*, on *Quercus petraea*. Abundant (n. 97 c, 98). — All specimens sterile.

Coll. G. Degelius: Loch Fyne, same localities, on *Fraxinus*. Sterile.

This species was described by Degelius 1935 who referred it to his omnivagously euoceanic lichens. It was then known only from a few localities in Norway, Ireland and Portugal. (Degelius 1935, map p. 134) Later it has been published from Jugoslavia (Croatia, Velebit) (Köfaragó-Gyelnik 1939 p. 44), the Azores (Degelius 1941 p. 15) and Madeira (Tavares 1952 p. 331). Degelius has further collected the species in Sicily, north of Spain, and Tunisia (unpubl.). Last year I could mention four new localities in western Norway (one of them leg. Ahlner) (Lindahl 1953). Lamb 1942 mentions five localities in England and Scotland, two of them in Argyll (shore of Loch Awe near North Porth Sonachan, leg. Lamb; Airds, Appin, leg. Crombie).

6. *Pseudocyphellaria crocata* (L.) Vain.

Island of Mull, Killichronan, Kellan Wood, on mossy trunks of old *Quercus*. Sparse. Sterile (n. 120).

Extremely oceanic distribution (Degelius 1935 p. 204, map p. 149). On the British Isles local in south-western England and southern Scotland, more frequent in the western Highlands, scarce in Ireland. Earlier

known from Argyll, viz. Inverary, Oban, and Head of Loch Awe (Smith 1918 p. 111). On the continent known from localities in western Norway (Degelius 1935 p. 146, Ahlner 1948 p. 70) and from two localities in Portugal (Tavares 1945 p. 498; 1947 p. 151). The last mentioned finds show that this species must be referred to the omnivagously euoceanic lichens.

7. *Pseudocyphellaria thouarsii* (Del.) Degel.

Dun Uabairtich (about 3 km south west of Oban), on grassy and mossy open ground on the slopes of the hills above the ferry. Rare (n. 22 a). — Loch Feochan, on the slopes of the hill "1032", about 1 km north-east of Knipoch, on big mossy boulders. Not rare (n. 41 a). — Kerrera, near Ardchoric, on mossy boulders. Rare (n. 52 b). — All specimens sterile.

Extremely oceanic distribution (northerly euoceanic) (Degelius 1935 p. 204). On the British Isles local and rare in the southern, western and northern districts of Great Britain and Ireland. Earlier known from Argyll, viz. Barcaldine, Oban, Falls of Brander and Inverary (Smith 1918 p. 110). On the continent a few localities in western Norway, one from Bretagne, and one from Faroe Islands (Degelius 1935, map p. 153).

8. *Sticta dufourei* Del.

Oban, Dunollie Woods, in mossy rock clefts on precipices. Sparse. Sterile (n. 13). — Portnan Cuilc (about 4 km south-west of Oban, by the road along Kerrera sound), on a mossy, shady rocky wall. Sparse. Sterile (n. 21 a).

Extremely oceanic distribution (southerly euoceanic). On the continent known from France, Corsica, Italy, Spain and Portugal (Degelius 1935 p. 204, map p. 193). On the British Isles known from south-western England, northern Wales, the western Highlands of Scotland and south-western Ireland. In Argyll known from Barcaldine (Smith 1918 p. 109). *Sticta fuliginosa, S. limbata,* and *S. sylvatica* are rather common and often very abundant in the investigated districts.

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Contributions to the Geological History of the Hepaticae

II. On a Fossil Member of the Marchantiineae from the Mesozoic Plant-bearing Deposits near Lago San Martin, Patagonia (Lower Cretaceous)

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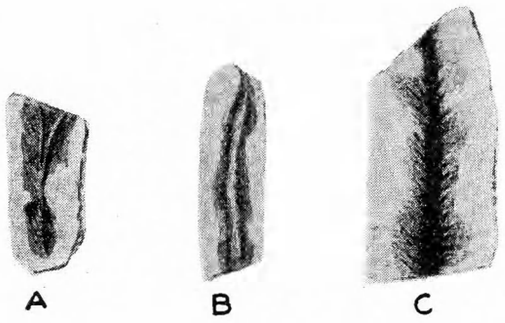
Introduction

In connection with previous work on Hepatics from the Mesozoic (Lundblad 1954), my attention was attracted by some fossil Liverwort material from the Lago San Martin district of Patagonia, kept at Riksmuseets Paleobotaniska Avdelning, Stockholm. It had been collected by T. G. Halle at Rio Fósiles during the Swedish Expedition to South America led by C. Skottsberg in 1907—1909. Halle (1913) described this flora at some length, giving the following list of species:

"*Marchantites*? sp.
Nathorstia alata n.sp.
Gleichenites cf. *micromerus* (Hr.)
Cladophlebis australis (Morr.) Sew.
Cladophlebis cf. *Browniana* (Dunk.) Sew.
Sphenopteris (*Ruffordia*?) *Goepperti* Dunk.
— *patagonica* n.sp.
— (*Onychiopsis*?) *psilotoides* (Stokes & Webb) Ward?
Asplenites lanceolatus n.sp.
Ptilophyllum acutifolium Morr.
Baiera cf. *australis* M'Coy
Arthrotaxites Ungeri n.sp.
Elatocladus sp."

There were no Dicotyledons. On the other hand, the flora was obviously not older than the Middle Jurassic. As to its age, Halle (1913, p. 46) came to the following conclusion: "Those of the species which



Text-fig. 1. Halle's original picture to his description of "*Marchantites*? sp." from the Lower Cretaceous of Rio Fósiles, Patagonia (1913). A, Halle's Fig. 14, Pl. 5; B, Fig. 15, Pl. 5; C, Fig. 16, Pl. 5.

are of the greatest value in this respect appear to indicate prevailing Wealden and Lower Cretaceous affinities." From a botanical point of view, Halle's description of the well-preserved material of the fern *Nathorstia alata* — material of this genus was known especially through finds from the Cretaceous of Greenland described by Heer and Nathorst — and his record of a Conifer later referred to the living genus *Athrotaxis* D. Don [*Athrotaxis Ungeri* (Halle) Florin; see Florin 1940, pp. 34—35] were of particular interest. It should perhaps be pointed out that Halle's ascription of a Lower Cretaceous age to the Lago San Martin flora was later confirmed by evidence obtained by Argentine geologists. The stratigraphy of the region has recently been summarized by Feruglio (1949, pp. 171—176; Stratigraphic Table pp. 188—189) in his monographical "Descripcion geologica de la Patagonia". Of considerable interest is the finding of the above-mentioned two species of fossil plants in the Lago Cardiel district (Piátnitzky), where they occurred in a horizon between layers of supposed Barremian and Aptian age. The plants of this so-called 'Zona con "Nathorstia alata"' belong, according to Feruglio, "muy probablemente al Aptense" (op.cit., p. 177).

It is now more than forty years since Halle (1913, p. 20, Pl. 5, Figs. 14—16; our Pl. 1, Figs. 1—3, and text-fig. 1) published the insignificant Liverwort fragments referred to as "*Marchantites*? sp." in the above list of species from the Lago San Martin district. One of the most important contributions to palaeobotany in the intervening period has been the introduction of transfer methods in the study of fossil plant remains (Walton 1923). They have been applied to Liverworts by Walton (1925), by Harris (1931, 1937, 1938, 1942), and by the author (Lundblad 1954). It seemed to me that a re-examination of Liverwort material previously described in literature might in many cases be well worth while, provided their state of preservation is favourable. Halle's

material from Patagonia offers a suitable object for an investigation of this kind.

I am indebted to Mr. K. E. Samuelsson for skilful technical assistance. He has made the balsam transfers for the investigation and taken all the negatives, including photographs in infra-red light. — Dr. Sigfrid Arnell has kindly read the manuscript, and I owe him some comments on the preservation of certain Liverwort spores (see below).

The Material

The material consists of sterile segments of thalli, mainly preserved as compressions of light brownish colour. The rock was characterized by Halle (op.cit., p. 18) as "a very hard siliceous slate of bluish grey colour". In the hand-specimens studied, the Liverwort segments were associated i.a. with *Athrotaxis Ungeri* (Halle) Florin.

The drawings of "*Marchantites*? sp." published by Halle (1913) are refigured in text-fig. 1. He describes them (op. cit., p. 20) as follows:

"The small fragments shown in pl. 5, figs. 14—16, are very similar to specimens figured from different Mesozoic strata and considered to represent thalli of Hepatics. The present specimens are impressions of flat protracted bodies with parallel but somewhat sinuous sides. In the middle they show the impression of a ridge, thicker but less sharply set off than the midrib of a frond. From the median ridge arise some indistinct arching lines which taper very abruptly and rapidly disappear. One specimen shows a dichotomous branching. It is possible that these fragments may be compared with specimens described as *Marchantites,* but they are much too poor to permit of forming any definite opinion."

Halle's description gives a good idea of the general appearance of the fossils. The specimens quoted by him have been photographed, and reproduced in natural size in Figs. 1—3, Pl. 1. Magnifications are shown in Fig. 4, Pl. 1, and in Figs. 2, 5, Pl. 3. In one of these fragments the rhizoids attached to the segment were visible when the compression was still lying on the rock (Fig. 5, Pl. 1). Two of Halle's specimens were made into balsam transfers according to Walton. Examination of the plant-bearing rock under xylol revealed some additional specimens worth closer study, of which the best ones are shown in Fig. 5, Pl. 2, and Fig. 6, Pl. 3.

No serious attempts were made to study the microflora of the rock containing the Hepatics described above. The spores of the *Marchantiaceae* are thin-walled and can therefore not be expected to resist strongly oxidative treatment. In nearly related groups (for instance *Targioniaceae*), however, the spores are thick-walled and may be resistant.

Nomenclature

A short review of the nomenclature of fossil Hepatics, with special reference to Walton's revision in 1925, was given in my paper on the fossil *Marchantiales* from the Rhaetic-Liassic of Scania (Lundblad 1954, pp. 385—387). As may be seen from the quotations from various authors (Seward, Walton, Steere) given there, the definition of the genus *Marchantites* is by no means exact.

Walton (1925, p. 564) suggested in his definition of *Thallites* that *Marchantites* should be limited to forms "such as *M. Sezannensis* Sap., which bear undoubted affinities to the *Marchantiaceae*". Steere (1946, p. 304) commented the genus "*Marchantites* Brongniart emend. Walton" by saying that "Fossil Hepaticae with the characters of the order Marchantiales are to be assigned to this form-genus."

In my opinion attempts should be made to separate fossil materials related to the *Sphaerocarpineae* or the *Ricciineae* from material of the *Marchantiineae* by means of a suitable nomenclature. (As for the systematic units used in the discussion I refer to Müller 1951, pp. 195—198). Being the only natural course to follow, this has already been practiced by the palaeobotanists working on well-defined fossil material related to the two first-mentioned groups. In the case of *Naiadita,* which Harris (1938, 1939) regards as closely allied to the family *Riellaceae* of the *Sphaerocarpineae,* there was no reason to suggest comparison with the *Marchantiineae.* As it was evident that the designation *Marchantites* would be inadequate and misleading for the *Riccia*-like forms from Skromberga discovered by me (op.cit.), a new generic name, *Ricciopsis,* was instituted for these.

Fossil Hepaticae with characters of the *Marchantiineae* should be assigned to the form-genus *Marchantites.* The most well-known family of the *Marchantiineae* is the *Marchantiaceae*; *Marchantia polymorpha* L. is known to every student of botany. The attention of palaeontologists should, however, be drawn to the fact that the *Marchantiineae* contain, beside the *Marchantiaceae,* a number of other families differing from it in rather important characters, such as sporogone shape and arrangement and air-pore structure. Nor is *Marchantia* itself the only genus of the *Marchantiaceae* to be considered for the interpretation of the fossil material.

The genus *Marchantiolites* Lundblad (op.cit.) was based on material which could not without reservation be compared to the *Marchantiineae* proper; it seemed indicated to express this in the naming of the material. *Marchantiolites* is intended for sterile material in a particularly favourable state of pre-

servation (cellular structure of thallus distinguishable), the air-pores of which resemble those characterizing the sub-ordo *Marchantiineae,* but which does not provide conclusive additional evidence to justify its assignment to the said group.

Description

Marchantites Hallei n.sp.

Pls. 1—2; Pl. 3, Figs. 1—8, text-fig. 1, 2 A.

Marchantites? sp., Halle 1913, p. 20; Pl. 5, Figs. 14—16.

The specimen seen in Fig. 2, Pl. 1 and in text-fig. 1 B (=Halle's Fig. 15, Pl. 5) is the one in which the distribution of the rhizoids is best shown. It is seen in magnification in Fig. 4, Pl. 1. The rhizoids were visible in patches when the specimen was still attached to the rock (Fig. 5, Pl. 1). To expose the lower side of the segment the specimen was made into a transfer. The distribution of the rhizoids is well shown in this preparation, where the presence of ventral scales is suggested, too (Pl. 1, Figs. 6—8). The specimen is regarded as the holotype of a new species, *Marchantites Hallei,* named for professor T. G. Halle, Stockholm, who discovered and first published the material.

The Holotype

Pl. 1, Figs. 2, 4—8; Pl. 2, Figs. 1—2; Pl. 3, Figs. 1, 8; text-fig. 1 B.

Coll. — T. G. Halle, January, 1909.

Occurrence. — Lago San Martin, Rio Fósiles (loc. *c* in Halle 1913).

Age. — Lower Cretaceous.

Diagnosis. — Fragmentary segment of Hepatic about 18 mm long, with a maximum width of about 4 mm. Margin of segment undulate. A median, thickened zone, less than 1 mm wide, resembling a midrib is present, from which arise ± arcuate lateral ribs towards the lateral margins. Air-pores are present, but their anatomical structure is unknown. A row of ventral scales, about 2 mm long, subovate in shape, occurs on each side of the midrib. The rhizoids, which are unicellular, occur in dense clusters attached to the proximal part of the lateral ribs. They attain a width of about 30 μ (average of five measurements 31 μ, range 23—38 μ). There are two kinds of rhizoids, viz. such as are apparently homogeneous and others, which show a central zone of dark material (about half as wide as the rhizoid itself). Elongated cells (about 70×20 μ) have been observed in the epidermis (traces only).

The Rest of the Material

Pl. 1, Figs. 1, 3; Pl. 2, Figs. 3—8; Pl. 3, Figs. 2—7; text-fig. 1 A, C; text-fig. 2 A.

The collector, place of occurrence, and age of the rest of the material are as stated above for the holotype.

The proximal part of a segment is shown in Fig. 5, Pl. 2. The preserved fragment is about 15 mm long; its distalmost portion must have been up to 6 mm wide. Very little is left of the carbonized substance of the lamina, but arcuate lateral ribs similar to those of the holotype are preserved. The presence of two distinct rows of ventral scales in the midrib-like portion is remarkable. They have been studied with the aid of infra-red light and transfers (Figs. 6—8, Pl. 2), and a reconstruction of the thallus segment based on these photographs is given in text-fig. 2 A.

In general appearance, the specimen shown in Halle's Fig. 16, Pl. 5 (text-fig. 1 C; refigured in Fig. 3, Pl. 1) is reminiscent of the holotype. As in the specimen described above, the median portion only is carbonized; the rest of the lamina is preserved only as an impression. In this case the material was not made into a transfer; there was accordingly no opportunity for a closer study of the rhizoids, but their presence was observed under magnification. The segment is shown magnified in Fig. 5, Pl. 3. The "arching lines" of Halle can be seen distinctly, and there are ventral scales on the midrib-like thickening as in the specimen shown in Figs. 5—8, Pl. 2 (this was verified by a photograph taken in infra-red light). — Another specimen of the same type as the holotype and the other specimen figured by Halle is shown in Figs. 6—7, Pl. 3. Its maximum length is 13 mm; the width of the lamina was about 4 mm. The segment shows distinctly the lateral arcuate ribs, to which clusters of rhizoids were attached, and the presence of ventral scales is also suggested.

The lamina of the dichotomizing segment seen in Halle's Fig. 14, Pl. 5 (text-fig. 1 A and our Fig. 1, Pl. 1) is more completely preserved than the rest of the material (Fig. 2, Pl. 3). The angle of the dichotomy is acute in its proximalmost part (about 13°) but widens later. The specimen was made into a transfer, in which the presence of air-pores is distinct (Figs. 3—4, Pl. 3). They seem to have been barrel- or cone-shaped, but their cellular structure is not preserved. The opening of a couple of the pores measured was about 40 μ in diameter.

General remarks

Arcuate lateral ribs similar to those in the segments of *Marchantites Hallei* are often seen in the *Marchantiales,* particularly among the *Marchantiineae.* In this group forms occur with strongly developed and persistent ventral scales attached along the ribs (cf. text-fig. 2 F).

As their cellular structure is not preserved, no satisfactory information on the architecture of the air-pores could be obtained, but their largeness excludes comparison with the *Ricciineae.*

The presence of ventral scales in two distinct rows on the midrib-like thickening, as seen in text-fig. 2 A, makes *Marchantites Hallei* comparable to the *Marchantiineae* rather than to the *Ricciineae,* where the scales arise from the ventral surface in a single row later split along the median line. The presence of appendages at the end of the ventral scales, similar to those in for instance the genus *Marchantia* itself (cf. our text-fig. 2 E), could not be verified in the photographs, but is not quite out of the question. Additional rows of scales (cf. text-fig. 2 F) were not observed with certainty in the fossil fragments, but the possibility that there might have been extra rows of ventral scales in the living plant cannot be entirely excluded. Negative evidence obtained from fossil material cannot always be considered a safe criterion of the complete absence of a given kind of structure, as this may depend on the preservation of the material.

The rhizoids of *Marchantites Hallei* present a puzzling problem. Owing to the unsatisfactory state of preservation it is impossible to say whether there were pores in the walls of some rhizoids or not. The most likely explanation appears to me to be that there may, as in most *Marchantiales,* have been two kinds — smooth-walled and tuberculate — of rhizoids. The former type would then be that represented in Fig. 1, Pl. 2, the latter those represented in Figs. 2 and 4, Pl. 2. In the latter the ± spiral thickenings of the wall (cf. text-fig. 3 and Fig. 9, Pl. 3) might during fossilization have caused changes in diameter of the kind

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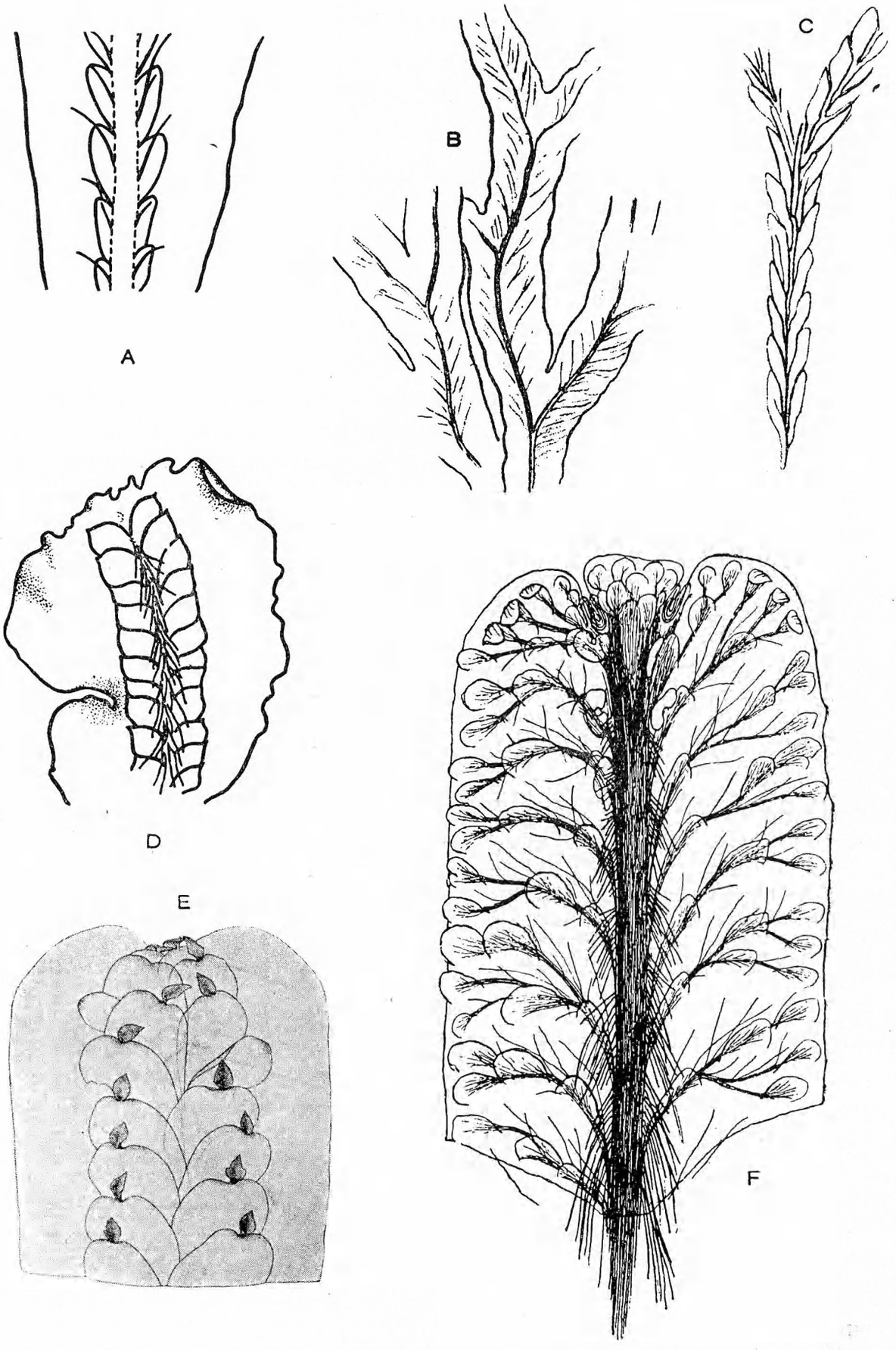
Text-fig. 2. A, *Marchantites Hallei,* reconstruction of thallus segment, showing ventral scales — 6/1; B, *M. sezannensis,* portion of thallus. From Saporta (1868, Pl. 22, Fig. 1); C, *M. sezannensis,* ventral scales from a thallus segment. From Saporta (1868, Pl. 22, Fig. 2 a); D, *Preissia commutata,* thallus segment with ventral scales After Müller (1906—11, p. 17: part of text-fig. 11, redrawn by K. E. Samuelsson) — 7/1; E, *Marchantia chenopoda,* distal portion of segment, showing ventral scales — 10/1. From Goebel (1930, p. 704, text-fig. 690); F, *Marchantia polymorpha,* distal portion of thallus segment, showing ventral scales and arcuate ribs. — Ca. 5/1. From Goebel (1930, p. 704, text-fig. 691).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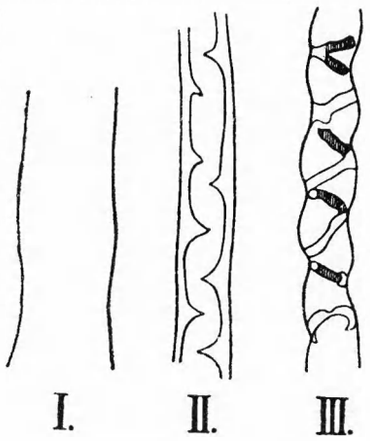

Text-fig. 3. *Marchantia polymorpha* L. Rhizoids of different kinds; figured at the same magnification. I, Smooth-walled rhizoid; II, transitional type to III, tuberculate rhizoid. From Goebel 1930 (p. 742, Fig. 740).

known from elaters, which have somewhat similar band-like structures (cf. Herzog 1925, text-fig. 91). Spiral thickenings reminiscent of those seen in elaters are actually suggested in the rhizoid figured in Pl. 3, Fig. 8, where the external parts of the wall may possibly have been removed, leaving but the central dark zone of the rhizoid).

Rhizoids of Hepatics not infrequently contain hyphae of fungi. Judging by pictures of *Marchantiaceae,* e.g. those published by Golenkin (1902, Pl. 11, Figs. 15—16), it would be far-fetched, however, to attempt to interpret a dark central zone in the rhizoids as due to fungal infection.

A comparison of the state of preservation of *M. Hallei* with that of the rest of the plant remains of the Rio Fósiles flora indicates the probability of *Marchantites Hallei* being a Mesophyte or a Hygrophyte, rather than a pronounced Xerophyte.[1]

Comparison

Marchantites Hallei is referred to the *Marchantiineae* on account of the shape of its air-pores and the presence of ventral scales of the type found in this group. The general habit of the plant, too, indicates clearly that it is affined to the *Marchantiineae* rather than to the *Ricciineae.*

From its external appearance, as well as from what is known of the

[1] In the light of previous remarks on xeromorphism in Liverworts (Lundblad 1954, p. 384) it should be noted that Ziegenspeck (1942), who studied the structure of the epidermis in several living representatives of the *Marchantiales,* was able to confirm that there is a true cuticula in many Liverworts; the cutinization of the air-pores was particularly mentioned.

finer structure of the plant, it is evident that the Patagonian material represents a type which differs considerably from the only hitherto described Mesozoic species with air-pores, viz. *Marchantiolites porosus* from the Liassic of Scania (Lundblad 1954, pp. 393—396, Pl. 3, Figs. 9—11; Pl. 4, Figs. 1—7; text-fig. 3). The segments of *Marchantites Hallei* are, for instance, much larger than those of the Swedish material. From a study under high magnification of its badly preserved air-pores it is also obvious that these represent a different, probably more elaborate type than those seen in the Liassic plant. The pores of the South American material are much wider, the diameter of their openings being about double that of the Swedish species.

A comparison with the ± unsatisfactorily known remains from the Mesozoic that have been supposed to represent remains of Liverworts is of little value. The following species may, however, be worth mentioning in this connection (cf. review in Lundblad, op.cit., pp. 407—410):

Hepaticites Wonnacotti Harris 1942. Middle Jurassic, England.
Thallites Rostafinskii (Raciborski) Harris. Raciborski 1888, 1894; Lower Lias, Poland.
Thallites Zeilleri (Seward) Harris. Seward 1894, Harris 1942; Wealden, England.
Thallites Sewardii (Berry) Lundblad. Berry 1920; Lower Cretaceous, U.S.A. (Maryland).
Thallites blairmorensis (Berry) Lundblad. Berry 1929; Lower Cretaceous, Canada.
Thallites Yabei (Kryshtofovich) Harris. Kryshtofovich 1929, 1933, Ôishi 1940, Harris 1942, Upper Jurassic-Lower Cretaceous, Siberia, Korea, Japan.
Thallites uralensis Kryshtofovich & Prynada 1933; Rhaetic-Lias, U.S.S.R. (Eastern Urals).
Thallites Jimboi (Kryshtofovich). Kryshtofovich 1918, 1929; Middle Cretaceous, U.S.S.R. (Sakhalin).

The general habit of *Hepaticites Wonnacotti, Thallites uralensis, Th. Rostafinskii, Th. blairmorensis* and *Th. Yabei* is of the type found in the *Marchantiineae* and in sub-ordo *Anakrogynineae* of the *Jungermaniales. Hepaticites Wonnacotti,* which is considerably larger than *Marchantites Hallei,* is of particular interest because it resembles our species in having arcuate ribs in the lamina (cf. p. 28). Harris noticed some surface-bulges, but these could not be definitely proved to represent air-chambers. — The presence of ± diffuse arcuate ribs in the thallus seems to have been observed in *Thallites uralensis* Kryshtofovich & Prynada, too, but the figures (op.cit., Pl. 1, Fig. 6; Pl. 3, Fig. 12; Pl. 4, Fig. 5; Pl. 5, Figs. 3, 6, 7 a, 9) are too indistinct to give any exact information on the kind of venation of the Cheliabinsk material. — *Th. Rostafinskii* and *Th. blairmorensis* are on the whole a little larger than *M. Hallei,* which they resemble in general habit. The first published material of *Th. Yabei* (Kryshtofovich 1929, Pl. 5, Fig. 3; Yabe 1905, Pl. 3, Fig. 11)

shows larger segments than the Patagonian species, but the specimens figured later (Kryshtofovich 1933, Pl. 6, Fig. 1; Ôishi 1940, Pl. 1, Fig. 1) are of about the same dimensions. The resemblance of *Th. Yabei* to Halle's specimens from Patagonia was mentioned by Kryshtofovich (1929, p. 146). The agreement is limited to externals, however, since nothing is known as to whether there are air-pores, ventral scales etc. in the material in question. One common feature is the presence of a midrib-like portion in the thallus. — *Thallites Zeilleri, Th. Sewardii,* and *Th. uralensis* are similar to our species in size and general habit, and they also have thickened, midrib-like portions.

Two forms of *Thallites* from the Far East, which were not mentioned in the review of records of Hepatics from the Mesozoic given in my 1954 paper, also show superficial agreement with the last-mentioned species and with *M. Hallei.* Kryshtofovich (1918, p. 34, text-fig. 4) originally referred *Thallites Jimboi* (Krysht.) from the Middle Cretaceous of Sakhalin to *Stenopteris,* but later transferred it to *Marchantites* on account of its resemblance to *Th. Yabei* and the discovery of new material in the same locality (Mgach coal mine). Its proper place is in *Thallites,* however, since there is nothing to prove definitely its affinites to the *Hepaticae.* Similar fossil material referable to *Thallites* has also been described by Sze (1933, p. 51, Pl. 12, Fig. 8) from the Mesozoic of China (Province of Fukien). Sze described the material as "*Problematica*" but recognized that it might possibly represent remains of a thalloid Liverwort.

The material of greatest interest to a comparison with *Marchantites Hallei* belongs to the Lower Jurassic flora of Victoria, Australia, which has very recently been reviewed and revised by Medwell (1954). After some general notes on the vegetative features of the thalli of the *Marchantiales,* this author describes the fossil material as follows: "Specimens from Bellarine and from Queen's Park, Geelong, seem best placed in this order of the *Hepaticae.* In these, the central axis of the dichotomously branching thallus is composed of elongate cells, at right angles to which are hexagonal or polygonal areas probably corresponding to the walls of the air chambers beneath. One specimen bears what appears to be two rows of scales on the ventral surface, and thus indicates affinity with the family *Marchantiaceae,* in which several such rows of scales occur, rather than with the *Ricciaceae,* in which one row only is found". Further data about this material are given in the specific descriptions. Part of it is described as "*Marchantites* cf. *Marchantites erectus* (Leckenby 1864) Seward 1900", part as "*Marchantites barwoni* n.sp.". No particulars are given of the material described under the first of these headings; the following is her diagnosis of the new species: "Thallus prostrate, dichotomously branching; surface wrinkled, margin regularly dentate. Upper surface divided into hexagonal areas more or less at right angles to the central axis, and

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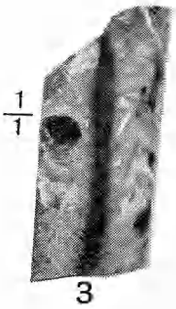

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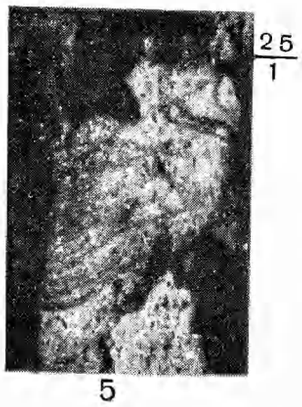

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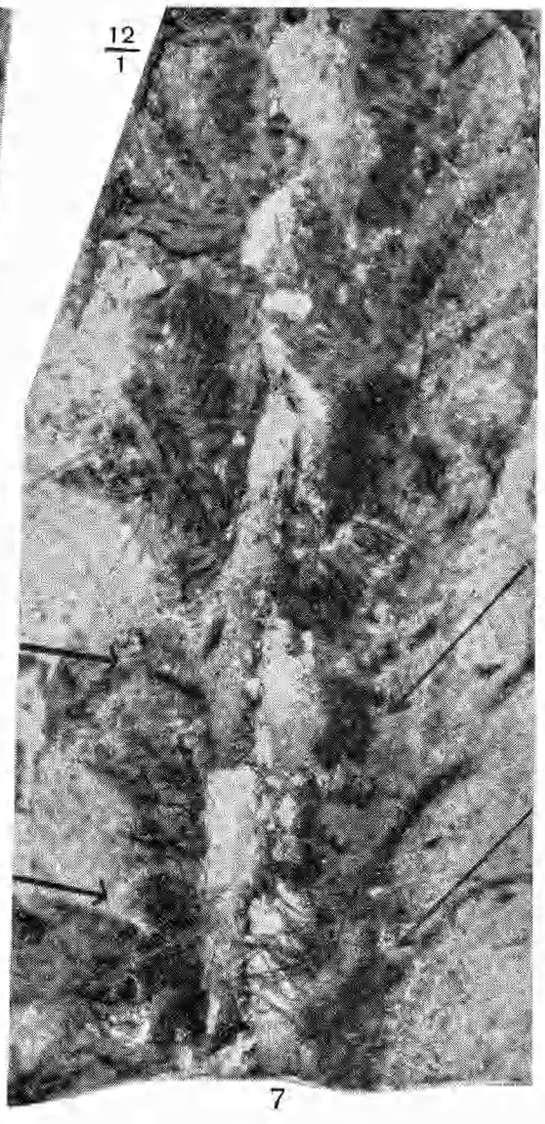

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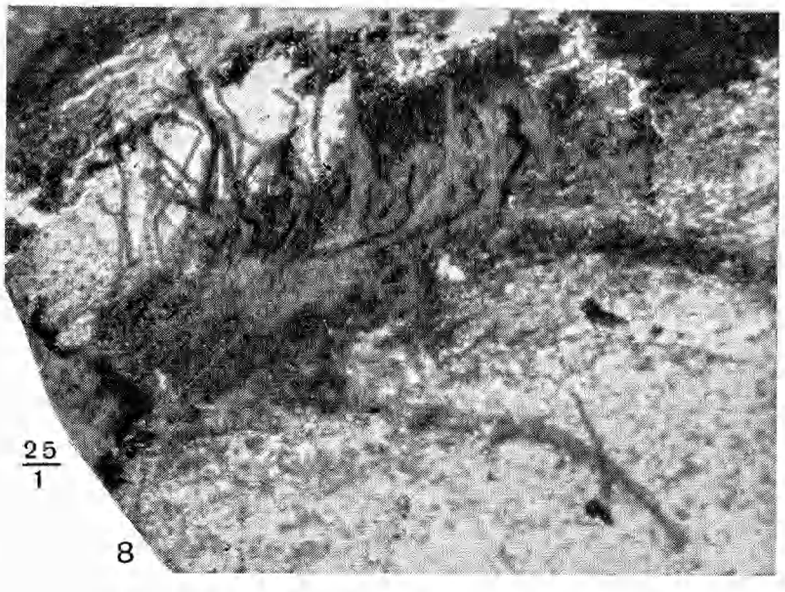

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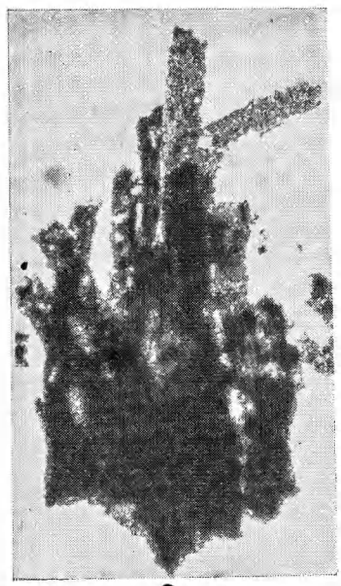

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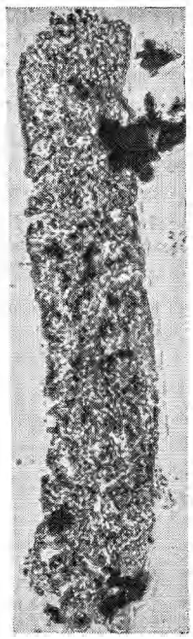

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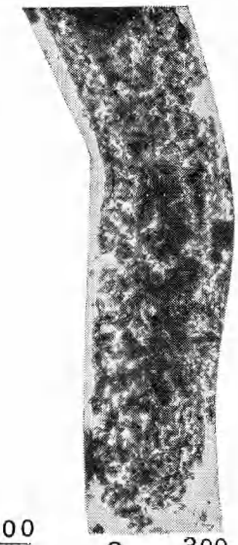

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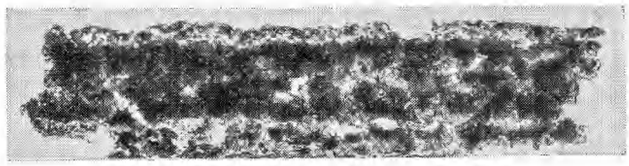

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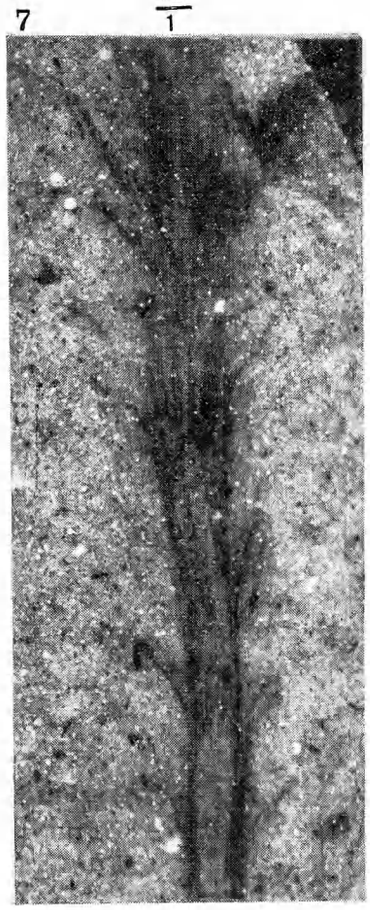

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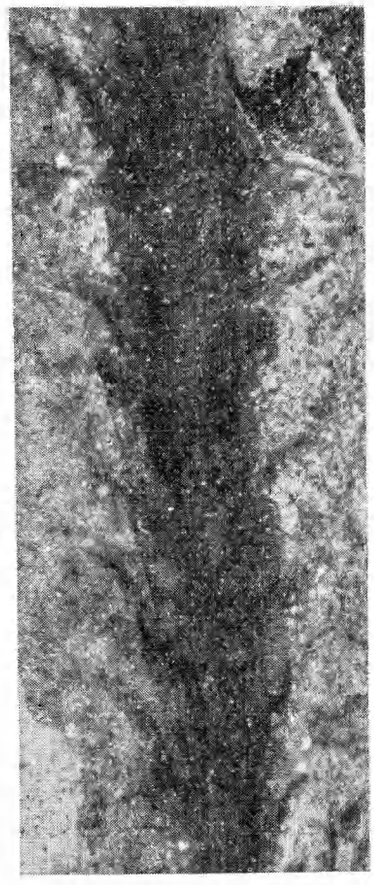

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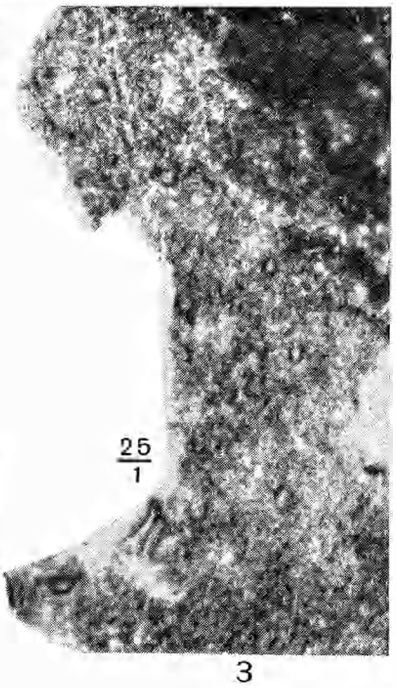
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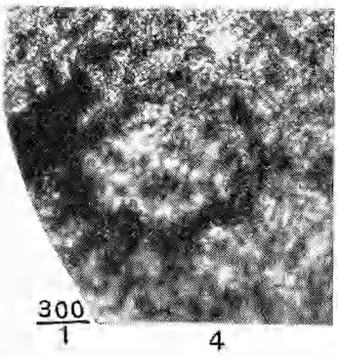
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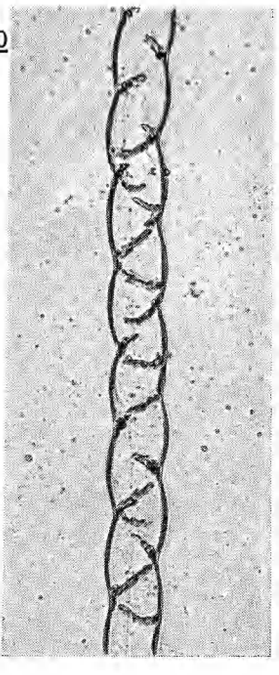
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area above axis formed of cells elongate in the direction of the axis." The author makes the following remark: "The specimens seem to be quite distinctive in the possession of a markedly dentate margin, and approach the type of thallus seen in the recent *Marchantia polymorpha*" (op.cit., pp. 79—80).

The only illustration accompanying Medwell's description (op.cit., Pl. 6, Fig. 21) shows none of the characters, given in the general and specific descriptions of the Australian material, that might prove conclusively that it should be ascribed to the *Marchantiineae,* e.g. the presence of ventral scales or the structure of the thallus. The specimen figured is a little larger than ours, and reminiscent of material described as *Thallites blairmorensis* and *Th. Yabei.* It is preserved as an impression. The new species should therefore be designated *Thallites barwoni* (Medwell) n. comb.

I need hardly point out that some of the species of *Thallites* mentioned here are very unsatisfactory from a botanical point of view, and are kept distinct mainly as a matter of convenience.

Marchantites Hallei agrees closely to the vegetative characters of the fertile *M. sezannensis* Brongniart (Saporta 1868, pp. 308—312; Pl. 1, Figs. 1—8) from the famous travertine of Sézanne in the Province of Marne in France (Eocene). In the French material, the dimensions of the dichotomizing segments are somewhat larger (text-fig. 2 B), but there are two rows of ventral scales (text-fig. 2 C) very similar to those seen in the Patagonian species (text-fig. 2 A). After a few words about "l'axe ou nervure médiane" of the segments, Saporta (op.cit. p. 309) describes the scales as follows: "Il est accompagné de deux rangées d'écailles distiques et obliquement insérées, sur lesquelles naissaient les radicules, et analogues à celles qu'on remarque sur le revers des frondes de la plupart des *Marchantiées,* mais plus courtes, mieux limitées et moins prolongées vers le bord que celles du *M. polymorpha* et plus sembables à celles des espèces exotiques à texture foliacée coriace qu'à celles de l'espèce indigène." — However, the old description — particularly the identification of the material with the genus *Marchantia* itself [1] — should probably be taken with some reservation.

[1] In my previous contribution to the knowledge of the fossil *Hepaticae* (1954, p. 411) I have stated that *M. sezannensis* clearly belongs to the *Marchantiaceae.* This was based on a statement by Walton 1925 (p. 564) that this plant "bears undoubted affinities to the *Marchantiaceae*". Following Müller's classification, it would be more correct to say that *M. sezannensis* clearly belongs to the subordo *Marchantiineae,* which, however, includes not only the *Marchantiaceae sensu stricto,* but also other

Discussion

Marchantites Hallei from the Lower Cretaceous of Patagonia constitutes a vertical connection between the Tertiary *Marchantites sezannensis* and the records of supposed Jurassic members of the *Marchantiineae*. Although the South American material is not fertile, serious objections can hardly be raised to its inclusion in the suborder in question. It is also of interest that new evidence confirming the assumed existence of true *Marchantiineae* in the Older Mesozoic is accumulating. Medwell's record of material with ventral scales and the habit of thalloid Liverworts in the Lower Jurassic of Victoria points in this direction. The observations on the material from Australia and on Liverwort remains from the Jurassic of England increase the probability that *Marchantiolites porosus* Lundblad from the Liassic of Scania, too, may rightly be regarded as a member of the *Marchantiineae* proper.

According to information by letter from Prof. T. M. Harris (9.2.1954) there is unpublished material of a similar type from the Jurassic of Yorkshire. He has found "one specimen of a rather ill-preserved thallus with Marchantia-like ventral scales", and thinks that "It is probable, but not quite certain, that there are air-chambers."

As suggested in my 1954 paper, I am inclined to believe that representatives of living families of Hepatics — even some modern genera — might be expected in Mesozoic floras. Since there is now evidence that the genus *Riccia* has survived fairly unchanged from the Older Mesozoic, it may readily be assumed that some genera of the *Marchantiineae* have also survived. Attention is drawn to the increasing number of remains of Jurassic vascular plants identified with living genera (*Selaginella, Equisetum, Dicksonia, Ginkgo*).

The Mesozoic material of *Marchantiineae* from the Southern Hemisphere is also of interest on account of its suggestions about the horizontal distribution of the *Marchantiineae* at that time. Since the previous records of supposed members of the group were all from the Northern Hemisphere, the new data seem to indicate that the *Marchantiineae* had a world-wide distribution in the Jurassic. This implies that the geological history of the group is very long.

In my previous contribution to the geological history of the *Hepatice* (op. cit., pp. 382—383) I drew attention to a publication by Fulford

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families with thalli of superficially rather similar appearance, e.g. the *Conocephalaceae* and the *Grimaldiaceae*.

on the distribution patterns of groups of living *Hepaticae* as a means of illustrating the past history of the group. Nothing was said, however, about the frequently cosmopolitan character of the distribution of many genera of Liverworts, an important factor in this connection.

Campbell (1907) has drawn attention to the fact that the cosmopolitan character of most genera, and many species of the *Hepaticae,* point to their being very ancient forms. Referring to a survey by D. H. Scott, he mentioned that too much stress should not be laid upon the negative evidence available from fossil botany at that time. Campbell points out that the *Hepaticae* contain a small number of genera of wide distribution, and that the Liverworts of to-day are not as a rule particularly adaptable, nor are they generally provided with very effective means of distribution. Herzog's treatment of the *Marchantiaceae* (cf. *Marchantiineae* of Müller 1951) in his "Geographie der Moose" (1926, p. 183) is based on similar observations:

"Bei den grossen Gattungen, die wie *Marchantia* und *Fimbriaria* je nahe an 100 Arten enthalten, kann es nicht weiter auffallen, dass sie zu den weltweit verbreiteten Verwandtschaftskreisen gehören, dass aber auch die kleineren Gattungen, ja selbst manche Monotypen, eine ähnlich weite Verbreitung gefunden haben, spricht entweder für einen sehr alten Stoff oder für die polytope Entstehung von Gattungen, deren Umgrenzung also nach konvergenten — wenn schon auf der Verwandtschaft eines weit verbreiteten Marchantiaceenstoffes beruhenden — Merkmalen stattgefunden hätte. Denn an eine besondere Leistungsfähigkeit der Verbreitungsmittel zu denken, ist angesichts der unvollkommenen Aussaatvorrichtungen und oft bedeutender Grösse der Sporen nicht wohl möglich."

The assumption that genera or species are of polytopic origin, including large scale convergence, seems to me very far-fetched:

"Es ist ganz unwahrscheinlich, dass bei den Arten der verschiedensten Familien durch Mutation, die bei Lebermoosen wohl allein artbildend in Frage kommt, in weit auseinanderliegenden Erdteilen immer gleichartige Individuen hervorgehen sollten." (Müller 1951, p. 209).

The evidence from the fossil material supports the general assumption that the geological history of the *Marchantiineae* is long, as the world-wide distribution of the group seems to have been maintained at least since Mesozoic times.

Note

During the Skottsberg expedition to South America in 1907—1909, T. G. Halle made a small collection of Palaeozoic plants in the Rio Grande do Sul district of Brazil. This was described by Lundqvist in 1919. The flora of the

locality Arroyo dos Cachorros contains fragmentary, dichotomously branching thalloid remains described as "*Marchantites* sp." (op.cit., p. 5, Pl. 1, Fig. 1). I have examined the rock specimen figured by Lundqvist, which is kept in the collections of Riksmuseets Paleobotaniska Avdelning, Stockholm. The material is of the *Thallites* type, and preserved as an impression only. The affinities of the poor remains are completely unknown.

Summary

This paper is a re-description of the Liverwort remains from the Mesozoic (Lower Cretaceous) of Rio Fósiles in the Lago San Martin district, Patagonia, described by Halle (1913, p. 20, Pl. 5, Figs. 14—16) as "*Marchantites*? sp.". They are now referred to *Marchantites Hallei* n.sp. The presence of air-pores and ventral scales similar to those of the *Marchantiineae* brings the Patagonian material into this group. The new species is of interest as a connecting link between the findings of supposed members of the *Marchantiineae* from the Jurassic (*Marchantiolites porosus* Lundblad etc.) and the fertile *Marchantites sezannensis* Brongniart from the French Eocene. The Southern Hemisphere record is further of interest from the point of view of plant geography. Together with some more doubtful remains recently described from the Lower Jurassic of Australia (Medwell 1954), it indicates that the *Marchantiineae* had a world-wide distribution already in Mesozoic time.

Addendum

The first part of the 12th edition of A. Engler's "Syllabus der Pflanzenfamilien" (1954), which appeared during the preparation of this paper, contains a systematic account of the *Bryophyta* (including the *Hepaticae*) by H. Reimers. This author keeps the sub-ordo *Sphaerocarpineae* of Müller (1951) as a separate order, the *Sphaerocarpales,* and divides the order *Marchantiales* into the "Familien-Gruppen" *Marchantiineales* and *Ricciineales*. The latter contains the *Oxymitraceae* and the *Ricciaceae*. The families included in the *Marchantiineales* are the same as in the *Marchantiineae* of Müller 1951. There are thus no fundamental differences from the system used in this paper.

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Explanation of Plates

The figured specimens and slides are the property of the Palaeobotanical Department of the Swedish Museum of Natural History, Stockholm. — K. E. Samuelsson phot.

Plate 1

Marchantites Hallei n.sp. — Lago San Martin, Rio Fósiles (loc. *c* in Halle 1913). Lower Cretaceous.

Fig. 1. Dichotomizing segment of thallus (Halle 1913, Pl. 5, Fig. 14). Xylol. — 1/1.
Fig. 2. Segment (Halle 1913, Pl. 5, Fig. 15). Holotype. Xylol. — 1/1.
Fig. 3. Segment (Halle 1913, Pl. 5, Fig. 16). Xylol. — 1/1.
Fig. 4. The thallus segment seen in Fig. 2 (the holotype) enlarged. — 4/1.
Fig. 5. Portion of the same, still more enlarged to show rhizoids. — 25/1.
Fig. 6. Balsam transfer of the thallus segment seen in Figs. 2 and 4, enlarged. Preparation immersed in water when photographed. — 6/1.
Fig. 7. The lowermost portion of the same segment, still more enlarged. The positions of some of the ventral scales are marked by arrows. — 12/1.
Fig. 8. A cluster of rhizoids from the same. — 25/1.

Plate 2

Marchantites Hallei n.sp. — Lago San Martin, Rio Fósiles (loc. *c* in Halle 1913). Lower Cretaceous.

Figs. 1—2. Rhizoids, obtained from the transfer of the holotype. — 300/1.
Fig. 3. Cluster of rhizoids. — 100/1.

Fig. 4. Rhizoid with a central zone of dark material (cf. Fig. 2). — 300/1.
Fig. 5. Proximal part of thallus segment with ventral scales. Xylol. — 4/1.
Fig. 6. Portion of the same, still more enlarged. Ordinary light (orthochromatic). Xylol. — 12/1.
Fig. 7. The same, photographed in infra-red light. Xylol. — 12/1.
Fig. 8. Balsam transfer of the segment seen in Figs. 5—7. Preparation immersed in water when photographed. — 12/1.

Plate 3

Figs. 1—8. *Marchantites Hallei* n.sp. — Lago San Martin, Rio Fósiles (loc. *c* in Halle 1913).
Fig. 1. Lateral portion of the transfer made of the holotype, enlarged to show air-pores (arrows). Preparation immersed in water when photographed. — 25/1.
Fig. 2. The segment shown in Fig. 1, Pl. 1, enlarged. (Halle 1913, Pl. 5, Fig. 14). Xylol. — 4/1.
Fig. 3. Part of the transfer made of the segment seen in Fig. 2, enlarged to show air-pores. Water. — 25/1.
Fig. 4. Air-pore from the segment seen in Fig. 2, enlarged. — 300/1.
Fig. 5. The segment shown in Fig. 3, Pl. 1, enlarged (Halle 1913, Pl. 5, Fig. 16). Xylol. — 4/1.
Fig. 6. Fragmentary segment. Xylol. — 4/1.
Fig. 7. Transfer of the same, still more enlarged. Preparation immersed in water when photographed. — 6/1.
Fig. 8. Rhizoid from the transfer of the holotype. A ± spiral structure is indicated (cf. Fig. 8). — 300/1.
Fig. 9. *Marchantia polymorpha* L. Recent.
Rhizoid showing a spiral structure in the wall. — 300/1.

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Some Remarks on the Structure of the Gametes and the Reproduction of Ulva lactuca

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The life cycle of *Ulva lactuca* is well known. There is an alternation between a haploid sexual generation, the gametophyte, and a diploid asexual one, the sporophyte. There is no difference in habit between individuals belonging to the two generations. It must be pointed out, however, that specimens belonging to the asexual generation are found only very rarely at the Swedish West coast. As female gamets are able to give rise to new plants without fertilization, it must be assumed that *Ulva lactuca* at least to a certain extent is reproduced by parthenogenetic female gamets in this area.

Gametes and Zoospores

The gametes are pyriform, two-ciliated, the male ones being somewhat smaller than the female ones. They are formed in a very distinct marginal fringe of the *Ulva* thallus, where the cells are transformed into gametangia, each one producing several gametes, which can be seen moving about inside the gametangium before liberation. Contrary to the female gametes the male ones do not contain any chlorophyll, the only pigments are carotinoids. Their colour is therefore yellowish. The female gametes are greenish. Due to the difference in colour of the gametes the fertile marginal fringe of a female plant is olive green, the one of a male individual yellowish. When liberated the gametes are moving very rapidly. They swim towards the light and are thus positively phototactic.

The zoospores are formed in a similar way as the gametes. They are four-ciliated, green, and slightly larger than the female gametes. Like the gametes the zoospores are primarely positively phototactic,

but after a while the reaction is changed, they swim from the light, settle down and lose their mobility. After about 24 hours the zoospores start to grow.

Studied in a polarizing microscope no birefringence could be observed in the surface of gametes or zoospores. But they do contain a very strongly birefringent body, the pyrenoid. Obviously the birefringence is caused by starch.

Swarmers were also studied with phase contrast optics. They are surrounded by a thin gelatinous coat. This corresponds to observations already made with spermatozoids of various fucoids (Levring 1952 p. 533).

Normal Fertilization

If suspensions of male and female gametes are mixed, the gametes immediately gather into small groups. The movements of the swarmers are also getting quicker. Group formation and increased mobility are thus the preliminary indications of an approaching copulation.

After a few minutes the male and female gametes have copulated two and two. In this stage the young zygots are four-ciliated like the zoospores, but contain two chromatophores and two eyespots. The movements are gradually getting slower and the phototactic reaction is changed to negative. The zygotes thus swim from the light, they soon lose their mobility and settle down. After 24 hours they start to grow. During this resting time they are surrounded by a wall, which increases in thickness. This is easely observed by using phase contrast optics. From the beginning the wall of the young zygote did not show any birefringence, but after about 24 hours a gradually increasing birefringence, negative in radial direction, could be observed. As the wall at this stage was stained blue by zinc chloride-iodine mixture the birefringence obviously is caused by the occurrence of cellulose with the molecules arranged tangentially to the surface of the zygote, which has a spherical shape. It is rather interesting to note that the wall of the young zygote primarely does not seem to contain cellulose. This substance is interstratified secondarely. After 24—36 hours the first signs of a cellular division can be seen.

The Occurrence of Polysaccharide Sulphates

In order to study the occurrence of polysaccharide sulphates, *i.a.* toluidine blue was used as a reagent. The colourless part close to the

cilia attachement of as well male as female gametes was stained metachromatically by toluidine blue. The gametes thus contain polysaccharide sulphates. According to Kylin (1946) the two substances ulvin and ulvacin occur in the cell walls of *Ulva* and *Enteromorpha* together with cellulose. Ulvin is a polysaccharide sulphate containing methylpentoses; the constitution of ulvacin is unknown. It seems therefore very likely that already the gametes contain ulvin or a related substance. It may also be of interest to point out that polysaccharide sulphates — probably fucoidin — occurs in the spermatozoids of fucoids (*cf.* Levring 1952 p. 533).

The zygotes — as well the membrane as the colourless parts of the cytoplasm — gave a very strong metachromatic reaction with toluidine blue. Especially the walls were stained very intensely. Obviously polysaccharide sulphates are released after the copulation. These substances are important constituents of the membrane, where they occur before the cellulose, which is introduced secondarely. A corresponding polysaccharide sulphate formation has been observed in eggs of fucoids after fertilization (*cf.* Levring 1952 p. 535).

Respiration of Gametes and young Zygotes

The respiration of gametes, copulating gametes and young zygotes were studied by means of the well known Warburg method. Thereby suspensions of male and female gametes were used. They were diluted so that the concentration, *i.e.* the number of gametes per cm^3, of male and female suspensions was made as equal as possible. The results of two such experiments are represented in Figs. 1 and 2. If the sum of the respiration of male and female suspensions is compared with the one of the zygotes, *i.e.* where the two suspensions have been mixed and copulation has thus taken place, it is obvious that the respiration intensity increases as soon as fertilization takes place. From Fig. 1, where readings were taken every 15 minutes, it can be seen that the oxygene consumption is especially high just after the mixing of the gametes, *i.e.* the period of group formation, increased mobility and copulation. As soon as fertilization has taken place the increased oxygene consumption is kept on a rather constant level. This increase is of the order of 50—60 per cent indicating an intensified cellular activity after fertilization.

In the experiment represented in Fig. 1 the respiration of single gametes and zygotes was calculated approximately. The oxygene con-

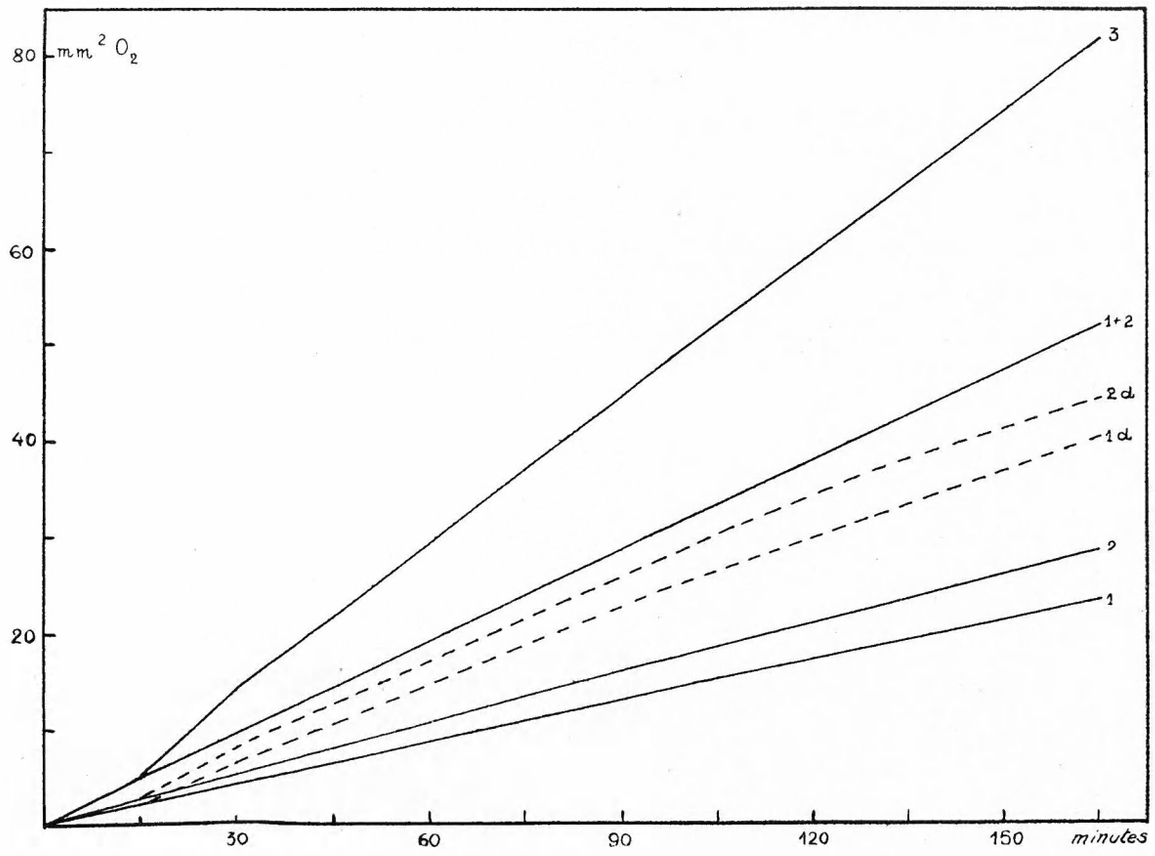

Fig. 1. Respiration of *Ulva* gametes and zygotes: Suspension of female (1) and male (2) gametes; combined respiration of male and female gametes (1+2); zygotes (3) — copulation at 15 minutes; respiration of female gametes with 0.001 % dupunol (1 d) and male ones (2 d) — addition of dupunol at 15 minutes.

sumption of a single male gamet was $0.7 \cdot 10^{-7}$ mm^3 per hour, and the corresponding figures for a female gamete $0.6 \cdot 10^{-7}$ and a zygote $2.0 \cdot 10^{-7}$.

Influence of Dupunol

The blocking effect of dupunol (a detergent with surface active features manufactured by the firm Du Pont in U.S.A.) on the fertilization of fucoids and other organisms is known before (*cf.* Levring 1949; 1952; Runnström *et al.* 1946). Dupunol is a mixture of sulphonates of long-chain aliphatic alcohols differing only in the length of the hydrocarbon chains (*cf.* Putnam 1948).

Male and female gamets were mixed in dupunol solutions of the following concentrations: 0.005, 0.002, 0.001, 0.0005 and 0.0001 per cent dupunol in sea water. In the first three cases no copulation or group formation took place. With 0.0001 per cent dupunol copulation

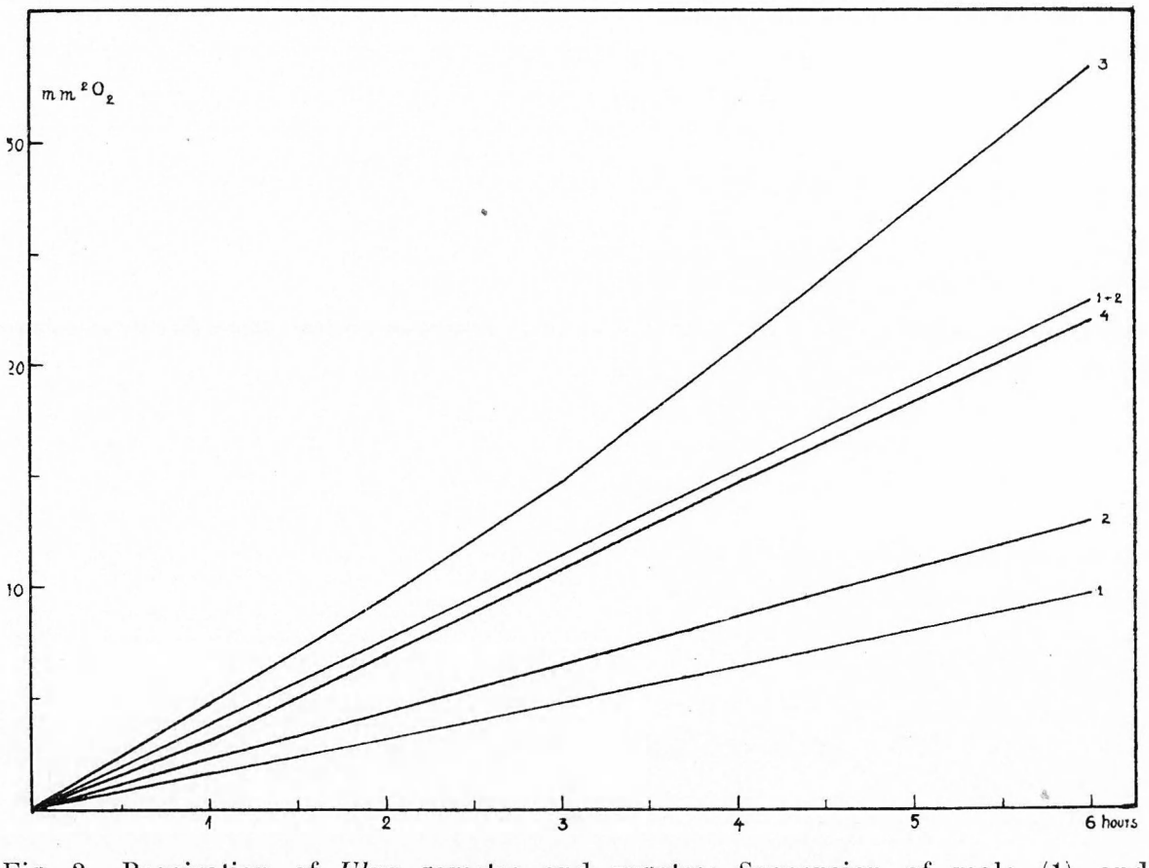

Fig. 2. Respiration of *Ulva* gametes and zygotes: Suspension of male (1) and female (2) gametes; combined respiration of male and female gametes (1—2); zygotes (3) — copulation at 0; respiration of female gametes with 0.001 % dupunol (4).

was normal, and 0.0005 per cent gave an intermediate effect. In another series of experiments male and female gametes were treated separately with dupunol solutions of active concentration (0.001 per cent) and then transferred into pure sea water (by centrifuging). Afterwards the suspensions were mixed. The result was that male gametes, which had been treated with dupunol, were able to copulate with untreated female ones. If untreated male and treated female gametes were mixed no group formation or any copulation could be observed. Treated male and treated female gametes did not copulate either. It is thus very interesting to note that if female gametes are treated with dupunol solutions (0.001 per cent) the surface of the gamet seems to react with the dupunol molecules, the surface is blocked and a normal fertilization thus made impossible. But a corresponding treatment of the male gamets has no such effect. The surface of male and female gametes must therefore have a different structure in some respects. Dupunol was also found to have another rather remarkable effect on

female gametes. During the treatment the phototactic reaction was gradually changed from positive to negative. Thus the same phototactic phenomenon could be observed as during normal fertilization. From Figs. 1 and 2 it can also be seen that the respiration is increased by about 40—60 per cent for both male and female gametes.

Obviously dupunol reacts with the surface of the female gametes and blocks normal fertilization. Thereby certain enzym systems are released or activated in a similar way as in normal fertilization. The result is an increased respiration intensity and a changed phototactic reaction. The surface of the male gametes seems to be of a different structure. However, respiration intensity is increased.

Summary

From the experiments described above it should be obvious that the preliminary effect of the copulation is a surface reaction. It is very interesting to note the corresponding effect of dupunol on female gametes. This preliminary effect is a blocking of the surface and an activation or release of certain enzym systems, which cause a changed phototactic reaction, formation of polysaccharide sulphates, and an increased respiration. At normal fertilization this effect may be caused by a surface active substance — like dupunol — which is released in connection with the copulation. Very soon the mobility is lost. The polysaccharide sulphates are important constituents of the wall formed after the fertilization. Cellulose is introduced secondarely.

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Some Determinations of Elementary Constituents in Mire Plants and Peat

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(Meddelanden från Lunds Botaniska Museum, Nr 109)

Contents

Introduction

One of the chief aims of plant ecology is to study how composition and production of vegetation are influenced by available nutrients and other substances taken up by plants from their substrate or medium. Of special interest are sites where vegetation and soil are undisturbed by man, as in many Swedish mires. There the soil as a rule consists of peaty deposits formed from the vegetation itself, with a small but

important addition of atmospheric material, and in fen sites, material dissolved by water having percolated through more or less minerogenous layers before its arrival in the fen. Our investigations in this field are now extended to chemical analyses of the mire plants.

As published investigations on chemical constituents in mire plants are few and of limited applicability in ecological discussion, we first had to gather some information to guide us in the selection of suitable plant material and to show the magnitude of variation in single species. This task was the main object of Sjörs' investigation. During a visit to North Sweden in the summer of 1953, he had opportunity to collect samples in a calcareous district in Jämtland and in a strongly contrasting and decidedly non-calcareous district in Dalarna. Malmer's samples from a Småland mire have been collected as part of an investigation which is still in progress. Although the material ready for publication is scanty, a preliminary discussion of some relations between the chemical composition of mire plants and their environment is in order, particularly in Malmer's part of the investigation — in which it was possible to compare the plant analyses with corresponding analyses of the substratum (peat and water). In view of the preliminary nature of the present results, detailed comparison with data in the literature would be premature.

The nomenclature of vascular plants follows Hylander 1941 (excepting *Oxycoccus*). *Sphagna* are named according to Waldheim (1944) and most other mosses according to Jensen (1939).

For terminology of the mires and their subdivisions, determined from phytosociological critera, reference is made to Du Rietz (e.g. 1949, 1954) and to Sjörs (1948, 1950, 1952).

Each author is responsible for his own data and the statements and conclusions derived from them. The sections on methods are written jointly, if pertaining to both authors' material. The chemical methods were tested in co-operation.

Each author has done his own field work; Sjörs was assisted by his wife, Fil. kand. Gunnel Sjörs. The analyses were made in the Laboratory of Plant Ecology in the Botanical Museum, Lund (Head of the Institute Professor Henning Weimarck). Miss Signe Kerwall and Mrs. Gunn Hansson gave technical laboratory assistance. Miss Inna Pärnamäe has performed the Kjeldahl analyses. Part of the Kjeldahl apparatus and the Beckman spectrophotometer were kindly placed at our disposal by Professor Hans Burström, Institute of Physiologic Botany, with whom we have also had the advantage of discussing some of our problems. The manuscript was also critically read, and the English revised by Dr. Eville Gorham, The Freshwater Biological Association,

Ambleside, England. We have received financial support from the Field Research Grants of the University of Lund and, for laboratory assistance, from the Swedish National Science Research Council. We wish to express our deep gratitude to all those who have aided in our investigation.

Methods

Sampling of Plants

Only a limited number of mire plants have been investigated. Most of them belong to the *Cyperaceae*; the others are *Menyanthes trifoliata* and some *Sphagnum* species. Thus, all woody species and all herbs except *Menyanthes* are unrepresented. Data in Mattson & Karlsson (1945), Tamm (1951 b), Mayer & Gorham (1951), and Gorham (1953 b) show that some of these differ considerably from the plants investigated by us in constituent proportions. *Eriophorum vaginatum* is similar to *Scirpus caespitosus* (lower in P, Tamm in press).

For practical reason those part of the plants were collected which could be obtained as a well-defined reasonably homogeneous sample, not too small in quantity. A little more than 2 g is the desirable minimum amount for analysis. Several of our samples proved to be much too small; in such cases dry ashing was omitted. We were also compelled to omit nitrogen analysis in most of these cases because this was made afterwards. It may be possible to analyse as little as 0.3 g for Na, K, Ca, Mn, Fe, P, N, and in some cases SiO_2. The production of dry matter is very low in many mire plants, so 2 g is not always easily gathered.

Our material was generally insufficient for comparison of various organs in the same plant. However, Malmer has analysed both fertile shoots and roots in *Rhynchospora alba*. The roots were rinsed in distilled water. The fertile shoots analysed include stem and leaves in *Rhynchospora alba* and *Eriophorum latifolium,* and stem in *Scirpus caespitosus, S. hudsonianus* and *Schoenus ferrugineus,* the stem being the only photosynthesizing organ in the latter plants. The inflorescence was removed, and in the *Scirpus* species also the uppermost part of the stem, which becomes dry at an early stage of fruiting. The soft, whitish basal parts were also removed. In *Menyanthes* only leaflets of fully developed leaves not yet subject to decay were used, i.e. the oldest and youngest leaf of a sterile shoot were mostly discarded. In sterile *Carex* shoots, leaves which were too old and dry or too young and soft were discarded, and the soft basal parts removed. The *Sphagnum* material consists of the uppermost, entirely living parts of the plants.

There is probably some variation with season or age (or both) in mire plants as well as in forage plants or in leaves of woody species (cf. Tamm 1951 a). This variation may be of some influence when we compare the material from Jämtland with that from Dalarna, because the latter was collected nearly one month later. However, the stage of development of the samples was not very different.

The plant samples were dried in a warm place indoors (not in the sun). Before analysis, they were cut into short pieces, which were well mixed. The homogeneity may be regarded as sufficient without grinding when 1 g or more

is subjected to dry or wet ashing. Grinding hardly promotes the ashing procedures, and may also involve iron contamination (a possibility not investigated by us). However, the small samples for nitrogen were taken from ground material. The handling of the material is likely to add small amounts of salt to it, which is a source of error when sodium is determined in small quantities. All samples were dried at 105° before analysis, to obtain dry weight.

Chemical Analysis of Plant Material

Dry ashing of about 1 g was performed in an electric furnace at about 550°. The ashes were dissolved in 0.1 *n* HCl, and the excess back-titrated with 0.1 *n* NaOH (mixed indicator, methyl red+bromocresol green) according to Gorham (1953 b p. 347). The titration is somewhat disturbed by manganate present in considerable quantity in some ashes, MnO_4^{2-} reacting both with HCl and with the indicator. The results are given as "excess base" in Tables 1—3 (m.equiv. per g dry plant material).

Total ash in a plant and its "excess base" are obviously dependent on so many variable terms that they are of limited significance, except as indication and check of other values and for comparison with published figures.

Wet ashing was carried out mainly according to Tamm (1953 p. 14), but the quantity of $HClO_4$ was reduced without inconvenience. One g of plant material, 25 ml conc. HNO_3, and 5 ml conc. $HClO_4$ were used. For *Sphagnum* and other plants of low ash content 2 g plant material and 35 or 40 ml HNO_3 are preferable; the amount of $HClO_4$ need not be increased. — To avoid bumping, the best procedure turned out to be as follows. Slow heating was begun immediately, and gas evolution kept at moderate speed; when the organic substance was almost dissolved heating was increased to maintain continuous boiling. The flask was covered by a small glass bowl containing water, in order to condense most of the nitric acid vapours and thus make it possible to boil for 20 minutes after the liquid was clear. Then the bowl was removed and the acids boiled off to nearly complete dryness (over-heating was avoided). 50 ml boiling water was added, and insoluble matter was filtered off, washed with 1 : 200 HCl, ashed at 550°, and weighed; it is regarded as (crude) SiO_2. The filtrate was diluted to 100 ml, and used for the other determinations mainly according to the procedure recommended by Toth, Prince, Wallace & Mikkelsen (1948).

Of the filtrate 25 ml were evaporated to dryness and heated to expel $HClO_4$ completely (cf. Tamm l.c.). The residue was heated with 2 ml 10 % HCl, evaporated to dryness, and again dissolved in 5 ml 0.1 *n* HCl and diluted to 25 ml; of this solution 20 ml were used for colorimetric determination of Mn after evaporation with phosphoric acid and oxidation with periodate to permanganate. The remaining 5 ml were sufficient for the spectrophotometric determination of Na, K, and Ca in a Beckman apparatus ("gasol"+oxygen flame). Blanks were run, and subtracted. They were very low, and of no importance except for Na. The possible influence of other ions was not investigated but may lead to low results in Ca.

Iron was determined colorimetrically in 5 ml or less of the original filtrate, using hydroxylamine hydrochloride and orthophenanthroline.

Phosphorus was determined in 2 ml of the original filtrate, after evaporation with nitric and sulphuric acids and with hydrochloric acid, according to Tamm (l.c.). The rest of the procedure followed Zindzadze's method (1935) according to Knutsson (1949).

Sulphur was determined by $BaSO_4$ precipitation from 50 ml of the original filtrate, adjusted to about pH 3. For complete precipitation, a more strongly acid reaction is objectionable.

Magnesium was determined by the thiazol yellow method (Toth & al. op.c., Mikkelsen, Toth & Prince 1948). This method is of low accuracy, and the determinations on Sjörs' samples had to be discarded because the colour became qualitatively different in the samples and standards. We have not yet had opportunity to try the practical convenience of other methods for determination of small quantities of Mg.

Nitrogen was determined in a ground portion of the original sample. Only 0.02—0.05 g were taken out for the micro-Kjeldahl procedure (50 ml flask; 1.5 ml H_2SO_4 and 0.5 g catalyst, composed of 100 K_2SO_4+10 crystalline $CuSO_4$+7.5 $HgSO_4$; also a few drops of 30 % H_2O_2 during heating). The samples were distilled in a Nicroma apparatus, the ammonia collected in 0.02 *n* HCl, which was titrated back with 0.01 *n* NaOH; a mixture of bromocresol green and methyl red was used as indicator. The determinations were made in closely agreeing duplicates, and a blank was run in each series; the small blank values were subtracted.

The accuracy of the published determinations is regarded as satisfactory except in very low figures for SiO_2 and Na and possibly concerning Ca. An extremely low value for Mn was checked in a duplicate (*Carex limosa,* Table 1 sample 519). Another very low value, for P in the same species, sample 529, could not be checked and is omitted. Most of the determinations on *Rhynchospora alba* shoots were duplicated with good agreement.

Analysis of Peat

For volumetric collection of peat samples a steel cylinder (height 9 cm and volume 450 cm^3) with lower cutting edge was used. The samples were taken between about 3 and 12 cm below the surface of the peat. After superfluous water had run off the samples were transferred to a polythene bag or a cardboard pot coated with paraffin for transport to the laboratory. Contact with air was avoided as much as possible. Immediately after arrival in the laboratory the samples were weighed in order to determine the weight/volume ratio. Subsamples were taken out and weighed separately for the following analyses. Living roots, larger branches etc. were avoided.

pH was determined with a glass electrode inserted directly into the peat. A calomel electrode with an asbestos plug in the tip was used as reference electrode. Several determinations were made on every sample. The differences between determinations on the same sample seldom exceed 0.1 pH-units.

Water content and dry matter were determined by drying at 100—110°C. Samples were ashed in a muffle furnace at 550°C. Some determinations were undertaken on the dry ashes. They were then treated with HCl according to

Piper (1950, p. 264—265). Various determinations on the solutions were made by means of the methods mentioned for plant samples (p. 49).

Total nitrogen was determined on dry peat by the micro-Kjeldahl method (duplicate samples). Cf. plant samples, p. 50.

Exchangeable ions were extracted with normal acetic acid (HAc) and normal ammonium acetate (NH_4Ac). Samples of about 35—40 g were taken from the wet peat and shaken for 2 hours with 250 ml of the extraction solution. After the samples had stood overnight, the change in pH of the supernatant liquid was measured to determine exchangeable metallic cations and exchangeable hydrogen ions in the HAc and NH_4Ac solutions respectively, according to Brown's method (Brown 1943; Gorham 1953 a, p. 129; Sjörs 1954, p. 73—74). The exchange capacity was calculated as the sum of exchangeable metallic cations and exchangeable hydrogen ions. The degree of neutralization (=percentage base saturation, Gorham op.c.) gives the exchangeable metallic cations as percentage of the exchange capacity.

These methods to determine exchangeable cations have been used by Gorham (1953 a, b) on English mineral soils and mires and by Sjörs (1954) on Swedish mineral soils. They are relatively rough methods, but with a good pH-apparatus and careful work they give satisfactory results. Above all they are rapid and permit a larger number of determinations than would be possible with the perhaps slightly more accurate but equally empirical methods which have been proposed e.g. by Schollenberger and Simon (1945) or Piper (1950). All ion-exchange methods are empirical and depend on nature and strength of the extractant. If the pH of the original HAc and NH_4Ac respectively is determined between every sample measurement, the change in pH can be measured with a deviation less than ± 0.02 pH-units. This corresponds to about ± 3 m.equiv. per 1000 ml wet peat. The ultimate pH is about 2.4—2.5 in the HAc-extraction while it is about 6.6 in the NH_4Ac-extraction. Thus the extractions are made at pH values which differ considerably from the pH of the peat.

The solutions were filtered for determination of the amounts of the separate ions. They were then clear but slightly brown-coloured by soluble humus. When the volume was measured, the solutions were evaporated to dryness and treated with conc. HNO_3 and 70 % $HClO_4$ (4 : 1) in order to destroy organic matter. The residues were dissolved in HCl and diluted to 50 ml. This concentration was suitable for determination of most of the ions with the same methods as were used for the plant samples (p. 49). As the samples were worked up in wet condition one had to make the necessary corrections for the dilution of the extraction solution in calculating the results.

In Table 4 the results of parallel extractions from five peat samples are put together. It appears from these determinations that similar amounts of Na, K, Mg and Ca are exchangeable with the two extraction solutions. However, large differences are met with concerning iron. These may depend on the fact that ferric iron is insoluble but ferrous iron soluble at the ultimate pH of the NH_4Ac extraction. At the ultimate pH of the HAc extraction both ferric and ferrous iron are soluble. Very probably more iron is extracted with normal HAc than is in the purely exchangeable state.

Especially in the analyses from site I (situated in bog vegetation) the sum

of exchangeable metallic cations calculated from the ionic determinations is somewhat higher than the same values calculated from the change of pH in the HAc-solution. This may be due to ± strong acids (probably organic) having in this case diminished the pH change. With these methods one determines the excess of exchangeable metallic cations and the sum of exchangeable hydrogen ions and free acids respectively.

During the determination of exchangeable ions with these methods, that fraction which is dissolved in the water is also measured. It is, however, evident from the results that among the cations only Na^+ is dissolved in the water to more than a few per cent of the total exchangeable content.

Analysis of Water

pH was determined with a glass electrode. On one water sample some ionic determinations also were made. After evaporating to dryness the sample in this case was treated with $HNO_3 + HClO_4$ (4: 1) in order to destroy all organic matter. Then the residue was dissolved in HCl. The succeeding determinations of the ions were made according to the methods described on p. 49.

Plant Samples from Dalarna and Jämtland

Sampling Sites in Dalarna

The material from Dalarna was collected by Sjörs on August 6 to 11, 1953. The district is situated around Lake Tisjön (430 m above sea-level) in Lima parish, NW part of the province, near Värmland and not far from the Norwegian boundary. This is in the part of the Scandinavian peninsula most distant from the sea, and the content of sodium and chloride (and probably magnesium) in the precipitation may consequently be assumed to be relatively low (cf. Emanuelsson & al. 1954). Actually, an analysis of superficial ombrotrophic bog hummock peat from Tisjökölen showed less Na (and Ca) but slightly more K per g dry matter than Malmer's analysis from site I: hummock (Table 5); neutralization was only slightly lower (13 %) but ash content as low as 1.3 % of the dry weight. However, the peats are not strictly comparable, because that from Tisjökölen has only half the content of dry matter per unit volume. Cf. also p. 56.

The bedrock is probably granitic but nearly always covered by glacial deposits. These consist of gravelly or sandy glaci-fluvial material around all the E and SE sides of the lake, and of rather coarse till (ablation moraine) rich in large boulders to the W of these deposits. The vegetation is of very poor types: pine forest with *Calluna* and lichens prevails on the glaci-fluvial deposits, and low-productive pine or spruce forest with *Vaccinium* and mosses on the till. Next to the lake

most of the country is flat and there are several extensive mires. Some of these are more or less sloping poor fens or intermediate fens (Sjörs 1952 p. 248); samples 170, 174, 508 were collected in these. Other mire sites are quite flat and more or less quaking fens along brooks, and still others (only on sandy plains) have developed into ombrotrophic bogs (Du Rietz 1954 p. 572). The latter are mostly eccentrically developed (Sjörs 1948) and may contain numerous pools replacing the normal hollows.

This is particularly the case on Tisjökölen (op.c. Plate 18 A), a large mire complex sloping gently from the W edge towards the N, E, and S. Large parts of this mire are purely ombrotrophic bog (samples 503, 518, 506, 502, 519). Other parts may be weakly influenced by water from mineral soil, but the botanical signs of this are somewhat ill-defined on this mire. A "soak" carries water across it and is distinct by its green colour and somewhat more lush vegetation, but of "Mineralbodenwasserzeiger" ("exclusive fen plants") in the sense of Du Rietz (1954), only *Carex pauciflora* and a few *Sphagna* are present; the chief vascular dominant is *Scheuchzeria palustris*. Sample 501 (Table 1) is remarkably luxuriant *Scirpus caespitosus* from a place where the soak is narrow and the flow of water may be appreciable between the *Scirpus* tussocks.

Some of the lower pools contain a few "exclusive fen plants", mainly *Sphagnum pulchrum* and *Menyanthes trifoliata*. It seems likely that there may be some connection with the water in the subjacent sand, although there is a peat layer below the pool bottoms. During July 1948, in one of the *Menyanthes* pools (E), two young of the red-throated diver (smålom, *Colymbus stellatus*) were constantly provided by their parents with small fish caught in Lake Tisjön. If the breeding is regular, this may imply a considerable addition of nutrients to this pool, as the young never leave the pool until they are able to fly (end of July). Pool D is too small and pool F too shallow for the diver. — Another peculiar thing was noticed (in August 1953) in pool E: elk (moose) had dug up and eaten *Menyanthes* rhizomes. *Menyanthes* appeared damaged in the pool, probably not only for this reason, and had diminished much since 1948.

Lake Tisjön (Sjörs 1949) has been used for water-storage by hydro-electric power plants since 1948, and the water level is raised during the vegetation period to a level about 130 cm above the former summer low water level. The effect on the shore vegetation is mainly destructive, but some plants continue to live. The *Menyanthes* sampled as

no. 171 grew originally on thin shore peat but was submerged, with only a few leaves reaching above water. A brook, Torsborån, tributary to Lake Tisjön, is also influenced by the high water. The fen alongside it is submerged, except for small islands of floating peat in places where the content of gases (and of intercellular air in living roots and rhizomes) had caused the peat to rise to the surface. In submerged places, only a few specimens of tall plants like *Carex lasiocarpa* (sample 510) and *Menyanthes* (520) reached above the water surface; most other vascular plants had been killed. The surviving plants were extremely luxuriant; *Carex lasiocarpa* reached on an average about 90 cm and maximally 110 cm above water, which was 60 cm deep in this place, and *Menyanthes* reached with its leaves above 80 cm of water. Note the absence of competition in this site as well as in no. 171. (Submerged parts were never used for analysis.)

Floating peat has also been formed from quagmires in several shallow bays of Lake Tisjön itself. As along the brook Torsborån, the original status of vegetation had been mainly that of a "moderately poor fen" (="transitional poor fen"). The plants (samples 511 and 521) had become markedly more luxuriant after the rise in water level, especially along the edges beside open water. This effect seems thus to be due to contact between root systems and circulating lake water, which is of course rich in oxygen but not in solutes; on the contrary this lake has a very low content of electrolytes ($\varkappa_{20} = 10 \cdot 10^{-6}$ before and $13 \cdot 10^{-6}$ after the damming; $13 \cdot 10^{-6}$ in Torsborån after the damming). See further p. 63.

Unfortunately the plant material from the Tisjön district cannot be accompanied by corresponding peat and water analyses; for various reasons those that were made cannot be published without supplementation and corroboration.

The following are some complementary notes on the vegetation of the sampling sites:

503 (A). Almost pure, apparently ombrotrophic *Scirpus caespitosus* "lawn" with *Sphagnum balticum*. *Scirpus* of medium size. 518 is near-by, at the edge of a wetter part of the hollow: *Carex limosa* "carpet" with *Sphagnum Dusenii* and *Lindbergii*; presence of some *S. papillosum* may suggest that conditions are not purely ombrotrophic here. (Contrary to conditions in the Åkhult mire, *S. Dusenii* is often present but *S. papillosum* generally absent on the ombrotrophic bogs in this district.)

506 (B). Small hollow. *Scirpus* rather small in size. Apparently ombrotrophic.

502 and 519 (C). Small hollow, apparently purely ombrotrophic. *Scirpus* (small size) and *C. limosa* in *Sph. Dusenii* "carpet" with scattered *S. balticum*.

504. *Scirpus* tall, in water oozing from a hollow above.

501. Soak; see above. *Scirpus* very tall.

507 and 523 (D). *Scirpus,* of medium size, growing together with *Eriophorum vaginatum, Andromeda polifolia, Sphagnum balticum, S. tenellum, S. Lindbergii,* etc.; and *Menyanthes,* the only "exclusive fen plant", mainly with *S. Dusenii,* at the edge of a small pool.

505 and 526 (E). Large pool, see above. *Scirpus* of medium size. Besides *Menyanthes* and very sparse *Carex pauciflora,* another "exclusive fen plant", *Sphagnum pulchrum,* occurs abundantly.

528, 529 and 522 (F). A large hollow, in part developed as a shallow pool. Extensive carpets mainly of *Sph. Lindbergii* and *Dusenii*; in certain parts *S. pulchrum. Scirpus,* of medium size, was collected along the edge of the pool, *C. limosa* in shallow water, and *Menyanthes* in a depth of about two dm.

510 and 520. Brook Torsborån, see above. *C. lasiocarpa, Menyanthes, C. rostrata, Utricularia intermedia* and *minor* and some submerged *Sphagna* were the only living plants.

511 and 521. Floating fen of 1.0—1.5 m peat (in 1.8 m water). *C. lasiocarpa* tall and fertile particularly at the edge; other dominants *Calamagrostis purpurea, Menyanthes, Carex rostrata, Sphagnum apiculatum, S. papillosum*; other plants *Betula nana, Salix lapponum, Oxycoccus quadripetalus, Peucedanum palustre, Potentilla palustris, Lysimachia thyrsiflora, Carex limosa, Sphagnum riparium, S. parvifolium, S. subsecundum, Drepanocladus fluitans, Calliergon stramineum* (the latter three only in the marginal zone).

170. Strongly sloping poor fen near the lake. Dominants *Menyanthes, Carex rostrata, Sphagnum pulchrum*; other plants *Andromeda, Oxycoccus quadripetalus, Eriophorum vaginatum, Carex chordorrhiza, C. pauciflora, Equisetum fluviatile, Sphagnum Lindbergii.*

508 and 174. Slightly sloping "intermediate" fen with some "flarks". Sample from "lawn" community: dominants *Scirpus caespitosus* (tall), *Molinia coerulea, Sphagnum warnstorfianum, parvifolium* and *subfulvum*; other plants *Pinus silvestris* (young), *Juniperus communis, Betula pubescens* (young), *B. nana, Calluna vulgaris, Andromeda polifolia, Oxycoccus quadripetalus, O. microcarpus, Menyanthes, Succisa pratensis, Viola palustris, Trientalis europaea, Potentilla erecta, Selaginella selaginoides, Drosera rotundifolia, Carex dioeca, C. lasiocarpa, C. echinata, C. pauciflora, Eriophorum angustifolium, E. vaginatum, Sphagnum teres, S. pulchrum, S. papillosum, S. magellanicum, Calliergon stramineum, Aulacomnium palustre, Pleurozium Schreberi, Polytrichum strictum, Scapania paludicola.*

The Sampling Site in Jämtland

The Jämtland samples were all collected on July 13, 1953, in the same locality, viz. the mire Forsflon W of the outlet of Lake Solbergsvattnet, Hammerdal parish. This mire is situated near the eastern border of the Cambro-Silurian of Central Jämtland. The country is covered by glacial drift rich in lime. All the mire is calcareous, although superficial deposits

containing $CaCO_3$ are rare in it, except for ephemeral thin crusts which are formed in the "flarks" in dry summers.

Electrical conductivity and Ca content have been determined in water from several parts of the mire ($\varkappa_{20}$ 191 to $286 \cdot 10^{-6}$; Ca 46 to 74 mg/l in waters with $\varkappa_{20}$ 198 to $286 \cdot 10^{-6}$). The water pH determinations were unsuccessful; in similar sites pH is as a rule well above 7 (Witting 1949, Sjörs 1952).

Two peat samples (not from the sampling site) showed much exchangeable Ca^{2+} and almost complete adsorptive neutralization (="base saturation"). One of these samples was from a site with *Cypripedium calceolus*, drier than that of the plant samples, and several hundred m away from the latter, and had a pH of 7.3 (fresh peat in water suspension) and 100 % neutralization. Total ash was 69 ‰, exchangeable Ca^{2+} c. 28 ‰ in fresh and c. 15 ‰ in dry peat, exchangeable K^+ 1.5 ‰ and Na^+ 0.35 ‰ in dry peat, all values in terms of dry weight. Values calculated per litre volume of fresh peat: dry weight 92 g; Ca^{2+} c. 130 or c. 70, K^+ 3.5, Na^+ 1.4 m.equiv. Thus Ca^{2+} is about 45—25 times that of the Tisjökölen bog hummock and 28—15 times that of the Åkhult bog hummock, if given per unit dry weight, or about 100—60 times and 30—16 times, respectively, if given per unit volume. The corresponding ratios are for K^+ 2 and 3 times per unit dry weight and 5 and 3.5 times per unit volume, and for Na^+ 3 and 1.8 times per unit dry weight and 5 and 1.5 times per unit volume. Although the figures from Forsflon are unfortunately not applicable to the actual sampling site, they show that the dominant position of the calcium ion is very strong but also that the absolute values for other cations may be distinctly higher in the calcareous fen than in ombrotrophic bogs. In ion uptake by plants, this supply is counteracted mainly by Ca^{2+} in the former and by H^+ in the latter (ion antagonism).

The Forsflon mire is mainly developed as "extreme rich fen", remarkable for a very rich flora (e.g. *Cypripedium calceolus, Epipogium aphyllum*), with several wooded islands and also numerous low hummocks made up in part of *Sphagnum fuscum*. These hummocks generally contain fen plants and are in this case best regarded as a drier, although obviously unstable stage in the successions occurring in the rich fen (cf. Du Rietz 1949 pp. 298, 304), not as isolated bog hummocks. Between them are "lawn" and some "carpet" and "mudbottom" communities. Regular "flarks" systems are not well-developed, probably because of a limited flow of water.

In the lower part of the mire, but well above the reach of high waters in Lake Solbergsvattnet, extensive but somewhat discontinuous "lawns" occur in which *Schoenus ferrugineus* is one of the chief dominants. The samples in Table 3 were gathered within a 20 m^2 plot in this area, and all species in the Table grew mixed together as co-dominants of medium size; moss dominant generally *Campylium stellatum*. Other species: *Pinus silvestris* (young), *Betula nana, Salix myrsinites, Empetrum* sp. (sterile), *Andromeda polifolia, Oxycoccus quadripetalus, Tofieldia pusilla, Pinguicula vulgaris, Melampyrum pratense, Drosera rotundifolia, Equisetum fluviatile, Carex dioeca, C. rostrata, Sphagnum warnstorfianum, Bryum pseudotriquetrum, Cinclidium stygium, Drepanocladus badius, D. intermedius, Fissidens adianthoides, Mnium Seligeri, Tomenthypnum nitens, Leiocolea Schultzii,* and *Riccardia pinguis.*

In addition, two samples of *Menyanthes* (Table 2) were collected near-by, on low hummocks. Sample 527 was taken from a community dominated by *Betula nana* and *Empetrum nigrum* (the dioecious, southern *Empetrum* has one of its northernmost known Swedish occurrences here); moss dominants *Sphagnum fuscum* and *Pleurozium Schreberi*; total flora 19 vascular plants and at least 18 mosses and lichens. Sample 524 was from an almost pure *Sphagnum fuscum* hummock, in part more bog-like, with *Rubus chamaemorus* dominant and *Empetrum nigrum* and *Betula nana* subdominant; total flora 16 vascular plants and a few mosses.

Variation between Species

The four species represented in Tables 1 and 2 show characteristic features. The content of all elements except Si differ remarkably little in *Scirpus caespitosus*. There is a distinct dominance of potassium (1/3 to 1/2 of total ash, if calculated as K_2O). Nitrogen, phosphorus, and usually sulphur and iron, are low. The chemical characteristics of *Scirpus caespitosus* stems are probably partly due to a low content of protoplasm and cell sap in relation to wall substance (*S. hudsonianus* and in part *Schoenus ferrugineus* show similar composition; see below). — Gorham (1953 b) and Mayer & Gorham (1951) found 27 ‰ ash and 0.23 m.equiv. "excess base" in *Scirpus caespitosus*; N was 16.1 ‰, Mn 0.43 ‰ and Fe 0.21 ‰.

Carex limosa is quite different. It shows large variation with regard to several elements investigated (note that leaves were analysed in this species). Both species grow in all kinds of mires, with regard to the "direction of variation" from ombrotrophic bog to calcareous extreme rich fen.

Carex lasiocarpa and *Menyanthes* are examples of exclusive fen plants, occurring in all kinds of fen, from extreme poor to extreme rich, but lacking in true bogs (Du Rietz opp.cc., Sjörs opp.cc., Gorham 1952). — Determinations in leaves by Gorham and by Mayer & Gorham (1951) are quoted below (p. 62).

The determinations on *C. lasiocarpa* leaves are too few and from too specialized sites for a definite judgment. They are able to take up much Si, and in the calcareous site, Ca almost reaches the K value (by weight).

Menyanthes leaves contain much mesophyll and have a higher content of protoplasm and cell sap than the *Cyperaceae* tissues. Ash and nitrogen contents are similar to those of many true aquatic plants (Gorham 1953 b), and the figures for most elements are much higher than in the preceding species, but there is a large variation. High values are often noted for K, P, N, and particularly for Mn. SiO_2 values are

Table 1. **Samples of three Cyperaceae species.**

Values in mg per g dry weight ("excess base" as m.equiv.).

Species and site	Ash Total	Ash "Excess base"	Na	K	Ca	Mn	Fe	SiO_2	P	S	N
Scirpus caespitosus, stems:											
503 Tisjökölen, bog hollow (A)	14	0.21	0.11	4.0	1.5	0.40	0.07	0.2	1.4	1.3	12
506 „ „ „ (B)	—	—	0.14	6.6	1.2	0.51	0.06	1.0	1.5	2.4	—
502 „ „ „ (C)	—	—	0.10	7.3	1.7	—	0.06	1.1	1.3	4.7	—
504 „ „ „ w. oozing water	15	0.18	0.09	5.7	1.2	0.38	0.07	0.0	1.2	1.2	15
501 „ soak w. *Carex pauciflora*	23	0.20	0.14	7.8	1.4	0.63	0.07	1.5	1.2	2.2	12
507 „ pool w. *Menyanthes* (D)	14	0.18	0.10	4.8	1.0	0.32	0.10	0.0	1.0	2.4	—
505 „ „ „ „ (E)	—	—	0.16	6.8	1.6	0.52	0.21	2.2	1.1	2.7	—
528 „ „ „ „ (F)	17	0.16	0.23	7.4	1.2	0.30	0.10	0.5	0.9	2.9	15
508 Örvikskölen, intermediate fen	32	0.16	0.09	7.4	2.0	0.68	0.13	2.3	1.4	1.5	16
500 Forsflon, calcareous extreme rich fen	32	0.25	0.10	10.0	2.1	0.52	0.11	6.8	1.2	1.8	14
Carex limosa, leaves of sterile shoots:											
519 Tisjökölen, bog hollow (C)	c. 17	c. 0.28	0.13	5.0	0.7	0.025	0.17	1.6	3.9	1.6	—
518 „ „ „ (A)	—	—	0.23	3.6	2.5	0.24	0.18	0.2	1.6	1.9	—
529 „ pool w. *Menyanthes* (F)	c. 25	—	0.24	12.5	1.5	0.16	0.11	1.0	—	1.4	—
515 Forsflon, calcareous extreme rich fen	31	0.33	0.10	8.2	4.6	1.16	0.24	8.5	2.2	1.2	20
Carex lasiocarpa, leaves of sterile shoots:											
510 Brook Torsborån	41	0.17	0.07	11.7	1.9	0.18	0.31	15.7	2.8	1.8	21
511 Floating fen in Lake Tisjön	39	0.22	0.14	10.9	2.7	0.76	0.15	14.4	2.5	2.8	19
509 Forsflon, calcareous extreme rich fen	41	0.31	0.06	6.5	6.0	0.76	0.14	16.3	1.9	1.6	20

Table 2. **Samples of Menyanthes trifoliata leaves.**

Values in mg per g dry weight ("excess base" as m.equiv.).

	Site	Ash	"Excess base"	Na	K	Ca	Mn	Fe	SiO_2	P	S	N
523	Tisjökölen, pool (D)	47	0.44	3.2	9.4	3.6	0.13	0.09	2.1	2.5	3.0	30
526	„ „ (E)	41	0.54	2.3	8.2	4.4	0.35	0.15	0.7	2.9	2.9	33
522	„ „ (F)	43	0.77	2.5	10.6	9.5	0.90	0.04	0.8	1.7	2.4	23
171	Lake Tisjön, shallow water, peaty bottom	61	0.77	1.9	20.7	5.2	0.50	0.31	0.3	8.7	2.3	52
520	Brook Torsborån	67	0.62	2.1	17.6	9.6	1.24	0.19	0.9	7.3	2.0	40
521	Floating fen in Lake Tisjön	67	0.47	2.2	22.7	10.8	3.63	0.15	1.8	2.3	2.0	22
170	Poor fen, sloping, w. much oozing water	68	0.56	2.7	17.6	12.8	0.69	0.13	4.7	2.9	2.6	18
174	Örvikskölen, intermediate fen	40	0.44	2.6	8.2	8.0	0.54	0.20	2.6	2.5	1.9	22
527	Forsflon, *Betula nana - Sphagnum fuscum* hummock in calcareous extreme rich fen	67	0.82	1.3	21.9	8.4	1.10	0.07	1.7	5.8	1.9	26
524	Forsflon, *Rubus chamaemorus - Sphagnum fuscum* hummock in do.	64	0.83	4.1	14.9	9.2	1.74	0.09	0.4	7.5	1.3	25
525	Forsflon, calcareous extreme rich fen	61	0.96	4.0	11.7	13.0	1.49	0.14	0.9	3.2	1.6	24

Table 3. **Samples of plants growing together in calcareous extreme rich fen, Forsflon, Hammerdal, Jämtland.**

Values in mg per g dry weight ("excess base" as m.equiv.). When fertile shoots (stems) were used, the inflorescence was removed.

	Species	Ash	"Excess base"	Na	K	Ca	Mn	Fe	SiO_2	P	S	N
500	*Scirpus caespitosus,* stems	32	0.25	0.10	10.0	2.1	0.52	0.11	6.8	1.2	1.8	14
513	— *hudsonianus,* stems	—	—	0.14	9.3	3.2	0.20	0.09	8.1	1.3	1.0	—
512	*Carex jemtlandica,* leaves of sterile shoots	—	—	0.06	5.7	4.2	0.70	0.15	7.6	1.3	1.3	—
515	*Carex limosa,* do.	31	0.33	0.10	8.2	4.6	1.16	0.24	8.5	2.2	1.2	20
517	*Eriophorum latifolium,* fertile shoots	27	0.29	0.12	5.9	5.1	0.75	0.10	7.6	1.4	1.4	9
516	*Carex panicea,* leaves of sterile shoots	48	0.38	0.17	13.6	5.7	0.23	0.15	18.9	0.8	1.8	16
509	*Carex lasiocarpa,* do.	41	0.31	0.06	6.5	6.0	0.76	0.14	16.3	1.9	1.6	20
514	*Schoenus ferrugineus,* stems	66	0.28	0.07	7.6	7.4	0.12	0.04	41.1	0.8	1.3	13
525	*Menyanthes trifoliata,* leaves	61	0.96	4.0	11.7	13.0	1.49	0.14	0.9	3.2	1.6	24

always low. Contrary to all other plants investigated by Sjörs, *Menyanthes* leaves contain considerable amounts of Na. Although moderate in comparison with that of some other plants, the Ca content may reach higher figures in *Menyanthes* than in the other plants investigated, and in the calcareous site even exceeds the content of K.

The properties of the other species investigated, in comparison with the four species mentioned, are evident from Table 3. This Table gives only results from an extreme rich fen. The species are arranged according to Ca content. Whereas such species as *Menyanthes, Schoenus, Carex lasiocarpa* and *Eriophorum latifolium* have a Ca content of the same magnitude as the K content, others, especially the two *Scirpus* species and *Carex panicea,* contain much less Ca than K. All the *Cyperaceae,* in particular *Schoenus ferrugineus,* contain large amounts of silica in this site, in contrast to *Menyanthes*; on the other hand, they all contain less Na, Ca, Mn, P, and N.

Of the species in Table 3, *Scirpus hudsonianus* and *Carex panicea* (in North Fennoscandia) are almost restricted to intermediate and rich fens, *Eriophorum latifolium* occurs mainly in rich fen, and *Carex jemtlandica* and *Schoenus ferrugineus* are confined to extreme rich fen. This ecological order is not reflected in the values in any general way, although the high Ca and Si contents of *Schoenus* are striking in relation to those of the two *Scirpus* species, otherwise similar, with respect to the other chemical constituents investigated as well as morphologically.

Variation between Plants from Different Sites

In Tables 1 and 2 the sampling sites are listed according to their approximate position in the series from bog to extreme rich fen, although several places in this series, e.g. moderately (transitional) rich fen, are unrepresented by samples.

It has been stated (Witting 1947, 1948, 1949, Du Rietz 1949) that this series runs parallel to increasing Ca content of the mire water, at least within one mire complex, although low conductivity values are found even in many North Fennoscandian intermediate and moderately rich fen sites (Sjörs 1946, 1948, 1952). The colloidal part of the substrate, the peat, seems to be better neutralized and less acid, and in most instances probably contains more exchangeable Ca^{2+} ions, as one follows this series. However, many more data are urgently needed, pertaining as much as possible to the peat conditions in the natural state.

It is not to be expected that the amounts of exchangeable metal ions should generally parallel the concentrations in the water. Differences in the quality of peats, in ion uptake and release by higher plants and microorganisms, in hydrogen ion concentration, and in mobility of water, may cause the dynamic equilibrium to be displaced in different ways. Only a small part of the available amount of potassium is actually in solution, and a still smaller part of the bivalent metals, in particular of Ca (see Malmer's part of this paper).

Potassium is strongly concentrated in living cells, as compared with the substrate; this is not the case with calcium. The peat colloids keep few K^+ ions adsorbed in relation to Ca^{2+} ions (and in bogs, H^+ and Mg^{2+} ions). Cf. Malmer. There is generally a higher amount of Ca adsorption in fens than in bogs, and in extreme rich fen than in other kinds of fen, also in proportion to exchange capacity, a calculation basis proposed by Jenny & Ayers (1939). This is reflected in usually somewhat higher values for Ca in plant tissues.

The supply of Ca (which is always much larger, especially in relation to uptake, than the supply of K) is probably always sufficient from a purely quantitative view (even in a bog), and the quantity of Ca present in the substrate can hardly be the limiting cause of plant distribution within the series discussed. But nevertheless, environments may be too poor in Ca (and at the same time too rich in hydrogen ions) for proper ionic regulation. Low ratios of Ca^{2+} to other exchangeable cations in the peat (or to some extent perhaps also the corresponding although numerically very different ratios in water solution) may be directly or indirectly critical or deleterious. For instance, K^+ uptake, according to Jenny & Ayers (op.c.), is much more lowered by H^+ ions than by Ca^{2+} ions. Partly similar questions pertain for instance to serpentine plants and soils (e.g. Vlamis 1949, Rune 1953, Whittaker, Walker & Kruckeberg 1954). From the very large more or less ecological literature on related problems, reference can also be made e.g. to Goodall & Gregory 1947, Wadleigh 1949, Olsen 1950, 1953, Heslep 1951, Baumeister 1952, Lundegårdh 1954, and Burström's reviews in Fortschritte der Botanik.

The large Ca content of poor fen *Menyanthes* in sample 170 (Table 2), closely similar to that in the calcareous rich fen, is of special interest. The obvious cause is the mobility of the water, which increases the supply of Ca^{2+} ions. (The N content of the leaves is very low here but growth is rather good.) In the floating fen, sample 521, the Ca content of *Menyanthes* is also rather high, but in this case K has been still more

augmented than Ca. In stagnant water, the zones next to the roots must be quickly depleted as to metal ions in solution, and the uptake comes almost exclusively from cations adsorbed on colloids (Jenny & Overstreet 1939), but moving water, even if very dilute, carries additional ions to the roots (Olsen opp.cc.).

A high potassium content is also found in plants from several other sites; the rise in K content along the previously mentioned phytosociological series is more irregular than in Ca and not sure in all species.

In cyperaceous plants response to this series is also obvious with respect to silica. Available silica is evidently very scarce in some bog sites (note their paucity in diatoms, Du Rietz 1954 p. 572), but increases with good water supply from mineral soil. Its solubility, which increases with pH, may be the chief regulator of uptake. In the circumneutral to slightly alkaline extreme rich fen, all plants that do accumulate silica contain much of it, *Schoenus ferrugineus* as mentioned above being foremost among the investigated species (about $^2/_3$ of total ash).

Iron and probably manganese are lower in plants from bogs than from fens. In plants from the latter these elements are variable, in response to other factors than those determining the poor fen — rich fen series. Mn and in most cases Fe are present in the bivalent state in the rhizospheres, where reducing conditions generally prevail (Malmström 1923, Pearsall 1938). Under reducing conditions Fe and Mn are abundantly available even at rather high pH, as evident from the high uptake in the Forsflon plants (Table 3). This deviates strongly from the conditions in aerated soils. The often very high Mn values (cf. Erkama 1947, Mayer & Gorham 1951) are striking. The figures for Fe are generally much lower (but Fe may remain in the roots as a result of oxidation there, cf. Malmer's values for *Rhynchospora alba* roots). In semiaquatic *Menyanthes* Gorham (1953 b) found as much as 101 ‰ of ash, ("excess base" 1.24 m.equiv.), 41.3 ‰ N, 0.28 ‰ Mn and 0.16 ‰ Fe (the latter two values from Mayer & Gorham 1951, but referring to the same sample). Values for *Carex lasiocarpa* were: 55 ‰ ash, 0.36 m.equiv. "excess base", 20.4 ‰ N, 0.46 ‰ Mn and 0.18 ‰ Fe.

The other elements (Na, P, S, and possibly N) show no correlations with the phytosociological series, except for an inverse trend for sulphur evident in *Menyanthes*. The samples are not sufficient in number to show if there are other weak trends, for instance towards higher P or lower N content in the bog plants.

Quantitative production studies are necessary to establish in a more definite manner the relations between environmental conditions and

substance uptake as a plant response. Only when multiplied by production can values for percentage of different elements give definite information about nutrient ecology. (Cf., for instance, Tamm 1953, where the difference in significance between percentage and total uptake is well illustrated.) Physical and chemical factors other than that under consideration may largely determine the extent to which increased availability of one substance may lead to increased growth and/or increased percentage in the plant. For example, it may be possible that such conditions as the capacity of the internal air spaces of roots and the redox potential at their surfaces against the more or less reducing surroundings ultimately limit ion uptake, because, according to Lundegårdh's theory, the necessary energy is delivered by anion respiration.

Some examples of a better growth than usual need special discussion:

Scirpus caespitosus is much more productive in site 501 (Table 1) than elsewhere. This is no doubt due to moving water, and shows that mire conditions in general are far from optimal for the growth of this typical, widespread and frequently dominant mire-species. Proportion of K is rather high, in spite of the much larger total weight of the tuft, and thus total uptake of this element is very large (cf. above, *Menyanthes* samples 170, 521). Nitrogen content is low, but total nitrogen as well as phosphorus uptake are certainly higher than in less well-developed plants. This larger uptake may be a result of the stronger root development, combined with the addition of free ions by moving water in the case of potassium, perhaps also to some extent with respect to calcium.

In the brook Torsborån, giant *Carex lasiocarpa* (510) and *Menyanthes* (520) are found (p. 54). These plants profit from changes brought about by submergence, and from lack of competition. In this case, K, P, and N are all rather high, at least in *Menyanthes,* and growth is probably limited only by climatic conditions. One could hardly imagine these species growing still taller; consequently the uptake of all nutrients must be regarded as optimal or over-optimal for growth. Still higher leaf values of K, P, or N, when found, are not parallelled by still better growth, but may on the contrary be characteristic of sites where growth is stunted by some factor.

The *Menyanthes* (171) found on submerged peat in Lake Tisjön was not so luxuriant; it may even be doubted that it will survive much longer, owing to the exposure of the site. It showed very high P and N values and was also higher in K than the preceding one. In these two cases the very good NPK nutrition may be due to the lack of competition, but also to changes of conditions in the peat when submerged under brook or lake water, for a better nutrition is also evident in surviving closed communities.

The last case to be considered is that of the floating peat (samples 511 of *Carex lasiocarpa* and 521 of *Menyanthes*; cf. above pp. 54, 55). Except for more Mn, the former species is similar in composition to the same species in the brook. *Menyanthes* shows very high values for K and Mn only, but

rather low values for P and N. Whether the high production of the total community is due mainly to excess of potassium ions, enlargement of root-systems due to access of circulating water and possibly oxygen, or to other causes, remains to be settled.

In contrast to these instances (similar to the "manuring with water" practised by farmers in the old days to improve the yield of mown fens and certain meadows), normal mires are generally poor in nutrients. The production potentialities of mire plants are largely unfulfilled both in bogs and in most fens. The familiar appearance of mire plants is, in fact, dwarfed, if compared with the potential size sometimes realized. ("Giants" have been observed also in *Eriophorum vaginatum, Carex rostrata, C. limosa, Scheuchzeria,* and submerged *Sphagna.*) Designation of the less acid "rich" fens as eutrophic or eutraphent is therefore inadequate. Probably deficiency is usually not restricted to one factor, and low availability of several elements (e.g. P, K, and N) may frequently participate in checking growth. Nutrient deficiencies (especially in phosphorus) become much more evident if one wishes to gain a higher yield from a mire than it produces in its natural state. But it would carry us too far to discuss these problems of agriculture and forestry here. Experimental work is going on e.g. in Finland (Valmari 1954), Sweden (Forest Research Institute: Romell 1950, Malmström e.g. 1949, 1952, Tamm in press) and Great Britain (Zehetmayr 1954).

There may also be essential deficiency in trace elements not determined by us. Copper deficiency may be common and was for instance demonstrated in oats by Lundblad (1950) on a soil derived from drained mire, in its natural state similar to, and situated not far from Forsflon in Jämtland. Experiments by Romell (Malmström 1949 pp. 95, 220, 1952 p. 13) probably show deficiency in boron.

In the natural state, plants compete successfully in a largely undernourished or even starving condition. Starving is indicated with regard to K, and very likely P and N. Tamm (in press) has demonstrated severe P starving. There may be degrees of resistance to starving, and probably more severe starving as to K and N in ombrotrophic bogs than in most fens. Need of P, K, and in many cases N fertilization during cultivation corresponds very well to certain mire types in the elaborate Finnish system (Valmari 1954). NPK fertilization was unsuccessful only on the most acid *Sphagnum fuscum* bogs. But there is no proof in our material that these nutrients should directly limit plant distribution with regard to the phytosociological m a i n types of mires according to Du Rietz. The problem of tolerance of environ-

mental conditions, reflected floristically in the differentiation into major plant communities, may probably be largely settled by other factors than those limiting growth and production. Again, the ecology of serpentine soils shows striking parallels. Whittaker, Walker & Kruckeberg (1954) have shown that, in spite of a general deficiency in NPK connected with a low production, the most specific property of temperate serpentine soils, a very low Ca/Mg ratio, is largely responsible for the peculiar floristic composition of the vegetation. Similarly, acidity and share of different components in the sum of exchangeable cations are probably the most efficient soil factors determining in the natural state the series from bog to extreme rich fen.

Peat and Plant Samples from Småland

Description of the Sampling Locality

All samples have been collected on the Åkhult mire in the parish of Aneboda, Kronobergs län. The mire is situated in the southernmost part of the South Swedish uplands, about 230 m above sea level. The bedrock and the glacial deposits of the surroundings contain very little lime but are rich in iron. The mean annual precipitation is about 725 mm.

The Åkhult mire covers only a bare km². About 70 % is covered by ombrotrophic bog vegetation. The rest consists of different types of poor fen influenced by mineral soil water. For preliminary sampling three sites (I, II, III) have been selected, which may be regarded as typical of the different plant communities on the mire. In the following brief description of the vegetation of the three sites Du Rietz's classification of mire vegetation has been used (Du Rietz 1949, 1950, 1954). In order to characterize the communities physiognomically Sjörs' classification (Sjörs 1950) into mudbottom, carpet, lawn and hummock communities is adopted.

Site I is situated within pure ombrotrophic bog vegetation. The lower hollow stage is composed of a *Rhynchospora alba - Sphagnum cuspidatum* mudbottom community; the upper hollow stage of a *Scirpus caespitosus - Sphagnum magellanicum* lawn community with locally dominant *Sphagnum balticum* and *S. papillosum*. The lower hummock stage consists of a *Calluna vulgaris - Sphagnum rubellum* hummock community. In the upper hummock stage secondary hummocks of *Sphagnum fuscum* as well as of *S. imbricatum* are found. This bog vegetation

is of a more or less western type. *Erica tetralix* occurs only in fen vegetation on the Åkhult mire.

Two peat samples have been collected at this site, one in the above-mentioned mudbottom hollow community and one in the lower hummock stage. Samples of *Rhynchospora alba, Sphagnum cuspidatum, S. magellanicum, S. papillosum* and *S. rubellum* have been collected in communities where each species dominates. The sample of *Rhynchospora alba* was collected on July 10, 1953, while the samples of *Sphagnum* and peat were collected on Dec. 28, 1953 immediately before the mire was frozen.

Site II is situated in a poor fen, with a row of pools connected by a small stream. These are surrounded by a *Menyanthes trifoliata - Sphagnum pulchrum* carpet community. The shallower pools are occupied by a *Rhynchospora alba - Sphagnum inundatum* mudbottom community. Among other species in these communities and the surrounding vegetation the following may be mentioned: *Erica tetralix, Narthecium ossifragum, Utricularia intermedia, U. minor, Carex lasiocarpa, C. limosa, Eriophorum angustifolium, Rhynchospora fusca* and *Calliergon stramineum*. From the above-mentioned mudbottom community one peat sample and one sample of *Rhynchospora alba* have been collected, while a sample of *Sphagnum pulchrum* has been collected in the carpet community.

About 30 m from this spot samples of *Sphagnum magellanicum* and *S. papillosum* have been collected in a *Calluna vulgaris - Sphagnum magellanicum* hummock community and a *Narthecium ossifragum - Sphagnum papillosum* lawn community respectively. In the lawn community one peat sample also has been collected. The vegetation on this spot is a little poorer in species, as *Menyanthes trifoliata, Utricularia intermedia, U. minor* and *Carex lasiocarpa* are lacking. As was the case at site I, *Rhynchospora alba* was collected on July 10, 1953 and the rest of the samples on Dec. 28, 1953.

Site III is situated in a narrow marginal fen (a lagg) where the vegetation is of the transitional poor fen type (moderate poor fen according to Du Rietz 1954). *Potentilla palustris, Carex dioeca, C. Oederi* ssp. *oedocarpa, C. panicea, C. rostrata, Sphagnum apiculatum, S. imbricatum, S. subsecundum* and *Riccardia pinguis* are to be found there but not at site II. Iron ochre occurs abundantly. Not far from this site there is a restricted occurrence of *Scirpus hudsonianus*. In a *Carex lasiocarpa - Sphagnum apiculatum - S. inundatum* community which

dominates the mudbottom vegetation at this site one sample of *Rhynchospora alba* was taken on July 10, 1953. One peat sample and one water sample were collected on June 15, 1954.

Peat Samples

The results of the peat analyses are presented in Table 4 (calculated per 1000 ml wet peat) and in Table 5 (calculated in ‰ of dry weight).

The distribution of the different mineralic constituents in extractable and nonextractable fractions can be studied in Table 5. Apart from the metals only P is soluble in measurable amount (maximally about 10 % of total P). Cl^- cannot be determined with the methods used, but the extractable amount is probably not much larger than the contents in the water, or 0.1—0.3 m.equiv./l (Malmer 1951, p. 59; the lower value mean from bog hollows). Nor can extractable S be determined owing to concentration being too low (probably < 0.7 mM per 1000 ml wet peat). The HCl-insoluble part of the ash (mainly SiO_2) did not contain any visible mineral grains. Total ash, after subtraction of Fe, P, S and Si (as oxides), agrees within the analytical and calculation errors with the sum of the exchangeable amounts of Na, K, Mg and Ca (as oxides). This suggests that on the whole the total contents of these elements in the peat are exchangeable with the methods used and that they in the main are adsorbed on the peat colloids (cf. Wilson & Staker 1935, p. 11).

Total ash as well as its contents of Fe, P, S and insoluble residue increase from site I to site III. The total nitrogen content of the peat is also higher in the fen sites than in the bog site. The values of N are similar to those mentioned by Gorham (1953 b) from English mires. It may be observed that the differences between the sites are most evident if the values are calculated per volume wet peat.

Both pH and the degree of neutralization of the peat are higher in the fen than in the bog, and both are highest in site III. These properties correlate well (cf. e.g. Wilson & Staker 1935, p. 6—7, Gorham 1953 a, p. 137, 1953 b, p. 355). The higher values are mainly dependent on higher contents of metallic cations relative to exchangeable hydrogen ions. The exchange capacity as well as the amounts exchangeable of hydrogen ions seem to be correlated with the degree of humification of the peat. Gorham (1953 b) has obtained similar values in comparable types of vegetation in English mires. Both his and my determinations show that the acid properties of the peat are reflected in the very high

Table 4. **Peat samples from the Åkhult mire.** Values calculated per 1000 ml wet peat.

Site	I								II								III		
Vegetation	Hummock community				Mudbottom community				Lawn community				Mudbottom community				Mudbottom community		
pH				3.2				3.3				4.2				4.7			5.2
Weight/volume ratio				1.05				1.05				1.04				1.06			1.08
Dry matter g				85				84				73				114			134
Nitrogen „				1.0				1.2				1.0				3.0			3.4
Ash „				2.6				2.8				3.7				6.6			16
Ash analyses:																			
P g				0.025				0.025				0.027				0.050			0.098
S „				0.17				0.13				0.23				0.33			0.43
Insoluble in HCl (SiO_2) „				1.4				1.9				1.2				3.5			10.0
Extraction analyses:																			
Extraction solution	NH_4Ac	HAc	HAc	Mean	NH_4Ac	HAc	HAc	Mean	NH_4Ac	HAc	HAc	Mean	NH_4Ac	HAc	HAc	Mean	NH_4Ac	HAc	Mean
Exch. H^+ m.e.	75	—	—	75	35	—	—	35	36	—	—	36	48	—	—	48	73	—	73
„ met. cations „	—	13	13	13	—	8.0	8.0	8.0	—	18	18	18	—	(65)	39	39	—	79	79
„ capacity for cations „	—	—	—	88	—	—	—	43	—	—	—	54	—	—	—	87	—	—	152
Degree of neutralization	—	—	—	15 %	—	—	—	19 %	—	—	—	33 %	—	—	—	45 %	—	—	52 %
Exch. Na^+ m.e.	0.68	0.78	0.76	0.74	0.68	0.55	0.62	0.61	0.69	0.70	0.80	0.73	0.83	0.93	0.95	0.90	0.59	0.57	0.58
„ K^+ „	0.88	1.01	1.14	1.0	0.74	0.48	0.52	0.6	1.11	1.09	1.09	1.1	0.70	0.75	0.71	0.72	0.64	0.68	0.66
„ Mg^{2+} „	15	14	17	15	14	15	12	13	14	12	11	12	19	16	16	17	—	—	—
„ Ca^{2+} „	4.4	4.0	4.3	4.3	3.0	2.8	3.2	3.0	4.7	6.1	5.8	5.6	18	18	22	19	25	26	26
„ Mn^{2+} „	—	0.06	—	0.06	—	0.05	—	0.05	—	0.11	—	0.11	—	0.23	—	0.23	(0.46)	0.76	0.76
„ Fe (as Fe^{2+}) „	(0.13)	0.63	0.65	0.64	(0.01)	0.29	0.31	0.30	(0.27)	3.9	4.1	4.0	(0.21)	3.3	3.7	3.5	—	13	13
Sum of analyzed met. cations „	—	—	—	22	—	—	—	18	—	—	—	22	—	—	—	42	—	—	—
HAc-soluble P [1] mM	—	—	—	—	—	—	—	0.11	—	—	—	—	—	—	—	0.06	—	0.05	0.05

Values within parentheses are not included in the mean values.

[1] Values from site I and site II from samples collected 15.6.1954.

Table 5. **Peat samples from the Åkhult mire.**

The same values as in Table 4 but calculated per g dry weight.

Site	I		II		III
Vegetation	Hummock	Mud-bottom	Lawn	Mud-bottom	Mud-bottom
Degree of humification [1]	H_4	H_3	H_2	H_{4-5}	H_6
Water g	11	12	13	8.3	7.1
Nitrogen mg	11	14	13	26	25
Ash „	30	32	50	58	116
Ash analyses:					
Fe mg	1.3	0.46	12	6.7	15
P „	0.33	0.33	0.37	0.44	0.72
S „	2.0	1.6	3.1	2.9	3.2
Insoluble in HCl (SiO_2) „	17	23	16	31	76
Not analysed constituents (as oxides) „	6	4	9	11	11
Extraction analyses:					
Exch. H^+ m.e.	0.89	0.42	0.50	0.40	0.55
„ met. cations „	0.15	0.09	0.25	0.34	0.59
„ capacity for cations „	1.04	0.51	0.75	0.84	1.14
„ Na^+ mg	0.20	0.17	0.23	0.18	0.10
„ K^+ „	0.48	0.3	0.59	0.25	0.19
„ Mg^{2+} „	2.2	1.9	2.0	1.8	—
„ Ca^{2+} „	1.0	0.72	1.5	3.4	3.9
„ Mn^{2+} „	0.020	0.017	0.042	0.055	0.15
„ Fe^{2+} (with HAc) „	0.21	0.098	1.5	0.84	2.8
HAc-soluble P „	—	0.036	—	0.021	0.012
Sum of extractive constituents (as oxides) „	6	5	9	10	—

[1] Scale 1—10 according to von Post 1924.

values of exchangeable hydrogen ions. These values are much higher than the H^+-concentration expressed as pH. It ought to be mentioned, too, that the pH of the peat is considerably lower than the pH of the free water (Table 6).

Whether the contents of exchangeable ions are calculated per unit dry weight or per unit volume, differences between the three sites exist above all with regard to Ca, Mn and Fe while Na, K and Mg do not show appreciable differences. The increase from site I to site III in the three first mentioned ions is very great.

Mn and Fe have been calculated as bivalent in Table 5. As to Mn it has been shown that in acid, organic soils it occurs only as Mn^{2+} (Leeper 1947; Mattson et al. 1948). It is more difficult to predict the degree of oxidation of Fe. However, it is probable that the main part of the exchangeable iron in these samples is in the ferrous state (cf. Pearsall 1938).

Table 6. **Composition of water samples from the Åkhult mire.**
Values calculated per 1000 ml.

Site		I	II		III
Sample number		1	2	3	4
(pH		4.1	—	4.9	5.9
$\varkappa - \varkappa_H +)_{20°} \cdot 10^{-6}$		26	—	42	37
Na^+	m.e.	0.11	0.15	0.20	0.11
K^+	,,	0.004	0.021	0.009	0.030
Mg^{2+}	,,	0.055	0.062	0.19	—
Ca^{2+}	,,	0.012	0.040	0.092	0.088
Mn	mM	—	0.0004	0.001	—
Fe	,,	—	—	0.05	0.021
P	,,	—	—	—	0.0007
Sum of analysed met. cations	m.e.	0.18	0.27	0.6	—

Sample 4 taken at the same time as the peat sample from site III. The pH and conductivity in samples 1 and 3 from water collected on Dec. 29, 1953. The analyses of Na, K, Mg, Ca and Mn in samples number 1—3 from Witting (1948, p. 132), who has kindly told me where her samples were collected. Sample 2 is taken at a site with the same vegetation as the lawn community of site II, while sample 3 is taken in the row of brook-pools. The Fe-value of sample 3 from Malmer (1951, p. 39).

The value of HAc-soluble P in samples from mudbottom communities is highest in the bog. Other samples have yielded similar results, but more detailed studies remain to be made.

The equivalent ratios between the different exchangeable cations in the samples are of great interest. The dominating exchangeable cation is H^+. Among the metallic cations Mg^{2+} and Ca^{2+} together make up about $^4/_5$. In comparison with these ions only Fe^{2+} is present in considerable amounts, and only in the fen samples. In the mire water the proportion is quite different. There Na^+ dominates among the metallic cations in all samples (Table 6). This is valid for the main part of Witting's samples from bogs and poor fens (Witting 1947, 1948). In the peat samples from the Åkhult mire there are, if calculated on equal volumes, 5—6 times as much Na, 25—150 times as much K, 90—240 times as much Mg and 150—300 times as much Ca as in the water samples. This can be expected as these ions are adsorbed in the following order:

$$Na^+ < K^+ < Mg^{2+} < Ca^{2+}$$

owing to decreasing volume and increasing charge.

The equivalent ratios Ca/Mg are particularly interesting. In the peat samples from site I the quotients are 0.29 and 0.23 while those from

site II are 0.47 for the lawn community and 1.12 for the mudbottom community. Mattson, Sandberg & Terning (1945) mention as a mean ratio 0.48 (minimum 0.26, maximum 0.99) from a vertical profile through ombrogenous peat from the Ramna bog on the Hallandsås. For soligenous fen peat in the bottom of the profile the mean is 1.38. In the water samples from the Åkhult mire the quotient is for site I 0.22 and for site II 0.65 and 0.49. Witting's as well as my own quotients mentioned above are low in comparison with determinations from other mires. Generally the quotients of Witting's (1947, 1948) water samples from other bogs in most cases range from 0.3 to 0.8 with very large variability. The ratios in the water samples from poor fens are higher than the ratios from adjacent bogs. They exceed in most cases 0.7. The samples from the poor fens of the Aneboda mires (Witting 1948, p. 132) show comparatively low values. In waters of rich fens the quotients are of course much higher. The ratio Ca/Na shows about the same variation as the Ca/Mg ratio. The Mg/Na ratio is fairly constant.

The Ca/Mg ratio of the bog waters varies between different regions. In 24 bog water samples from Britain the mean ratio is about 0.7 (Gorham, private communication). In water samples from the bog of the Skattlösberg Stormosse in Dalarna (Sjörs 1948, p. 100, samples 1 and 2) the ratios are 1.2 and 1.5. In this case the Na-content of the water is also remarkably low (0.03 m.equiv./l) or about $^1/_3$ of that of the Åkhult mire. The Skattlösberg Stormosse is situated far from the sea and consequently gets a lower supply of Na and probably also of Mg through the precipitation. As to Na this is shown by Emanuelsson et al. (1954). A further discussion of their results would be outside the scope of this paper.

Consideration of the ways in which substances are supplied to the mire will serve to explain the above-mentioned differences in mineral contents between bog and fen. A bog is furnished with mineral substances only by precipitation and various forms of airborne drift. A very important supply of mineral substances is brought to a fen by water from the mineral soil (mineral soil water or "Mineralbodenwasser" according to Thunmark 1942). As sea water is probably the most important source of metallic cations in the precipitation, Na^+ and Mg^{2+} play a greater part in the peat and water of an ombrotrophic bog than is normal for inland soil and water. From mineral soil water the fens obtain an addition of different mineral substances, the amount of which depends on the character of the mineral soil and the hydrological conditions. The addition of calcium (as bicarbonate) is especially important.

Owing to the high adsorptive capacity of the peat for Ca^{2+} ions the ionic balance in the fens is adjusted to conditions normal for inland soils. The increase of the degree of neutralization in the fen also depends chiefly on this higher content of Ca^{2+}. The increase of Mn and Fe in the fen in comparison with the bog must likewise depend on influence from the mineral soil. The remaining metallic cation analysed, K^+, shows no differences between bog and fen in spite of the greater supply to the fen from the mineral soil. It is however evident from the plant analyses (p. 73) that K is very much concentrated in the plants. This may be the reason for the low, constant contents of K^+ in the peat, as plant production seems usually to be higher in fens than in bogs. Concentration in the plants may also explain the lower values of HAc-soluble P in the fen sites. However, it could be due to a greater degree of fixation in an insoluble state. The higher values of total P and Kjeldahl-N in the fen sites may depend on supply through mineral soil water but the decay of the peat and the activity of micro-organisms have effect, through increasing the concentration. Finally influence from the mineral soil explains the higher contents of SiO_2 in the fen peat.

Plant Samples

The plant material comprises three samples of *Rhynchospora alba* (shoots and roots) and seven samples of five *Sphagnum* species (Table 7).

The analyses on the roots of *Rhynchospora alba* are unique among the samples. However they have been made on very small samples and have therefore a low accuracy. Particularly noticeable is the very high concentration of Fe in the roots in comparison with the shoots of *Rhynchospora alba*. Also Na and Ca are comparatively much more concentrated in the roots. K and P, on the other hand, seem to be concentrated in the shoots. Owing to the low accuracy of the determinations on these root samples, further discussions seems inadvisable.

The *Sphagnum* species have about the same contents of Na, Mg, Ca and Mn as the shoots of *Rhynchospora alba*. Ash is lower throughout in the *Sphagnum* samples. The low amount of protoplasm in *Sphagnum* is reflected in low concentration of K, P, S and N. There seems, however, to be slightly more Fe in the *Sphagnum* than in the *Rhynchospora alba* shoots. In site I there is also more SiO_2 in the *Sphagnum* species, but in site II there is much more SiO_2 in *Rhynchospora alba*. According to Anschütz & Gessner (1954) all cations in the *Sphagnum*

Table 7. **Plant samples from the Åkhult mire.**
Values in mg pr g dry weight.

Species	Site	Ash	Na	K	Mg	Ca	Mn	Fe	SiO_2	P	S	N
Rhynchospora alba, shoot..	I	22	0.9	7.3	1.6	0.9	0.12	0.1	2.6	1.3	2.0	11
— — —	II	42	0.8	8.7	0.9	1.1	0.30	0.2	21	0.9	1.3	11
— — —	III	45	0.9	10.3	1.0	1.4	0.16	2.7	15	1.0	1.8	14
— — root	I	33	4	6	1	1	0.03	0.4	9	0.6	—	—
— — —	II	49	8	7	2	2	0.2	5	18	0.7	—	—
— — —	III	95	8	6	4	4	0.2	10	25	0.8	—	—
Sphagnum cuspidatum	I	18	0.8	5.2	0.9	0.8	0.07	0.3	4.4	0.3	0.9	—
— *pulchrum*	II	27	1.4	6.4	0.7	1.8	0.05	0.7	4.0	0.4	1.0	—
— *magellanicum*	I	18	0.8	3.9	0.6	0.8	0.11	0.4	3.2	0.3	0.9	5.0
— —	II	19	0.9	4.1	0.5	1.0	0.41	0.3	4.6	0.3	0.8	6.2
— *papillosum*	I	16	1.2	3.1	—	0.7	0.15	0.3	4.4	0.3	0.8	—
— —	II	19	1.0	5.0	0.8	1.1	0.12	0.4	3.2	0.5	1.0	—
— *rubellum*	I	—	0.5	6.4	1.5	0.6	0.10	0.4	4.1	0.2	—	—

are not really taken up. They can also be fixed in an exchangeable state.

In this case a rough comparison of the concentration of the different elements in the living plants and the substrate is possible on the basis of the dry weight of peat and plants. A comparison between Table 5 and Table 7 shows that Na, K and P are concentrated particularly in the plants. For K the concentration effect is very great, e.g. for *Rhynchospora alba* from site III 50 times. This is valid also for P if the calculations are based on the HAc-soluble P of the peat. Calculated on total P in the peat the degree of concentration is very low. The concentration of Na is comparatively slight and evidently not characteristic of most of the plants analysed by Sjörs (p. 60). Mn is concentrated by the plants only in sites I and II. The remaining elements — Mg, Ca, Fe, N, S and SiO_2 — are not concentrated in the living plants if this basis of calculation is used. For instance concerning Ca and N on site I, and some Fe-values, there are much the same values in peat and plant. As to N, S and SiO_2 the higher contents usually found in the peat probably depend on the fact that these elements occur in such a manner that the plants have difficulty in taking them up (cf. P above!). Available Mg is, however, probably in excess and this is also probably the case with Ca at least in sites II and III. Total ash is constantly higher in the peat, especially in the fen sites.

On comparing the mineral contents of the plants from different

sites, the values of K, Ca, Fe, Mn, and SiO_2 are in most cases higher in the samples from site II and site III than in those from site I. With the exception of K these elements show the most important differences in the peat, too. The differences are very large especially as to Fe and SiO_2 in the samples of *Rhynchospora alba*. In most cases the differences are not as large as in the substrate. However, the relative increase in K from site I to site III is no less than that in Ca, although there is no increase of K in the peat. This may be due to a larger supply of K in sites II and III than in site I. It is, however, taken up by the living plants. The standing crop of living plants — macrophytic as well as microphytic — is considerably larger in the fen than in the bog. Nitrogen is slightly higher in the fen sites than in the bog site. In all three sites, Na and Mg show on the whole the same concentration in the plant samples as in the peat. The content of P and S in the shoot samples of *Rhynchospora alba* is highest in site I and throughout somewhat lower in site II and site III. In the *Sphagnum* samples the contents of P are slightly higher in site II than in site I while S shows no important differences. The ash contents are considerably higher in the fen sites than in the bog, particularly in the case of *Rhynchospora alba*. Here the higher values are mostly due to SiO_2.

Mayer and Gorham (1951) and Gorham (1953 b) have published some analyses of plant samples from English mires. They include total ash, nitrogen, Mn and Fe. No important differences are found between their values and ours.

The extremely low equivalent ratio Ca/Mg in the bog is reflected in the plant samples. The Ca/Mg ratio for *Rhynchospora alba* from site I is 0.35, from site II 0.78 and from site III 0.84. Among the *Sphagnum* samples from site I *Sphagnum rubellum* has the lowest value (0.27) and *Sphagnum magellanicum* the highest (0.78). On site II *Sphagnum papillosum* has the lowest (0.84) and *Sphagnum pulchrum* the highest (1.56). This species is the only exclusive fen plant in the samples and it shows a noticeably high content of Ca. These ratios are equal to or slightly higher than the corresponding values in the peat samples. Mattson, Sandberg & Terning (1945, pp. 104 ff.) found the Ca/Mg ratios 1.38 for *Sphagnum acutifolium* and 1.17 for *Eriophorum vaginatum* on samples from the Annersta mire in SW Småland. In the same locality the ratio in the peat is 0.87. From the Ramna bog (cf. p. 71) where the Ca/Mg ratio in the uppermost layer is 0.84 they have found for *Sphagnum fuscum* 1.33 and *S. cuspidatum* 1.20.

In Table 8 are given some values on length and weight of shoots

Table 8. **The vegetative development of Rhynchospora alba in the samples from the Åkhult mire.**

Site	Number of shoots	Length of the shoots in cm			Number of individuals per g dry weight
		Mean	Standard deviation	Standard error	
I	208	20.5	± 3.5	± 0.21	19.2
II	375	24.0	± 4.7	± 0.24	18.3
III	69	30.1	± 3.6	± 0.43	11.0

in the analysed samples of *Rhynchospora alba*. There are very important differences. The mean length is about 50 % larger and the mean weight about 75 % larger on site III than on site I. There were, however, no significant differences in fertility calculated as number of spikelets per individual. The degree of cover of the total field and bottom layers was the same in all sites, but the *Rhynchospora alba* specimen grew more scattered and among luxuriant *Carex lasiocarpa* on site III. For detailed studies there are many other important factors to take into account.

On the basis of the values given in Table 8 it is possible to calculate the volume of wet peat which contains the same amount of different mineral nutrients as the shoots of *Rhynchospora alba* on the three sites. Calculated per 100 individuals the corresponding volume of wet peat is for K 1.6 dm^3 on site I, 1.7 dm^3 on site II and 3.7 dm^3 on site III. For P (the HAc-soluble part) the values of the volume are respectively 1.9, 2.6 and 5.8 dm^3. The values are lower calculated on total P (0.3—0.9 dm^3). Similar values are found for Na (0.2—0.7 dm^3) and Mn (0.07—0.6 dm^3). For Mg, Ca, Fe, SiO_2 and S the values are 0.1—0.01 dm^3 of wet peat per 100 individuals of *Rhynchospora alba*. Calculated on Kjeldahl-N in the peat the values are about 0.01 dm^3 on all three sites. It is very difficult to estimate that part of N which is included in the turnover. It may be possible that it is so low (about 0.5 %) that the volume-values will be similar to those of K and P mentioned above. To phosphorus but not to nitrogen alone strong responses have come out from fertilization experiments with other plants on mires (Tamm in press; Zehetmayr 1954).

Discussion

The investigation on the Åkhult mire deals especially with the ecological differences between bog and fen. One cannot come to definite

conclusions from this preliminary study, and therefore only some more noticeable differences will be briefly considered. There is no reason to suppose that only one ion or a single factor should determine the differentiation of the vegetation within the series from bog to poor fen. On the contrary this differentiation may be the result of co-variation in several factors. Nor is it probable that all the exclusive fen plants are limited by the same factor or set of factors. Most striking in the bog is perhaps the low degree of neutralization and the high concentration of hydrogen ions with their various effects upon the vegetation (cf. e.g. Lundegård 1954, pp. 438 ff.). The hydrogen ion concentration as such may exclude many species from the bog. In addition the distribution between K, Mg and Ca is very abnormal, with a great excess of Mg. This is of particular importance to the vegetation when it is combined with a low concentration of mineral nutrients (cf. Olsen 1950). In the fen, running parallel to increasing pH and degree of neutralization, this abnormal distribution balance seems to be compensated, more normal values resulting from addition of Ca from the mineral soil. In this way a fen also gets other mineral substances (cf. pp. 71—72), among which probably also several micronutrients not analysed here may well be of importance. It is difficult to calculate the part of K in the above-mentioned balance, because a large fraction of total K is accumulated in the living plants. The potassium analyses show in particular the importance of analysing the living plants to facilitate a better understanding of mineral nutrient ecology. Among the mineral nutrients analysed potassium together with phosphorus appear to be the most probable ones to limit production. Nitrogen may also be of great importance. Without reliable calculations of the total production per unit area it is difficult to draw definite conclusions. It is perhaps not necessary that a factor which limits production also limits the distribution of a plant.

Differences in microbiological metabolism in the peat, (probably quantitative as well as qualitative) may well be of importance to the higher vegetation. A slower chemical and microbiological decay of the peat under the ecological conditions prevailing in a bog may be a prerequisite for its increase in height and isolation from the mineral soil water. The rate of microbiological metabolism influences the amounts of nutrients available to the plants. This is of special importance concerning N, P and S, which to a predominant extent are fixed in organic compounds in the plants, and therefore have to be mineralized in the course of peat formation before they are available anew to living plants.

Summary

Samples of mire plants (shoots or leaves, roots in one species) and a few peat samples were analysed for most of the following constituents: total ash, "excess base" of ash, Na, K, Mg, Ca, Mn, Fe, SiO_2, P, S, and N. Methods of sampling and analysis are described. Variation between species, relation to plant communities, and influence of some environmental conditions are briefly discussed.

Menyanthes is rather rich in Na, but does not accumulate much silica. The *Cyperaceae* accumulate silica only in less acid environments, and in large quantities in a weakly alkaline environment (Table 3). Rather high values for K and particularly for Mn may sometimes occur in mire plants. Percentages of Ca, Si, and possibly K show a relation to the bog — poor fen — rich fen series of Du Rietz, and Fe and Mn are usually higher in fens than in ombrotrophic bogs.

The need for productivity studies is emphasized. A few cases of unusually high production are due to submergence of the substrate (all nutrients high in the plant) or to influence of moving water (potassium and sometimes calcium values raised, nitrogen and phosphorus remain low). In "normal" sites the plants seem to be more or less under-nourished, probable frequently with respect to more than one of the elements K, P and N, but compete successfully in this state. Thus the problem of tolerance, reflected in establishment of floristic plant communities, may be different from that of growth and production, and different sets of factors are probably decisive.

Our peat analyses indicate that a bog differs from a fen in H — K — Ca — Mg proportions as well as in lower peat pH and lower content of several mineral substances. Most of these are highly concentrated in the adsorbed form on the peat, in comparison with the mire water. A considerable part of the total K in a mire is accumulated in the living plants. Attention is drawn to the significance of micro-biological metabolism, and to the importance of analysing plants as well as peat and water.

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Studies on Myxobacteria

III. Organic Factors in Nutrition

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Introduction

Investigations on the physiology of myxobacteria have mostly been carried out on agar substrate although such a technique has certain drawbacks. Oxford (1947), using a liquid medium showed that a total acid hydrolysate of casein, free from polypeptides and carbohydrates was quite satisfactory as a source of carbon and nitrogen for *Myxococcus virescens*. In his basal medium, Oxford used the casein hydrolysate in a concentration of 0.06 % but he could observe a better growth

at higher concentrations though no accurate method of growth determination was used. The present author worked out a better method of cultivation of myxobacteria in liquid, which allowed a more exact determination of the rate of growth as well as the amount of growth, these values being determined photometrically (Norén 1952). It was further demonstrated that not only *Myxococcus virescens* but also *M. fulvus* and *Chondrococcus coralloides* were able to use casein hydrolysate for their carbon and nitrogen nutrition though growth was less good than in *M. virescens*. However, in no case did the casein hydrolysate seem to provide an ideal source of both carbon and nitrogen. An interesting result was also that a mixture of amino acids, which was considered to correspond to a complete casein hydrolysate, gave rise to a growth considerably inferior to that of the casein hydrolysate itself. These results led to further experiments concerning both the relationship of myxobacteria to amino acids, and the carbon requirements of these microorganisms. However, when only in their preliminary stages these investigations led to a study on the requirements of vitamins and other growth substances. On both of these subjects the information in literature appeared to be incomplete, though valuable contributions have in recent years been published by Kühlwein (1950), Finck (1950), Oetker (1953) and Clark (1954). Since their experiments were performed on agar substrate it was also felt to be of value that their findings were supplemented with observations in liquid medium.

A. Material and Methods

In the present investigation three species of myxobacteria were employed: *Myxococcus virescens, Myxococcus fulvus* and *Chondrococcus coralloides*. The strains previously mentioned (Norén 1952, p. 326) were used.

The general methods used have been described in a previous paper (Norén 1952, p. 327—330). The chemicals used were of the quality "pro analysi", the casein hydrolysate was "Difco" Bacto casamino acids, and the vitamin-free casein hydrolysate was "Difco" Bacto vitamin-free casamino acids. Both are casein completely hydrolyzed in acid to the amino acid stage. In the following, the term "casein hydrolysate" will refer to the non vitamin-free casamino acids.

As a basic medium for the following experiments, a solution corresponding to Nutrient solution III (Norén 1952, p. 345) was used. It has the following composition:

Casein hydrolysate	2.5 g	NaCl	1.0 g
Asparagine	2.5 g	$MgSO_4 \cdot 7H_2O$	0.1 g
K_2HPO_4	2.0 g	$CaCl_2$	10.0 mg

$MnSO_4 \cdot 4H_2O$	1.0 mg	Distilled water	1000 ml
Ferric citrate	3.0 mg [1]		

This solution — Nutrient solution III B — was sometimes supplemented with vitamins, the composition of the mixtures are given under the appropriate experiments.

The method of inoculation has also been described in the previous paper (Norén 1952, p. 329). However, to obtain an homogeneous inoculum of *M. virescens* a somewhat improved technique was used. In order to eliminate the risk of contamination, the myxobacteria were grown in test tubes on agar slopes. As the pseudoplasmodia with fruiting bodies developed they were scraped off, suspended in 0.9 % NaCl, and vigorously shaken together with numerous glass beads. This suspension was then filtered through glass wool. The inocolum, obtained in this way, contained microcysts and vegetative cells, the latter occurring either singly or in small groups of 2—5 cells. By this method the number of cells per ml could be determined more exactly than in the inoculum used earlier, where the vegetative cells were often forming larger units.

In this investigation, to each tube with 7 ml — in Expt. 13, 9 ml — of nutrient liquid, was added 1 ml of inoculum, containing approximately 50 millions cells. As a rule, developing pseudoplasmodia were visible on the glass walls after 1 day's growth.

In the experiments myxobacteria were grown in liquid media in test tubes, these being mechanically shaken during the incubation. In the majority of cases the tubes were placed at a slightly inclined position with the same side directed downwards throughout an entire experiment. With such a technique the pseudoplasmodia developed first on the downwards side before slowly spreading upwards over the glass wall. As a result relatively abrupt changes between various determinations appeared, thus giving the growth curves characteristic "knees" (Norén 1952 p. 336—338). In order to avoid this disadvantage, the tubes were turned one quarter of a circumference every day in some experiments with the result that the pseudoplasmodia developed with a more uniform thickness around the glass wall. In this way higher *Z*-values were obtained and the differences in growth in various nutrient solutions were accentuated. However, a pseudoplasmodium is easily loosened from the glass wall and the thicker it is the more easily it is detached. Therefore, by turning the tubes during the incubation the pseudoplasmodia tend to loosen before the maximum growth is reached. For this reason, this method was used in only a few experiments (Expts. 3, 4, 13, 14), and the fact has been mentioned accordingly.

In this investigation as in the earlier one, the development of the pseudoplasmodia which appeared on the glass walls of the culture tubes was followed photometrically, the extinction being determined in white light by means of the photometer used by Åberg & Rodhe (1942). The increasing *Z*-values were taken as a measure of the growth of the myxobacteria. In agreement

[1] Ferric citrate was added after sterilization, together with citric acid, as a concentrated solution.

with Rodhe (1948) the Z-values are calculated according to the formula $Z = (e_\gamma - e_0) \cdot 10^3$.

It seemed advisable that the growth figures obtained with the photometrical method should be compared with the figures for growth obtained in another way. For this several methods were available but the simplest one was to determine the pseudoplasmodial dry weight as this would give an objective measure of growth. Several such experiments were performed, and in all cases the development up to the growth maximum was followed photometrically. The pseudoplasmodia were then scraped off the glass walls of the test tubes and the contents of all the tubes from one series — the number of replicates was conveniently about 5 — were passed through a glass filter with a pore size of 90 to 150 μ. When using filters of a smaller size the pseudoplasmodial slime tends to fill the pores and stop up the passage of the liquid. Before the determination of their dry weight the pseudoplasmodia were washed repeatedly in distilled water. These dry weight values were found to be very low, but it was evident that the growth differences recorded at the photometrical determinations were also found at the dry weight determinations. A tube with a Z-value of 100 corresponded to a dry weight of approximately 0.5 mg.

B. Utilization of Carbohydrates

1. *Earlier observations*

The influence of carbohydrates on the growth of myxobacteria has been examined in several investigations. Jahn (1939), summarizing the facts then available, expressed the opinion that besides starch, and ultimately agar, myxobacteria in their metabolism were able to utilize monosaccharides as sources of carbon. As regards starch his assumption has been confirmed but in the case of the monosaccharides some later works have disagreed. Details of investigations concerning the capability of myxobacteria to utilize various carbohydrates will be given in connection with the appropriate carbohydrate.

Generally the previous experiments have been carried out on agar media and after some days the diameter of the colonies developing from the inocula (fruiting bodies) have been determined. The values from one, or occasionally from two successive determinations are usually recorded. Such a method, however, has the drawback that the yields are compared on the basis of a fixed time of examination, thus an effect on the rate of growth might not be apparent. This must be regretted. In the case of fungi the diameter of a colony may be a very poor measure of the amount of growth (cp. for instance Fries 1943) and this also may be the case with the myxobacteria.

Agar is not an inert medium for myxobacteria as it contains certain growth substances (Robbins 1939, Day 1942) which will influence their growth (see below). In addition, the agar seems also to be available as a source of nutrient. The observation of Beebe (1943) that myxobacteria are able to grow on plain agar has also been verified by Oetker (1953) and other workers. According to Oetker the myxobacterial growth would be made possible by the food reserve attached to the inoculum, while the agar itself would be left intact. However, it seems questionable if this is the correct view. It is true that the trans-

formation of microcysts into vegetative cells, as well as the outward migration of these cells over an agar surface will occur with a rate which is independent of the presence of sucrose in the agar (Norén 1952). Later on, when the reserve food of the inoculum has probably been consumed the cells are reduced to the necessity of utilizing the nutrients in the agar and then the differences in growth on sucrose-containing and sucrose-lacking agar media will become obvious. But growth will still occur on the sucrose-lacking agar. In addition, if a weak agar is used, a certain corrosion of the agar surface is visible, particularly under the fruiting bodies, where small cavities appear. It has further been demonstrated that *M. virescens* does not grow in a nutrient solution containing a complete mineral salt supply including $NaNO_3$ when a C-source is lacking, though in the identical nutrient solution supplemented with sucrose pseudoplasmodia are formed, appearing as very small and thin flakes on the walls of the tubes (Norén 1952). These facts seem to support the ideas that at least some myxobacteria are able to utilize agar as a source of carbon, and in these cases the utilization of other carbohydrates may be affected (Horr 1936, Norkrans 1950).

In some points the results recorded from previous investigations appear somewhat conflicting. For instance, in respect to the effect of glucose on the growth of *M. virescens* the following records are given: glucose acts as an inhibitor (Beebe 1943), weakly stimulating (Oetker 1953), or not at all (Solntzeva 1940), and when glucose is added to a peptone agar it greatly stimulated the growth (Finck 1950), only weakly (Oetker 1953) or gave poorer growth than did peptone without glucose (Clark 1954). In some cases these conflicting results might be a result of variations in the inoculum. The quality of the fruiting bodies being used might possibly vary from one experiment to another, or even within the same experiment (Norén 1950). Recently Clark (1954) has demonstrated that the inhibitory effects of monosaccharides obtained by Beebe (1943) were due to the sterilization of the media by autoclaving. When Seitz-filtered the same carbohydrates did not produce any inhibition. However, in Clark's experiments, better growth was obtained from carbohydrate-free media than when carbohydrates were supplied, a fact which was explained by Clark to be a result of the unusual susceptibility of myxobacteria to slight increases in the osmotic pressure of the medium.

2. *Experiments*

In the present study the experiments have been performed in a casein hydrolysate medium, to which the various carbohydrates were added. Even though the casein hydrolysate would supply the myxobacteria with nitrogen, carbon and energy for growth (Oxford 1947, Norén 1952) it was assumed that a utilizable carbohydrate also present would be an advantage. It was also assumed that in the event of an acid production from one carbohydrate or another, the pH-changes of the culture solutions would be influenced (Liu 1952).

In an early experiment the effect of ribose was tested on the growth

of *M. virescens, M. fulvus* and *Ch. coralloides.* To Nutrient Solution III B containing 1 g/l of casein hydrolysate was added 1 g/l of d-ribose. The growth of neither *M. virescens* nor *M. fulvus* was influenced but that of *Ch. coralloides* was slightly improved. The presence of ribose did in no case influence the pH-changes of the media.

More detailed experiments were later carried out with *M. virescens* as the only test organism.

The experimental procedure was always as follows: Nutrient solution III B was used as the basic medium with the amount of casein hydrolysate reduced to 1 g/l. In this medium quite a measurable growth could be expected. In the investigation the following carbohydrates were employed: l-arabinose, d-xylose, d-glucose, d-galactose, d-mannose, d-fructose, lactose, maltose, cellobiose, sucrose, raffinose, starch, dextrin, glycogen and inulin. These were autoclaved separately and then added to the sterilized basic solution. As a rule, each carbohydrate experiment consisted of 4 series, each comprising 3 replicates:

Series I, no carbohydrate added;
„ II, carbohydrate added in the concentration of 0.2 g/l;
„ III, „ „ „ „ „ „ 1.0 g/l;
„ IV, „ „ „ „ „ „ 5.0 g/l.

The initial pH of the culture solutions was constantly about 7.5. The final pH are given in the table below.

Series	Ara 1.	Xyl 1.	Glu 1.	Gal 3.	Man 1.	Fru 1.	Lac 2.	Mal 3.	Cel 2.	Suc 2.	Raf 2.	Sta 2.	Dex 2.	Gly 1.	Inu 2.
I	7.8	7.8	7.8	8.0	7.8	7 8	8.0	8.0	8.0	8.0	8.0	8.0	8.0	7.8	8.0
II	7.9	7.8	7.8	8.0	7.7	7.6	8.0	7.8	8.0	7.9	8.0	8.0	8.1	7.8	8.1
III	7.8	7.6	7.6	7.8	7.6	7.5	7.9	8.0	8.0	8.0	8.0	8.1	8.1	7.9	8.1
IV	—	—	7.5	7.1	—	—	7.7	8.0	7.7	8.0	8.0	8.0	8.0	—	8.3

1. Time of incubation at 30°C was 6 days.
2. „ „ „ „ „ „ 8 days.
3. „ „ „ „ „ „ 9 days.

Arabinose (Fig. 1): Only the arabinose concentrations of 0.2 g/l and 1 g/l were tested. At both concentrations a certain increase in the rate of growth was obvious, and in addition the total yield of living matter was increased. This last effect was most accentuated in Series III. The effect of arabinose on the growth of myxobacteria has previously been studied by Solntzeva (1940), Beebe (1943) Kühlwein (1950) and Clark (1954) but only in the case of *M. fulvus* (Kühlwein 1950) a utilization has been demonstrated.

Glucose (Fig. 1): It is clear from the curve that glucose in the con-

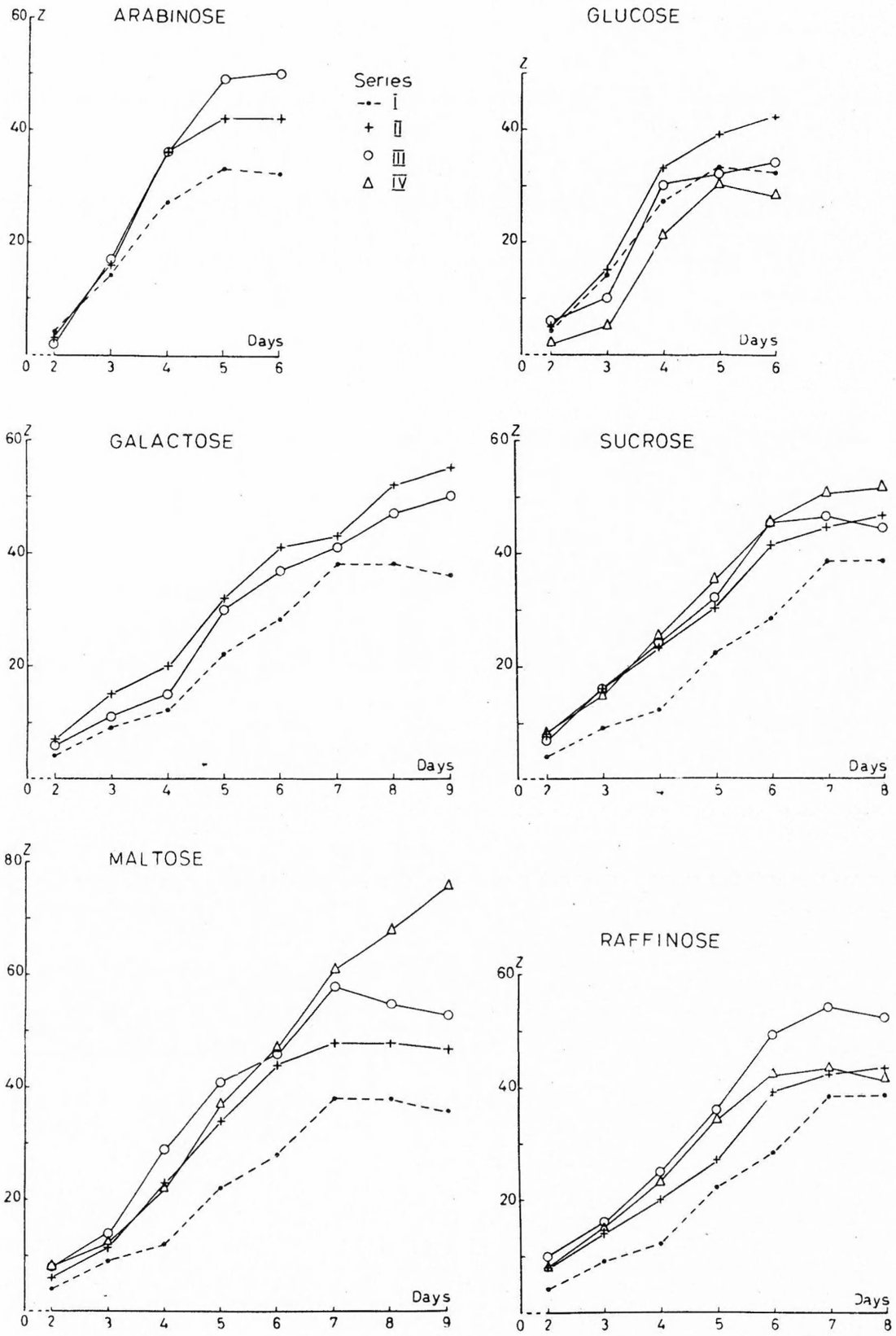

Fig. 1. The effect of carbohydrates on growth (*Z*-values) of *Myxococcus virescens*. 0, 0.2, 1.0 and 5.0 g carbohydrate per l. added in Series I, II, III, and IV respectively.

centration of 5 g/l produced a weak inhibition on the growth of *M. virescens*. At 1 g/l the growth obtained was equivalent to the control. As seen from the table above a certain influence on the changes of pH was also obvious. The pH of the Series 5 g/l and 1 g/l, in spite of relatively good growth, did not rise as much as the control. At the lowest glucose concentration the pH-change was equal to that of the control and here the total amount of growth appeared slightly better.

As previously mentioned, the effect of glucose on the growth of *M. virescens* has already been studied in several investigations, the results being somewhat diverging (Solntzeva 1940, Beebe 1943, Kühlwein 1950, Finck 1950, Oetker 1953, Clark 1954). However, in combination with an organic source of nitrogen such as peptone (Finck 1950, Oetker 1953), or amino acids (Kühlwein 1950, Finck 1950, Oetker 1953) a better effect was obtained with glucose. Yet Solntzeva (1940) reported "no effect" and according to Clark (1954) better growth occurred on peptone agar than on peptone-glucose agar.

Galactose (Fig. 1): As would be expected from the occurrence of this sugar in agar (Pigman and Goepp 1948), galactose was utilized by *M. virescens*. However, at the concentration of 5 g/l only a poor growth was obvious — only thin pseudoplasmodia developed which could not be measured in the photometer — but the pH of the culture solution was modified in an acid direction to 7.1, a not very suitable pH-level for the growth of *M. virescens* (Norén 1952). It was thus evident that owing to the activity of the myxobacteria an acid production occurred which was sufficient not only to mask the ammonia production from casein hydrolysate but also to cause a pH-decrease. In the remaining galactose series, slightly higher *Z*-values were constantly obtained than in the control. At the end of the experiment, the differences became more accentuated, thus indicating increased amounts of growth in the sugar containing tubes.

The availability of galactose as a source of carbon for myxobacteria has been tested by Kühlwein (1950) who observed a marked positive effect on the growth of *M. fulvus*. The same is obvious from the records of Solntzeva (1940, Table III) and Clark (1954, Table 2). As regards *M. virescens,* however, the results of Beebe (1943) and of Clark (1954), seem to indicate a certain inhibitory effect, and from the result of Solntzeva (1940), no effect.

It is perhaps significant that l-arabinose possesses the steric configuration identical with that of d-galactose.

The remaining monosaccharides tested did not, on any occasion,

significantly improve the growth of *M. virescens*. However, certain effects on the pH-changes could be observed. *Fructose,* even at its lowest concentration of 0.2 g/l gave a final pH lower than did the control. The same effect, though to a lesser extent, was produced by *mannose* and also *xylose*. From these facts it may be concluded that *M. virescens* is capable of a certain attack on these sugars. None of these three carbohydrates have evidently been found to promote the growth of *M. virescens* (Solntzeva 1940, Beebe 1943, Kühlwein 1950 and Clark 1954), but in the case of *M. fulvus,* Solntzeva (1940) obtained a slight growth stimulation with xylose and fructose.

Sucrose (Fig. 1): As seen from the curve, the positive effect of sucrose involved an increase both in the rate and in the amount of growth, and although the effect was never very high, the differences obtained appear to be significant. It is worth noting that the 5 g/l concentration was not much better than that of the 0.2 g/l.

According to Solntzeva (1940), Beebe (1943) and Oetker (1953) the presence of sucrose produces neither a stimulating nor an inhibitory effect on the growth of *M. virescens*. This was also stated by Clark (1954), though he did record a somewhat better growth on the control plates than on those plates containing 0.5 % sucrose. As a matter of fact, these results are not surprising, since the slight improvement of growth produced by sucrose and visible on Fig. 1 might hardly be detectable on the agar media used by these investigators. On the other hand Yoshii (1926) found that *M. virescens* grew well in a peptone-sucrose solution and by using a liquid medium the present author (1952) showed that sucrose is available as a source of carbon. This sugar was also shown by Kühlwein (1950) to improve the growth of *M. fulvus*.

Maltose (Fig. 1): It is clear from the curves that the addition of maltose had a positive effect on the growth. In all the concentrations tested this disaccharide both increased the rate of growth and the total yield of living matter. While the *Z* max. of the control remained at 38, in the series containing 5 g/l, 1 g/l and 0.2 g/l of maltose it reached 76, 58 and 48 respectively. The initial growth of 1 g/l was slightly better than that of 5 g/l. This might possibly indicate that the supply of 5 g/l was a little too high, but after being consumed by the myxobacteria, the maltose concentration reached a level suitable for a luxuriant growth. At 0.2 g/l the pH rose a little more than at 1 g/l which equalled that of the control, but at the highest concentration, in spite of the good growth, the pH did not rise to the same level as in the control.

The role of maltose for the growth of various myxobacteria has been

investigated by Solntzeva (1940), Beebe (1943), Kühlwein (1950) and Clark (1954). With *M. virescens* whereas no positive effect has been observed, negative results were obtained by Beebe and Clark. This is a remarkable fact since in the present experiment the positive effect of maltose was very clearly demonstrated. It is possible that this effect was due to certain substance(s) present in the medium and necessary for the utilization of this sugar (see below p. 92). In connection Kühlwein (1950) observed a better effect of maltose on the growth of *M. fulvus* in the presence of an organic source of nitrogen such as asparagine or urea.

Lactose had a slightly positive influence on the growth of *M. virescens* while in the case of *cellobiose* no effect was observed. But in both cases, at the concentration of 5 g/l the pH rose only to 7.7 (in the control to 8.0), and this fact seems to indicate that the myxococci were able to a certain attack on these sugars. In the investigations of Solntzeva (1940) and Clark (1954) none of these carbohydrates were found to improve the growth of myxobacteria.

Raffinose (Fig. 1): The addition of raffinose had a positive effect on *M. virescens*. In all the carbohydrate series the growth was improved though in the Series II and IV the *Z* max. obtained were not very superior to that of the control series. At 1 g/l, however, the *Z* max. was definitely higher. The influence of raffinose on the growth of *M. virescens* was studied by Solntzeva (1940), who observed no effect, and by Clark (1954) who obtained poorer growth with raffinose than without. From the data presented by Clark, however, it seems that the inhibitory effect of raffinose would be reduced in the presence of peptone.

Among the polysaccharides tested, the effect of *inulin* was somewhat uncertain. It speeded the growth rate slightly, increased the change in pH as compared with the control but did not increase the amount of growth. On the other hand starch, and the starch related compounds dextrin and glycogen, greatly improved the growth of *M. virescens*.

Starch: With the exception of the concentration of 0.2 g/l the nutrient liquids containing starch were not clear and as a consequence accurate *Z*-values could not possibly be obtained. The supply of 0.2 g/l produced only a slight increase of the *Z*-values.

Starch as a source of carbon for *M. virescens* has been tested in several investigations and all workers have proved the ability of the myxococci to utilize it (Jahn 1939, Solntzeva 1940, Beebe 1943, Kühlwein 1950, Oetker 1953, Clark 1954). (In the case of Solntzeva and Clark, however, the positive effect is not very clearly demonstrated in the tables.) The results from the present investigation is in keeping

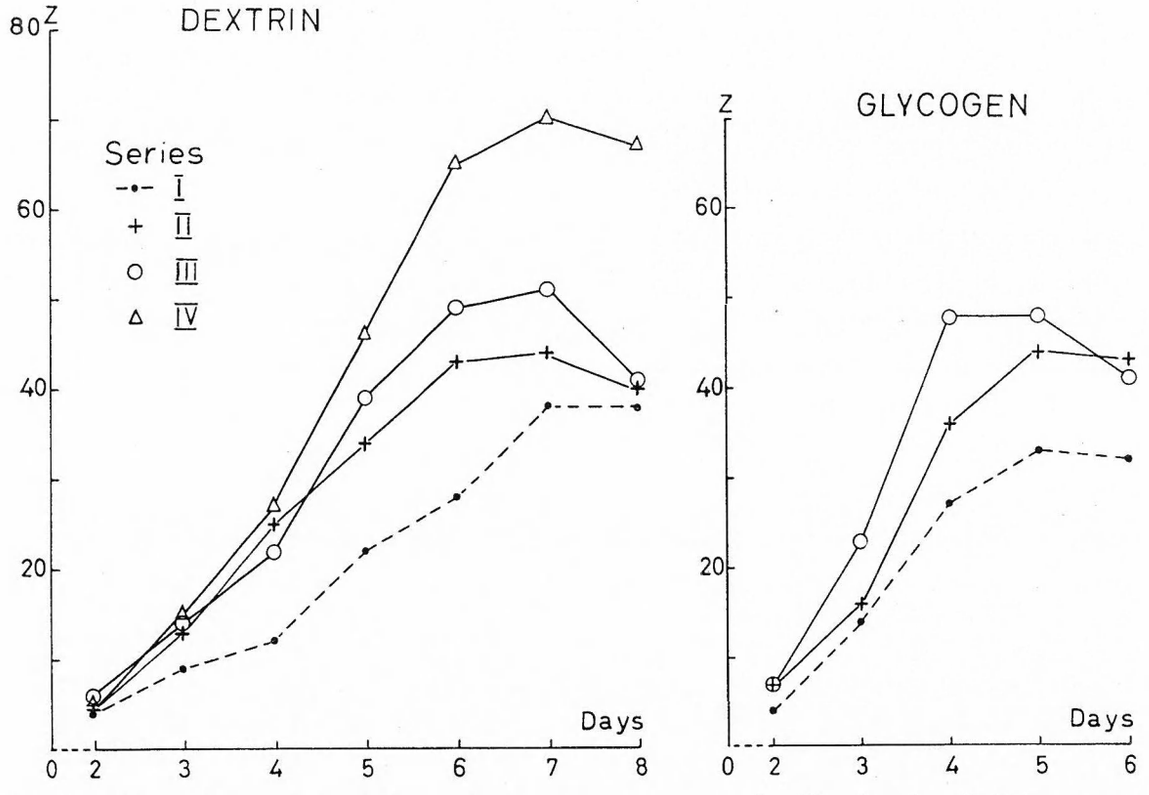

Fig. 2. The effect of dextrin and glycogen on growth (*Z*-values) of *Myxococcus virescens*. 0, 0.2, 1.0 and 5.0 g carbohydrate per l. added in Series I, II, III, and IV respectively.

with these findings. The growth in the starch-tubes appeared much better than that of the control and hydrolysis of the starch occurred.

In the presence of starch the pseudoplasmodia and the later fruiting bodies were observed to develop a greenish colour. At 5 g/l the colour was marked, at 1 g/l it was less accentuated and at 0.2 g/l only a faint touch of green colour was visible. No other carbohydrate tested produced such an effect. As yet, little is known of the factors controlling the pigmentation of the pseudoplasmodia and the fruiting bodies of myxobacteria. As regards *M. virescens* the Ca and Fe content of the medium is of some importance (Norén 1952) and in view of the present results, also the source of carbon.

Dextrin (Fig. 2): In all series dextrin increased the growth rate. Up to the 4th day the development was roughly equal in all dextrin series, but then some differences appeared. At 5 g/l the heavy growth continued and a *Z* max. of 70 was reached after 7 days. At 1 g/l and 0.2 g/l the *Z* max. was also reached after 7 days, the values being 51 and 44 respectively. Though the last value is not much higher than that of the control, the course of the curve will make clear the positive effect of

dextrin. The effect of dextrin on the growth of myxobacteria has been studied only once, by Solntzeva (1940) who did not find any improvement in growth of either *M. virescens* or *M. fulvus*.

Glycogen (Fig. 2): The addition of glycogen promoted a good growth of *M. virescens*. It is seen from the curves that even 0.2 g/l produced both a definite increase in amount and in the rate of growth. This was even more accentuated at 1 g/l. At this concentration the pH change was somewhat higher than in the control. Solntzeva (1940) who tested the influence of glycogen, found it inactive with *M. virescens* while *M. fulvus* was stimulated.

3. *Discussion*

The results of the present investigation will make it clear that *M. virescens* is able to attack a great number of carbohydrates. As a matter of fact, a greater number of carbohydrates acted positively on the growth of *M. virescens* than could be expected from earlier records. This might be due to the use of a more exact examination of growth as compared with previous studies — the positive effect for example sucrose would hardly be observed on an agar medium — and the cultural medium used. No carbohydrate can be utilized if the medium is lacking in any essential element or compound. In the case of *M. virescens,* which evidently is an organism with highly specific nutritional requirements (Kühlwein 1950, Finck 1950, see also below), it is quite probable that even if a utilizable carbohydrate is added, an agar medium with inorganic mineral salts and inorganic N-source may be deficient in certain essential substance(s) which the organism is able to synthesize only slowly or not at all. Under such conditions not the carbohydrate, but the substance(s) lacking, may be the factor(s) which limit(s) the growth. On the other hand, the casein hydrolysate medium used in the present investigation is a fairly rich medium, on which *M. virescens* grows well. It apparently contains all the essential metabolites that this organism needs for growth, but since carbon appears to be available only in insufficient amounts (Norén 1952) the growth promoting effect of an added utilizable carbohydrate may be reflected more distinctly than on an agar medium supplied with inorganic nutrients. It has also been shown that the effect of glucose and starch on *M. virescens* was improved in the presence of peptone or aspartic acid (Finck 1950, Oetker 1953) and in the case of *M. fulvus* a supply of an organic source of nitrogen such as asparagine or urea improved the positive effect of maltose (Kühlwein 1950).

M. virescens was not able to utilize all the carbohydrate tested to the same degree. The best growth promoting effect was produced by starch, the starch related compound glycogen and the hydrolytic products of starch, dextrin and maltose. These carbohydrates did greatly improve the growth but they did not seem to influence the pH-changes of the culture solution, at least not significantly. However, the effect on the pH-changes might possibly be greater than evident from the results obtained. It is known from several experiments that a better growth of *M. virescens* in a casein hydrolysate medium will usually produce a higher pH-increase of the medium. It is thus quite possible that acidic intermediate(s) were excreted into the medium but that the pH-depressing effect of these became completely masked by an increased alkali production from casein hydrolysate.

As mentioned above, hydrolysis of starch and the stimulation of growth by this compound have been reported by several workers (Jahn 1939, Solntzeva 1940, Beebe 1943, Kühlwein 1950, Oetker 1953, Clark 1954) and the result obtained in the present investigation is thus in keeping with their findings. However, records on the effect of glycogen, dextrin and maltose are rare and never any positive effect have been observed. (Solntzeva 1940, Clark 1954).

In view of the positive effect of maltose the results obtained with glucose were somewhat surprising. However, it is known that in some bacteria the incorporation of this carbohydrate into culture media causes a general reduction of deaminase activity (Epps and Gale 1942, Boyd and Lichstein 1951). It is quite possible that in the present experiment a similar effect was at least partly responsible for the decreased growth at the glucose concentration of 5 g/l and to the small pH-changes at 5 g/l and 1 g/l. It is however also possible that an excretion of acidic intermediates from the cells into the media was contributary to the pH-effect. It is an interesting fact that at the lowest concentration the inhibitory effect of glucose was completely overcome by the myxobacteria. The result seems to indicate even a slight growth-stimulative effect of the glucose addition, though the differences were not significantly established. Up to the present, the influence on deaminase activity of such a low glucose concentration as used here has not been investigated. In view of the results obtained such a study would appear to be profitable. From all these facts obtained one would be inclined to assume that *M. virescens* is able to utilize glucose though the uptake may follow a slow rate. Such a view is also supported by the results by Finck (1950) and Oetker (1953). On the other side, however,

Solntzeva (1940) found that in spite of good growth no glucose was consumed by this myxobacteria grown in a peptone containing medium.

Arabinose, galactose, sucrose, and raffinose did also improve the growth of *M. virescens* though to a lesser extent than did the starch and the compounds related to starch. Previously, positive effect has been recorded only with sucrose (Yoshii 1926, Norén 1952). It was also noted that the pH of the nutrient media containing galactose did not increase as much as that of the control. This possibly indicates that acidic intermediate(s) from the dissimilation process were excreted into the nutrient solution. As yet, no details concerning these acidic products are known. At the concentration 0.2 g/l however, the final pH of the sugar series was equal to that of the control. This might possibly mean that at this concentration only small amounts of acid(s) were produced which were completely masked by the simultaneous formation of ammonia from casein hydrolysate.

Xylose, mannose, fructose, lactose, and cellobiose did not, on any occasion, significantly improve the growth of *M. virescens*. However, their presence in the nutrient solutions had certain effects on the pH-changes which in the sugar containing media were less than in the controls. From this fact it might be concluded that the organism was able to attack all these sugars and as a result acids were formed.

C. Relations to Amino Acids

The investigations on the role of the amino acids in the growth of myxobacteria are few. Solntzeva (1940) reported that at the concentration of 0.5 %, none of alanine, d(?)-leucine, l(?)-cystine, glycine, tyrosine and asparagine produced growth of *M. virescens* or *M. fulvus* when they were used as nitrogen sources. Using a casein hydrolysate medium Oxford (1947) showed that at 0.4 % concentration, sodium glutamate, arginine, histidine and lysine could be substituted for asparagine, while glycine, alanine and cystine hydrochloride, separately or admixed, were inhibitory. On the other hand, Kühlwein (1950) found glycine as well as asparagine at 0.5 % being available as nitrogen sources to *M. virescens* and *M. fulvus*. Finck (1950) tested the effect of aspartic acid, glutamic acid, leucine, l-cystine and β-alanine and found all but aspartic acid and β-alanine inhibitory to growth of *M. virescens*. A mixture comprising aspartic acid, leucine, cystine and β-alanine produced only a weak growth, but the additional supply of glutamic acid greatly improved the result. Good effect of aspartic acid on growth of *M. virescens* was demonstrated by Oetker (1953). The present author (1952) showed that the individual amino acids of casein hydrolysate were utilizable as nutrients for *M. virescens* and that the best growth was produced by leucine, glutamic acid, methionine

and arginine. However, in no case was an optimum, or even a good growth obtained.

M. virescens will grow better in casein hydrolysate than in a mixture of amino acids considered to be completely corresponding to casein hydrolysate (Norén 1952). A reason for this has been proved to be the content of vitamins in the hydrolysate used (see below). However, the better growth could not be explained as a pure vitamin effect. Even a vitamin free casein hydrolysate will give a growth superior to that of the corresponding amino acid mixture (See Fig. 4).

1. *Experiments*

The following experiment studies the effect of the individual amino acids in combination with casein hydrolysate. It was assumed that if the casein hydrolysate was used in a low concentration, the stimulating or inhibitory effect of an added amino acid would be reflected in the growth.

Expt. 1. *The effect of nineteen amino acids on the growth of Myxococcus virescens, grown in a casein hydrolysate medium* (Fig. 3).

As a basic medium Nutrient solution III B was used with a vitamin-free casein hydrolysate content of 1 g/l. The amino acids were used in the proportions found in casein hydrolysate (Schmidt 1944) and calculated on the basis of 1 g casein hydrolysate. The basic nutrient solution was supplemented as follows:

Series	Addition per l basic solution, containing 1 g/l vitamin-free casein hydrolysate	Final pH
I	None	8.0
II	Casein hydrolysate, vitamin-free 1 g	8.2
III	Glycine 5 mg	8.0
IV	l(+)-alanine 19 „	8.0
V	l(+)-valine 79 „	8.0
VI	l(—)-leucine 49 „	8.0
VII	l(+)-isoleucine 49 „	8.0
VIII	l(—)-phenylalanine 39 „	8.0
IX	l(—)-tyrosine 66 „	8.0
X	l(—)-tryptophane 22 „	8.0
XI	l(+)-glutamic acid 323 „	8 2
XII	l(+)-aspartic acid 41 „	8.1
XIII	l(—)-proline 90 „	8.1
XIV	l(—)-hydroxyproline 2 „	8.0
XV	l(+)-serine 5 „	8.0
XVI	dl-threonine 35 „	8.0
XVII	l(—)-cystine 3 „	8.0
XVIII	l(+)-methionine 34 „	8.0
XIX	l(+)-arginine 38 „	8.1
XX	l(—)-histidine 25 „	8.0
XXI	l(+)-lysine 60 „	8.0
XXII	All amino acids (III—XXI) combined 984 „	8.1

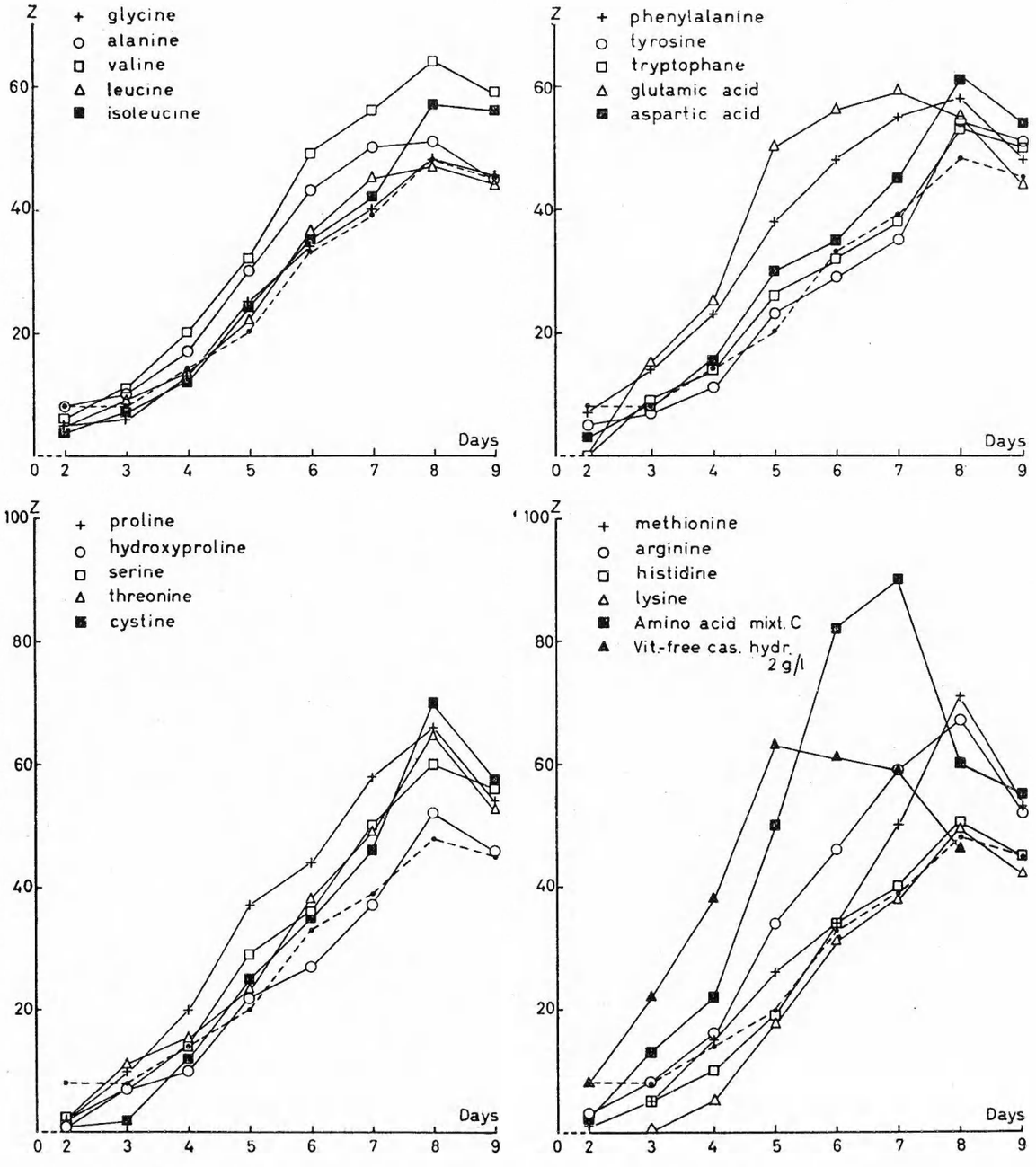

Fig. 3. The effect of 19 different amino acids on growth (*Z*-values) of *Myxococcus virescens*, grown in a casein hydrolysate medium (Expt. 1).

Each nutrient medium was supplemented with 200 µg riboflavin, 100 µg Ca-pantothenate, 200 µg folic acid, 100 mµg biotin, 1000 µg choline per 1 of solution. In all series the pH was adjusted to 7.5. The cultures were incubated at 30°C for 9 days.

When studying the results obtained which are summarized in Fig. 3 it must be born in mind that the addition of an amino acid to the casein hydrolysate represented a supply to the medium of a source of carbon,

nitrogen and energy which might be available to the organisms. In view of this it was not surprising that the addition of glutamic acid greatly improved the growth since this compound was used in a rather large quantity. Glutamic acid is in fact the chief constituent of casein hydrolysate. It seems also reasonable to ascribe partly the other positive results to an increased nutrient and energy supply. However, the growth promoting effects cannot entirely be explained only on this basis. Positive effects were obtained by additions being only 1/10 to 1/200 of the quantity of the casein hydrolysate present in the medium. It was also striking that though no one speeded the rate of growth as much as glutamic acid, the amount of growth was increased to the same extent — or even somewhat more — by other amino acid used in much smaller quantities.

Due to their effect on the growth of *M. virescens* the amino acids tested could be divided into five groups. The first group, including valine, phenylalanine, glutamic acid, proline and arginine acted greatly stimulatory. They both speeded the rate of growth and increased the amount of growth, though in the case of phenylalanine and glutamic acid this last effect was not so obvious. A second group consisting of aspartic acid, threonine, cystine, and methionine stimulated only the later phase of growth, and as a result higher *Z*-values i.e. higher total yields of growth were obtained. The positive effect of aspartic acid was not pronounced. It is possible that serine and isoleucine are to be included in this group. Alanine which was the only member of the third group, without actually affecting the amount of growth speeded the rate of growth, though not to any great extent. The fourth group, also consisting of only one member, lysine, produced an inhibition, visible only in the slightly retarded early phase of growth. The amino acids of the fifth group, which comprised glycine, leucine, tyrosine, tryptophane, hydroxyproline, and histidine did not influence the development in any detectable way, when they were added to casein hydrolysate.

It would be mentioned that several experiments similar to that above have been carried out. Although these always showed the same tendencies, certain differences appeared in some cases. Thus, by the addition of either aspartic acid, hydroxyproline or histidine a slight retarding effect on the rate of growth has been observed and in the case of serine and threonine less pronounced increases of the amount of growth have been obtained.

The mixture of amino acids had a remarkable effect when added

to the casein hydrolysate (Series XXII). At first, the growth was not as good as with casein hydrolysate alone (2 g/l, Series II) but this weak initial growth was followed by a rapid development resulting in a higher *Z* maximum. The reason of this effect is not known. Since acid hydrolyzed casein contains no tryptophane and only a small quantity of cystine it could be expected that the presence of one or both of these amino acids in the mixture would be responsible for the result. As a matter of fact the addition of cystine to casein hydrolysate yielded a higher amount of growth. However, such a view is not supported by the results obtained in Expt. 2.

As in Expt. 1 only a slight inhibitory action of one amino acid was obvious it was not possible to give an explanation for the poorer growth in the amino acid mixture as compared with that in casein hydrolysate. Therefore, a further experiment was carried out to test the effect of the individual amino acids in this mixture.

Expt. 2. *The growth of Myxococcus virescens in an amino acid mixture C, corresponding to a complete casein hydrolysate, and in C modified in successive cases by the removal of one of the amino acids* (Fig. 4).

Nutrient solution III B without casein hydrolysate was used. To the media was added, per litre, 200 µg riboflavin, 100 µg Ca-pantothenate, 200 µg folic acid, 100 mµg biotin and 1000 µg choline. The experiment comprised 21 series, and the additions per litre basic medium were made according to the following table:

Series	Substances added		Final pH
I	Casein hydrolysate, vitamin-free	2.5 g	8.1
II	Amino acid mixture C	2.46 g	8.0
III	C without glycine	12.5 mg	7.5
IV	C „ l(+)-alanine	47.5 „	8.0
V	C „ l(+)-valine	197.5 „	7.6
VI	C „ l(—)-leucine	122.5 „	7.5
VII	C „ l(+)-isoleucine	122.5 „	7.6
VIII	C „ l(—)-phenylalanine	97.5 „	8.0
IX	C „ l(—)-tyrosine	165.0 „	7.7
X	C „ l(—)-tryptophane	55.0 „	8.0
XI	C „ l(+)-glutamic acid	807.5 „	7.7
XII	C „ l(+)-aspartic acid	102.5 „	8 0
XIII	C „ l(—)-proline	225.0 „	8.0
XIV	C „ l(—)-hydroxyproline	5.0 „	8.0
XV	C „ l(+)-serine	12.5 „	7.8
XVI	C „ dl-threonine	87.5 „	7.9
XVII	C „ l(—)-cystine	7.5 „	7.9
XVIII	C „ l(+)-methionine	85.0 „	7.7
XIX	C „ l(+)-arginine	95.0 „	7.7
XX	C „ l(—)-histidine	62.5 „	8.1
XXI	C „ l(+)-lysine	150.0 „	8.1

In the mixture C, the amino acids were combined in the same proportions as found in casein hydrolysate (Schmidt 1944), and in amounts calculated on the basis of 2.5 g of casein hydrolysate. In all series pH was adjusted to 7.4. The cultures were incubated at 30°C for 11 days.

During the course of the experiment the nutrient media became slightly yellowish, the appearance of this colour varying with the different series. In the series III and V—VII it did not appear at all. On the 9th day it was noted in the series IV, VIII, XIII, XX, and XXI and on the 11th day in the solution where glutamic acid was removed. In all the remaining series the colour was apparent on the 10th day. Since the *Z*-values obtained with the coloured liquid do not adequately reflect the pseudoplasmodial development these figures have not been marked out on the diagrams.

It is evident from Fig. 4 that the development up to the 6th day was definitely slower in the amino acid mixture than in the casein hydrolysate. The rate of the following development, however, was about the same until the 8th day where the growth maximum for the casein hydrolysate series appeared. In the amino acid mixture the *Z*-values for 9 days did not indicate any cessation of the growth. Here, however, the readings were influenced by the yellowish colour of the culture liquid. An examination after two further days showed that in the tubes with casein hydrolysate the pseudoplasmodia were much thicker and spread out over a greater area, than in the tubes with the amino acid mixture. Thus, in comparison with a corresponding amino acid mixture the vitamin-free casein hydrolysate does not only give the best initial growth but it seems also to give the best total yield of living matter. From this fact it might be concluded that the casein hydrolysate, used in my first experiments (Norén 1952) stimulated the growth of *M. virescens* not only by its content of vitamins but also in some other way. The effect can only partly be due to the tryptophane content of the mixture since during the first 7 days the growth was definitely better on the vitamin-free casein hydrolysate than on the tryptophane-free amino acid mixture.

The removal of an amino acid from the mixture implied a certain change in the carbon, nitrogen and energy supply to the organisms, this change being particularly great in the case of glutamic acid. However, the effects obtained cannot entirely be explained on this basis since the quantities omitted were always very small as compared with the total amino acid content of the medium.

A particularly strong stimulation of the growth was obtained in the

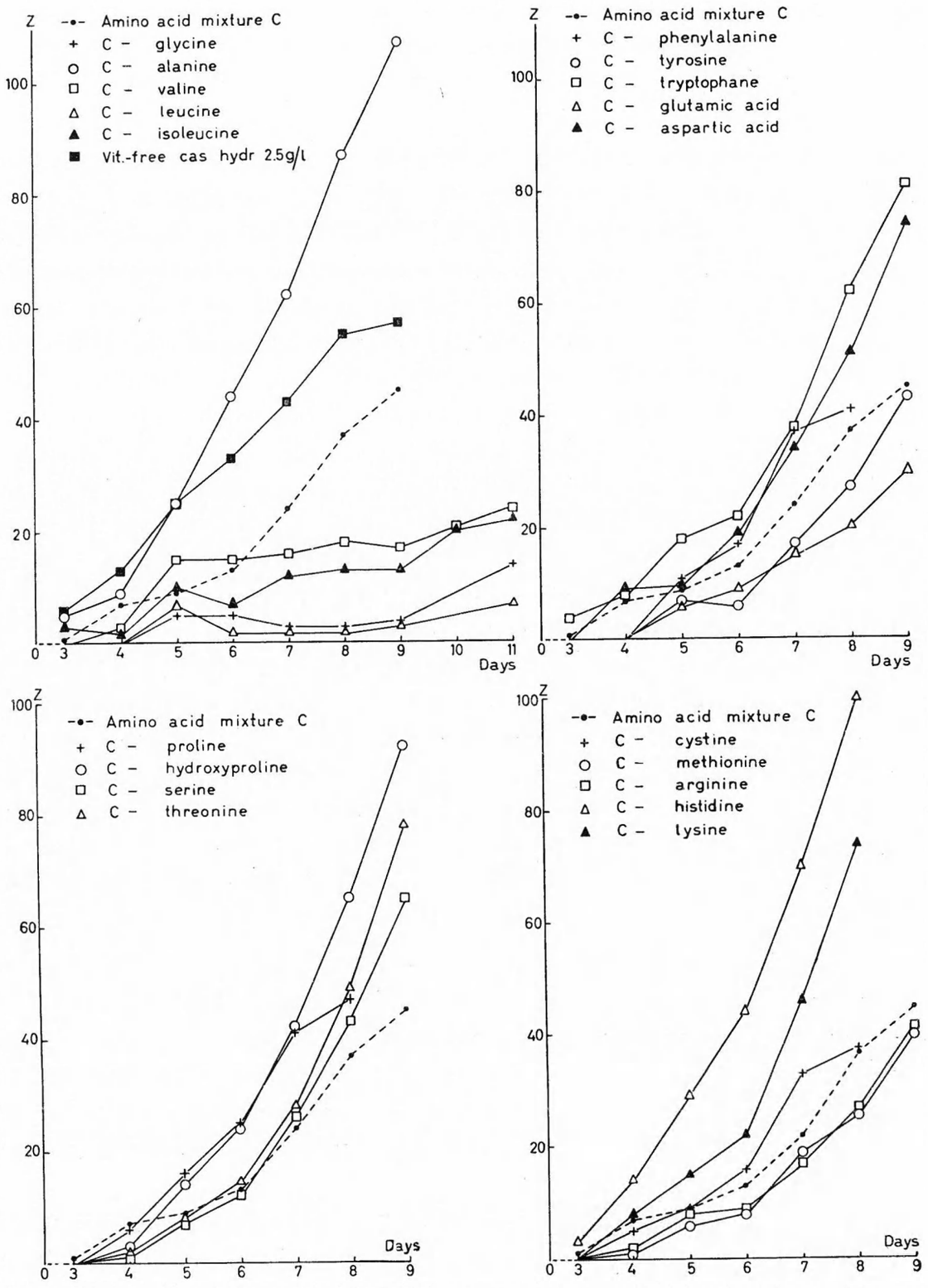

Fig. 4. Growth (*Z*-values) of *Myxococcus virescens* in an amino acid mixture C, complete and reduced in turn by each single amino acid. The amino acids used in quantities corresponding to those found in casein hydrolysate (Expt. 2).

absence of alanine and of histidine. In both these cases the effect was most obvious in the yield of growth although the rate of growth was also increased. In fact, both the rate and the total amount of growth was considerably greater than in Series I (with casein hydrolysate)! An increase in the growth rate, though not to the same extent as above, was also obtained by the omission of tryptophane, aspartic acid, proline, hydroxyproline, and lysine. Here, too, with the exception of proline, the total amount of growth was superior to that in the casein hydrolysate medium. It would be noted that the addition of proline to casein hydrolysate also produced a considerable growth improvement.

The removal of either serine or threonine did not, to any great extent, affect the rate of growth but the amount of growth was definitely increased. The values of the *Z* max. obtained in these series were even greater than those of Series I. Both these amino acids acted stimulating on growth when they were added to casein hydrolysate.

In other cases the removal of an amino acid from the complete mixture C induced an inhibition of the growth of *M. virescens*. This was particularly obvious by the omission of either glycine or leucine by which a certain growth occurred although the pseudoplasmodia formed were extremely thin and transparent. A somewhat better growth was obtained in the absence of either valine or isoleucine. In the medium without valine the development apparently stopped after 5 days, with no *Z*-increase from the 5th to the 9th day while in the medium without isoleucine a slow growth seemed to occur right through the experiment. Notable is the slight increase of the *Z*-value between the 9th and 11th day appearing in all these series.

A retarding effect on the growth was obvious when either glutamic acid, tyrosine, methionine or arginine were omitted. In the case of the three last amino acids, however, after an initial inhibitory phase, the organisms grew sufficiently well to give a *Z* max. about equal to that in Series II. The removal of glutamic acid, on the other hand gave a somewhat inferior total yield of growth. In this experiment the development was followed for 11 days, and in a repeated experiment for 3 weeks. At the final examination in both cases the pseudoplasmodial films appeared definitely thicker in the complete amino acid mixture.

The omission of phenylalanine produced only a slight tendency for an inhibition of the initial growth and cystine was removed without any obvious change in growth whereas the increase in pH of the culture medium was not as great as in Series II.

2. *Discussion*

On the basis of the present experimental data it appears not possible to draw any definite conclusions concerning the amino acid requirements of *M. virescens*. However, some facts can be noted.

In the amino acid mixture considered to correspond to casein hydrolysate the presence of glycine, valine, leucine and isoleucine is necessary to produce a good growth of *M. virescens*. The removal of one of these amino acids resulted in a strong growth inhibition. However, when added to casein hydrolysate (in a low concentration) only valine improved the growth. It is not known if these amino acids are produced in insufficient quantities by the organism or if they counterbalance the inhibitory effect of one or more other amino acids present in the mixture. The effect of a simultaneous omission of valine, leucine and isoleucine (see Gladstone 1939, Bonner 1946, Norkrans 1950, Dien et al. 1954 and others) was not studied. The positive effect of the addition of valine to casein hydrolysate may possibly indicate a slow synthesis of this compound. On the other hand, leucine as the sole source of carbon and nitrogen produced a much better growth (Norén 1952). Solntzeva (1940), however, found this amino acid to be inactive and Finck (1950) found it even inhibitory to the growth of *M. virescens*.

In view of the fact that glycine is involved in biological reactions concerning choline (Jukes 1947) and in the formation of purines (see Shive 1951 for review) the result obtained with this amino acid is remarkable. As shown below both choline and adenosine are stimulating the growth of *M. virescens*. However, Solntzeva (1940) did not obtain any effect with glycine and Oxford (1947) found it even inhibitory when it was added to a casein hydrolysate medium at the concentration of 0.4 %. On the other hand, at the concentration of 0.5 % glycine was utilized as a source of nitrogen in the experiments of Kühlwein (1950) and the present author obtained growth by using only 12.5 mg/l of glycine as the sole source of carbon and nitrogen (Norén 1952).

Glutamic acid, arginine and methionine are truly stimulatory to the growth of *M. virescens* and their presence in the amino acid mixture is necessary to produce good growth. Phenylalanine and tyrosine also possibly have a certain stimulating influence on the growth, but the effect of these amino acids was not so clearly demonstrated as in the above cases. The results obtained with glutamic acid might possibly be ascribed to the changes in nutrient supply in the medium but the

effects with arginine and methionine cannot be explained in this simple way, since the total amino acid content of the medium was here 25 or 30 times greater than the removals or the additions. While Finck (1950) found glutamic acid completely inhibitory when added to a glucose agar at the rate of only 50 mg/l, Oxford (1947) reported that in a casein hydrolysate medium, both glutamate and arginine were tolerated at the rate of 4 g/l. When used as sole sources of carbon and nitrogen glutamic acid, arginine and methionine induced better growth than all other amino acids except leucine (Norén 1952).

The removal of alanine or histidine from the amino acid mixture corresponding to complete casein hydrolysate caused a particularly great growth stimulation. In fact, in both these cases the growth was much better than in the medium with vitamin-free casein hydrolysate. An improved growth was also obtained in the absence of lysine, tryptophane, aspartic acid, hydroxyproline or proline. The amount of growth was increased when threonine or serine were removed. Thus, in some way or other each of these amino acids inhibited the growth of *M. virescens*. This result was somewhat unexpected since none of them appear to be inhibitory in themselves. When used separately, each of them can be utilized as a sole source of carbon, nitrogen and energy (Norén 1952). From this fact it is evident that the inhibitory effects are produced only when the amino acids in question are included in the amino acid mixture. However, when added to casein hydrolysate, only lysine produced a negative effect. The other were proved to be inactive or even stimulatory. In literature, the only record of inhibition by one of these amino acids is given by Oxford (1947), who found alanine at the concentration of 0.4 % inhibitory to the growth of *M. virescens*. On the other hand, the same author also found that at the same concentration asparagine, lysine and histidine were tolerated by the myxococci. Asparagine or aspartic acid have been recorded to have been utilized (Kühlwein 1950, Finck 1950, Oetker 1953) or inactive (Solntzeva 1940), but never inhibitory to *M. virescens*.

The effects obtained in Expt. 1 with the "inhibitory" amino acids appear to disagree with the effects obtained in Expt. 2. In considering the results, however, it must be remembered that the relationship between the single amino acids in a mixture is rather complicated. To produce a good growth of an organism the components must necessarily occur in the correct proportions. An imbalance can affect the utilization of one or more of the other amino acids present in the mix-

ture, or the synthesis of products from them (Christensen 1953, McCoy et al. 1954). Due to inhibitory interactions between the different amino acids the removal or the addition of a single amino acid can result in a growth inhibition. In literature, several records are given on antagonistic effects between amino acids of similar chemical structure. (Gladstone 1939, Bonner et al. 1943, Robbins and McVeigh 1946, Bonner 1946, Meincke and Holland 1948, Norkrans 1950, Dien et al. 1954, and others). However, a removal or an addition of a single amino acid can also cancel the imbalance of a mixture in which the constituents initially do not occur in adequate concentrations. Such a change in the composition will have a growth-stimulative effect.

Casein hydrolysate produces a relatively good growth of *M. virescens*. However, Expt. 1 and Expt. 2 have shown that the growth will be improved when a single amino acid is added to casein hydrolysate or removed from the amino acid mixture corresponding to casein hydrolysate, i.e. when the balance between the amino acids in the mixture is changed. From this it is clear that the casein hydrolysate does not contain the amino acids in proportions suitable for an optimum growth of *M. virescens*. The imbalance is more or less reduced either by the removal of alanine, histidine, lysine, tryptophane, aspartic acid, hydroxyproline, proline, threonine or serine or by the addition of certain amounts of phenylalanine, proline, aspartic acid, threonine or cystine. The best growth-stimulative effect had the removal of alanine or histidine.

Since acid hydrolyzed casein does not contain tryptophane the results obtained for this amino acid in Expt. 1 and Expt. 2 are not quite comparable. In the former only 22 mg/l were added but in the latter 55 mg/l were removed. However, from the results it would seem reasonable to assume that a dose of 55 mg/l tryptophane is growth-inhibiting, and that of 22 mg/l is inactive, for the growth of *M. virescens*. Such a view appears to be supported by the results from a supplementary experiment, in which the addition of 44 mg/l to the vitamin-free casein hydrolysate decreased the rate of growth but did not affect the amount of growth. However, in a medium with a non vitamin-free casein hydrolysate the amount of growth was not affected by the addition of 55 mg/l (Norén 1952). Fries (1950) tentatively explained a growth-inhibiting effect of tryptophane on some Hymenomycetes as possibly due to a conversion of tryptophane to 3-indolacetic acid, which has been shown to be inhibitory to several microorganisms (Richards 1949).

D. Vitamin Requirements

1. *Earlier observations*

In previous investigations the cultivation of myxobacteria was performed on media of rather complex composition, such as dung decoction agar, which would contain several unknown substances. Because of this it was not possible to obtain adequate informations concerning the exact requirements of nutrients and growth substances. Even now, when more defined substrates are used, the myxobacterial capability of synthesizing the vitamins necessary for their growth has by no means been clarified.

Nigrelli-Hutner (1945) recorded that *Chondrococcus columnaris,* a myxobacterial pathogen on fish, seemed to require no unknown growth factors. Finck (1950) tested the vitamin requirements of 8 species of *Myxococcus* using an agar medium, containing either only glucose, or glucose with a mixture of aspartic acid, glutamic acid, leucine, l-cystine, and β-alanine, to which medium vitamins were added. On the whole negative results were obtained. Riboflavin, pyridoxine and nicotinamide caused an almost complete inhibition of growth of *M. virescens,* while p-aminobenzoic acid produced no change. Ca-pantothenate improved the growth but pantothenic acid acted as a growth inhibitor. On the other hand β-alanine, which is known to be an integral part of the pantothenic acid molecule stimulated the growth.

As a basal medium Oetker (1953) used an agar with the cells of *Escherichia coli* incorporated as the sole source of nutrition. Only nicotinamide had a slight growth promoting effect on *M. virescens,* while aneurin, riboflavin, Ca-pantothenate, pyridoxine (separate or in mixture) and B_{12}, p-aminobenzoic acid and ascorbic acid were either slightly inhibitory or without effect. It was concluded that the growth substances tested were already present in the basal medium in amounts sufficient for good growth. In the light of the results recorded below, however, it seems possible that the added vitamin concentration was too high.

As mentioned, both the experiments of Finck and Oetker were performed on agar media. The agar itself, however, may contain active elements such as aneurin and biotin (Robbins 1939, Day 1942) which might have influenced the results. Oxford (1947) used a liquid medium with casein hydrolysate as the sole source of carbon and nitrogen, and to this medium was added a vitamin mixture consisting of nicotinic acid, riboflavin, pyridoxine, Ca-pantothenate, aneurin and biotin. No effect was observed either on the growth of *M. virescens* or on the production of antibiotic substances. A further test of the vitamin effect on the same species and on *M. fulvus* and *Ch. coralloides* was made by the present author (1952). A vitamin mixture comprising aneurin, nicotinic acid, pyridoxine, Ca-pantothenate, riboflavin, p-aminobenzoic acid and biotin was added to a casein hydrolysate medium in various concentrations. *M. virescens* and *Ch. coralloides* did not appear to be influenced, but in the case of *M. fulvus* a certain stimulative effect was obvious. This positive effect was still more accentuated if a further supply of choline and folic acid was made. However, in this investigation, as apparently in that of Oxford, a non vitamin-free casein hydrolysate was used.

In all experiments on the vitamin requirements of myxobacteria, the organisms had been able to develop without a supply of any exogenous vitamin source. But on the other hand, it was apparent that no experiment had been carried out with a completely vitamin-free medium. Consequently, it was not possible to make any definite statements on the basis on the results existing. This fact in itself was reason enough for a further study on the subject. But there was also another point. Myxobacteria, which grow well in a casein hydrolysate medium, give a weaker growth if this is substituted for a mixture of amino acids in the proportions as found in casein hydrolysate (Norén 1952). The possibility existed that the growth differences were due to a lack of certain growth stimulating substances, for instance vitamins, in the mixture.

2. *Experiments*

The experiments were carried out using liquid media, in which a vitamin-free casein hydrolysate ("Difco" Bacto vitamin-free casamino acids) provided the sole source of carbon and nitrogen. During the course of the investigations it appeared necessary to concentrate the work on the one species *M. virescens,* although on two occasions *Ch. coralloides* was used as well. Here will be considered the effect of vitamins on the growth. Some observations on their influence on the production of bacteriolytic substances will be recorded in a later paper.

The organisms were first tested whether they could be considered independent of or completely or partly dependent on the presence of vitamins.

Expt. 3. *A comparison of the growth of Myxococcus virescens and Chondrococcus coralloides grown in vitamin-free and in non vitamin-free medium* (Fig. 5).

As a basic medium, Nutrient solution III B without casein hydrolysate was used. The experiment included 6 series with 5 replicates in each. The basal medium was supplemented according to the table below.

Series	Casein hydrolysate		Amount of			*Myxococcus virescens*		
						pH		
	non vitamin-free g/l	completely vitamin-free g/l	each vitamin in mixture B µg/l	biotin mµg/l	choline µg/l	start	7 days	14 days
I	—	2.5	—	—	—	7.5	8.1	8.6
II	—	2.5	50	50	250	7.5	8.2	8.7
III	—	2.5	100	100	500	7.5	8.3	8.7
IV	—	2.5	200	200	1000	7.5	8.1	8.7
V	—	2.5	400	400	2000	7.5	8.1	8.7
VI	2.5	—	—	—	—	7.5	8.1	8.8

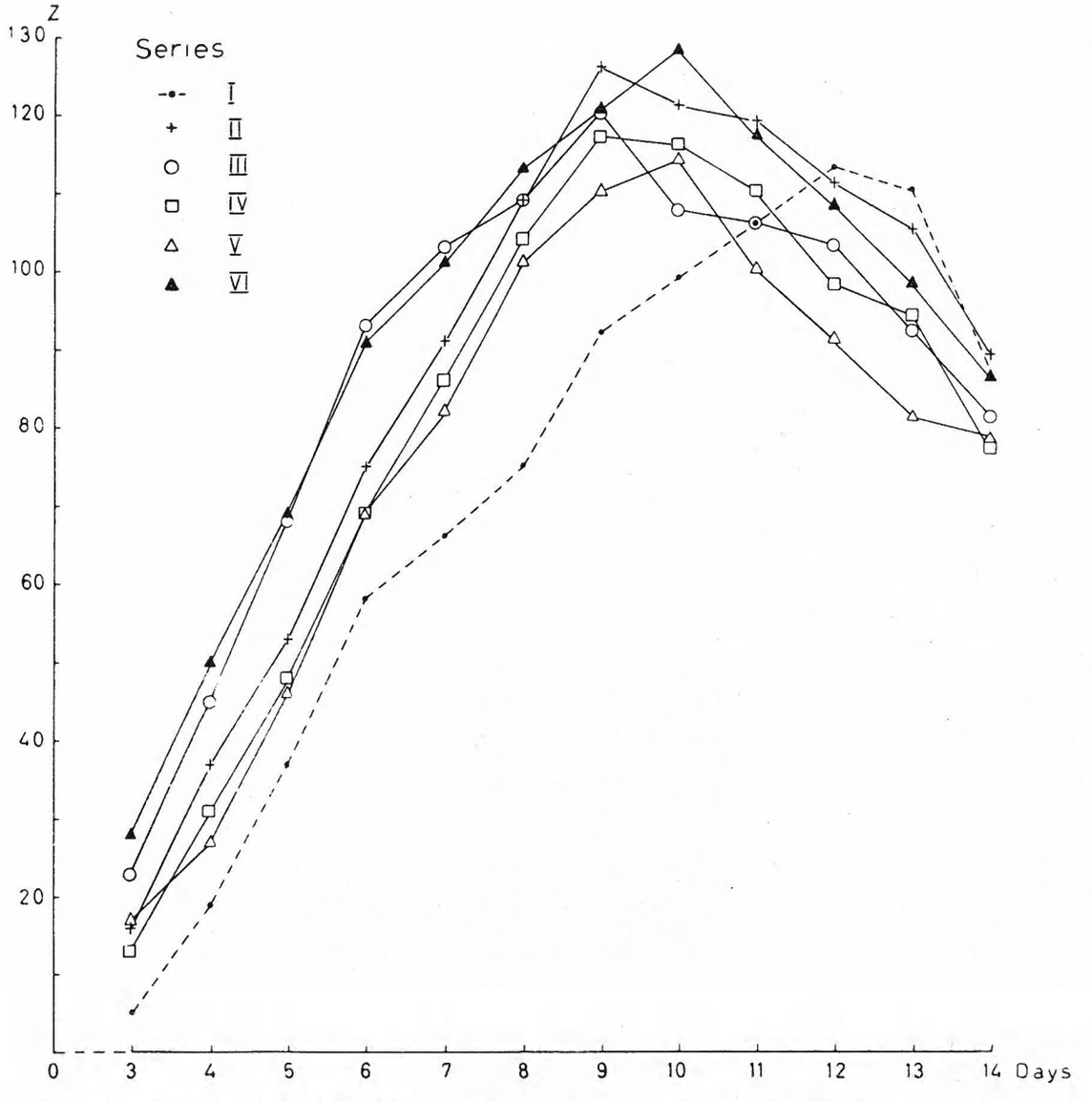

Fig. 5. Growth (*Z*-values) of *Myxococcus virescens* in a vitamin-free casein hydrolysate medium (Series I), in the same medium supplemented with a vitamin mixture in different concentrations (Series II—V) and in a medium with a non-vitamin-free casein hydrolysate (Series VI) (Expt. 3).

The vitamin mixture B consisted of aneurin, riboflavin, nicotinamide, pyridoxine, Ca-pantothenate, folic acid (pteroylglutamic acid) and p-aminobenzoic acid. The initial pH of the nutrient media for *Ch. coralloides* was 6.8. *M. virescens* was incubated at 30°C for 14 days, and *Ch. coralloides* at 25°C for 28 days, during which time the tubes were turned a quarter of a circumference per day (see above p. 83).

In all series *M. virescens* grew well. In Series VI the growth was speedy and here the curve reached its maximum after 10 days, but later, when autolysis set in, a decline of the curve occurred. The vita-

min-free casein hydrolysate yielded a much slower growth, and all through the experiment the development of Series I was about two days behind that of Series VI, the growth maximum being obtained after 12 days. The maximum of growth was also a little higher in Series VI though the difference was not very marked, the maximum of Z in Series VI being 128 and that of Series I 113.

The vitamin mixture added to a medium containing vitamin-free casein hydrolysate greatly improved the growth. The positive effect was shown mainly by the increased rate of growth, rather than by the amount of growth which was less influenced. In Series III where each of the vitamins aneurin, riboflavin, nicotinamide, pyridoxine, Ca-pantothenate, folic acid, and PABA was added to give a concentration of 100 µg/l, biotin 100 mµg/l and choline 500 µg/l, a growth almost equivalent to that in Series VI was obtained. At higher concentrations the growth improvement was not so good, indicating some inhibitory effects. This effect was particularly accentuated in Series V, where the vitamin addition was four times as large as that of Series III.

In the case of *Ch. coralloides* no responses in growth at the various vitamin concentrations could be observed.

Experiment 3 showed the following:

1. *M. virescens* is capable of assimilating a completely vitamin-free casein hydrolysate medium without any exogenous vitamin sources being added. This might mean that under the cultural conditions existing this organism is able to bring about a synthesis of all the vitamins necessary for its growth.

2. The more rapid development in the non vitamin-free casein hydrolysate indicates that though a synthesis of all the essential vitamins occurs, the synthesis at some stage(s) may follow such a slow rate that growth is limited.

3. The addition of vitamins to the vitamin-free medium produced an increased rate of growth while the amount of growth was less or not at all affected. A suitable supply gave rise to a growth equivalent to that in the non vitamin-free casein hydrolysate. In concentrations too high the positive effect was diminished.

4. The growth-stimulative vitamin mixture comprised aneurin, riboflavin, nicotinamide, pyridoxine, Ca-pantothenate, folic acid, p-aminomenzoic acid, biotin, and choline. It could thus be concluded that one or several of these vitamins may act as the limiting factor(s) for growth in the vitamin-free medium.

It could thus be concluded that *M. virescens,* under the culture con-

ditions used, is deficient with respect to one or several of certain vitamins, the deficiency, however, being only partial. In order to elucidate which vitamin(s) could be the active factor(s) in the mixture of Expt. 3, an experiment was carried out where the vitamins were tested separately. Since the best growth promoting effects were obtained in Series III of Expt. 3, the vitamins were used in the same amounts.

Expt. 4. *The effect of vitamins, added separately, on the growth of Myxococcus virescens and Chondrococcus coralloides* (Fig. 6).

Nutrient solution III B was used, the casein hydrolysate was vitamin-free. The experiment comprised 10 series, each with 5 replicates.

Series	I	No vitamin added.	
,,	II	Aneurin	100 µg/l of the vitamin added in each case.
,,	III	Riboflavin	
,,	IV	Nicotinamide	
,,	V	Pyridoxine	
,,	VI	Ca-pantothenate	
,,	VII	Folic acid	
,,	VIII	P-aminobenzoic acid (PABA)	
,,	IX	Biotin, added in amount of 100 mµg/l	
,,	X	Choline, added in the amount of 500 µg/l.	

M. virescens was incubated at 30°C for 14 days, and *Ch. coralloides* at 25°C for 28 days. In this experiment, as in Expt. 3, the tubes were turned a quarter of a circumference per day during the incubation. The culture media for *M. virescens* had the initial pH of 7.5. The final pH was 8.6 in all series except for Series IX and X where it reached 8.7. In the experiment with *Ch. coralloides* the media had the initial pH of 6.8. Here the pH did not change during the incubation.

Ch. coralloides did not give any evident growth response on the addition of the various vitamins.

The growth of *M. virescens* in the vitamin-free Series (broken line in Fig. 6) showed almost the identical features as in the previous experiment. The growth maximum, which appeared after 12 days, showed a *Z*-value of 119, this value, however, being about the same as *Z* at 10 days (116).

In accordance with their action, the individual vitamins of the mixture used in Expt. 3 could be divided into two groups, those that produced a stimulated growth of *M. virescens* and those that did not. The vitamins of the second group were aneurin, nicotinamide, pyridoxine and p-aminobenzoic acid, and their effects are summarized in Fig. 6.

Aneurin: This vitamin appeared to exert a certain inhibitory influence on the development during the first days, the inhibition being

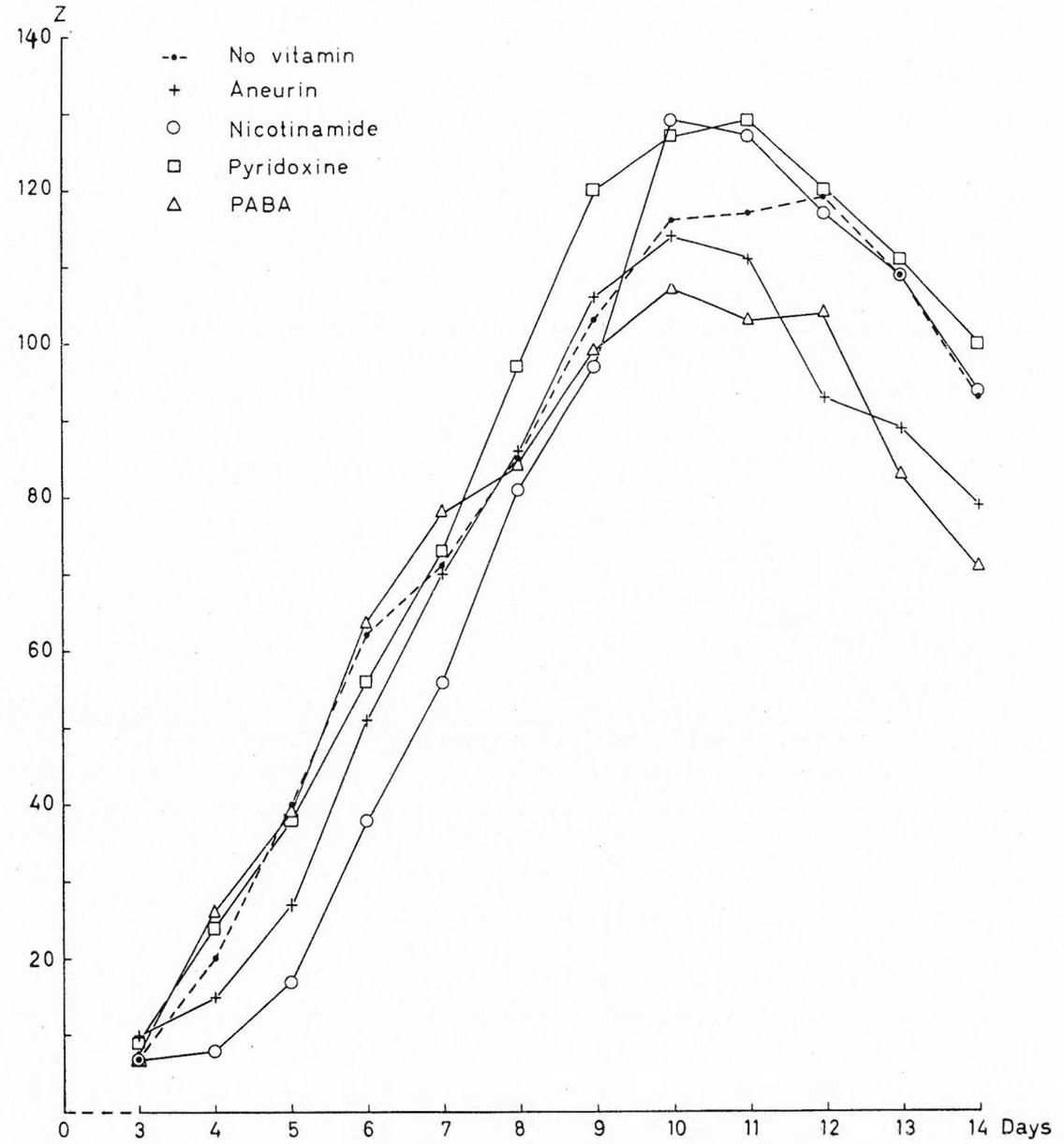

Fig. 6. The effect of some vitamins on growth (*Z*-values) of *Myxococcus virescens* (Expt. 4).

only slightly reflected on the curve. Later the growth apparently was in keeping with that of Series I. The growth maximum was reached after 10 days. The steep decrease of the curve after the maximum seems to indicate a rather rapid autolysis.

Nicotinamide: As in the case of aneurin, nicotinamide retarded the growth during the first days. The inhibition was more accentuated here than in the previous case, but was evidently overcome after 5 days when a rapid development occurred. As a result, the growth maximum appeared after 10 days, the *Z* max. being 129.

By heating for 48 hours a solution containing asparagine and glutamic acid, nicotinic acid or a substance with the similar properties is formed (Bovarnick 1943). Though in my experiment the experimental conditions quite differed from those of Bovarnick, the possibility must not be disregarded that a small amount of a growth factor with a nicotinic acid effect was present in the medium even before the addition of nicotinamide. It is, however, noteworthy that tryptophane which is involved in the formation of niacin (see Mitchell 1950 for review) does not produce stimulated growth of *M. virescens* (Norén 1952, see also above).

Pyridoxine: This vitamin did not affect the initial phase of growth but after 8—9 days a slightly stimulating effect was visible, the stimulation, however, being not significantly proved (*Z*-max. 129).

p-Aminobenzoic acid: During the first 9 days no influence could be noted but in the later phase of development a tendency to inhibition appeared. The growth ceased after 10 days, the maximum value then (107) being somewhat lower than that of the control series. The rapid decrease of the curve might indicate an intense autolysis.

The other group of vitamins tested comprised riboflavin, folic acid, Ca-pantothenate, biotin and choline. Their effect, being more or less obvious, implied an increase in the rate of growth and in some cases a slight increase in the amount of growth (cp. Figs. 7—11).

Ca-pantothenate was a special case in that it considerably increased the growth rate but after 9 days the growth ceased at $Z=106$ — lower than that of the control — and then Z decreased. It is further notable that folic acid, but not p-aminobenzoic acid, had a positive effect on the growth.

Experiment 4 thus revealed that under the conditions prevailing *M. virescens* might be considered as self-sufficient with respect to aneurin, pyridoxine and p-aminobenzoic acid, and probably to nicotinamide. On the other hand the synthesis of the vitamins riboflavin, folic acid, pantothenic acid, biotin and choline seemed to be too slow to provide an optimum growth of the organism. The supply of one of these substances to the medium, i.e. the supply of an exogenous vitamin source to the cells, had a growth promoting effect. Thus, in these cases a partial deficiency evidently exists.

Further details concerning the action of the growth promoting vitamins are given in the following experiments (Expt. 5—9) where their effect in various concentrations were tested.

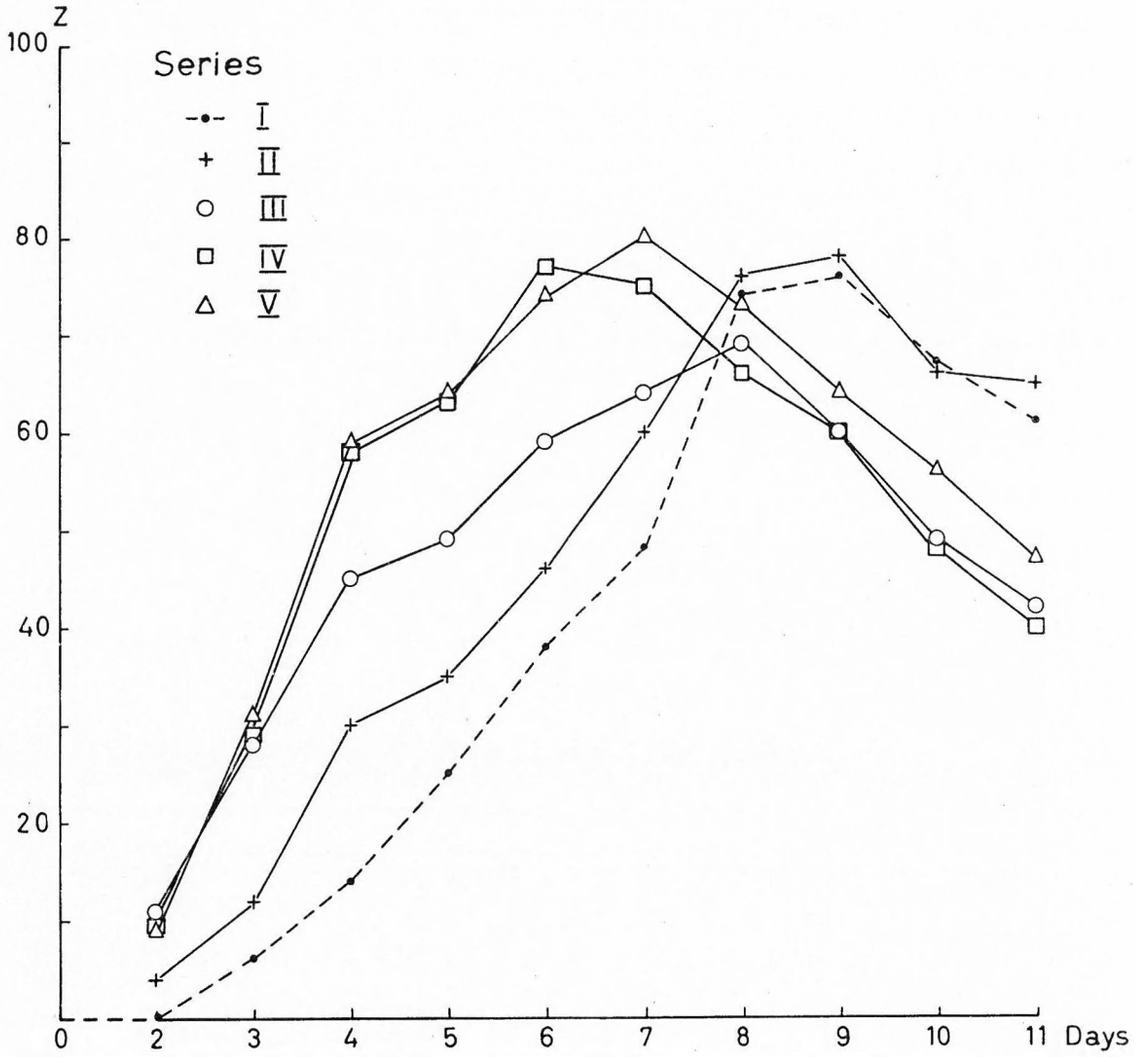

Fig. 7. The effect of concentration of riboflavin on growth (Z-values) of *Myxococcus virescens*. 0, 50, 100, 200 and 400 µg riboflavin per l. added in Series I, II, III, IV, and V respectively (Expt. 5).

Expt. 5. *The effect of riboflavin concentration on the growth of Myxococcus virescens in a casein hydrolysate medium* (Fig. 7).

Nutrient solution III B was used, the casein hydrolysate being vitamin-free. The experiment comprised 5 series, each with 4 replicates. The amount of riboflavin added was: Series I, none; Series II, 50 µg/l; Series III, 100 µg/l; Series IV, 200 µg/l; and Series V, 400 µg/l. The cultures were incubated at 30°C for 11 days. In each series, the initial pH of the culture medium was 7.5, and the final pH 8.7.

An addition of riboflavin in the concentration of 50 µg/l gave a slight increase in the rate of growth, this increasing effect being further accentuated with 100 µg/l. A supply of double this amount, i.e. 200 µg/l improved the growth still more already producing a growth maximum

of $Z=77$ after 6 days. This dose appeared to be the optimum one. In any case further improvement was not obtained with 400 µg/l, and it may be possible that the later growth maximum here — after 7 days — indicated a tendency to inhibition as compared with the previous series. The differences obtained were however too small to allow any definite conclusions.

As a result, under these conditions riboflavin had a markedly stimulating effect on the rate of growth. A concentration of 200 µg/l appears to be adequate to give an optimum effect.

Expt. 6. *The effect of Ca-pantothenate concentration on the growth of Myxococcus virescens in a casein hydrolysate medium* (Fig. 8).

Nutrient solution III B, with vitamin-free casein hydrolysate was used. Five series were set up, each with 4 replicates. The amounts of Ca-pantothenate added was: Series I, none; Series II, 50 µg/l; Series III, 100 µg/l; Series IV, 200 µg/l; and Series V, 400 µg/l. The cultures were incubated at 30°C for 11 days. In each series the initial pH of the medium was 7.5. The final pH of Series I was 8.7 and in Series II—V, 8.8.

The addition of 50 µg/l gave no increase in the rate of growth and had a slightly depressing effect on a later phase of the development. At higher concentrations, 100—400 µg/l an increased rate of growth was first visible, but after 4 days the development became greatly retarded and from this point the growth of the vitamin series were considerably slower than that of the vitamin-free control cultures. The total yield of growth appeared to be unaffected at the concentration of 100 µg/l but at the two higher concentrations of Ca-pantothenate it was somewhat decreased.

Thus, as pointed out in connection with Expt. 4, this experiment revealed that Ca-pantothenate produces a twofold effect on *M. virescens,* i.e. during the early phase of growth it is stimulating but later a marked inhibitory action is obvious. Evidently an amount of 100 µg/l produces the stimulating effect but does not depress the total amount of growth.

Expt. 7. *The effect of folic acid concentration on the growth of Myxococcus virescens in a casein hydrolysate medium* (Fig. 9).

Nutrient solution III B was used, the casein hydrolysate being vitamin-free. The experiment comprised 5 series, 4 replicates in each. Folic acid was added in the following amounts: Series I, none; Series II, 50 µg/l; Series III, 100 µg/l; Series IV, 200 µg/l; and Series V, 400 µg/l. The cultures were incubated at 30°C for 11 days. In all series the initial pH of the medium was 7.5. The final pH was 8.7 in Series I, II and V, and 8.8 in Series III and IV.

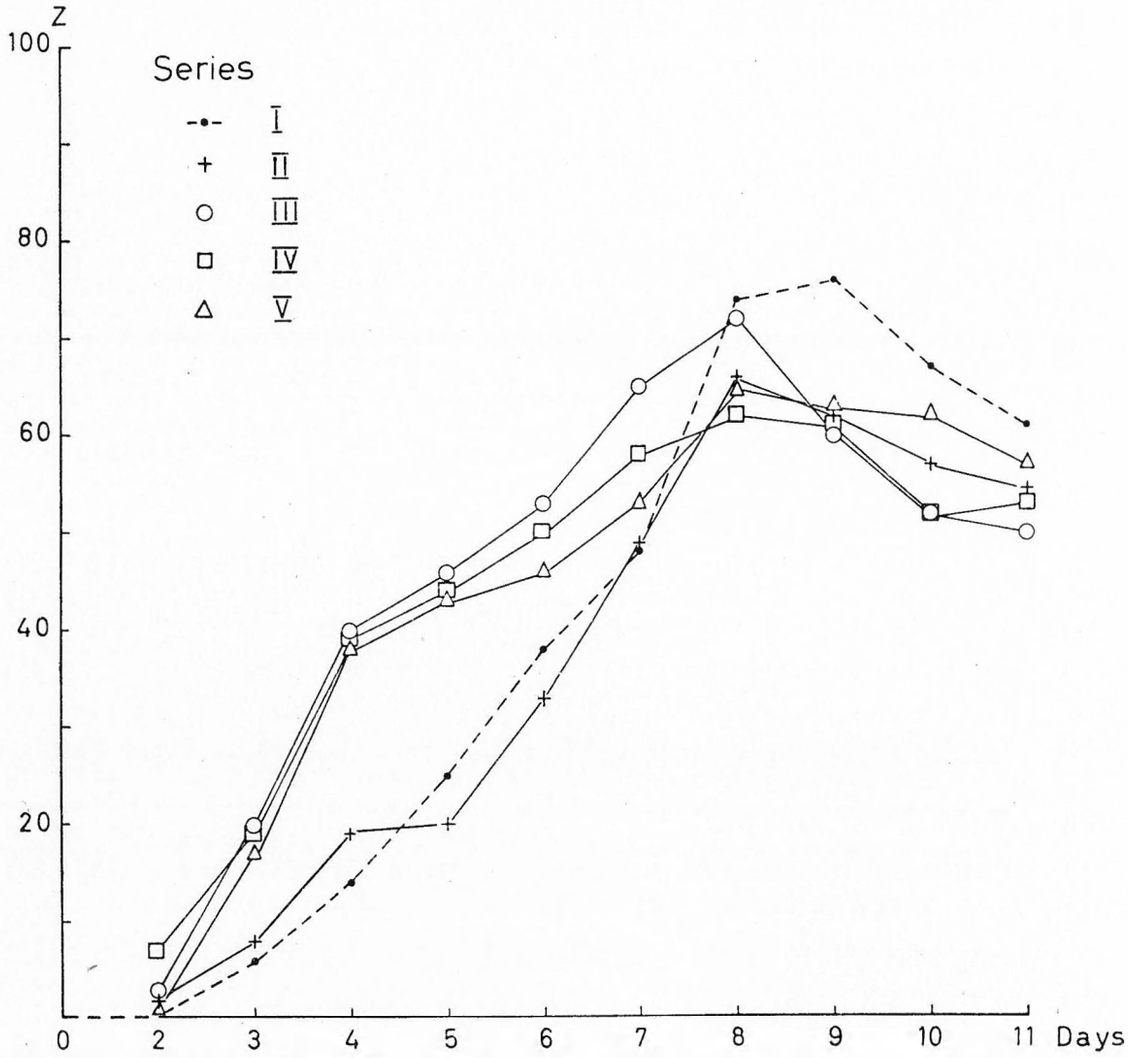

Fig. 8. The effect of concentration of Ca-pantothenate on growth (*Z*-values) of *Myxococcus virescens*. 0, 50, 100, 200, and 400 µg Ca-pantothenate per l. added in Series I, II, III, IV, and V respectively (Expt. 6).

In all cases, the addition of folic acid caused an increase in the rate of growth. The growth was only slightly affected, however, by a supply of either 50 µg/l or 400 µg/l. In the first case the vitamin concentration appears to have been too low, while in the second case it might have been too high. Series III and IV, 100 µg/l and 200 µg/l respectively, provided good growth, the development being much more speedy than in the control series. However, in no case was there detected any increase in the amount of growth.

Expt. 8. *The effect of biotin concentration on the growth of Myxococcus virescens in a casein hydrolysate medium* (Fig. 10).

Nutrient solution III B was used, the casein hydrolysate being vitamin-free. Five series were set up, each with 4 replicates. Biotin was added in the

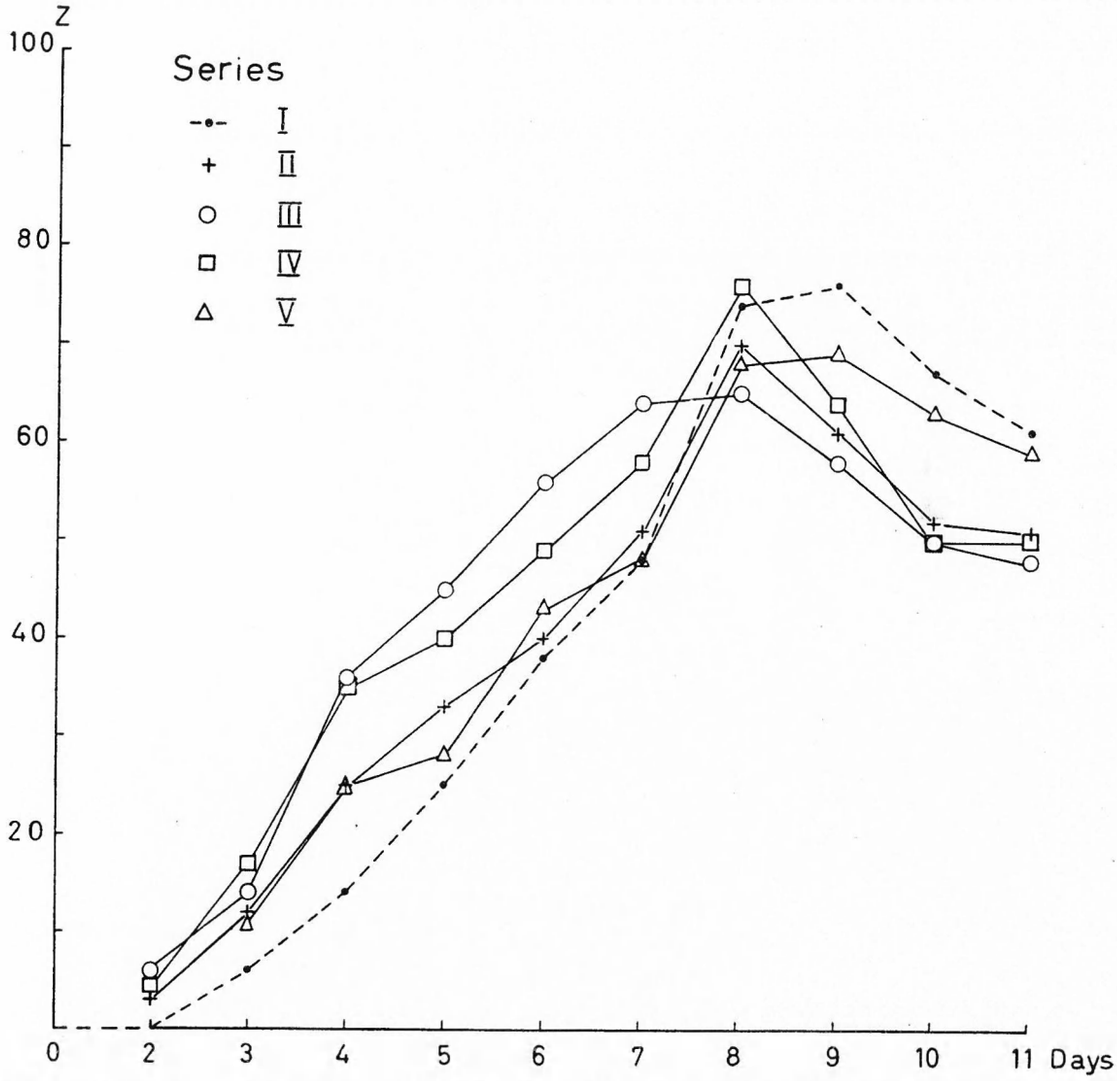

Fig. 9. The effect of concentration of folic acid on growth (*Z*-values) of *Myxococcus virescens*. 0, 50, 100, 200, and 400 µg folic acid per l. added in Series I, II, III, IV, and V respectively (Expt. 7).

following amounts: Series I, none; Series II, 50 mµg/l; Series III, 100 mµg/l; Series IV, 200 mµg/l; Series V, 400 mµg/l. The cultures were incubated at 30°C for 11 days. In each series the initial pH of the media was 7.5. The final pH was 8.7 in Series I, IV and V, and 8.8 in Series II and III.

Fig. 10 shows that the addition of 50 mµg/l of biotin only slightly improved the growth, but at double this concentration, 100 mµg/l the stimulating effect was most accentuated, resulting in an increased rate of growth. The maximum value of *Z* lies somewhat higher than in the control series but it is uncertain whether the difference may be considered significant or not. At the next concentration tested, 200 mµg/l, inferior *Z*-value was obtained and the *Z*-value of the highest concentra-

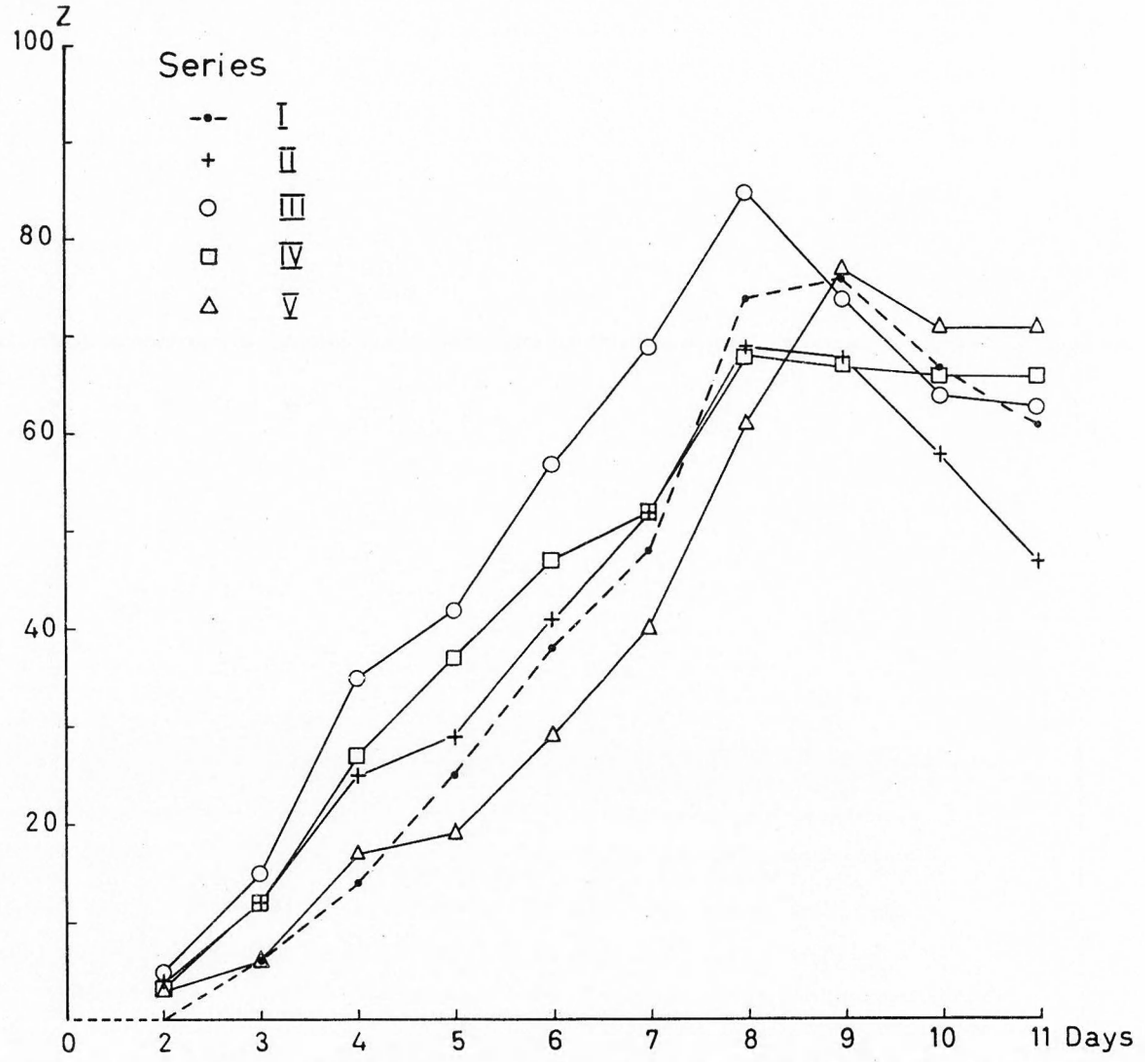

Fig. 10. The effect of concentration of biotin on growth (*Z*-values) of *Myxococcus virescens*. 0, 50, 100, 200 and 400 mμg biotin per l. added in Series I, II, III, IV, and V respectively (Expt. 8).

tion, 400 mμg/l even lies below those of the vitamin-free series. Thus, the organism reacts rather sensitively to the amount of biotin present in the culture liquid, when even a concentration of 400 mμg/l is somewhat inhibitory.

The experiment demonstrated that biotin is active at a greater dilution than the other vitamins tested, the optimum concentration being apparently 100 mμg/l.

Expt. 9. *The effect of choline concentration on the growth of Myxococcus virescens in a casein hydrolysate medium* (Fig. 11).

Nutrient solution III B was used, the casein hydrolysate being vitamin-free. Five series were set up, each with 4 replicates. Choline was added in the following amounts: Series I, none; Series II, 250 μg/l; Series III, 500 μg/l;

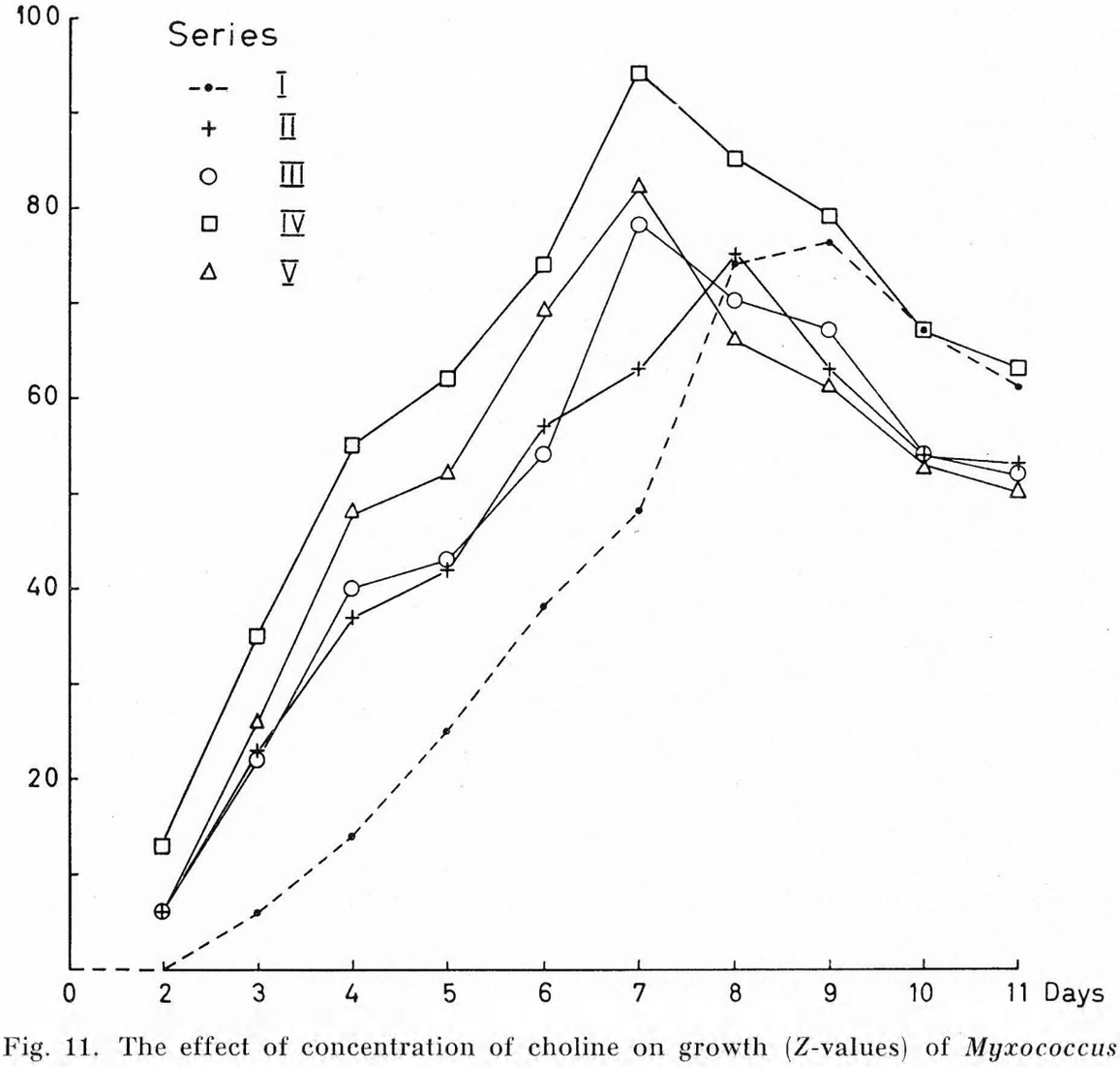

Fig. 11. The effect of concentration of choline on growth (*Z*-values) of *Myxococcus virescens*. 0, 250, 500, 1000 and 2000 µg choline per l. added in Series I, II, III, IV, and V respectively (Expt. 9).

Series IV, 1000 µg/l; and Series V, 2000 µg/l. Incubation time was 11 days at 30°C. In each series the initial pH of the medium was 7.5. The final pH was 8.7 in Series I, IV and V, and 8.8 in Series II and III.

It can be seen from Fig. 11 that the growth was greatly affected by the presence of choline which produced the strongest response of all the vitamins tested. The optimum effect was obtained by the addition of 1000 µg/l, when the growth rate was considerably increased. The development was about 3 days ahead of the vitamin-free series, and the maximum was reached in 7 days. However, not only the rate of growth was increased, but also the total amount. In the case of riboflavin and biotin the vitamin addition yielded slightly higher maximum values of *Z*, but the differences were always too small to allow conclusive state-

ments. Choline, however, produced a maximum value definitely higher than that of the vitamin-free control series. Lower choline concentrations, Series II and III, did not produce such a strong effect as the concentration of 1000 µg/l, but even so the rate of growth was stimulated considerably. On the other hand, a supply of 2000 µg/l appeared to be slightly too high, since a small but definite decrease was obvious in the rate of growth as compared with that of 1000 µg/l, and the maximum of *Z* was also reduced.

Expt. 10. *The effect on the growth of Myxococcus virescens of a vitamin mixture D, added in various amounts to a casein hydrolysate medium* (Fig. 12).

The nutrient solution III B with a vitamin-free casein hydrolysate was used. Five series were set up, each with 4 replicates. The vitamin mixture D comprised riboflavin, Ca pantothenate, folic acid, biotin and choline. The vitamins were added to the culture solution as shown in the table below.

Series	Each of riboflavin Ca-pantothenate and folic acid	Biotin	Choline	pH	
				Initial	Final
I	—	—	—	7.5	8.7
II	50 µg/l	50 mµg/l	250 µg/l	7.5	8.8
III	100 „	100 „	500 „	7.5	8.8
IV	200 „	200 „	1000 „	7.5	8.8
V	400 „	400 „	2000 „	7.5	8.7

The cultures were incubated at 30°C for 12 days.

Fig. 12 shows that an addition of the vitamin mixture D considerably promoted the growth of *M. virescens*. Yet, on the whole, the improvements of the series II and III were equivalent to those produced by choline alone. In Expt. 9 the *Z*-values of series III after 4, 5, and 6 days were 40, 43 and 54 respectively and in Expt. 10 with the identical choline addition, the corresponding figures were 39, 43 and 55. On the other hand, a comparison between the optimum series of the two experiments — in both cases series IV — shows that from the 5th day the *Z*-values in Expt. 10, where the medium was supplemented with the vitamin mixture, became gradually higher than the values in Expt. 9. The growth in series V was depressed as compared with series IV. This would be expected from the results obtained in the previous experiments, since the addition of the single vitamins in equivalent amounts produced certain inhibitory effects.

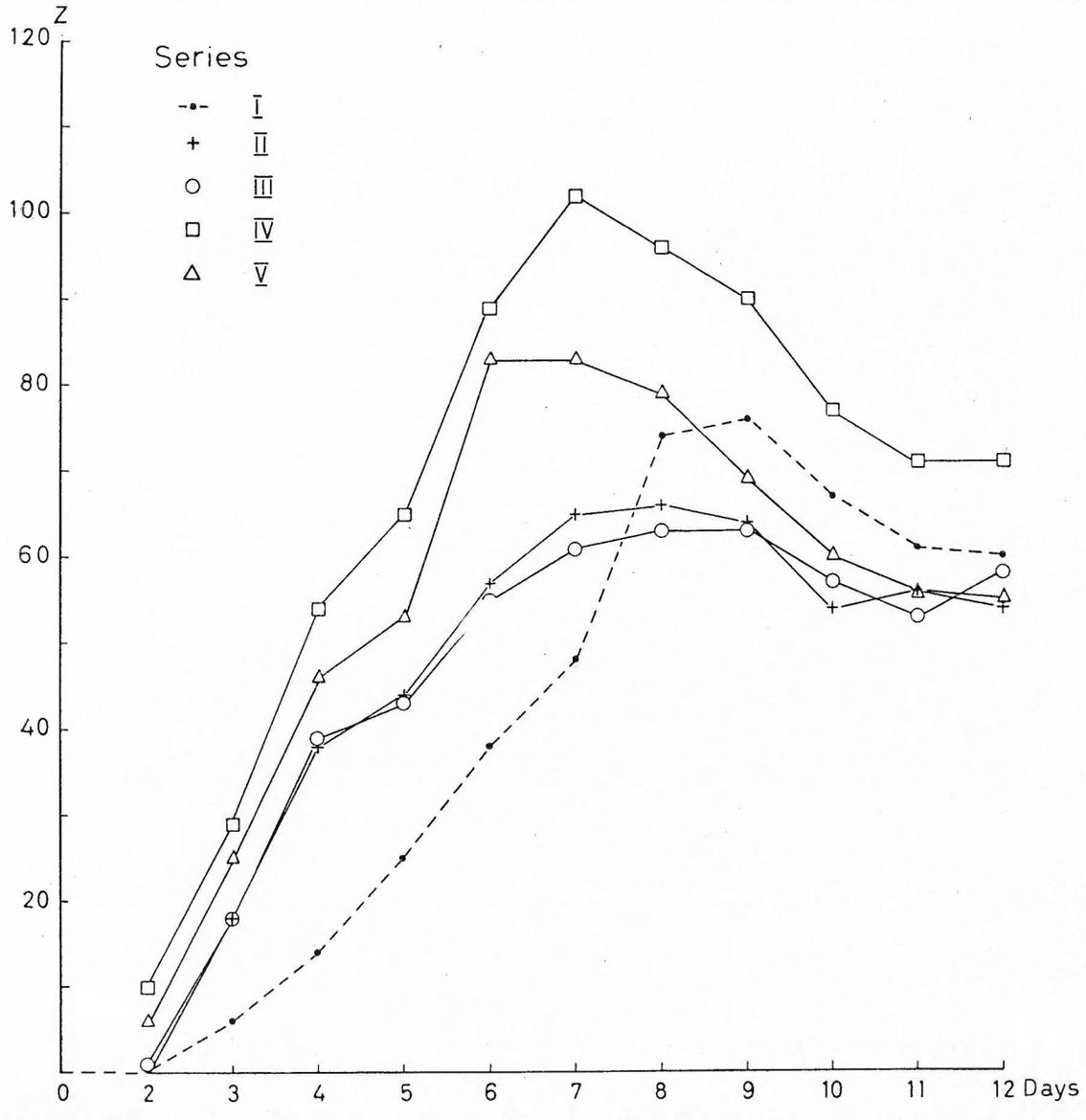

Fig. 12. The effect of concentration of a vitamin mixture D, comprising riboflavin, Ca-pantothenate, folic acid, biotin and choline, on growth (*Z*-values) of *Myxococcus virescens* (Expt. 10).

Thus on the development of *M. virescens* the addition of vitamin mixture D produced a definite stimulation, involving an increase both in the rate and the amount of growth.

3. *Discussion*

Since *M. virescens,* under the cultural conditions used was able to utilize a completely vitamin-free medium, and yet respond positively to the addition of each of the vitamins riboflavin, Ca-pantothenate, folic acid, biotin and choline, the organism appears to be partly deficient with

respect to these vitamins, i.e. they may be synthesized at such a slow rate that they can act as limiting factors for growth.

The degree of the deficiency is evidently variable, and it is most pronounced in the case of choline. This vitamin not only considerably increased the rate of growth but also the total yield of growth. This is a noticeable fact. Choline is a substance of well established importance to animals (see Jukes 1947 for review) but to micro-organisms it has been shown to act as a growth promoter only in the case of certain *Neurospora* mutants (Horowitz and Beadle 1943), pneumococci (Rane and Subbarow 1940, Badger 1944) and a halophilic *Sarcina* (Katznelson & Lochhead 1952).

In view of the fact that choline can be replaced by methionine in certain animals (see Jukes 1947 for review) and *Neurospora* mutants (Horowitz et al. 1945) it is interesting that methionine has a stimulating effect on the growth of *M. virescens* (see above).

Among the remaining stimulating vitamins, riboflavin, used in the concentration of 200 µg/l, produced an effect which was somewhat superior to that of Ca-pantothenate, folic acid or biotin, the optimum doses of these being about 100 µg, 200 µg and 100 mµg respectively per litre of nutrient solution. These vitamins acted chiefly on the rate of growth, but in the case of riboflavin and biotin slight tendencies to an increase of the amount of growth appeared as well.

It is worth noting that folic acid had a positive effect on growth whereas PABA had not, though these two vitamins are closely interrelated metabolically (cp. Shive 1951). In this respect *M. virescens* thus agrees with certain lactobacilli and enterococci (Lampen and Jones 1946). In light of the generally accepted view on the function of folic acid (Shive 1951, Woods 1953, Bessey et al. 1953) it is an interesting fact that the growth of *M. virescens* is stimulated also by adenosine and thymine (see below) and that an exogenous supply of methionine appears to be of certain importance for its growth (see above).

Like several other myxobacteria, *M. virescens* is abundant in dung. In view of this it is interesting that coprogen, a growth factor present in dung and active for some *Pilobolus* species (Hesseltine et al. 1953), may bear some metabolic relationship to the folic acid group of vitamins (Ritter et al. 1953).

The action of Ca-pantothenate is difficult to understand. It is possibly due to the slow formation of one of the moieties in the pantothenic acid molecule — either β-alanine or pantoic acid, — while the synthesis of the remaining part may occur at a greater rate. As a result, one part

may be produced in an excess, and at a certain point of development this excess may interfere with other metabolic processes within the cell, resulting in an inhibition of the later growth. Under such conditions it is possible to understand the rapid increase in the rate of the early growth on the addition of an exogenous supply of this vitamin. In connection with this it is interesting that Finck (1950) found pantothenic acid acting as an inhibitor whereas Ca-panothenate stimulated the growth of *M. virescens*. She also found that β-alanine stimulated some *Myxococcus* species, among them *M. virescens*.

A mixture comprising the five stimulating vitamins greatly improved the growth. At lower concentrations, the effect was very similar to that of choline alone, and at first at higher concentrations its stimulating advantage was demonstrated. On the other hand, the most concentrated supply of vitamins produced a slight but definite decrease in the effect. Such inhibitory effects were also observed when the vitamins in question were used separately, and in case of biotin the growth at the highest concentration was even poorer than that of the vitamin-free control series. It is thus evident that the organism reacts sensitively to an excess of vitamins, a fact which possibly may have influenced the results obtained by Oetker (1953).

As regards to *Ch. coralloides* the experiments performed have not revealed any conclusive data concerning the vitamin requirements.

E. Influence of Nucleic Acids and Nucleic Acid Components

1. *Experiments*

Up to the present, the only record in literature on the activity of a nucleic acid component on myxobacteria is given by Finck (1950). An addition of adenine — the exact amount not recorded — to a glucose-agar, did not affect the growth of the myxobacteria except in the case of *M. virescens,* where the growth was almost totally inhibited. However, when considering the important role of the nucleic acids and their constituents on the metabolism of the organisms it seemed desirable to carry out further work on these substances, especially as the experimental conditions of the investigation of Finck widely differed from those of the present study. Though the immediate interest was centred on their possible activity as growth factors, the first experiments were on the ability of the myxobacteria to utilize nucleic acids as the sole source of energy, carbon and nitrogen in a liquid medium.

Expt. 11. *The growth of myxobacteria in a nutrient solution with nucleic acids as the sole source of carbon and nitrogen.*

Nutrient solution III B, without casein hydrolysate and asparagine was used, supplemented with a vitamin mixture comprising aneurin, riboflavin, nicotinamide, pyridoxine, p-aminobenzoic acid, Ca-pantothenate and folic acid, 100 µg/l of each, 100 mµg/l biotin and 1.5 mg/l choline. Three series were set up, each with 5 replicates and the addition to each was as follows: Series I, casein hydrolysate 1 g/l+asparagine 2.5 g/l; Series II, yeast ribonucleic acid (RNA) (The British Drug Houses Laboratory, London) 0.1 g/l; Series III, desoxyribonucleic acid (DNA) (Krishell Laboratories, Portland) 0.1 g/l. For *M. virescens* and *M. fulvus,* the pH was adjusted to 7.4 and the cultures were incubated at 30°C for 21 days. In the case of *Ch. coralloides* the pH was adjusted to 6.9 and the incubation was 30 days at 25°C.

The development of *M. virescens* in series I was normal, reaching its maximum growth after 6 days ($Z=41$). In the culture liquids containing RNA or DNA extremely thin and transparent pseudoplasmodia developed on the test-tube walls below the level of the broth, the films being too thin to be photometrically examined. However, the pseudoplasmodia in the DNA-series were definitely thinner than those of the RNA-series.

In the case of *M. fulvus* and *Ch. coralloides,* the same tendencies as for *M. virescens* were obvious. Thus, the best growth occurred in series I with casein hydrolysate and asparagine, and the development in RNA was markedly better than that in DNA.

Thus, the myxobacteria tested are capable of utilizing RNA, and to a lesser degree DNA, as their sources of carbon, nitrogen and energy. However, in the concentrations used neither of the two substances produced a heavy growth.

Expt. 12. *The growth of myxobacteria in a nutrient solution with nucleic acid constituents as the sole cource of carbon an nitrogen.*

As a basic medium, Nutrient solution III B was used without casein hydrolysate and asparagine and supplemented with the same vitamin mixture as in Expt. 11. Five series were set up, each with 5 replicates, and the addition to each was as follows: Series I, casein hydrolysate 1 g/l+asparagine 2.5 g/l; Series II, adenosine 0.1 g/l; Series III, guanosine 0.1 g/l; Series IV, cytidine 0.1 g/l; Series V, thymine 0.1 g/l. The initial pH of the culture solution for *M. virescens* and *M. fulvus* was 7.4 and the cultures were incubated at 30°C, the former for 21 days, and the latter for 30 days. The cultures of *Ch. coralloides,* with an initial pH of 6.8 were incubated at 25°C for 28 days.

In all series, pseudoplasmodia developed on the walls of the glass tubes. Although they were too thin and transparent to give significant Z-values the experiment proved the fact that *M. virescens, M. fulvus* and

Ch. coralloides are able to utilize for their growth adenosine, guanosine, cytidine and thymine as sources of carbon, nitrogen and energy. Some differences in growth between the various series could be observed but the differences were too uncertain to allow any definite comparison of the nutritive value of various compounds.

It was now decided as a matter of interest, to test the influence of RNA and DNA on the myxobacterial growth in a casein hydrolysate medium. For the further studies, only *M. virescens* and *Ch. coralloides* were used.

Expt. 13. *The effect of RNA and DNA on the growth of Myxococcus virescens* (Fig. 13).

Nutrient solution III B was used, with a casein hydrolysate content of 1.25 g/l, and supplemented with the same vitamin mixture as in Expt. 11. Each tube contained 9 ml of nutrient solution. Four series were set up each with 5 replicates and they were supplied as follows: Series I, no addition; Series II, casein hydrolysate 1.25 g/l, (thus making the total amount of casein hydrolysate 2.5 g/l); Series III, RNA 0.1 g/l; and Series IV, DNA 0.1 g/l. In each series pH was adjusted to 7.4. The cultures were incubated at 30°C for 14 days. The tubes were turned a quarter of a circumference every day (see above p. 83).

As the inoculum was not very virulent, the growth started slowly in all series. After 7 days, however, the growth became more rapid in the control series, indicated by the steeply rising curve. In series I, II and III, the pseudoplasmodia loosened from the glass walls of the tubes during the 15th day. The culture liquid of Series II became slightly yellowish after 13 days and more markedly so after 14 days. As a result, the *Z*-values then obtained were high, particularly on the 14th day, and they do not adequately reflect the pseudoplasmodial development.

In Series I the pH of the medium rose to 8.3 and in Series II and III to 8.4 and in Series IV to 8.7.

The addition of RNA gave an increased rate of the initial growth, this phase was about two days shorter than in the control (Series I). The steep rise of the curve indicating heavy growth started after 5 days and in the control series after 7 days. Up to the 7th and 8th days the development was somewhat faster than in the control though after the 8th day it appeared to be about the same. After the 10th day growth started to cease and the *Z* max. obtained was not higher than that of series I, indicating that RNA did not produce any increase in the total production of living matter.

The experiment thus revealed that under the cultural conditions used, RNA was stimulating to *M. virescens*, speeding up its initial growth. The

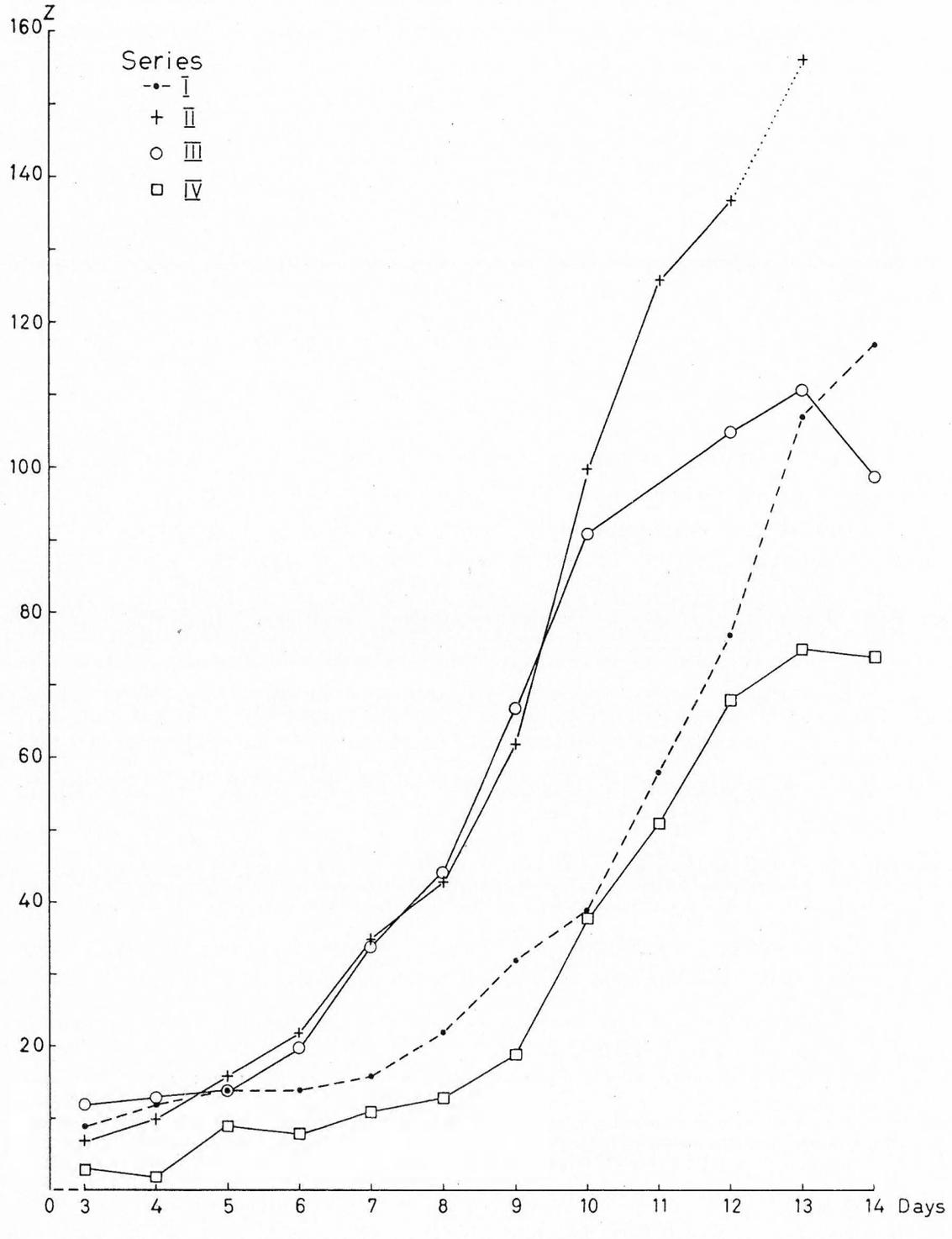

Fig. 13. The effect of RNA (Series III) and DNA (Series IV) on growth (Z-values) of *Myxococcus virescens,* grown in a casein hydrolysate medium. Series I control and Series II with the double concentration of casein hydrolysate (Expt. 13).

stimulation might not entirely be ascribed to the further supply of a carbon-nitrogen source to the medium as this probably would also have produced an increased amount of growth. It was also shown (Expt. 11) that RNA alone is capable of producing only a weak growth of *M. virescens*. Thus the result seems to indicate that this organism is capable of synthesizing RNA or some of its constituents only with difficulty, and that this may be a limiting factor to its growth.

It is a remarkable fact that up to the 10th day the growth of Series III was about the same as that of Series II, i.e. an addition of 0.1 g/l of RNA produced an effect equal to 1.25 g/l of casein hydrolysate both speeding the initial growth. Series II, however, gave a greater yield of living matter.

While the addition of RNA had a stimulating effect on the growth of *M. virescens,* the addition of DNA caused an inhibition. The *Z*-values of the DNA-series were always somewhat lower than those of the control series, indicating a slight but definite depressing effect by this substance on the rate of growth. The maximum value of *Z* after 13 days was 75, indicating the inhibiting effect even more clearly.

It is probable that the RNA-molecule is first split into its constituents, before it can be assimilated by the myxobacteria. In an attempt to show which of these constituents were responsible for the stimulating effect of RNA, the following experiment was carried out. Since it was assumed that a better total effect would be obtained with the nucleosides than with the free bases (Loring & Pierce 1944, Fries 1946,1949) adenosine, guanosine and cytidine were used in the examination. In addition the constituent thymine from DNA was also tested. It was chosen in preference to thymidine as this substance contains desoxyribose.

Expt. 14. *The effect of nucleic acid constituents on the growth of Myxococcus virescens* (Fig. 14).

As a basic medium Nutrient solution III B was used, with a casein hydrolysate content of 1.25 g/l and supplemented with the same vitamin mixture as in Expt. 11. Six series were set up, each with 5 replicates, and they were supplied as follows:

Series	I,	No addition		
,,	II,	Casein hydrolysate	1.25 g/l	(total amount thus 2.5 g/l)
,,	III,	Adenosine	0.1 g/l	
,,	IV,	Guanosine	0.1 g/l	
,,	V,	Cytidine	0.1 g/l	
,,	VI,	Thymine	0.1 g/l	

In each series, the pH of the culture solution was adjusted to 7.4

The cultures were incubated at 30°C for 14 days, and the tubes were turned a quarter of a circumference each day (see p. 83).

As in the previous experiment the growth of the control series started slowly. On the 7th or 8th day, as seen on the curve, a more rapid development occurred. The medium of Series II (2.5 g/l of casein hydrolysate) became yellowish after the 12th day affecting the *Z*-values obtained for the 13th and 14th day. On the 15th day the pseudoplasmodia of all but series IV (guanosine) had loosened from the glass walls of the tubes. During the incubation, the initial pH of the culture media of 7.4 rose to 8.3 in Series I, to 8.0 in Series IV and to 8.4 in Series II, III, V, and VI.

It is evident that the addition of adenosine, cytidine and thymine to the casein hydrolysate medium greatly improved the growth of *M. virescens*. The stimulation, however, seems to differ somewhat with each substance.

Adenosine produced a substantial increase in the rate of growth during the first 8 days, while the later development was about the same as that of the control series. After 10—11 days the curve fell off and the *Z* maximum, obtained was no higher than that of series I. Thus, the stimulating effect of adenosine is evidently limited to a promoting effect of the initial phase of growth, while the total production of living matter is not increased, and in this effect it corresponds to RNA. The addition of 0.1 g/l of adenosine improved the growth up to the 9th day as much as an addition of 1.25 g/l of casein hydrolysate.

Cytidine also improved the growth of *M. virescens,* though the response produced during the initial phase was not as good as in the case of adenosine. Later, however, the rate of growth increased considerably and after 9 days the cytidine curve rose more steeply than those of the other two substances. The maximum value of *Z* was definitely higher than that of the adenosine series.

Thymine produced a growth which up to the 8th day was about identical to that of cytidine, but the following development was somewhat slower. The maximum values of *Z* were almost equal in the two series.

Only guanosine had any inhibitory effect. For the first 7 days, the development was about the same as in the control series, but from the 8th—12th day it was definitely slower. In the final phase of growth, however, the rate increased, finally equalling that of the control series.

Experiments 13 and 14 were also carried out with *Ch. coralloides* as the test organism. However, in order to get as good a growth as possible,

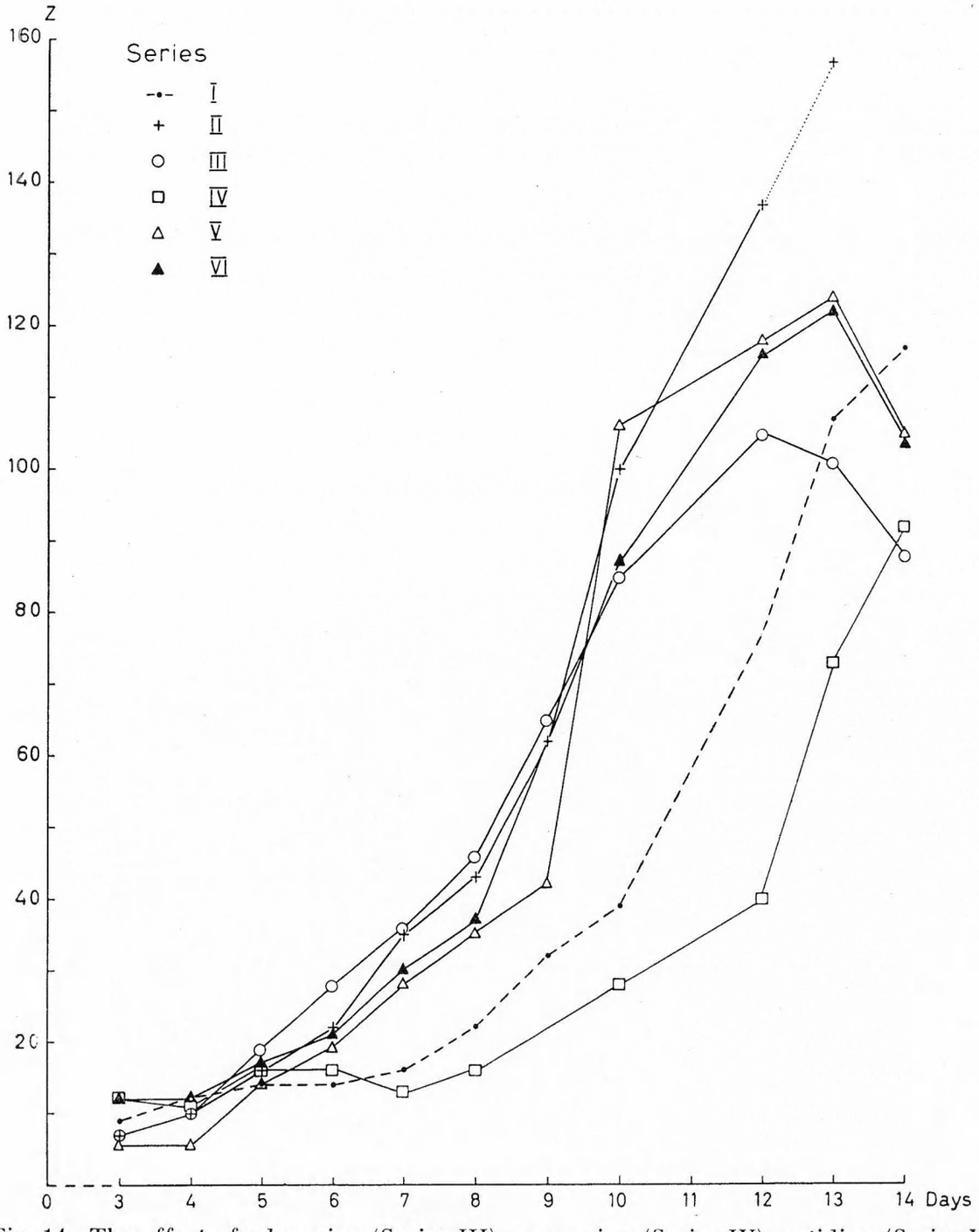

Fig. 14. The effect of adenosine (Series III), guanosine (Series IV), cytidine (Series V) and thymine (Series VI) on growth (*Z*-values) of *Myxococcus virescens* grown in a casein hydrolysate medium. Series I control and Series II with the double concentration of caseinhydrolysate (Expt. 14).

some changes in the experimental conditions were made. The concentration of the casein hydrolysate was constantly 2.5 g/l, the initial pH was adjusted to 6.8 and the temperature of incubation was 25°C (See Norén 1952). Growth occurred in every series. Some differences could be

observed. A supply of RNA, adenosine or guanosine seemed to give rise to a better growth than the other compounds tested. However, the pseudoplasmodia developing were always too thin to give significant extinction values in the photometer and consequently the differences cannot permit any conclusions.

2. *Discussion*

The results recorded above make it evident that *M. virescens, M. fulvus* and *Ch. coralloides* all are able to utilize RNA, DNA, adenosine, guanosine, cytidine or thymine as the source of energy, carbon and nitrogen. However, in the above experiments (Expts. 11 and 12) they were possibly used in a too low concentration to produce good growth. The pseudoplasmodia formed were very thin and consequently the differences in growth, which certainly existed between the various series, could not be easily observed. Only in the case of DNA it was possible to record a definitely inferior growth as compared with that of the remaining compounds. A similar utilization of pyrimidines has been demonstrated for some other soil bacteria by Lara (1952) and Wang & Lampen (1952).

The experiments showed clearly that when adenosine, cytidine or thymine was added to a casein hydrolysate medium there occurred a definite stimulation to the growth of *M. virescens*. Although such an addition supplied an extra carbon-nitrogen source to the medium the strong positive effects obtained cannot possibly be ascribed to this, as these substances, as shown in Expt. 12, are only poorly utilized. The results seem to indicate that the organism is able to carry out synthesis of these substances, but only at such a slow rate that they act as factors limiting the growth. Since ribose does not appear to influence the growth of *M. virescens* (see above) the active part of the nucleoside molecules might be the purine and the pyrimidine residues. Therefore, this organism can be added to the group of bacteria with partial requirements for RNA and DNA constituents (Snell & Mitchell 1941, Pelczar & Porter 1943, Niven 1944, Niven & Sherman 1944, Katznelson & Lochhead 1948, Krueger & Peterson 1948).

Adenosine chiefly increased the rate of the initial growth phase, while the following development apparently was little affected. Fries (1954) found that adenine influenced the growth of some Hymenomycetes in a similar way. (For the interpretation of this effect, see Fries 1954, p. 576). The speeding effect on the initial growth was obtained not only

by the addition of 0.1 g/l of adenosine to the 1.25 g/l of casein hydrolysate but also by a doubling of the casein hydrolysate concentration. Which of the numerous compounds of casein hydrolysate was (were) responsible is not known. The casein hydrolysate used in these experiments was non vitamin-free and it seems to contain a mixture of vitamins stimulating the growth (see Expt. 3) and when doubled, these substances might produce an increased rate of the initial growth. Some amino acids (Expt. 1) have also been shown to shorten the initial phase. However, from Expt. 1 it is evident that none of the tested synthetic amino acids, separately or ina mixture produced such a great effect on the initial growth as the vitamin-free casein hydrolysate. This experiment seems to indicate that the casein hydrolysate contains substance(s) other than amino acids and vitamins which act(s) shortening on the initial growth phase.

The positive effects of cytidine and thymine differed somewhat from that obtained with adenosine. These compounds improved the initial growth somewhat less but increased the rate of the following development somewhat more than adenosine. These effects were most pronounced in the case of cytidine. Higher total yields of growth were also produced.

Organisms requiring pyrimidines have been reported by several workers, e.g. Snell & Mitchell (1941), Pelczar & Porter (1943), Merrifield & Dunn (1948). In the case of *M. virescens* the deficiency is only partial. The organism responds strongly to both cytidine and thymine.

The effect of guanosine is somewhat surprising since in the majority of the purine requiring micro-organisms, adenine and guanine are more or less interchangeable (Möller 1939, Pappenheimer & Hottlé 1940, Snell & Mitchell 1941, Katznelson & Lochhead 1948). However, in some cases guanine has been shown not to be effective in replacing adenine (Mueller & Miller 1942, Fries 1949, Pomper 1952) and even to be inhibitory (Krueger & Peterson 1948). As yet, nothing is known of the reason for the inhibiting action of guanosine. As its structure is closely related to that of adenosine, it is possible that it may competitively inhibit some step in the synthesis of adenosine or in the utilization of this substance.

As mentioned above, *Ch. coralloides* is able to utilize RNA, DNA, adenosine, guanosine, cytidine, and thymine as sources of carbon, nitrogen and energy. So far, however, it is not known whether these substances can also act as growth factors for the organism.

Summary

The object of this work has been to study the influence of some organic substances on the growth of certain myxobacteria. The main test organism was *Myxococcus virescens,* but in some cases *Myxococcus fulvus* and *Chondrococcus coralloides* were also investigated. All experiments were carried out in liquid media according to a method earlier described. Some improvements in the method are recorded.

1. The myxobacterial capability of utilizing agar as a source of nutrient is discussed.

2. It is proved that *M. virescens* is readily able to utilize starch and glycogen and their hydrolytic products, dextrin and maltose. The addition of one of these sugars to casein hydrolysate medium greatly improves the growth. The utilization of glucose is discussed. Arabinose, galactose, sucrose, and raffinose are also shown to be available to the organism as sources of energy and carbon but they improve the growth to a lesser extent than do starch and compounds related to starch. Xylose, ribose, fructose, mannose, lactose, cellobiose, and inulin appear also to be attacked but they don't improve the growth. The growth of *M. fulvus* is not influenced by an addition of ribose while in *Ch. coralloides* a slightly positive effect is produced.

3. The concentration of starch present in the medium is shown to influence the pigmentation of the pseudoplasmodium and the fruiting bodies of *M. virescens.*

4. *M. virescens* will grow better in casein hydrolysate than in a mixture of amino acids considered to correspond to a complete casein hydrolysate. This may only partly be ascribed to the tryptophane content of the mixture.

5. The growth of *M. virescens* in a casein hydrolysate medium is more less stimulated by the addition of valine, phenylalanine, glutamic acid, proline, arginine, aspartic acid, threonine, cystine or methionine. Alanine, isoleucine, and serine have only a slight positive effect, while lysine is slightly inhibitory. Glycine, leucine, tyrosine, tryptophane, hydroxyproline and histidine have no obvious influence on growth.

6. The growth of *M. virescens* is greatly inhibited when glycine, valine, leucine or isoleucine is removed from an amino acid mixture corresponding to complete casein hydrolysate. The growth is also reduced by the single omission of glutamic acid, methionine, arginine or tyrosine. On the other hand the presence of alanine, tryptophane, aspartic acid, proline, hydroxyproline, histidine or lysine has an inhibitory effect on the growth. The inhibition is particularly great in the case of alanine and histidine.

7. Under the given experimental conditions *M. virescens* has been proved to be heterotrophic for the vitamins riboflavin, folic acid, Ca-pantothenate, biotin, and choline. The inability to synthesize respective substances is not absolute but only partial. The degree of deficiency is greatest for choline. Aneurin, nicotinamide, pyridoxine, and p-aminobenzoic acid do not improve the growth. *Ch. coralloides* did not respond positively to any of the vitamins tested.

8. It has been shown that *M. virescens, M. fulvus* and *Ch. coralloides* are capable of utilizing RNA, adenosine, guanosine, cytidine, thymine and to a lesser extent DNA as sole sources of energy, carbon and nitrogen.

9. When added to a casein hydrolysate medium, RNA greatly improves the initial growth of *M. virescens,* while DNA acts as an inhibitor. *Ch. coralloides* responds only weakly to these substances, the inhibitory effect of DNA however, was quite visible.

10. The effect of RNA on *M. virescens* is also produced by its component adenosine. Cytidine and thymine are also growth-stimulative. Guanosine has an inhibitory influence on the growth. Under the cultural conditions used the growth of *Ch. coralloides* is only slightly affected by these substances and no differences in their effect are detectable.

11. The addition of 0.1 g/l of RNA or adenosine to a medium containing 1.25 g/l of casein hydrolysate improves the growth of *M. virescens* as much as does an addition of 1.25 g/l of casein hydrolysate.

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Smärre uppsatser och meddelanden

Cuscuta australis funnen i Kungälv

I augusti i år fann trädgårdsmästare Tor Ohlén i sin handelsträdgård i Kungälv en *Cuscuta*-art, vilken senare av docent H. Hjelmqvist, Lund, bestämdes till *Cuscuta australis* R. Br. Den var utbredd över en yta av c:a ½ m² och parasiterade huvudsakligen på *Callistephus chinensis,* men den hade spritt sig till några vilda växter i närheten (9 arter). Trädgårdsmästare Ohlén lyckades överföra den till *Dahlia* och *Anthemis.* Meningen var att försöka få den att övervintra i växthus eller inomhus. Blomningen var riklig och frukterna såg ut att utveckla sig normalt. Den första frostnatten, som kom i början av oktober, förstörde dock alla exemplar av *Cuscuta,* innan några frukter hunnit mogna och innan något exemplar bringats i säkerhet.

Cuscuta har troligen kommit in med *Callistephus*-frön. Ursprungligen parasiterade den nämligen endast på *Callistephus* och övergick senare spontant till 9 andra arter och överfördes till två. Värdväxterna voro följande 12, varav de 7 första Compositéer:

Callistephus chinensis (ursprungsangrepp)
Dahlia variabilis (överförd)
Anthemis tinctoria (överförd)
Artemisia vulgaris
Matricaria inodora
Sonchus asper
Taraxacum sp.
Atriplex patulum
Capsella bursa-pastoris
Medicago lupulina
Plantago major
Trifolium repens

Cuscuta australis har förut tillfälligt inkommit i Sverige och insamlats men i allmänhet bestämts som *Cuscuta campestris* (Hjelmqvist 1953). De flesta lokaluppgifterna (5 st.) äro från Skåne. I Västsverige är den funnen på två ställen: Göteborg (1943)[1] och Tegneby (1944).[1] Dessutom har den insamlats i Södermanland, Västmanland och Jämtland (Hjelmqvist 1953 sid. 102).

I samtliga fall torde *Cuscuta australis* ha inkommit med utländskt frö, troligen från Holland, som i sin tur fått växten från Amerika. I inget fall har den iakttagits mer än några få år i följd på samma lokal.

Som värdväxter för *Cuscuta australis* i Sverige anför Hjelmqvist följande 19 arter:

[1] Fries 1945 sid. 354 som *C. campestris.*

Acer platanoides
Allium cepa
Asperugo procumbens
Atriplex sp.
Callistephus chinensis
Calendula officinalis
Cannabis sativa
Capsella bursa-pastoris
Chenopodium album
Feoniculum officinale
Phaseolus vulgaris
Poa annua
Polygonum aviculare
Satureja hortensis
Senecio vulgaris
Sinapis arvensis
Stellaria graminea
— *media*
Veronica agrestis.

Av ovanstående arter äro endast *Callistephus chinensis, Capsella bursa-pastoris* och eventuellt även *Atriplex* sp. gemensamma med dem i Kungälv.

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Kungälv, den 20 november 1954.

FOLKE LUNDBERG

Om förekomsten av Botrychium matricariifolium i östra Närke

Botrychium matricariifolium (Retz.) A. Br. ap. Koch har anträffats på följande lokaler:

Svennevad socken: I en grusgrop vid infartsvägen till Bottorp, där arten växte i ett bestånd på sex exemplar. Upptäckare av denna fyndplats var folkskollärare G. Kjellmert, Arboga.

På en sandås till höger om vägen c:a 200 m N Skepphultatorp. Arten växte där i ett ensamt exemplar tillsammans med ett litet individ av *B. lunaria* (L.) Sw.

Sköllersta socken: I den s.k. Gropahagen Ö Fall. Arten uppträdde där i ett litet bestånd på sex exemplar, tillsammans med ett par, mycket små individ av *B. lunaria.*

Beläggexemplar av arten finns från samtliga lokaler.

ULF STARBÄCK

Notes on South African Hepatics I

1. Species new for South Africa

Adelanthus sphalerus (H. et T.) St.: Cape Prov.: Table Mt., steep wet rock on the south side, about 900 m s. m., Arnell, n. 961.

Earlier known from Tierra del Fuego, W. Patagonia and Juan Fernandez.

Calypogeia longifolia St.: N. Transvaal: Zoutpansberg, Blaauwberg, 29° E, 23° S, 5 500 ft, Esterhuysen, n. 21 564.

Differs from *C. bidentula* by narrower leaves with apex usually entire. — Earlier known only from Madagascar.

Clevea spathysii (Lindenb.) K. Müller: South West Africa: Windhoek, 1 mile E of Windhoek. Locally common in shaded crevices in schist outcrop, 5 700 ft, leg. Schelpe.

Earlier known from the Mediterranean area of Europe and N. Africa, Palestina and the Canarias.

Jamesoniella grandiflora (L. et G.) Spr.: Cape Prov.: Worcester, Witteberg, Slanghoek Mts, 3 000 ft, n. 22 263; Upper Wellington Sneeuwkop, 3 000 ft, n. 21 505; Table Mt., Saddle Face area, 2 000 ft, n. 22 150. All collected by E. Esterhuysen.

Differs from *J. colorata,* i.a. by smooth cuticle. — Earlier known from Tristan da Cunha, S. America and Tasmania.

Jamesoniella paludosa St.: Cape Prov.: Worcester, Fonteintjiesberg, 5 000—5 500 ft, Esterhuysen n. 20 036, on wet rock surfaces, where water trickles in the summer, flows strongly in the winter.

In my opinion this "species" is only an aquatic modification of *J. grandiflora.* Intermediate forms occur. — Earlier known from Tristan da Cunha and S. America.

Lepidozia carnosa St.: Natal, Bergville, Drakensberg, Mt. Weni area, on steep rocky slope, SE aspect, in a kloof, 7 000 ft, Esterhuysen, n. 21 614.

Earlier known from Congo.

Lepidozia lacerata St.: N. Transvaal: Zoutpansberg, Blaauwberg. On damp, shaded bank above stream; with *Hymenophyllum, Herberta capensis, Bazzania adnexa.* Alt. 6 000 ft, Esterhuysen, n. 21 567.

New for S. Africa, earlier found on Kilimandjaro (Uhlig).

Lopholejeunea subfusca (Nees) St.: Natal, Drakensberg, Tugela Gorge, among other bryophytes on rocks near a stream, leg. Cholnoky.

Common from W. Africa to Tanganyika, also found in Madagascar, Indonesia, Malaya, Brasilia and Florida.

Microlejeunea africana St.: Cape Peninsula, Noordhoek Peak, 700 ft, on bark, Garside, n. 6 553.

Earlier known from W. Africa.

Plagiochila tridenticulata Tayl.: Natal, Drakensberg, Tugela Gorge. On rocks under *Podocarpus,* together with *Plagiochila capensis* St., *Lejeunea caespitosa* Ldbg., *Metzgeria limbato-setosa* St., *Radula boryana* (Web.) Nees, *Frullania silvestris* Sim., leg. Cholnoky.

It is earlier found in Great Britain, Norway, France and the Canarias.

2. Remarkable Localities

Calypogeia fusca (Lehm.) St.: Cape Prov.: Worcester, Witteberg, Slanghoek Mts., 3 000 ft, Esterhuysen, n. 22 266.

Earlier known from Cape Prov., Table Mt., and Tristan da Cunha.

Cheilolejeunea convexa S. Arn. (=*Lejeunea convexa* S. Arn.): Cape Prov.: Worcester, Upper Wellington, Sneeuwkop, 3 000 ft, Esterhuysen, n. 21 504.

Earlier known only from Cape Prov., Table Mt.

Adelanthus unciformis (Tayl.) Mitt.: Cape Prov.: Worcester, Witteberg, Slanghoek Mts., 3 000 ft, Esterhuysen, n. 22 263.

Earlier known only from Cape Prov., Table Mt., Tristan da Cunha and S. America.

Cephalozia crassicaulis St.: Cape Prov.: Ceres, Upper Witels Kloof, Esterhuysen, n. 21 849.

Earlier known from Congo and S. Africa, Cape Prov., Table Mt. and Knysna Forest.

Frullania brunnea (Spr.) St.: Cape Prov.: Worcester, Witteberg, Slanghoek Mts., 3 000 ft, Esterhuysen, n. 22 264.

Earlier known only from Table Mt. and Steenbras Valley in the Cape Province.

Lejeunea microlobulata S. Arn.: Natal, Bergville, Mnweni area, Drakensberg, 9 000 ft, Esterhuysen, n. 21 617.

Earlier known only from Cape Province, Knysna.

Lophocolea subulistipa Herz.: Cape Prov.: Caledon, Rivier Zonder Einde Mts. Deep kloof on south side, near Greyton. On wet rock surfaces near stream, 2 000 ft, Esterhuysen, n. 21 103.

Earlier known only from the type locality, Cape Prov., Table Mt.

Lophocolea vermicularis Herz.: Cape Prov.: Caledon, Rivier Zonder Einde Mts., Schilpad Kop, Esterhuysen, n. 21 102; Worcester, Upper Wellington, Sneeuwkop, 3 000 ft, Esterhuysen, n. 21 504.

Earlier only known from Cape Prov., Table Mt.

Metzgeria limbato-setosa St.: Zululand, N'kandhla, Zndeni forest, 5 000 ft, Miss Nixon, n. 133; Drakensberg, Tugela Gorge, leg. Cholnoky.

New for Natal, earlier known from Cape Province, Congo and Ruanda — Urundi.

Schistochila alata (Lehm.) St.: Cape Prov.: Worcester, Upper Wellington Sneeuwkop, Esterhuysen, n. 21 803; Witteberg, Slanghoek Mts., 3 000 ft, Esterhuysen, n. 22 269.

Earlier known only from Cape Prov., Table Mt.

SIGFRID ARNELL

Palynology in South Africa

Third report, covering the activities during 1952 and 1953

Research on palynology was continued during the years 1952 and 1953 in the various centres in South Africa and has been concentrated on some of the most urgent problems.

The lack of sufficient knowledge of the pollen and spore morphology of the vegetation of this country is still felt and hampers the progress of the research. An extensive pollenanalytical study of the peat deposits of the coastal region of the Cape and of the Transvaal is, nevertheless, being carried out and work on air-borne pollen is continued. Atmospheric pollen is studied both from the medical and from the botanical point of view.

International cooperation amongst palynologists is of very great importance

and palynologists in South Africa are, because of their isolated position, very much in favour of the cooperation outlined by the "Commission Internationale de Palynologie". Colleagues in South Africa are also in great need of a good circulation of reprints of current work done in other countries.

Palynological work has been carried out at the following centres:

Johannesburg. Dr H. Friede (6 Judith Road, Emmarentia) published a paper on "Palynology and History of Tree Vegetation" in which he discussed the possibilities of palynology in South Africa.

In cooperation with Mrs M. E. Norwood-Young, Dr Friede prepared a report on "The Vlei-Soils of the Transvaal Highveld and their Pollen-analytical Aspects". The origin of these soils has been discussed and the organic matter and pollen content of six deposits has been investigated. No tree pollen grains have been found. Thus the Highveld has likely been unforested during the formation of the soils investigated.

Mrs Norwood-Young and Dr Friede are studying the pollen morphology of some liliaceous plants and some gymnosperms.

Dr D. Ordman is in charge of the Allergy Laboratories of the South African Institute for Medical Research at Johannesburg where the diagnosis and treatment of allergic diseases is carried out. The investigation of the pollen and fungus spore content of the atmosphere is continued.

At the Department of Pharmacology and Therapeutics of the University of the Witwatersrand research on atmospheric pollen is carried out under the guidance of Prof. J. M. Watt. A paper on "Pollen Observations in the Atmosphere of Johannesburg" by Mrs Breyer-Brandwyk and Cremer, illustrated with photomicrographs, is under preparation.

Pretoria. Dr C. K. Brain of the Transvaal Museum is carrying out research on pollen in Transvaal cave deposits. A breccia of Swartkrans, containing delicate rodent bones in an excellent state of preservation, seems to be promising.

Grahamstown. Mr A. R. H. Martin from the Rhodes University at Grahamstown (Cape Province) is studying the valuable palynological material he collected in the Cape Province at the London University College.

Mr Martin has chiefly concentrated his research on the peat deposits of the coastal region of the Cape Province, e.g. in the Knysna area, the Table Mountain, and the Cape Peninsula. His study also includes the ecology of the area. Soil samples have been analysed and the relations of the fen vegetation to soil and biotic influences is being studied.

Reference collections of pollen grains (700 slides), seeds, fruits, and rhizomes have been established for the identification of the fossil material.

Pollen grains in moss cushions in the Knysna forest have been largely identified. Their frequency has been compared with the known percentage composition of the forest.

The analysis of one of the most important cores (20 ft. in length) collected near Knysna is in progress. The diatoms in the samples give important information about a marine transgression in the Holocene.

A statistical analysis of the size of the pollen grains of the different South

African species of *Podocarpus* has been carried out and an investigation on the pollen grain size in *Olea* is planned.

Bloemfontein. The palynological research work in Bloemfontein is carried out by Miss J. A. Coetzee and the compiler of this report in the Department of Botany of the University of the Orange Free State with financial aid of the South African Council for Scientific and Industrial Research.

A collection of 1 700 microscope slides of acetolyzed pollen grains and spores is being studied. The results of this study are published in "South African Pollen and Spores" of which part I has been issued and part II is completed. Part III, chiefly dealing with the Leguminosae pollen, is under preparation. The study of the South African fern spores has been started.

Miss Coetzee worked for some time during 1953 in the Palynological Laboratory, Stockholm-Bromma, and also visited the colleagues in Bergen, Velp, Utrecht, and Cambridge.

Eight pollen traps have been in operation in various types of natural vegetation. The standard Durham type of trap (cf. Journal of Allergy, vol. 17, p. 79, 1946) did not prove to be efficient and had to be altered. The vegetation surrounding the traps in Zululand and at Jonkershoek (Cape Province) has been studied.

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E. M. VAN ZINDEREN BAKKER

Recherches palynologiques en URSS

Le développement de la palynologie en URSS est étroitement lié à l'économie nationale.

Les travaux, dans le domaine de la palynologie, sont effectués dans les Instituts de Botanique et de Géologie des Académies des Sciences des Républiques soviétiques, dans les établissements du Ministère de la Géologie et Production minière, ainsi qu'au Ministère de l'Industrie du Pétrole.

Actuellement on compte en URSS plus de 70 laboratoires d'analyses polliniques et environ 350 palynologistes travaillant dans ce domaine. La palynologie est particulièrement utile en stratigraphie.

La détermination des pollens fossiles se fait actuellement suivant la classification naturelle; le système artificiel n'étant utilisé que pour le Paléozoïque. En ce moment on tente de mettre en évidence les pollens des grands groupes de la classification dans les formations du Paléozoïque également.

Ainsi l'analyse pollinique s'étend à tous les niveaux sédimentaires, du Paléozoïque inférieur jusqu'au Quaternaire compris.

Par suite d'une large utilisation de l'analyse palynologique l'étude de la morphologie des pollens se développe aussi; la palynologie est non moins utile à la phylogénie et à la systématique des plantes. Cela se confirme par la publication de toute une série de travaux de Zauer, Monoszon, Sladkov, Avedisian, etc. Vient de paraître le 1er tome de la "Flore d'Arménie" où la description des genres est complétée par des caractéristiques palynologiques.

Le travail d'ensemble le plus important, dans le domaine de la palynologie est l'ouvrage: "L'analyse pollinique" publié sous la direction de I. M. Pokrovskaia (1950). La bibliographie palynologique de M. I. Neustadt (1952) dans "Methody sporo-polliniques en URSS est aussi un ouvrage fondamental. Au deux congrès palynologiques de 1948 et de 1953 la situation de la palynologie a été largement discutée et les résultats des travaux ont été échangés.

Les travaux du Laboratoire d'analyses sporo-polliniques de l'Institut de Géographie de l'Académie des Sciences de l'URSS sont régulièrement publiés dans "Matériaux sur la Géomorphologie et la paléogéographie de l'URSS" (Travaux de l'Institut de Géographie de l'Académie des Sciences d'URSS).

Récemment les palynologistes d'URSS ont constitué une commission spéciale rattachée à la Société botanique d'URSS; le président de cette commission est M. I. Pokrovskaia, le vice-président L. A. Kouprianova et le secrétaire-responsable V. V. Zauer.

L'une des réunions de la Commission a été consacrée à la discussion du dernier livre du Dr Erdtman et a provoqué un échange de vues animé au sujet de la terminologie qu'il propose.

Nous espérons que les travaux de la section palynologique du VIII^{e} Congrès botanique international permettront de fixer une terminologie et une nomenclature unifiées, ce qui faciliterait un développement rapide de la palynologie dont les applications dans différentes branches de la Science sont de plus en plus nombreuses.

Les palynologistes soviétiques ont appris avec plaisir la création d'une commission palynologique internationale rattachée à la section botanique de l'Union international des Sciences biologiques. Ils tâcheront d'établir des échanges de leurs travaux avec ceus des palynologistes des autres pays.

L. A. KOUPRIANOVA

On the Fine Structure of the Pollen Wall in Clivia miniata

Electron micrographs of acetolyzed exines of *Lycopodium clavatum* have been discussed in a previous paper (Afzelius, Erdtman, and Sjöstrand 1954). Further investigations by electron microscopy have confirmed that two main types of fine structure are met with in acetolyzed pollen grains and spores: the sporopollenine (i.e. the substance constituting the most resistant part of

the sporoderm) being either laminate [each individual lamella (50—60 Å thick) consisting of a single layer of granules] or amorphous, granular (cp. electron micrographs published in The Year Book of the Swedish Natural Science Research Council, No. 8, 1953/54). In acetolyzed as well as in non-acetolyzed pollen grains of angiospermous plants the laminate structure has, so far, only been found in the endonexine.

The present note is based on an investigation of acetolyzed and non-acetolyzed pollen grains of *Clivia miniata*. The non-acetolyzed material was fixed in a buffered solution of osmium tetroxide, pH 7.2. Ultrathin sections were made using the technique described by Sjöstrand and the microtome designed by him (Sjöstrand 1953 a, 1953 b).

A section through the extra-nexinous part of a non-acetolyzed pollen wall exhibits the presence of two components, viz. sporopollenine and another substance (Plates 1, 2). The former is acetolysis-proof whereas the latter is destroyed by acetolysis.

Both materials are granular. The average diameter of the sporopollenine-granules (about 50—60 Å) is the same in acetolyzed as in non-acetolyzed material. As for the other component the granular structure rather points to the absence of cellulose, a substance otherwise known to be present in the sporoderm.

The non-acetolysis-proof material is found in the lumina and the interbacular interstices of the endosexinous parts of the muri. It also forms a more or less thin cover on top of the ectosexinous parts of the latter. It thus seems easy to explain why Sitte (1953), when making his instructive replicas of pollen grains and spores, found a great difference between acetolyzed and non-acetolyzed material. (Sitte ascribed this to a change of the sporopollenine by acetolysis.)

The nexine, as it seems, consists exclusively of sporopollenine. In many acetolyzed pollen types so far examined by electron microscopy the most apparent difference in structure is to be found between the endonexine and the rest of the exine, the endonexine being of less compact structure (cp. also Afzelius 1954). Similar observations have been made on non-acetolyzed pollen grains (Plate 1). A lamination of more or less the same type as that in the spores of *Lycopodium clavatum* has been observed. Small portions of a material with a fine structure rather similar to that of the endonexine are sometimes seen between the nexine and the substance, non-resistant to acetolysis, mentioned above (cp. e.g. the portions at the bottom of the central and the right hand side lumina, Plate 1).

The existence of an endonexine has often been doubted. The presence of an endonexinous layer was first indicated by Erdtman [cp. Figs. 26 and 27 in "Pollen- och sportyper i Sveriges kvartära lagerföljder" (Västerås 1939)]. The term endonexine was coined in 1948 [cp. e.g. "Algunos aspectos de la palinologia", Anal. Inst. Esp. Edaf. VII: II ("La parte más interna y delgada de la nexina, la endonexina, es más refringente que la capa externa más gruesa, la ectonexina")].

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February 28th, 1955

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B. M. AFZELIUS

Pollen Grains of cf. Ctenolophon from Tertiary Deposits in India

The surmised Miocene lignites from Warkalli, Travancore, contain a rich pollen and spore flora described by Rao and Vimal (Preliminary observations on the plant microfossil contents of some lignites from Warkalli in Travancore. Current Science 21: 302—305, 1952) and by Vimal [Tertiary spores and pollen from Warkalli lignites, Travancore. Proc. Indian Acad. Sci., 28 B: 195—210, (1953) 1954]. Four illustrations of pollen grains in the first of these papers are reproduced here (Figs. 2—5) together with an illustration of a pollen grain of *Ctenolophon englerianus* Mildbr. (Angola, Gossweiler 9188; reproduced from Erdtman, Pollen Morphology and Plant Taxonomy. I. Uppsala 1952).

Pollen identifications should not as a rule be based exclusively on illustrations. In the present case, however, it seems justifiable to point at the striking resemblance between the pollen grains in *Ctenolophon englerianus* and those in *Septa-* and *Octacolpites* described by Rao and Vimal (cp. also the grains quoted as *Hexa-* and *Septacolporites* in Vimal l.c.). All of them are about the same size (equatorial diameter in *Ctenolophon englerianus* about 42 μ, in *Septacolpites* 45 μ, in *Octacolpites* 41 μ) and offer similar apertures. According to Erdtman l.c. the grains in *Ctenolophon englerianus* are 7—9-colp(oroid?)ate. Further investigations of the scanty material at hand have shown that each colpus is provided with a small lalongate os; this is also the case in the 6—7-colporate pollen grains of *C. grandifolius* (Penang, Oliver 721). According to Rao and Vimal there are two distinct layers in the exine in *Septa-* and *Octacolpites*. The same features can also be seen in *Ctenolophon englerianus* through a good immersion objective. In *C. grandifolius* the study of the exine stratification meets with no difficulty. The pollen grains in *Septa-* and *Octacolpites* come definitely more close to those in the African *Ctenolophon englerianus* than to those in the Malayan *C. grandifolius*.

Ctenolophon was originally described by Oliver in 1873 and referred by him, as later by Ridley (The Flora of the Malay Peninsula. I. 1922), to the Olaca-

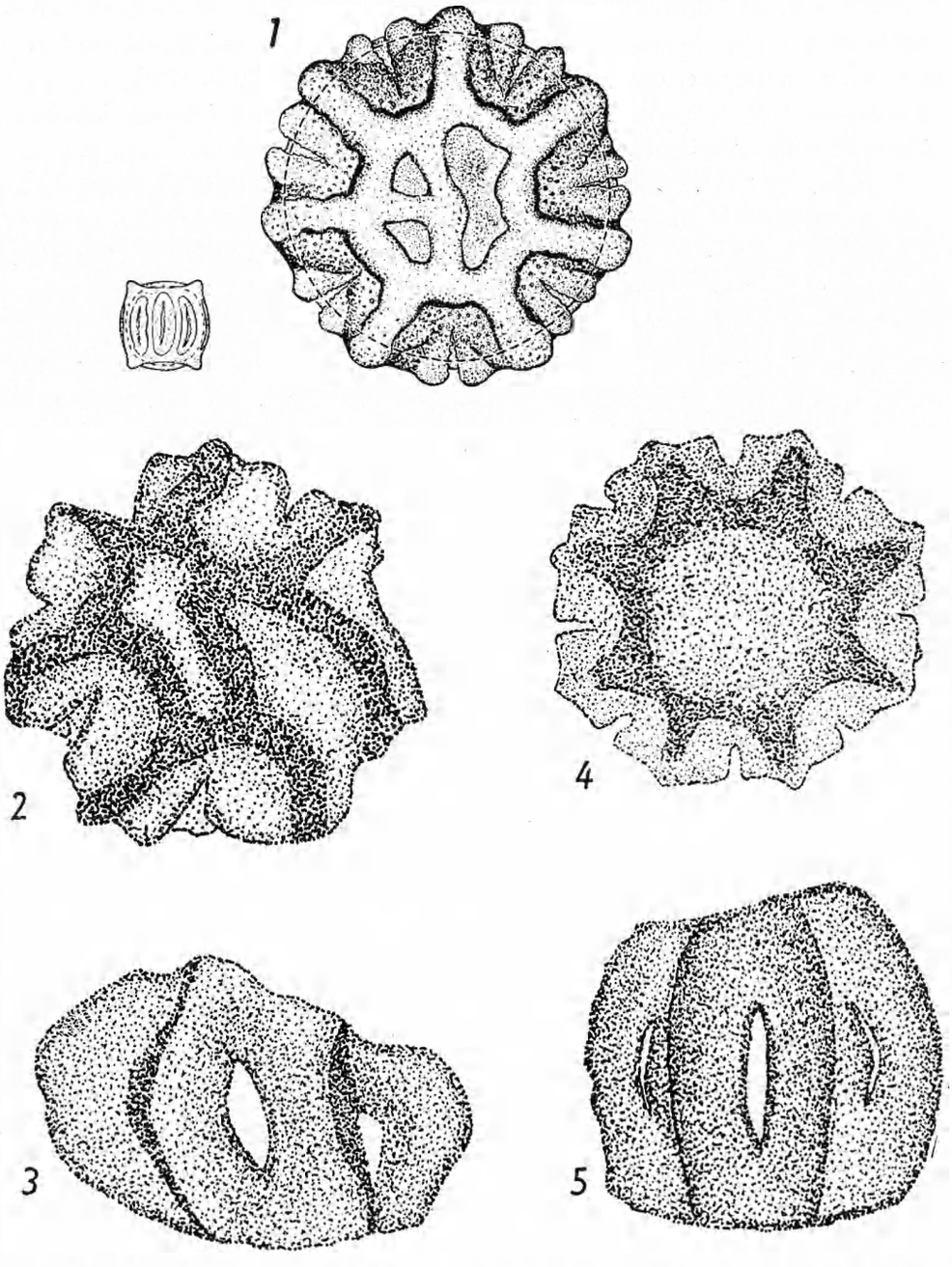

Fig. 1. *Ctenolophon englerianus* (Angola, Gossweiler 9188; from Fig. 143, p. 244, Erdtman l.c.). — Figs. 2, 3. *Septacolpites* (from Fig. 18, 19, Rao and Vimal l.c.). — Figs. 4, 5. *Octacolpites* (from Fig. 20, 21, Rao and Vimal l.c.). — ×1000 (Fig. 1, lower left hand detail figure ×250).

ceae. By Winkler (Engler-Prantl, Natürliche Pflanzenfamilien, 2nd Ed., Vol. 19 a, 1931) it is referred to the Linaceae. Exell and Mendonca (Conspectus florae angolensis, 1: 2, 1951) place *Ctenolophon* in a family of its own, the Ctenolophonaceae, between the Linaceae and Erythroxylaceae. According to Metcalfe and Chalk (Anatomy of the Dicotyledons. I. 1952, p. 271, 272) the

marked differences between genera strongly suggest that the Linaceae is not a sound natural group, and — further — that *Ctenolophon,* which has a much more primitive structure than the other genera except possibly *Indorouchera,* bears a close resemblance to the Humiriaceae. The pollenmorphological data so far at hand do not speak in favour of the reference of *Ctenolophon* to the Olacaceae, nor do they contradict the surmised relationship with the Humiriaceae.

G. ERDTMAN

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Från Lunds Botaniska Förenings förhandlingar 1954

Den 17 februari. Ett från föregående termin vilande förslag om ökning av antalet styrelseledamöter utan särskild funktion från tre till fem antogs. Till nya styrelseledamöter valdes docent Robert Lamm och amanuens Nils Malmer.

Till ersättare för v. ordföranden, docent H. Virgin, vilken ännu inte återvänt från sin amerikavistelse, utsågs docent Hugo Sjörs.

Föreståndaren för den naturvetenskapliga stationen i Abisko, laborator Gustaf Sandberg, höll föredrag om »Klimatfluktuationer och vegetationsförändringar i fjällen».

Sedan 1700-talet har tendensen i stort sett varit den, att klimatet blivit mildare med en särskilt markant stegring av medeltemperaturen under de senaste decennierna. Detta har medfört att glaciärerna dragit sig tillbaka och att busk- och trädvegetationen kunnat förflytta sig högre upp på fjällsluttningarna. Föredragshållaren drog en jämförelse mellan den nuvarande vegetationens fördelning och den som Thore Fries tidigare iakttagit. Av intresse är, att kalkängsvegetationen på många ställen ersatts av gråvidesnår. Björkskogsgränsen har höjts betydligt på senare tid, på vissa håll 30—40 m. På platser med några få gamla, knotiga träd växer nu nya björkar upp, tätt och frodigt.

Professor Eric Hultén, Stockholm, visade därefter ett stort antal magnifika närbilder av fjällväxter i färg.

Den 9 mars. Docent Ove Almborn uppläste revisionsberättelser över kassörens och styrelsens förvaltning under 1953. Full och tacksam ansvarsfrihet beviljades.

Fil. mag. Henry Rufelt demonstrerade negativ geotropism hos rötter. Detta fenomen, som inte tidigare med säkerhet påvisats, hade han fått fram genom att tillsätta ett antiauxin, *α-indolylisosmörsyra,* i koncentrationen 10^{-4} mol/l till unga veterötter.

Docent Ove Almborn höll föredrag om »Botaniska strövtåg i Sydafrika». I detta redogjorde han för de intryck han fått av vegetation och flora i denna del av Afrika under ett halvt års botaniska studier där.

Föredragshållaren behandlade inledningsvis Sydafrikas olika vegetationsregioner. Kapregionen är ett bergland, vars klimat kännetecknas av vinterregn och en därav betingad macchiavegetation. Karoo utgöres av högslätter med låg nederbörd och starka växlingar i temperatur mellan dag och natt. Här dominerar en stäppvegetation med karaktärsväxter tillhörande de artrika släktena

Mesembryanthemum och *Aloë*. Längre norrut utbreder sig Kalahari-platån, som dock inte är en öken utan en grässtepp med acacior och liliacéer. Södra delen av Transvaal täckes av en trädfattig s.k. »Highveld»-vegetation, vilken norrut ersättes av en »Bushveld»-vegetation, dominerad av bl.a. caesalpiniacéer och rhamnacéer. I östra Transvaal har utbildats en s.k. »Lowveld»-vegetation med acacior och andra tropiska träd. Den östra kustregionen i Natal utgöres av ett smalt subtropiskt bälte med sommarregn, vilket utmärkes av ständigt gröna lövträd och palmer. Drakensbergen slutligen är ett högt bergland med torr gräsvegetation och bl.a. *Helichrysum*-arter som karaktärsväxter. Där uppträder även ett holarktiskt element med *Ranunculus* och *Anemone*.

Docent Almborn visade därefter ett stort antal utmärkta färgbilder, som han tagit under resan genom de olika områdena. Dessa gav en god uppfattning om de utmärkande dragen hos de olika vegetationstyperna och om de stora kontrasterna mellan floran i olika delar av Sydafrika.

Den 6 april. Till revisorer för växtbytet omvaldes fil. lic. A. Almestrand och fil. mag. H. Rufelt.

Museiintendent Tycho Norlindh demonstrerade några biologiskt intressanta arter från Rhodesias savanner. Föredragshållaren framhöll, att de flesta träden på dessa savanner, vilka ligger inom det tropiska bältet, är bladfällande på grund av speciella klimatiska förhållanden. Vegetationen får nämligen där genomlida en sammanhängande torrperiod på c:a 7 månader. Savannen kan uppdelas i skogs-, busk- och grässavann. På skogssavannen är i regel ärtträden, bl.a. *Brachystegia* och *Pterocarpus* helt dominerande. Det senare släktet, som har bredvingade, diskuslika, enfröiga ärtbaljor, är känt som tertiärfossil ända uppe i Rhenlandet. Där märkes vidare det skönblommiga polygalacéträdet *Securidaca,* vars vingade frukter äro förvillande lika lönnens, och den brandhärdiga liljeväxten *Vellozia,* vars skenstam är uppbyggd av tätt packade, hårda bladslidor.

Professor A. I. Virtanen, Helsingfors, höll föredrag över ämnet »Kvävenäringens inverkan på den enzymatiska aktiviteten och sammansättningen av olika kvävefraktioner i mikroorganismer och gröna växter».

Under enzymforskningens tidigare skeden ansåg man, att endast en liten bråkdel av protoplasmans äggviteämnen utgjordes av enzym, sade föredragshållaren. Nyare forskningsresultat, som bygger på renframställning av ett stort antal enzym och bestämning av deras aktivitet, tyder emellertid på att praktiskt taget hela proteinmängden i cellerna utgöres av enzym eller lätt kan omvandlas till dylika. Det är därför naturligt, att kvävebrist leder till nedsatt enzymaktivitet i samma mån som den påverkar proteinhalten. Alla enzym är emellertid inte lika känsliga för minskad kvävetillförsel. Det är i första hand aktiviteten hos de s.k. *adaptiva enzymen,* vilka är nödvändiga endast under speciella näringsförhållanden, som minskar kraftigt vid minskad kvävehalt. De *konstitutiva enzymen,* vilka är absolut nödvändiga för livets upprätthållande, bibehåller däremot sin aktivitet tämligen oförändrad i det längsta. Först vid kvävehalter så låga, att organismerna dukar under, får man en kraftig minskning även av dessas aktivitet.

Den 5—7 juni. Exkursion till Öland under ledning av docent H. Sjörs och fil. lic. O. Andersson. — 26 deltagare.

Solens och vindarnas ö visade sig i synnerhet från den soliga sidan och tog emot i sin praktfullaste försommarskrud. Inkvarteringen skedde i Vickleby, beläget vid landborgen, gränsen mellan den bördiga kustremsan i väster och Stora alvaret. Detta var en ypperlig utgångspunkt för kortare utflykter, som naturligtvis främst ägnades alvaret med dess egenartade vegetation. En enorm rikedom på färger tjusade här ögat. Den lilla ölandssolvändan med sin klara, gula färg förekom i massor, en ljusare gul färgton svarade *Oxytropis campestris* för, medan den annars gula getväpplingen här visade alla skiftningar från ljusgult till purpurrött. Andra färgrika inslag utgjorde *Primula farinosa, Orchis mascula* och *O. sambucina,* den klarblå globularian och blommande gräslök i mängd. Mera oansenliga botaniska rariteter tilldrog sig även intresse såsom *Artemisia laciniata, Plantago tenuiflora* och *Agrostis interrupta.*

Den 6 juni, pingstdagen, ägnades åt en bussfärd runt mellersta Öland. Efter ett besök vid Mysinge hög, där blomsterprakten beundrades och fotograferades, gick färden tvärs över Stora alvaret och därefter norrut på östra sidan av ön. Anhalter gjordes vid en lokal för *Medicago minima, Festuca polesica* och *Koeleria glauca* söder om Gårdby och vid en riklig förekomst av *Carex ligerica* och *Carex obtusata* en km söder om Runsten. Några km öster om Ismanstorp besöktes ett område, där *Orchis ustulata, O. militaris* och *O. mascula* växte massvis, varjämte även *Ophrys myiodes* och *Neottia nidus-avis* förekom. I närheten av Ismanstorps fornborg hittades den vita *Cephalanthera longifolia.* Efter ett besök på Karums alvar gick färden till Halltorp på Ölands västsida, där en rundvandring gjordes i avenbokskogen, Sveriges största bestånd av någorlunda ren avenbok.

Den 26 september. Svampexkursion till Snogeholms-trakten under ledning av fröken Maja-Lena Nilsson, Ystad.

En stor del av dagen ägnade deltagarna åt att i större eller mindre grupper ströva omkring i skogsområdena norr och väster om Snogeholmssjön, där man fann åtskilligt med svamp. Vid Snogeholms slott, där lunch intogs, hade en svamputställning ordnats, och fröken Nilsson demonstrerade de talrika arter som hittats. Bland dessa kan nämnas *Craterellus cornucopioides, Cordyceps militaris, Coprinus picaceus* och *Mutinus caninus.*

Den 28 september. Docent Ove Almborn demonstrerade ett 30-tal kärlkryptogamer från Sydafrika.

Cand. mag. Erik Bille Hansen, Köpenhamn, höll föredrag över ämnet »Sporespiring og Frugtlegemdannelse hos Storsvampene».

Föredragshållaren gav en översikt över metoderna för odling av svamp och behandlade de faktorer, som påverkar sporernas groning och fruktkroppsbildningen. För en fullständig bestämning av dessa faktorer fordras det, att man lyckas med att odla svampen på ett syntetiskt medium, i vilket man känner de ingående komponenternas kemiska natur. Föredragshållaren redogjorde så för en serie försök över olika näringsfaktorers inverkan på fruktkroppsbildningen hos fem *Coprinus*-arter. Dessa försök visade, att det kan föreligga betydande skillnader även mellan arter inom samma släkte.

Den 22 oktober. Docent Erik Rennerfelt, Stockholm, höll föredrag om »Svampskador på skog och virke».

Föredraget var utformat som en översikt över det arbete, som bedrives vid Statens Skogsforskningsinstitut för att komma tillrätta med de svampsjukdomar, som åstadkommer de allvarligaste förlusterna för skogshushållningen. Bland dessa märkas barrost på gran, vilken i synnerhet de senaste åren förorsakat allvarliga skador, törskatesvampen, som angriper tallen, samt rotrötan på gran. Särskilt har ett omfattande arbete lagts ned på att finna metoder att bekämpa den sistnämnda, dock utan större framgång. Den förorsakas av *Polyporus annosus,* som huvudsakligen sprides via rotkontakter mellan närbelägna träd. Luftinfektion av stubbar har emellertid även påvisats. — Skador på stock och stormfälld skog åstadkommes i synnerhet av blåytesvampen, vilken ger upphov till missfärgning av virket. Dess spridning står i samband med insektsangrepp och kan förhindras genom sprutning av stockarna med insektsbekämpningsmedel.

Amanuens Rolf Dahlgren visade färgbilder från en fotvandring i Lule Lappmark, vilken han tillsammans med några kamrater företagit sommaren 1954.

Den 19 november. Val av styrelse för år 1955 förrättades enligt följande: Ordf. fil. dr Asta Almestrand; v. ordf. docent Hugo Sjörs, nyval; sekr. amanuens Lennart Eliasson, v. sekr. amanuens Rolf Dahlgren, styrelseledamöter utan särskild funktion, proff. Hans Burström och Henning Weimarck, dir. K. E. Flinck, docent Robert Lamm samt amanuens Nils Malmer. Till revisorer omvaldes lektor Oscar Palmgren och docent Ove Almborn med docent Bertil Hylmö och assistent Anders Kylin som suppleanter.

Docent Tore Arnborg, Uppsala, höll föredrag om »Växtgeografiska studier i västra Nordamerika — från Grand Canyon till Mount McKinley».

Föredraget, som illustrerades med färgbilder, skildrade en studieresa, som docent Arnborg företagit huvudsakligen för att studera barrträdsvegetationen. Resan hade gått från Arizona över Sierra Nevada ut till stillahavskusten, vidare genom de nordvästra delarna av Förenta Staterna till Klippiga Bergen samt genom västra Canada upp till Alaska. Dessa delar av Amerika får sin prägel av bergskedjorna och de nederbördsfattiga högplatåerna. Här förekommer rikligt med barrskog, kännetecknad av sin stora artrikedom. De olika arterna är anpassade för mycket skiftande klimatiska förhållanden, vilket bl.a. belyses av arternas fördelning på bergskedjornas västsluttningar. På Klippiga Bergen växer sålunda *Pinus ponderosa* lägst, därefter följer i ordning *Pseudotsuga, Abies lasiocarpa* och *Picea engelmanni.* I Idaho finns vackra bestånd av *Larix, Tsuga* och *Thuja.* I områden, där barrträden har svårt att bilda sammanhängande vegetation, förekommer rikligt med asp. Denna har även börjat dominera i åtskilliga tidigare barrträdsområden, en följd av de kraftiga avverkningarna, som ofta skett utan att man sörjt för återväxten. I Canada består kustskogen av *Pinus contorta* och *Picea sitchensis,* medan det inne i landet förekommer *Picea glauca, Larix lyalli* och *Abies balsamea.*

Den 29 november. Dr Per Wendelbo, Bergen, höll föredrag över ämnet: »Tirich Mirs flora. De botaniske resultater av den norske Himalayaekspedisjon 1950».

Expeditionen bestod huvudsakligen av bergsbestigare, men även några få

vetenskapsmän medföljde. Tirich Mir är den högsta toppen i bergsmassivet Hindukush och belägen i provinsen Chitral i nordvästra delen av Pakistan. Området är mycket nederbördsfattigt och endast i dalgångarna är det möjligt att genom konstbevattning odla kulturväxter. Floran i det otillgängliga området är mycket ofullständigt utforskad. Den är trots de ogynnsamma klimatiska förhållandena mycket artrik och har anknytning både till Himalayas flora i öster och Afganistans i väster. Ända upp till snögränsen 5 000 m över havet finns en omväxlande vegetation, lokaliserad till de fläckar på sluttningarna där fuktigheten tränger i dagen.

Den 10 december. Ordf. meddelade, att föreningens kassör, akademiträdgårdsmästare Axel Törje, avgått och att styrelsen till hans efterträdare utsett fil. lic. Anders Kylin. Till ny revisorssuppleant efter den nyvalde kassören valdes fil. lic Henry Rufelt.

Fil. lic. Artur Almestrand uppläste revisionsberättelse för årets växtbyte. Full ansvarsfrihet beviljades för bytesföreståndaren.

Amanuens Lars Gösta Dahl talade om termofila mikroorganismer och demonstrerade några kulturer av dylika. Dessa organismer, som förekommer praktiskt taget överallt, trivs i allmänhet bäst vid 50—70° C och kan klara sig vid sterilisering vid betydligt högre temperatur, varför de utgör ett problem för konservindustrin. Både deras ursprung och deras fysiologi är gåtfulla och erbjuder intressanta problem för forskningen.

Professor H. Weimarck höll ett kåserande föredrag med titeln »Resa till England». Resan, som företagits under sommaren 1954, hade huvudsakligen gällt ett besök vid universitetet i Leicester, från vilket en del glimtar gavs. Det fuktiga och milda klimatet medför en hel del skillnader mellan den brittiska och svenska floran. Sålunda betraktar engelsmännen sådana växter som *Anemone hepatica* och *Corydalis cava* som rena rariteter medan t.ex. *Viola lutea, Ilex* och *Arum maculatum* är vanliga där.

LENNART ELIASSON

Litteratur

F. E. WIMMER, Campanulaceae — Lobelioideae. II. Teil. — Das Pflanzenreich Regni vegetabilis conspectus. Im Auftrage der Deutschen Akademie der Wissenschaften zu Berlin herausgegeben von A. Engler (†)-L. Diels (†) fortgesetzt von H. Stubbe und K. Noack. Red. R. Mansfeld. IV. 276 b (107. Heft). VIII+553 Seiten mit 622 Einzelnbildern in 57 Figuren. Akademie-Verlag Berlin. 1953. — Pris 70: — DM.

Som första del av »Pflanzenreich» efter kriget föreligger nu fortsättningen på monografien över *Campanulaceae — Lobelioideae*. Det är över tio år sedan ett häfte av »Pflanzenreich» utkom och en del förändringar i utgivandet av detta verk ha inträffat. Deutsche Akademie der Wissenschaften zu Berlin står numera som finansiell understödjare och Akademie-Verlag Berlin har efterträtt Engelmanns förlag. Med det utgivna 107:e häftet är ungefär 1/4 av fanerogamfamiljerna mer eller mindre komplett behandlade i »Pflanzenreich» sedan starten 1900.

Prof. Wimmer vid Naturhistorisches Museum i Wien har ägnat nära 30 år åt studiet av »Lobeliaceae». Första delen av hans stora monografi över familjen utkom 1943 och andra delen förelåg i färdigt skick redan två år senare. Emellertid kunde den utgivas från trycket först under de sista dagarna av 1953.

Den enda tidigare tillgängliga monografi, omfattande hela *Lobeliaceae,* utgjordes av DeCandolles bearbetning 1839. Detta arbete behandlar 27 släkten med 358 arter. Wimmer däremot räknar med 29 släkten och över 1 150 arter.

Vid jämförelse med Dalla-Torres Index finner man att följande släkten slagits samman med andra: *Palmerella* och *Isotoma* (nu sect. under *Laurentia*) samt *Rhizocephalum* (nu subgenus under *Lysipomia*). Under de senaste åren ha emellertid några nya släkten tillkommit: *Legenere* (N. och S. Am.), *Unigenes* (S. Afr.), *Dielsantha* (trop. Afr.), *Dominella* (S. Am.) samt *Phyllocharis* (Nya Guinea), som dock beskrevs redan 1919. *Diastatea* (gen. incertae sedis) föres nu definitivt till *Lobelioideae*.

Beträffande indelningen av lobeliacéerna påpekar förf. de stora svårigheterna att uppställa klart avgränsade grupper. Till grund för indelningen lägges fruktbyggnaden. Första delen av monografien behandlar alla lobeliacéer med bärfrukt (Tribus I. *Delisseeae*) och den mer omfångsrika andra delen alla med kapsel (Tribus II. *Lobelieae*). Förutom en bestämningstabell grundad på frukternas byggnad finnes en examinationsnyckel, som i görligaste mån tager hänsyn till andra karaktärer än dem frukten uppvisar. Detta är av mycket stort värde vid arbete med herbariematerial, som ju ofta saknar mogna frukter. I fråga om artavgränsningen har förf. sökt en medelväg genom att använda varken för snävt eller för vidsträckt artbegrepp.

Vid uppdelningen av *Lobelieae* har förf., på grund av materialets omfång och mångformighet, fått använda sig av ett flertal taxonomiska enheter (nycklar finnes till samtliga). Av nio subtribus är den första den viktigaste och största. Här möter man inom två olika grupper de stora släktena *Siphocampylus* och *Lobelia*. Det förra med 207, det senare med 383 arter. Inom *Lobelia* ha vi underfamiljens enda europeiska representanter, *L. dortmanna* och den sydvästliga *L. urens*. För notblomstret uppges »Südliches Schweden bis über Stockholm» som utbredningsområde i Sverige. Om härmed avses även mellersta delen av landet samt norrländska kustlandet är det rätt. Jämte de trädartade lobeliorna i trop. Afrika och Hawaiis dvärgrosetträd hör säkerligen släktet *Brighamia* (monotypiskt) från Sandwichöarna, till de egendomligaste av alla lobeliacéer. Ett litet förbiseende i fråga om ett geografiskt namn under *Lobelia dregeana* (p. 513) borde ha tillrättalagts. Vid behandling av släktet *Downingia* borde kanske angivits att detta namn är uppfört som nomen conservandum.

I ett förelöpande meddelande (Ann. Naturhist. Mus. Wien 56: 317—374. 1948.) har Wimmer publicerat diagnoserna till de flesta nya arterna i monografien men ytterligare 32 (varav 24 till *Lobelia*) ha nu tillkommit förutom ett stort antal varieteter, former och nykombinationer. Tyvärr har förf. underlåtit att angiva typexemplaren för nya taxa.

Supplement tyckas vara oundvikliga. Wimmers monografi avslutas med två. Ett tillägg till första delen av arbetet med bl.a. tre nya *Cyanea-* och *Clermontia*-arter samt ett till senare delen, upptagande nya arter och varieteter, som tillkommit under tiden monografien låg i press.

44 figurer med över 600 detaljteckningar samt 13 foton av originalexemplar komplettera framställningen. Slutligen register till båda delarna, även ett över inhemska namn.

Upplagan av första delen av *Lobelioideae*-monografien, som kom 1943, är tyvärr uppbränd men ett nytryck kommer inom kort.

Om denna värdefulla monografi kan man instämma i författarens önskan i avslutningensorden till förordet »dass diese Arbeit eine grosse Lücke ausfüllt und zur besseren Kenntnis dieser artenreichen Familie beiträgt».

Bo Peterson

Willi Christiansen: Neue kritische Flora von Schleswig-Holstein. — Buchverlag Heinrich Möller Söhne, Rendsburg 1953. 532 sid. — Pris 24 DM.

Det floristiska utforskandet av Schleswig-Holstein har en snart tvåhundraårig historia. Grundläggande arbeten utfördes mot 1700-talets slut och under 1800-talets första decennier av G. H. Weber (Primitiae Florae Holsatiae, 1780), Ch. W. Ritter och E. F. Nolte. Åtskilligt material från området redovisas i Flora Danica och i Rafns, Hornemanns och Langes danska floror. Floristikens storhetstid i slutet av 1800-talet avspeglas i tillkomsten av två sammanfattande arbeten, nämligen P. Knuth, Flora der Provinz Schleswig-Holstein (1888) och P. Prahl, Kritische Flora der Provinz Schleswig-Holstein (1890). De senaste femtio åren har medfört ett intensivt utforskande av områdets flora under ledning av främst bröderna Albert († 1917) och Willi Christiansen och den först-

nämndes son Werner Christiansen. I fråga om grundlighet torde denna inventering ha få motstycken på något annat håll i Europa. De båda sistnämnda författarna har tidigare utgivit Flora von Kiel (1922) samt åtskilliga andra undersökningar av provinsens flora och växtgeografi.

Av Willi Christiansen föreligger nu en koncentrerad sammanfattning av det väldiga material av fyndortsuppgifter och utbredningskartor som samlats av »Arbeitsgemeinschaft für Floristik» i Kiel. Det är ej fråga om en flora i egentlig mening, eftersom boken ej har diagnoser på släkten och arter (men väl på infraspecifika taxa; jfr nedan). Framställningen av varje art innefattar fem skilda avdelningar. — 1. En formel innehållande mycket korta uppgifter om artens allmänna växtgeografiska ställning, spridningsbiologi, livsform (enligt Raunkiaer) och kromosomtal. — 2. Ståndortsekologi, ofta med den växtsociologiska terminologi som utbildats av R. Tüxen. — 3. Utbredning inom området. Blott för sällsyntare arter anges lokaler. Ofta nämnes blott nummer på de distrikt varifrån arten är känd. För 240 arter anges utbredningen inom provinsen med kartor. I regel har förekomst inom ett distrikt markerats schematiskt med ett kryss; i ett mindre antal fall har lokalernas lägen angivits närmare med ringar. Det är att beklaga, att det ej varit möjligt att publicera mera av det jättematerial som ligger till grund för uppgifterna (c:a 30 000 småkartor angivande flertalet arters utbredning inom varje distrikt). — 4. Variation inom arten. Det verkar något förbryllande att se så många underarter, varieteter, former (och t.o.m. subformer!) beskrivna, då arterna, som nämnts, helt saknar diagnoser. Exempelvis har *Anemone pratensis* 11 infraspecifika taxa och *Potentilla argentea* 13. Beskrivningarna torde i regel vara kompilerade efter Aschersons och Græbners eller Hegis floror. Förf. följer ej de vid Stockholmskongressen stadfästa reglerna om upprepning av artepitetet för huvudtypen utan har — liksom Hylanders nordiska flora — talrika namn av typen *eupratensis,* var. *genuina* etc. Förf. menar att arternas avgränsning numera är i stort sett klarlagd, medan man ofta ägnat ringa intresse åt variationen inom arterna. Att avgöra i vad mån den ymniga formrikedom som här redovisas, är av genotypisk eller fenotypisk art fordrar ju ingående genetiska undersökningar. Säkerligen har den livaktiga cytologskola som i Kiel vuxit fram under G. Tischlers ledning, väsentligen bidragit till att ställa den infraspecifika variationen i blickpunkten. — 5. Uppgifter om när arten första gången nämnts i litteraturen från Schleswig-Holstein, om huruvida den är ursprunglig eller införd (ett stort antal adventivväxter är medtagna) och om eventuell fridlysning.

Nomenklaturen följer i allmänhet Mansfeld, Verzeichnis der Farn- und Blütenpflanzen Deutschlands (1941) och avviker alltså i fråga om släkten och arter ej mycket från den som skandinaviska botanister fått vänja sig vid under de senaste femton åren.

Det är uppenbart att denna flora har mycket att bjuda även för nordiska florister, främst i Danmark och södra Sverige, där flertalet arter är gemensamma med Schleswig-Holstein. För dem som hos oss sysslar med inventering och kartering av vegetationen, har det sitt givna intresse att se hur man löst likartade problem i ett närbeläget utomnordiskt område.

OVE ALMBORN

W. O. JAMES: Plant respiration. — 282 sid. Oxford University Press, Oxford 1953. — Pris 30 sh.

Det intensiva arbete som de senaste decennierna lagts ned på att utreda naturen hos de energifrigörande processerna i levande celler har främst varit koncentrerat till mikroorganismer och djurvävnad. Att åtskilligt uträttats även när det gäller andningsprocesserna hos högre växter, ger emellertid föreliggande monografi belägg för.

De första kapitlen handlar om storleken av koldioxidutveckling resp. syreförbrukning under olika förhållanden, hur detta gasutbyte förändras med tiden hos hela plantor eller avskurna växtdelar och om andningskvotens storlek under olika förhållanden. På dessa områden har andningsforskningen mycket gamla anor inom växtfysiologin, och framställningen består till stor del av sammanställningar av experimentella data från olika epoker alltifrån Saussures försök i början av 1800-talet. Begreppet andning har i dessa kapitel använts i sin äldre betydelse, nämligen såsom gasutbyte mellan omgivning och organism. Efterhand som man lyckats klara upp de processer i protoplasman, som ligger bakom detta gasutbyte, har emellertid betydelsen hos denna term utvidgats, så att den kommit att innefatta även de biokemiska processer i cellen, som sker vid nedbrytningen av olika substrat till koldioxid och vatten. Det är härvid fråga om komplicerade enzymkemiska förlopp, intimt sammanvävda med det biokemiska skeendet i cellen i sin helhet. Dessa aspekter av andningen behandlas i bokens senare hälft. Det är denna del av boken, som har den största aktualiteten. I ett kapitel om olika andningsmaterial finns ett avsnitt om hur kolhydraterna genom fosforyleringsprocesserna indragas i andningsmekanismen. Så kommer ett kapitel om glykolysen, det bäst utforskade avsnittet av kolhydratnedbrytningen. Även om den reaktionskedja, som leder från glykos till pyrodruvsyra, huvudsakligen har fastställts med jäst och djurvävnad som försöksmaterial, så kan det numera anses visat, att reaktioner efter samma schema även äger rum hos de högre växterna. Mekanismen för överförandet av vätet på luftsyre under medverkan av olika oxidassystem ägnas ingående uppmärksamhet. På detta område har många nya fakta tillkommit under de senaste årtiondena inte minst från försök med växtmaterial. Däremot ges Krebs cykel — en kedja av reaktioner, som ombesörjer nedbrytningen av pyrodruvsyra till koldioxid och vatten — en mer undanskymd behandling, även om allt tyder på att den förekommer åtminstone hos vissa växtvävnader.

Ett kapitel med rubriken »Oxygen Effects» handlar om syrgaskoncentrationens betydelse för andningen och om de ur växtfysiologisk synpunkt viktiga problemen om syrgasdiffusionen genom växtorgan. Medan det inom djurriket har utbildats komplicerade och högeffektiva andnings- och cirkulationsorgan för upptagning och transport av syre, är varje växtcell även i det inre av massiva vävnader helt beroende av syrgasens diffusion för sin andning. Detta kapitel, som delvis bygger på opublicerat material, ger exempel på synnerligen långt driven teoretisk analys av experimentella data. Inspirationskällan härvidlag torde vara den framstående engelske växtfysiologen F. F. Blackman, vilken vid bearbetningen av sina experimentella resultat utarbetade komplicerade metoder för tolkningen av andningsvärdena. Genom detta rent fysiologiska sätt att angripa problemen har Blackman och hans skola kommit fram till en

del allmänna slutsatser om andningsprocessernas förlopp, vilka åtminstone bitvis stämmer överens med vad biokemisterna funnit genom sina mera direkta metoder.

I inlednings- och slutkapitlen ägnar författaren en del resonemang åt den mera filosofiskt betonade frågan om det kan finnas liv utan andning, ett problem som av allt att döma sysselsatt gångna tiders fysiologer åtskilligt. Någon form av aktivt liv utan energifrigörande processer är inte känd, men författaren tycks ändå inte vilja utesluta möjligheten, att någon sådan form av liv åtminstone skulle kunna tänkas. Kanske det. Men varför då inte lika gärna liv utan vatten och utan äggviteämne? Som hos kommande tiders perfekta elektronrobotar, om någon skulle vilja tänja ut begreppet liv så långt. Men utan energi i någon form torde väl knappast de heller kunna ge prov på någon aktivitet, som kan jämställas med levande organismers livsyttringar.

En bibliografi på bortåt tusentalet referenser avslutar boken. Den bör kunna bli till stort gagn för forskare, som har anknytning till detta centrala fält inom fysiologisk forskning, och för dem som av andra orsaker är intresserade av en utförlig framställning över vad som gjorts för att utreda andningsmekanismen hos växter.

LENNART ELIASSON

JOHN GILMOUR & MAX WALTERS: Wild Flowers. Botanising in Britain. — The New Naturalist 5. Collins. London. 1954. — Pris 20 sh.

Med serien *The New Naturalist* har brittiska naturvetare visat sin vilja att hålla en rik naturhistorisk tradition levande in i våra dagar. John Gilmours och Max Walters' *Wild Flowers* med den talande undertiteln *Botanising in Britain* är en bok fylld av fin kultur, sunt förnuft och äkta känsla för naturen. Den brister ej heller i kritisk insikt och skärpa, där den närmar sig den vetenskapliga botanikens frontlinje i våra dagar. Var denna frontlinje går i modern botanik ger boken många fina tips om.

Illustreringen är rik och står både då det gäller färgfotona och svart-vitbilderna i toppklass. Man frapperas av hur väl arternas väsentliga karaktärer är återgivna. Även den naturliga miljön kring den enskilda arten kommer utmärkt fram trots en ofta starkt beskuren bildyta.

Bokens huvuddel upptas av skildringar av de viktigaste brittiska växtsamhällena och deras karakteristiska arter. Den föregås av några inledande kapitel. Det första av dessa försöker ge ett svar på frågan: Varför blir man fältbotanist? Gilmour menar att det beror på »the irresistible passion» som vägledes och tyglas av strävan efter kunskap. Den förklaringen tror jag att alla fältbotanister är villiga att underskriva.

I dessa sammanhang kommer Gilmour också in på vilka krav man kan ha rätt att ställa på en god fältbotanist och amatör och hur dessa krav kan uppfyllas. Som en hjälp för den enskilde pekar han här på de lokala naturhistoriska föreningarnas omistliga betydelse, då det gäller att uppamma och sprida fältbotaniskt kunnande i vidare kretsar. Dessa föreningars medverkan i att utföra ett detaljerat växtgeografiskt forskningsprogram pointeras också.

Den brittiska florans upptäcktshistoria skildras på ett litterärt och fackligt

förnämligt sätt av Gilmour. Han går ända från 1600-talets första trevande försök och fortsätter med John Ray och hans grundläggande arbete, som kulminerade med *Synopsis stirpium brittannicarum*. Av 1700-talets stora botanister fäster man sig främst vid den ingående behandlingen av William Curtis, grundare av *The Botanical Magazine* och författare till *Flora Londinensis*. I verkningsfull kontrast till adertonhundratalets senromantiska högflod på gott och ont inom naturhistorien står vårt århundrades nyktert sakliga, men samtidigt friska och framgångsrika fältekologiska forskning i England. Kapitlet är säkert det, som ger den svenska botanisten mest av nya, intressanta fakta. Det skänker också många paralleller till den svenska botanikens historia.

De övriga inledande kapitlen ge översikter över den brittiska florans biologi och invandringshistoria. Biologi-kapitlet är mycket innehållsrikt och behandlar i samband med växtens ontogenetiska utveckling sådana frågor som groningsbiologi och de faktorer som inverka på blombildning och fröproduktion, däribland pollinationsbiologin. Även livsformerna enligt Raunkiærs system får sin beskärda del. Förhållandet mellan växtindividen och det växtsamhälle, vari den ingår samt de faktorer, som tänkas reglera växtsamhällets utformning och förändringarna i dess struktur skildras också. Man får här en översikt över de principer som engelska växtsociologer arbetar efter. Max Walters behandlar i kapitlets slut i korthet art- och formbildningsproblemen ur moderna genetiska synpunkter.

I kapitlet om invandringshistorien får man även en intressant skildring av den brittiska florans växtgeografiska gruppering ur arealgeografisk och ekologisk synpunkt.

Skildringarna av vegetationstyperna och de i dem ingående arternas biologi ge en mycket god popularisering av de engelska ekologernas arbeten. De av Arthur Tansley, Georg Salisbury och W. H. Pearsall och deras lärjungar utformade undersökningsmetoderna och vunna resultaten ger en säker grund för framställningen.

Indelningen av de brittiska växtsamhällena är ej floristisk-sociologisk efter samhällenas artinnehåll som i de moderna mellaneuropeiska och skandinaviska arbetena. I stället tillämpas, vilket i en populär anglosachsisk framställning torde vara både nödvändigt och oundvikligt, en uppdelning och kapitelindelning efter ståndorten och samhällenas morfologiska struktur. Rubriker som: *Woods and hedgerows, Mountains, Moors, heaths and commons* och *Bogs, fens and marshes* ge exempel härpå. De olika vegetationstypernas uppbyggnad och förekomst på de brittiska öarna skildras målande. Deras art-bestånd behandlas ingående med redogörelser för arealen i England och det övriga Europa, bundenheten till viss miljö, särpräglade drag i livscykeln och utnyttjandet av människan. Uppgifter om arternas postglaciala och senare historia i relation till människans ingrepp i och utnyttjande av markerna fogas här in. Ett särskilt fint drag åt skildringarna ge de vackra prov på engelsk naturlyrik, som flätats in för att återge stämningarna över de olika naturtyperna.

Genom sin up to date förda litteraturförteckning samt ett fylligt register står boken som ett mönster för svenska arbeten på det populärvetenskapliga området. Registret lyser hos dessa alltför ofta med sin frånvaro.

Wild Flowers ger en ypperlig översikt över modern fanerogamekologi med

många utblickar till systematik och fysiologi. Den bör vara mycket givande för övergångsstadiets och gymnasiets biologilärare, som ju på svenska sakna en sådan översikt, som motsvarar de nyaste kursplanernas fältbiologiska intentioner. De brittiska öarnas artbestånd är också så nära överensstämmande med Sveriges, att boken helt igenom behandlar för svenska läsare välbekanta arter och släkten. Avgränsningen av de systematiska enheterna är också gjord med nödvändig vidsyn.

TORSTEN HÅKANSSON

Notiser

Statens Växtskyddsanstalt. Till professor och chef för Statens Växtskyddsanstalt i Stockholm har utnämnts fil. dr Ingvar Granhall, Balsgård.

Växtförädlingsanstalten i Balsgård. Till föreståndare för Balsgård har styrelsen för Föreningen för växtförädling av fruktträd antagit fil. lic. Nils Nybom, Lund.

Uppdrag i utlandet. Professor Hans Burström har inbjudits att i maj eller juni gästföreläsa vid universiteten i Leicester och Tübingen.

Forskningsanslag. Statens naturvetenskapliga forskningsråd har utdelat bl.a. följande anslag: till prof. Hans Burström 6.700 kr. för anskaffande av en koronahygrometer för transpirationsbestämningar, till prof. G. Erdtman 5.700 kr. för elektronmikroskopiska studier av pollen och sporväggar, till doc. H. Hjelmqvist 750 kr. för undersökning av sädeskornsavtryck från förhistorisk tid i Sverige, till prof. A. Müntzing 6.000 kr. för genetisk-biokemiska undersökningar över effekten av embryoendospermympningar hos vete och råg, till laborator T. Nilsson 3.000 kr. för pollenanalytisk bearbetning av provserier från Ageröds mosse i Skåne, till fil. lic. B. Norén 4.000 kr. för undersökningar över myxobakteriens näringsfysiologi och bakteriolytiska aktivitet, till prof. C. Skottsberg 7.000 kr. för undersökningar rörande Juan Fernandez-öarnas växtvärld, till doc. O. Almborn 1.200 kr. för att utarbeta en lavflora över Sydafrika, till fil. lic. A. Kylin 615 kr. för undersökningar av jonupptagningens fysikaliska och metaboliska komponenter, till prof. H. Lundin 15.000 kr. för undersökningar rörande vitamin B_{12}-faktorer i havsalger.

Kungl. Lantbruksakademien har ur fonden för främjande av forsknings- och försöksverksamheten på jordbrukets område till fil. mag. Arne Gustavsson utdelat 14.000 kr. för sex månaders studier i USA av frågor rörande svartrostens biotyper och vetesorternas resistens mot dessa.

Ur stiftelsen Lars Hiertas Minne har bl.a. fil. mag. Tord Ingmar erhållit ett anslag på 1.500 kr. för undersökning av myrområdet »Floran» i norra Uppland.

Från Magnus Bergvalls stiftelse har bl.a. följande anslag utdelats: till doc. J. Ekdahl 3.100 kr. för studier över rot- och rothårstillväxten hos kulturväxter, till fil. kand. S. Ellerström 1.800 kr. för undersökning av konkurrensförmågan hos olika kromosomtalsraser av timotej i blandbestånd, till fil. dr I. Granhall och fil. lic. L. Ehrenberg 6.000 kr. för fysiologiska undersökningar över sambandet mellan vilotillstånd och strålningskänslighet hos fruktträdens vinterknoppar samt frön hos olika växtslag, till agr. lic. P. E. Nilsson 8.000 kr. för fortsatta undersökningar rörande relationen mellan växten och markens mikrobflora.

Från Knut och Alice Wallenbergs stiftelse har bl.a. utdelats till Lantbruksakademien 95.000 kr. för prof. Åke Gustafssons mutationsforskningar.

K. Fysiografiska sällskapet i Lund har utdelat bl.a. följande anslag: till fil. dr Asta Almestrand 1.700 kr. för naturvetenskapliga undersökningar av mikrofytvegetationen (exkl. bakterier) i de biologiska oxidationsdammarna vid följande reningsverk: Lund, Eslöv, Höör, Findus (Bjuv) och Säbyholm, till prof. Heribert Nilsson 1.200 kr. för revision av försöksmaterial av *Salix*-bastarder på försöksfält i och kring Lund, till fil. lic. Nils Olof Bosemark 5.460 kr. för fortsatta undersökningar över accessoriska kromosomer hos vissa gräsarter, till fil. lic. Arne Lundqvist 4.818 kr. för fortsatta undersökningar över självsterilitet och inavelseffekt hos råg.

Från Hierta-Retzius' stipendiefond har K. Vetenskapsakademien utdelat: till lektor S. Rönnerstrand 1.500 kr. till arvode åt biträde vid undersökningar över oxydassystemen hos röd- och brunalger, till prof. Å. Gustafsson 2.500 kr. för forskningar över de skandinaviska björnbärens formbildning och systematik, till doc. Hugo Sjörs 2.300 kr. för växtekologiska laboratorie- och fältstudier i Storbritannien, till doc. M. Waern 2.020 kr. för algologiska undersökningar vid Sveriges kuster och vissa inlandsvatten, till fil. lic. L. Ehrenberg 2.900 kr. för undersökningar över den joniserade strålningens biologiska verkningsmekanism genom analyser av enzym och auxin under groningen av korn, till fil. mag. M. Jaarma och fil. lic. L. Ehrenberg 1.500 kr. för undersökning av biologiska jämvikters beroende av vattenhalten i kärnor av korn och vete, till doc. Birgitta Norkrans 3.000 kr. för undersökning av cellulosans och ligninets enzymatiska nedbrytning, till fil. lic. C. A. Wachtmeister 2.400 kr. för undersökningar inom lavkemien, till prof. A. Tiselius och fil. lic. H. Leyon 1.800 kr. för köp av plåtar och förbrukningsmaterial för elektronmikroskopiska studier över kloroplasternas struktur, till dr K. von Wettstein 1.100 kr. för elektronmikroskopiska och kemiska studier över klorofyllsyntes och plastidutveckling i lägre och högre växter, till doc. M. Waern 400 kr. för algologiska undersökningar vid Sveriges kuster och i vissa inlandsvatten, till fil. kand. P. O. Nyman 700 kr. för bryologisk undersökning i västra Pite lappmark, till fil. lic. O. Hedberg 1.500 kr. för herbariestudier, huvudsakligen i Florens, för en undersökning av den afro-alpina kärlväxtflorans ursprung och differentiering, till doc. M. Fries 900 kr. för avlöning av medhjälpare vid pollenanalytisk undersökning av västsvenska *Trapa*-förande fornsjösediment och till Riksmuseets paleobotaniska avdelning 6.490 kr. för undersökning av charofyter från Podoliens downtonian.

Liljewalchska stipendienämnden har till fil. mag. T. Willén utdelat 1.500 kr. för fortsatta algologiska undersökningar i Uppland.

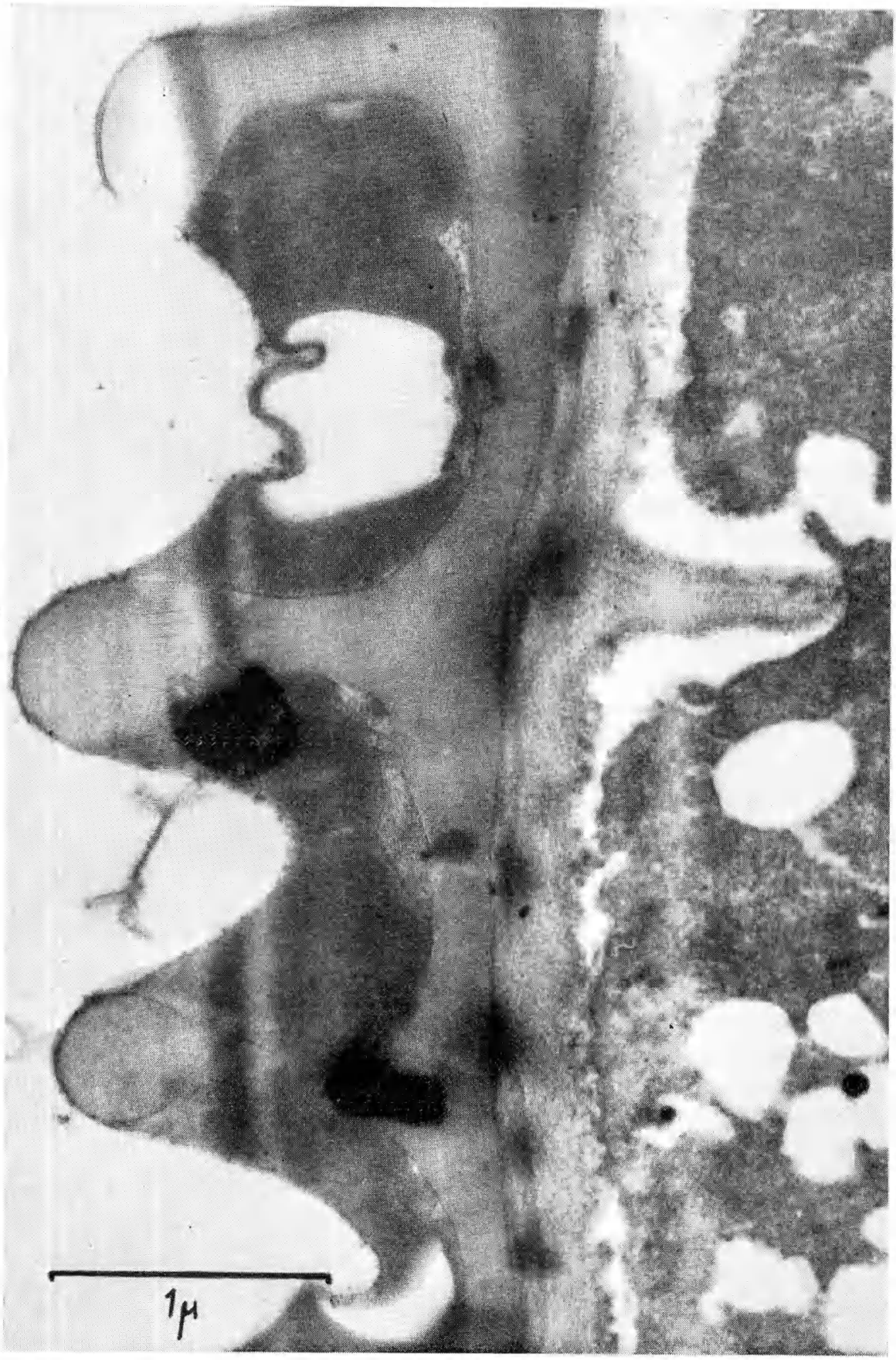

Plate 1. Section through part of the wall of an osmium-fixed pollen-grain of *Clivia miniata*. In one spot the intine penetrates into the cytoplasm. — ×37 000.

Plate 2. Section through part of the wall of an osmium-fixed pollen grain of *Clivia miniata*. — ×68 000.