complete hydrolysis. The difference between the amounts of amino nitrogen produced by acid hydrolysis of the residues at two successive stages may be regarded as a measure of the amino nitrogen which gradually passed into solution as the katabolic reactions in the tissues proceeded. The residues from the fresh leaves yielded 104 gm of total amino nitrogen, those from the leaves that had been cured for 12 days yielded only 53 gm, consequently in this period 51 gm of potential amino nitrogen must have passed into a soluble form. But, instead of a corresponding increase of 51 gm of amino nitrogen in the extract from the 12-day cured leaves, this increase amounted to only 26 gm; 25 gm of amino nitrogen disappeared as such. It was found, however, that the accumulation of ammonia and amide nitrogen that occurred amounted together to approximately 28 gm, which corresponds satisfactorily to the observed deficit of amino nitrogen. It is clear, then, that at least half the potential amino nitrogen of the protein that underwent enzymatic hydrolysis in the first 12 days was further converted to ammonia and amide nitrogen.

The approximate constancy of the quantities of ammonia, amide and amino nitrogen in the extracts from the more fully cured specimens of leaves suggests some sort of an equilibrium condition in the relationships between these forms of nitrogen. In the period from 12 to 51 days the sum of the three forms diminished by only 5 gm. The unassigned or "rest" nitrogen likewise diminished by about 5 gm in spite of the fact that, during this interval, some 23 gm of nitrogen, originally present in the form of coagulable protein, were converted to a soluble form. Extensive changes took place in the forms in which the nitrogen is combined in the leaves and the data suggest that, although many side reactions doubtless occurred, the *essential* sequence of reactions was, protein nitrogen → amino acid nitrogen → ammonia nitrogen → amide nitrogen. Whether the nitrogen that escaped from the tissues, presumably in the form of ammonia, was derived from subsequent hydrolysis of the amides or directly from the deamination of amino acids was not ascertained. Probably both sources were available since the presence in the leaves of enzymes that hydrolyze amides was suggested by the extensive decrease in amide nitrogen that ensued during fermentation of the fifth lot of leaves.

Perhaps the most significant result of these studies is the demonstration of the rapidity of the katabolic reactions during the early stages of curing. Three quarters of the loss of water and of soluble carbohydrate and more than half of the loss of organic solids and of ether soluble constituents occurred during the first 12 days; more than three quarters of the quantity of protein digested underwent this process in the same period. It is obvious that far-reaching chemical changes set in very shortly after leaves are detached from the plant.

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POLLEN-STATISTICS: A NEW RESEARCH METHOD IN PALEO-ECOLOGY

THE number and importance of the papers on ecology in American botanical literature clearly testify to the popularity of ecology among American botanists. Yet the domain of Paleo-ecology has been almost untouched, although researches along such lines would, in many cases, lead to a better understanding of actual ecological problems. In Europe no other branch of Paleo-ecology has been more popular during the last years than pollen-statistics, or the method of tracing the history of the forests from the occurrence of the fossil tree pollen grains in peats and sediments, which was elaborated some twenty years ago by the Swedes G. Lagerheim and L. von Post. There are signs that an activity in this field similar to that in Europe is not far off in this country, and it has been considered appropriate, therefore, to give here a brief description of the working methods, together with some practical hints which might prove useful to beginners.

THE FIELD WORK

The field work is chiefly confined to the summer. During the winter, however, when the lakes are frozen over, samples of bottom sediments could be obtained by means of borings through the ice. Several types of boring rods are in use. The more obsolete ones are being supplanted more and more by the Hiller peat auger, manufactured by the Beus and Mattson Company of Mora, Sweden. This company offers the auger in two different models; a smaller one with extension rods of 100 cm each, which is kept in a leather case and carried by a strap over the shoulder, and a bigger one with extension rods of 150 cm each. The approximate cost of the two models is about twenty-five and forty-five dollars, respectively. If the field work is carried out with the help of an assistant, preference should be given to the heavier auger, which is more reliable than the smaller one. The field apparatus also includes a spade, a big knife, forceps, glass tubes in which to keep the samples (about 7.5 cm long and 1.3 cm inside diameter and corked at both ends), diopter compass, and a geodetical set for taking levels and distances.

At the boring place a big sod of the surface material is first removed with the spade. From the walls of the sod the first samples are taken, say, from 2, 5, 10, 15 and 20 cm below the surface. Then the auger

is put in the hole left by the sod, and forced down in the peat. Meanwhile the handle of the auger should be kept turning slightly to the right (clockwise) to prevent the opening of the container. Then the container is opened, and a good and compact core is obtained if the handle is turned, swiftly, about eight times (four revolutions), counter-clockwise. It is then closed by a couple of turns to the right, and the auger is pulled up out of the peat during a continued slight revolution to the right. Samples are taken with the forceps at regular intervals, *e.g.,* at every fifth cm, out of the core of the container, since the outer layer of the core, which might be contaminated, has been removed with the knife. The forceps should be nickeled and have smooth ends.

It is useful to have the spade standing nearby the boring place and to put the lower end of the auger with the container through its handle when the samples are taken. Fig. 1 shows, schematically, a

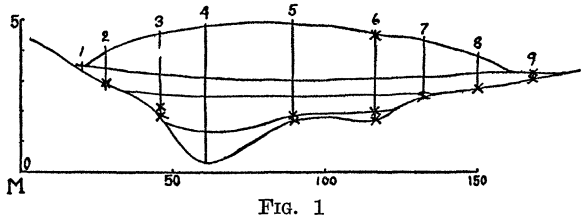

FIG. 1

section of a bog, the stratification of which has been made out from serial borings. A series of peat samples would most suitably be gathered from point 4, and additional samples for the study of the growth of the bog and the composition of the sub-recent pollen flora, etc., from the places marked with a cross.

THE LABORATORY WORK

When preparations for microscopical investigation are to be made, a small amount of peat is taken from each end of the substance enclosed in a glass tube, laid on a slide and mixed with 10 per cent. caustic potash. The slide is held with a clothes pin and the mixture is carefully boiled over a small alcohol flame until the greater part of the water has evaporated. Some drops of glycerine are then added and mixed with the peat, and a part of the mixture is removed to another slide and covered with a cover-glass. The pollen-grains are then counted by the use of the micrometer stage of the microscope. A magnification of about 200 times is required, and then a high power lens for the study of the finer details of the exine. Some sediments need no boiling and preparations are simply made by mixing a part of the substance with distilled water. Calcareous material is treated with dilute hydrochloric acid and minerogene earths, even rather coarse sand, could be subjected to pollen-analysis if centrifugated and treated with hydrofluoric acid.

Trustworthy percentages are obtained if about 150 pollen-grains are counted. The frequency of the pollen-grains of hazel, willows, and other species which are more or less confined to the under-growth of the forests, is calculated separately and expressed as a percentage of the sum of the pollen-grains of the forest trees proper. Thus, a willow pollen frequency of 138 per cent. indicates that the number of willow pollen-grains in that preparation was bigger than the sum of the pollen grains of the forest trees. The frequency of *Sphagnum* spores, tetrads of *Ericaceae,* etc., is expressed in the same way. It is useful, too, to have a record of the PF, or pollen frequency per square cm, from each preparation.

By means of the percentage figures, a *pollen-diagram* is constructed. The relative frequency numbers, which are produced for the pollen-species found in a sample, constitute the *pollen-spectrum* of the sample. On the basis of a series of pollen-spectra from a boring in a bog, a pollen-diagram may be constructed, with the depth of the peat as ordinate and the pollen percentages plotted down on abscissae corresponding to those levels from where the samples were taken. In a pollen-diagram the curves for the single species or for a group of species give both a visual representation of the composition of the pollen flora and the oscillations as regards frequency which have taken place reciprocally between the pollen-curves during the formation of a bog.

Great difficulties are often encountered in identifying the pollen grains. Illustrations should not be relied on, but everyone working with pollen-statistics should have access to reference preparations of pollen-grains from recent trees. Such preparations could be made directly from fresh material or from boiling stamens of herbarium specimens with 10 per cent. caustic potash and mounting the pollen grains in glycerine jelly. After some practice it would be possible also to identify pollen grains of different species within the same genus; for instance, to distinguish the pollen of *Picea canadensis* from the slightly smaller one of *Picea mariana,* that of *Pinus murrayana* from that of *Pinus banksiana,* etc. In some cases, complete variation statistical analyses must be made. As to *Pinus* and *Picea,* it might be useful to calculate and plot down in a reference table the limits within which the dimensions of the pollen grains vary. As in the preparations some pollen grains are only shown from above, obliquely, or contorted, or, even in mere fragments, as isolated wings, not only the breadth of the pollen grains should be measured, but also the height and depth of the pollen grain proper, and the breadth, height and depth of the wings.

Notes on the finer structure are desirable; also notes on the color, which might vary according to the chemicals used.

After the study of recent pollen grains, it would be advisable to search samples from the surface of the peat for pollen grains. The pollen grains found among the branches of living *Sphagnum* and in the moss cover of stumps and fallen trees give a picture of the composition of the contemporaneous pollen grains. That would give a key to the conclusions which can be drawn from fossil pollen in general, but, in this respect, too much care can not be exercised. We know, for instance, that pollen grains can be carried by wind for very long distances, so that coniferous pollen might be encountered in the peats of Greenland; and, further, that the pollen grains of some trees might be under-represented in the pollen-spectra, owing to their being distributed at a time when the lakes and the peat surfaces are still frozen, or from other causes are not as fit for catching and preserving the pollen grains as at a later season. Because the delicate *Populus* pollen grains may not be preserved in peat, it is understandable, too, that a virgin Cordilleran coniferous forest would produce somewhat the same pollen-spectra in the mountain bogs as do some of the poplar forests, with scattered conifers, in the muskegs of the Great Western Plains. I do not mention this to discourage any one wishing to take up pollen-statistics. Its renown as a good and helpful paleontological research method can not be jeopardized, as shown by its success in Europe. I only mention it because, in my opinion, a thorough study of the recent and sub-recent pollenflora of American bogs would be more valuable as a start for pollen-statistical investigations in America than the presumably rather hazardous task of identifying a multitude of pollen types from old deposits with material often much decayed and altered.

Further information on pollen-statistics can be obtained from the papers listed in "Literature on Pollen-statistics published before 1927" (Geol. Fören. Förh., 49, 196–211, Stockholm, 1927) and "Literature on Pollen-statistics published during the years 1927–1929" (ibid., 52, 191–213, 1930).

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STRUCTURAL AND FUNCTIONAL VARIATIONS OF FIBROBLASTS IN PURE CULTURES

A MEDIUM has already been described[1] for the long-continued cultivation of mesenchyme cells under conditions which allow of very limited cell proliferation. Instead of the cells being fed upon the growth-promoting substances contained in embryonic tissue juice, they are treated with adult blood plasma. Cultures which are so nourished grow very slowly and can be kept in good condition over a much longer period of time than cultures which are allowed to proliferate at their maximum rate.

The experiments here reported have given additional information concerning the properties of cells as manifested under conditions of slow growth. The material consisted of various pure strains of mesenchyme cells which were isolated simultaneously from an embryo chick. These strains were derived from heart muscle, skeletal muscle, the perichondrium of cartilage and the periost of bone, respectively. Although these cell types exhibit striking differences in their nutritional properties,[2] it has not yet been possible to distinguish them morphologically. Until used for the experiments, which were made in flasks, the strains were carried by the hanging drop method on media favorable for the maximum proliferation of the various cell types. Each series of experiments was made at the same time from strains of the same age. Although regularly washed and treated with blood plasma, a number of cultures which have been allowed to remain in the same flasks for as long as one hundred days without being disturbed have continued to show appreciable growth over the entire period. In the case of those cell strains which become early adjusted to the plasma medium, growth becomes progressively more and more active from passage to passage. Sufficient heparin is added to the plasma to prevent its coagulation during each period of treatment. In the concentration used, appropriate experiments have shown, however, that the heparin has no appreciable effect upon the rate of growth of fibroblasts. When the cultures are subdivided and transferred, the new clots are allowed to coagulate spontaneously without the customary addition of tissue juices, and the same manner of treatment is resumed. Hence, we can definitely state that these cells are able to live and multiply at the expense of the food substances contained in the plasma alone.

It is undoubtedly true that the process of adaptation to the plasma treatment begins from the moment that the fibroblasts are transferred from an environment of ample food to one which is deficient in readily available food substances But while certain cell colonies are able to adjust themselves to the new environmental conditions without very pronounced structural changes in the cells themselves, it does not follow that this is always the case, even among sister

[1] A. Fischer and R. C. Parker, *Arch. f. exper. Zellforschung,* 8, 325, 1929.

[2] R. C. Parker, *Arch. f. exper. Zellforschung,* 8, 340, 1929.