"On the Depletion of the Endosperm of *Hordeum vulgare* during Germination." By HORACE T. BROWN, F.R.S., and F. ESCOMBE, B.Sc., F.L.S. Received December 11, 1897,—Read March 3, 1898.

[PLATE 1.]

In an account given by one of us in 1890 of the results of an investigation of the histological and physiological changes which take place in the seeds of the Grasses during germination,* a prominent position was given to a discussion of the relations existing between the endosperm and embryo, and to the part played by each in the preparation of the reserve materials of the seed for the nutrition of the young plant. This branch of the inquiry was much facilitated by the discovery that the embryo, when separated from the other parts of the seed, is capable of an independent existence, providing it is supplied with a suitable artificial nutriment in the form of certain carbohydrates, its own store of proteids being sufficient to supply the nitrogen requisite for the production of young plants of a considerable size.

The carbohydrates most favourable to rapid growth in such cases are sucrose, dextrose, and maltose; but it was also found that the embryo, when deprived of such readily assimilable material, acquires the power of dissolving solid starch to a very notable extent, a function which was subsequently localised in the columnar epithelium of the scutellum.

The endosperm itself was also subjected to examination with a view to determine if it possesses any power of acting on the reserve materials contained within its cells, and of bringing about any self-depletion which is independent of the influence of the embryo.

This question was attacked in two different ways. In the first place, endosperms, after being degermed, were placed under favourable conditions for the full play of any metabolic activity which might be possessed by any portion of their tissue, every facility being afforded for the rapid outward diffusion of the products of change; and, secondly, advantage was taken of a fact which had previously been established, that an embryo may be transferred from one endosperm to another without materially affecting its power of subsequent growth; thus affording an opportunity of subjecting an endosperm to such treatment as may reasonably be supposed capable of destroying any residual vitality in its cells, and of then observing how this affects the subsequent development of a fresh embryo "grafted" upon it.

For the full details of these experiments we must refer to the

* Brown and Morris, 'Chem. Soc. Journ.,' vol. 57, p. 458.

original paper, and here merely quote the general conclusions which were drawn from them.

Although the peripheral layer of the endosperm, the so-called "aleurone-layer," or "Kleberschicht," undoubtedly consists of living cells, no evidence could be obtained of the existence of any residual vitality in the amyliferous cells, which constitute by far the greater portion of the endosperm.

No changes were observed in the isolated endosperms in the direction of self-depletion which were comparable in intensity with those produced when the embryo was attached; and when those changes did occur they were always preceded by an invasion of bacteria and moulds in the culture-medium, the disintegration and dissolution of the endosperm-contents in such cases proceeding in such a manner as to suggest that they were conditioned entirely by the organisms.

When living embryos were "grafted" on endosperms which had remained in alcohol for six months, and in which it was then reasonable to suppose that any residual vitality had been effectually destroyed, all the usual phenomena incident to normal germination were observable in those endosperms. Hence it was concluded that the idea of any co-operation on the part of the endosperm-cells was superfluous, and that the determining factor in the normal endospermous changes is the embryo itself, which, by independent experiment, had been shown to possess the power of dissolving starch and of initiating those phenomena of cytohydrolysis which are amongst the earliest exhibited in natural germination. According to this view the endosperm of *Hordeum*, and probably of all the Grasses, is, as far as its starch-containing cells are concerned, a *dead* storehouse of reserve material, whose stores can be converted to the use of the plant by the action of the embryo only, and that this, for a limited period of its existence, lives a truly saprophytic life.

These conclusions are in accord with the views of Van Tieghem, that, whilst an endosperm, such as that of *Ricinus*, containing oil and aleurone as reserve materials, is endowed with a vital activity of its own, by virtue of which it is capable of digesting the reserve material in preparation for the embryo, the endosperm of seeds, whose reserve materials, on the other hand, consist of starch and cellulose, remains passive during germination, the digestion of its reserves being in this case effected by the embryo.

Two years after the appearance of the above-mentioned paper, Pfeffer gave a brief description of some work by B. Hansteen, 'Über die Ursachen der Entleerung der Reservestoffe aus Samen,'*

* 'Ber. der Königl. Sächs. Gesellsch. d. Wissenschaften zu Leipzig,' 1893, p. 421.

which was followed the year afterwards by a more detailed paper on the same subject by Hansteen himself.*

Hansteen strenuously opposes Van Tieghem's division of endosperms into "active" and "inactive," and asserts that the latter was led into error on this point by not taking precautions to put his endosperms under conditions favourable for the rapid removal of the products of change. Although reference is made to the Brown and Morris paper of 1890, the author does not appear to have made himself thoroughly acquainted either with the details of the experiments described or with the conclusions drawn from them.

Hansteen's principal experiments were made with the seeds of *Zea Mays* and *Hordeum vulgare*, but he also made observations on the mucilaginous endosperm of *Tetragonolobus purpureus*, and the cotyledons of *Lupinus luteus* and *Helianthus annuus*. For our present purposes it is only necessary to consider the experiments on barley and maize. The seed was, in the first instance, soaked in water for two days, and the embryos, including the scutellum, were removed. To the isolated endosperms there was then applied a mixture of plaster of Paris and water, so as to form a small plaster column, which occupied the original position of the embryo.

The little plaster columns, with the endosperms attached, were then put into glass dishes containing a sufficient amount of water to reach half-way up the columns. In order to avoid the disturbing influence of micro-organisms the seeds were placed for two hours in a 1 per cent. solution of copper sulphate; all the materials and vessels used were carefully sterilised, and the experiments were performed under strict antiseptic conditions in a cultivation chamber so arranged as only to admit germ-free air. The author states that he has been able in this manner to maintain his cultures sterile for at least a month. When there was a sufficient amount of water in the culture-dishes, and the conditions were thus favourable for a rapid outward diffusion of the products of change, Hansteen found that, within from ten to thirteen days of commencing the experiment, the isolated endosperms of both maize and barley had given rise to a very considerable self-digestion of the cell-contents. In the immediate neighbourhood of the plaster the cells had quite lost their starch, whilst the starch-granules, even at a distance, were more or less corroded, and the partially depleted endosperm had become soft and disintegrated. In the case of barley these visible changes were very strongly marked indeed, and simultaneously with them sugar could be detected in the water into which the small plaster columns dipped.

In those experiments in which the amount of water had been much reduced, but very little starch-erosion took place at the point

* 'Flora,' vol. 79, 1894, p. 419.

of contact with the plaster, a fact which the author attributes to the accumulation within the endosperm of an excess of soluble products, which thus exercise an unfavourable influence on the continuous chemical change of the solid reserve substances.

In view of Haberlandt's assertion that the cells of the "Kleberschicht" have a distinct diastase-secreting function, Hansteen experimented in a similar manner with endosperms which had been deprived of this layer, and he found the same indications of self-depletion as before. He therefore concluded that the dissolution and depletion which he had observed are due to a special activity of the inner starch-bearing cells of the endosperm. The question is then discussed whether, during germination, the embryo does or does not secrete an enzyme, and the conclusions arrived at are in accord with those of Brown and Morris, and Grüss, that such a secretion does take place. Hansteen, again agreeing with the former observers, regards this secretion of diastase as conditioned by the falling off in the supply of readily soluble carbohydrates; whether, however, the diastase so produced plays an important part in normal depletion, or whether the asserted self-depletive power of the endosperm-cells is sufficient, in normal germination, to account for all the observed results, the author leaves an open question.

In a long memoir entitled "Beiträge zur Physiologie der Keimung,"* J. Grüss discusses the question of the appearance of ferments in the endosperms of maize and barley after excising the embryos and filing off the "Kleberschicht" ("aleurone-layer"). From experiments made by burying fragments of such endosperms for a few days in sterilised moist sand, he concludes that the starch-bearing cells have the power of producing spontaneously within themselves a diastase, the presence of which he determined, in the first place microscopically by the extremely doubtful guaiacum-reaction, and secondly by the increased action of the endosperm-tissue on thin starch-paste.

The most recent contribution to the subject is a paper, taking the form of a preliminary communication, by K. Puriewitsch, "Ueber die selbstthätige Entleerung der Reservestoffbehälter,"† followed since by a more detailed paper, entitled "Physiologische Untersuchungen über die Entleerung der Reservestoffbehälter."‡ Making use in the main of Hansteen's method of experiment, Puriewitsch examined, amongst other seeds, the isolated endosperms of *Zea Mays*, *Triticum sativum*, *Hordeum distichon*, *Secale cereale*, and *Oriza sativa*, and he extended his observations to the cotyledons, bulbs, rhizomes, and roots of various other plants, a list of which is given in his paper.

* 'Landwirtschaft. Jahrbücher,' 1896, p. 385.

† 'Ber. Deut. Bot. Gesell.,' vol. 14, 1896, p. 207.

‡ 'Pringsheim's Jahrb.,' vol. 31, 1897, p. 1.

In the case of *maize* he found the first indications of action in the cells lying next the scutellum, and this gradually extended along the periphery of the endosperm until, within fourteen or fifteen days, this was completely emptied of its contents, with the exception of a few cells in the central portions. He states that this action is not due to any direct influence of the plaster, as suggested by Grüss, since it takes place also in contact with water only. In the cotyledons of *Lupinus* the depletion takes place even with greater rapidity than in normal germination, and no difference is observed whether the cut surface in contact with the water or gypsum, as the case may be, is on the side adjacent or opposite to the axial organs. With the isolated endosperms of maize and wheat, on the other hand, Puriewitsch states that the case is different, since self-depletion proceeds much more rapidly through the surface originally in contact with the scutellum than it does from the opposite side. The author also found that the depletion of the endosperm is much retarded in the case of maize and wheat by the presence in the water of 2 per cent. of dextrose or glycerine, or by 3 per cent. of cane sugar, and that it is completely arrested by 1·5 per cent. of sodium chloride or potassium nitrate. The results on the whole are regarded as contradicting the conclusions of Brown and Morris that the endosperm is merely an inactive storehouse of reserve material, and Puriewitsch considers that this is further borne out by the behaviour of isolated endosperms in an atmosphere of water and by the action of anæsthetics such as ether and chloroform. Under these latter conditions, he states that the endosperms of maize and wheat remain unchanged, but that the depletive action recommences as soon as the disturbing influences are removed. Attempts were made, by applying food material in the form of weak sugar solutions, to induce a re-deposition of reserve material in the self-depleted tissue. These attempts were wholly unsuccessful in the case of maize and wheat, but the emptied cotyledons of *Lupinus albus* and *Phaseolus multiflorus*, the bulbs of *Hyacinthus orientalis*, and the rhizomes of *Curcuma amada* and *Iris germanica* were all capable of re-forming starch within their cells.

It will be noticed that in the recent work of Hansteen, Pfeffer, Grüss, and Puriewitsch, there is a general agreement that the amyliferous cells of the endosperm of the Grasses have a definite power of digesting their reserve materials, this power being entirely independent of any influence of the embryo, and the only necessary condition for its exhibition being that the products of metabolism shall not be allowed to accumulate within the endosperm. The conclusion is, in fact, that the starch-bearing endosperm-cells are still living units, just as are the cells of the cotyledons of *Lupinus*, *Phaseolus*, and *Ricinus*, which are admitted on all hands to have self-depletive power.

As these conclusions are in many respects opposed to those arrived at by one of us a few years ago, we have considered it necessary to institute a further series of experiments, and to re-examine the whole question of the mutual dependence of the embryo and endosperm. In doing this we have endeavoured to free our minds of any bias which might, even unconsciously, have been given by our previous experiments, and to subject those experiments to the strictest possible criticism.

Broadly speaking, the question resolves itself into a consideration of the various causes at work in bringing about the solution of the reserve material of the seed in preparation for its absorption by the scutellum of the young plant, and the due apportionment of this work to (1) the embryo itself, (2) the amyliferous cells, and to (3) the peripheral cells of the endosperm, the so-called "aleurone-layer" or "Kleberschicht."

In addition to this, we have to take into account the possibility of some of the changes being brought about by enzymes pre-existent in the amyliferous cells, which may be altogether independent of the present life of the cytoplasm. We have, further, to determine the part played by micro-organisms accidentally brought into action during the experiments, and to eliminate the changes due to their influence alone.

In work of this character we can only attain to results of any value by a great multiplication of experiments made in such a manner as to admit of the close and frequent comparison of different series performed under every conceivable variation of conditions.

All our new work was conducted on barley only, and the results are based on very many hundreds of experiments, extending over a period of more than twelve months, during which time various possible sources of error were gradually excluded.

As long as we confine our attention to intact seeds the disturbing influence of micro-organisms is but small, but the case is different when the seed envelopes have to be cut through and the embryo removed, the endosperm, thus bared and deprived of its protective coatings, being then open to the attack of bacteria and moulds, which thrive in the culture-medium employed, and by their action induce changes in the contents and cell-membranes of the endosperm-cells which it is almost impossible to distinguish from those initiated by the cells themselves, supposing them to be living and active units.

At the outset of the investigation we spent a considerable time in endeavouring to find some antiseptic agent possessed of such a differential action as to inhibit, or at any rate to materially retard, the growth of micro-organisms, whilst not interfering with the normal growth of vegetable organs. Many various reagents were tried,

commencing with extremely dilute solutions, which were gradually increased in strength until their influence on the germinative power of the seed was just perceptible. The germicidal effect of such a solution was then tested on degermed grains in water-culture. At one time extremely dilute solutions of formaldehyde and of acid potassium fluoride offered some hope of success in this direction, but neither of these substances on further investigation gave a sufficient differential action to be of any practical use.

In the experiments of 1890 (Brown and Morris, *loc. cit.*) the disturbing effect of micro-organisms was minimised by restricting the time of the experiment as far as possible, and by sterilising the culture-media, and we have seen that Hansteen relied on killing the adherent germs with a solution of copper sulphate, and on the employment of strict antiseptic methods, even to the extent of carrying out all the operations in a germ-free atmosphere.

We have made experiments in order to see how far such a treatment with copper sulphate effects sterilisation of the integuments, the grain after such treatment being incubated in contact with vegetable infusions. The results have clearly shown us that although such a procedure may retard the subsequent development of *Bacteriaceæ* and moulds, it is impossible by means of it to ensure a complete destruction of all the germs adherent to the paleæ, unless the treatment is sufficiently prolonged to destroy, or at any rate to materially reduce, the germinative power of the embryo.

Since any process which will affect the vitality of the embryo cannot be without some similar influence on the endosperm, there is thus introduced an element of uncertainty into all subsequent processes which may be devised for determining whether the amyliferous cells are living or dead.

Extreme refinements for avoiding air-sown organisms are obviously of little efficacy when complete initial sterilisation of the exterior of the grain cannot be ensured. Nevertheless, many of our experiments have been carried on with precautions of this kind, but have not yielded better results than those made in covered dishes with sterilisation of the culture-media and apparatus.

In all experiments with endospermous seeds deprived of their embryos both Hansteen and Pfeffer have, very properly, laid great stress on the necessity for providing for a rapid removal of any possible products of change in the isolated endosperm as fast as they are formed, but these observers have apparently entirely overlooked the fact that this was fully insisted upon and provided for in the earlier experiments described by one of us in 1890.* The plan adopted was to insert the proximal ends of the degermed grains into small holes made in a thin mica plate, which was then floated

* 'Chem. Soc. Journ.,' 1890, Trans., p. 481.

on water in such a manner as to just immerse that portion of the endosperm which had been in contact with the embryo.

This method really affords much greater facilities for outward diffusion from the endosperm than does Hansteen's plan of fixing the degermed seeds on small columns of plaster partially immersed in water, and it is also free from the objection of any possible disturbing influence due to the solubility of the plaster. Moreover, the mica-raft method is easier of manipulation, and whilst giving perhaps better facilities for sterilisation, also allows the detection of the very first appearance of micro-organisms.

The barley used in our experiments was *Hordeum vulgare* (var. *distichon*), derived from two sources. One, with which most of the work was done, was a well-matured Chilian barley, of the Chevalier type, the other an English Chevalier barley grown on light land in Northamptonshire, both samples being well matured and well harvested.

It will be convenient in the first place to consider the visible changes which can be induced in the endosperm when this is completely deprived of its embryo, and is put under such conditions as to ensure the speedy removal of any soluble and diffusible products which may result from any self-digestive processes initiated by any portion of the endosperm tissue.

Some of our experiments on this point were made in the following manner:—

The grain was, in the first place, steeped from one to two hours in a 1 per cent. solution of copper sulphate, and after being washed with sterilised water was steeped, also in sterilised water, for a period of from twenty-four to forty-eight hours. From the corns selected for experiment the paleæ and embryos were then removed with antiseptic precautions, this process being conducted in a glass-fronted sterile operating chamber, furnished with "sleeves." The degermation was performed with a small scalpel, taking care to thoroughly remove all traces of the scutellum, and to lay bare the "depleted layer" of the endosperm.*

The isolated endosperms were then put in position in small holes made in a very thin mica-raft which was floated on sterilised water in a Petri's dish, or in a glass vessel of somewhat similar construction.

* The nature and origin of this "depleted layer" can only be understood by following the developmental history of the endosperm and embryo, and this has been so fully described in the Brown and Morris paper of 1890 (*loc. cit.*) that it requires but a passing notice here. The "depleted layer" is made up of several thicknesses of cell-membrane, which originally formed part of the amyliferous cells of the young immature endosperm. During the later stages of development of the grain, and some time before maturation, the contents of these cells are used up for the nutrition of the young embryo, but the cell-membranes persist and become squeezed together by the gradual encroachment of the scutellum.

In those cases where comparisons had to be made between endosperms treated in different ways the mica-rafts were made to carry twelve corns, the two series of six each being placed on either side of the raft. In this manner there was an exactly equal chance of the two sets being infected to the same extent by extraneous organisms, an important condition, which often enabled us in a long series of experiments to differentiate changes due to the influence of organisms from those due to other causes. Latterly we found these extreme antiseptic precautions unnecessary for the reasons already given, and we also found it undesirable to previously steep the grain before degermation, since the embryo may readily begin to function slightly during the softening process, especially when the temperature is high. In such cases there is a danger of the projection of a small quantity of enzymes from the embryo into the proximal portions of the endosperm, and these enzymes, after degermation of the grain and the floating of the endosperms on the rafts, may give rise to certain changes in the endosperm which may be wrongly attributed to a self-digestive power of the endosperm-cells themselves, whereas they have a different origin altogether.

It is true that this source of error may be minimised by reducing the period of steeping, and by keeping the temperature of the water low; but it is much more satisfactory to degerm the grain *whilst still in its dry resting condition*, a process which does not present any difficulty. It must, however, be performed with the aid of a lens, so as to ensure the complete removal of the scutellum and the whole of its limiting epithelial layer.

If endosperms thus treated are soaked in water for from twenty-four to forty-eight hours, and are then transferred to the perforated mica-rafts in such a manner as to immerse the whole of the depleted layer, we observe the following changes to take place.

Within two or three days from the commencement of the experiment the peripheral, tripartite layer of the endosperm, the so-called "aleurone-layer" ("Kleberschicht"), shows an increasing tendency to separate from the adjacent amyliferous cells. This is noticeable in the first instance at the proximal end of the endosperm, on the dorsal side,* where the "aleurone-layer" is intersected by the "depleted layer," and whilst it is to some extent traceable for some distance round the periphery towards the ventral fold, it extends much more rapidly in a distal direction along the dorsal side. Where there is this megascopic indication of the separation of the "aleurone-layer," it is always found that the amyliferous cells, immediately underlying,† show indications of change. In the first

* The *dorsal* side is that immediately opposite the ventral suture. The terms *proximal* and *distal* are used with reference to the position of the embryo.

† The outermost layer of the amyliferous portion of the endosperm consists of

place, the cell-contents become hyaline in appearance, owing to the protoplasmic matrix losing its granularity and acquiring a refractive power approximating to that of the embedded starch-granules. Later on these hyaline portions imbibe water and swell up enormously, ultimately becoming very elastic and ductile, and capable of extension into sticky, stringy masses, very similar in appearance to the gluten of the wheat-endosperm. We shall in future refer to this change as "gluten-formation." At the same time the cell-membrane of the peripheral starch-cells swells up considerably, and as the action progresses the cell-walls undergo disintegration, with all the indications of cytohydrolysis as described by Brown and Morris.

It is to this cytohydrolysis that the separation of the "aleurone-layer" is due, and the disintegration due to this cause proceeds centripetally into the endosperm and extends round the periphery nearly to the ventral fold, whilst it advances more rapidly in a distal direction on the dorsal side. The extent to which this cytohydrolysis has proceeded is always evidenced megascopically by the reduction of the endosperm-contents to a "mealy" consistency, but even after the lapse of seven or eight days the actual amount of depletion is small, as long as micro-organisms are absent, or present only in comparatively small numbers. If, however, as is frequently the case, masses of *Bacteriaceæ* in the zoogloea-state attach themselves to the mutilated surface of the endosperm, a very distinct removal of some of the endosperm-contents may take place.

The erosion of the starch-granules is generally not very pronounced under these conditions, but when it does occur it always commences at the same point as the cytohydrolysis, that is, on the dorsal side, at the angle of intersection of the "aleurone-layer" and the "depleted layer," and extends distally just as does the cytohydrolytic action.

The starch-erosion produced in this manner under the "aleurone-layer" is, in the main, very different in character from that observed immediately under the scutellum of a grain germinating normally with its embryo attached. Whilst in the latter case the action commences by the formation of numerous minute "pits," this preliminary pitting is rarely observable in the eroded granules lying under the "aleurone-layer," which show the production of large rifts, and a general concentric dissolution of the various layers. We shall in future refer to these different modes of attack on the starch-

cells differing in general appearance from the more deeply seated cells. They are smaller, are packed with far smaller starch-granules, and the proportion of starch-granules to proteinic contents is less. These peripheral cells constitute the youngest part of the starchy endosperm, and may be regarded as having been arrested in their development by the falling off in the supply of formative material at the period of maturation.

granule as "sub-aleuronic" and "sub-scutellar" respectively; for although occasional instances may occur where one form of attack merges insensibly into the other, yet, looked at generally, they differ so much from each other as to suggest that the transforming agents are essentially different.

The accompanying photographs (Plate 1) illustrate these differences far better than can any mere description.

It appears to us that the phenomena which are observed when the endosperms of *Hordeum* are deprived of their embryos, and are treated in the manner we have described, must be attributable to one or more of the following causes:—

1. They may be the result of micro-organisms originating in the culture-medium, and gradually invading the endosperm-tissues, which undergo progressive alteration either by the direct action of the organisms or in virtue of their secreted enzymes projected into the endosperm.

2. The phenomena may be due to residual enzymes, cytohydrolytic, amylohydrolytic, and proteohydrolytic, left in the endosperm at the time of maturation and desiccation of the grain.

3. They may be due to the revival of metabolic activity of still living cells of the endosperm when these are placed under favourable conditions of moisture and temperature, and facilities are afforded for the removal of the products of change. If this is the correct solution the active cells may be those of (*a*) the "aleurone-layer," or (*b*) the amyliferous portions of the endosperm.

We must now consider these three possibilities in detail.

We have already stated that, no matter how careful we may be in sterilising the apparatus and culture-medium, the appearance of micro-organisms is only a question of time, unless we employ antiseptic methods of so drastic a nature as to seriously imperil the vitality of the endosperm-tissue, a course which would render it impossible to get the answer we require as to the respective parts played by organisms and by autonomous changes in the endosperm-cells themselves. We can, however, arrive at certain conclusions by making a large number of experiments and by confining our observations to the period prior to the appearance of organisms, a period which, under favourable circumstances, may extend to about eight days. When this is done we find that the changes originating in the first place under the "aleurone-layer" of the degermed seeds so far precede in point of time the appearance and multiplication of the *Bacteriaceæ* and moulds as to render it in the highest degree improbable that the two sets of phenomena are causally related to each other.

A much more satisfactory proof of the truth of this proposition may be obtained in an entirely different manner. Endosperms of

barley which have been degermed in the dry state are, in the first place, steeped in a saturated aqueous solution of chloroform for twenty-four hours. After having freed the endosperms from adherent moisture, they are warmed gently for a few hours in a flask connected with a water-pump, and are then steeped for a further period of twenty-four hours in running water, every trace of chloroform being thus removed. The endosperms are then floated in the usual manner on a mica-raft, alongside other degermed endosperms which have been merely steeped in water for forty-eight hours. The two sets of endosperms are thus under exactly similar conditions as regards their liability to attack by micro-organisms, and if the described "sub-aleuronic" changes of the endosperm are due solely to the direct or indirect influence of extraneous organisms the same results ought to be given by the two series, whereas, if the vitality of any portions of the endosperm is a determining factor, evidence of this ought to be forthcoming, since one set of endosperms has been under conditions which would completely arrest the vital functions of any of their component cells.*

When such an experiment is performed we find very considerable differences between the two sets of endosperms at all stages. Whilst the series merely steeped in water go through the ordinary cycle previously described in detail, the series made up of the chloroformed endosperms show no internal changes for a considerable period of time. In the latter case the "aleurone-layer," which so speedily separates under ordinary conditions, retains its unbroken continuity with the subjacent amyliferous cells, which in their turn preserve their cell-walls and cell-contents intact. Until the growth of micro-organisms has progressed to a very considerable extent the endosperm-contents of the chloroformed grains show no megascopic or microscopic change, except in the direction of a more hyaline appearance of the contents of the starch-cells, a change which is apparently the first stage of the "gluten-formation," to which reference was made in an earlier part of the paper. There is neither cytohydrolysis nor amylohydrolysis apparent in the tissues until the micro-organisms which have attached themselves in a zoogloœa state to the outside of the "depleted layer" have attained to a very luxuriant growth, and even then the tissue-changes differ in some important particulars from those produced in a "living" endosperm. It is in fact possible,

* In our earlier experiments it was assumed that a treatment with chloroform-water, sufficient to destroy the vitality of the embryo, would also be sufficient to kill the aleurone-cells. This, however, is not the case, the embryo being much more sensitive to the chloroform than the peripheral cells of the endosperm. We have satisfied ourselves, however, that a twenty-four hours' steeping of the *dry* endosperms in chloroform-water at a temperature not less than 15° C. will permanently destroy the functionating power of *all* the cells of the grain.

by such comparative experiments, to differentiate with certainty the modifying action of micro-organisms from the autonomous action of the endosperm-cells themselves.

The action due to extraneous organisms always commences at the surface of the "depleted layer," the cell-membranes of which this is made up being softened, swollen, and ultimately disintegrated. This cytohydrolytic action then gradually extends to the membranes of the amyliferous cells, and the proteid contents of the cells are also involved in the change, which ultimately permeates the whole of the endosperm.

There is, however, a striking difference between the mode of progression of this bacterial action from that observed in "living" degermed endosperms. In this latter case, as we have already noted, the action is essentially centripetal, commencing under the "aleurone-layer" on the dorsal side, where this layer is intersected by the "depleted layer," and extending peripherally and axially, but more rapidly on the dorsal side. In the degermed "dead" endosperms, on the other hand, there is no differential progression of this kind, since the action, whilst progressing in an axial direction, does not extend more rapidly along the peripheral than the central parts, and does not show the slightest tendency to more rapid extension on the dorsal side, a tendency which is so strongly marked in "living" degermed endosperms in water-culture, or in intact grains of barley undergoing ordinary germination. It is only when the disintegration of the endosperm-contents under the action of micro-organisms has proceeded to a very considerable extent that any notable amount of erosion of the starch-granules is observable. This sometimes does not occur for many days, a fact probably due to the bacteria not secreting any special starch-dissolving enzyme as long as they are well supplied with readily assimilable food material from other sources.

So far the conclusions are altogether opposed to the view that the normal phenomena of endosperm solution and depletion, as they occur in degermed endosperms in water-culture, can be explained by the action of extraneous micro-organisms. It is true that, under certain circumstances, the mixed growths of *Bacteriaceæ* which attach themselves to the mutilated surface of the endosperm can induce changes in the subjacent tissues by the projection into them of certain enzymes, the products of their growth, but this action can, with due care, be clearly differentiated from the normal action, which is of quite a different character, and must be in some way self-induced by the endosperm-cells themselves.

Before considering how far the normal changes are dependent on the vitality of any particular portion of the endosperm, we must inquire if the phenomena are in any way due to enzymes pre-existent

in the endosperm-cells, and this inquiry is the more necessary since we know that even the distal portions of the endosperms of the barley-grain contain a certain amount of a feeble diastase, and in most cases also a distinct amount of a cytohydrolytic enzyme.*

In the first place we satisfied ourselves that both the amylohydrolytic and cytohydrolytic enzymes of barley are not appreciably weakened in their respective actions by a saturated aqueous solution of chloroform.† A number of grains of barley were degermed, and, after being softened by a sufficiently long steep in chloroform-water, were placed in the usual manner on a mica-raft, which was floated on water kept fully saturated with chloroform during the whole of the experiment. Under these conditions bacterial growth was quite inhibited, as was also any autonomous action due to the endosperm-cells, but the pre-existent enzymes, on the other hand, were allowed full play to produce any alterations of which they were capable.

Not even the feeblest action of any kind could ever be detected in the endosperm-tissue placed under these conditions, even after the lapse of several weeks, and we must therefore regard such experiments as fatal to the view that *pre-existent enzymes* exercise any appreciable influence in bringing about the well-marked and definite changes in the endosperm such as we have described.

We are thus led to what appears to be the only conceivable explanation remaining,—that the phenomena are dependent on the metabolic activity of some portion of the endosperm itself; and if this is the case, it follows that during normal germination the endosperm is not wholly passive, but takes some share with the embryo in preparing the reserve materials for the use of the young plant.

It now remains to ascertain how far it is possible to localise the particular part of the endosperm-tissue which is active in producing these changes, an inquiry which resolves itself into an examination of the respective functions of the "aleurone-layer" and amyliferous cells respectively.

The observations of Tangl,‡ and more recently those of Haberlandt, have shown that each of the "aleurone-cells" possesses protoplasm with the usual reticulation of fine strands, enclosing a well-defined nucleus, and presenting all the usual cytological evidences of activity. As far as we are aware, no one who has ever carefully

* 'Chem. Soc. Journ.,' 1890, p. 507; *ibid.*, 1892, p. 362.

† These facts were determined by estimating the diastatic and cytohydrolytic powers respectively of extracts of the grain made under similar conditions, in the one case with water only, and in the other with a saturated aqueous solution of chloroform. The determinations of diastatic activity were made by Lintner's method, and those of the cytohydrolytic by the times necessary to produce visible action on the cell-membranes of thin sections of the grain immersed in the two liquids.

‡ 'Sitzungsber. d. Wiener Akad.,' vol. 102, 1885.

examined these cells during the germinative period has ever doubted that they are actually *living* units.*

The cytological evidence as to the state of the amyliferous cells is not so clear, and we have been unable to find any record of a systematic examination of the appearances presented by their protoplasmic contents.

The difficulties of examination are, of course, much greater here than they are in the case of the "aleurone-cells," owing to the tightly packed starch-grains, which must be removed by some method incapable of acting on the other cell-contents, which they completely obscure. The ordinary reagents which are used for this purpose, such as acids and alkalis, are quite inadmissible, and although much better results are obtained with cold water extracts of malt, or of animal pancreas, acting for some time at 40—50° C., there are objections to both of these agents. The malt extract often possesses some cytohydrolytic power, which acts on the more delicate portions of the cell-membrane, and destroys the coherence of the tissue, and even when this objection is removed by previously heating the malt extract to 60—65° C. for some time, malt-proteids are often precipitated in a finely granular form within the sections, and confuse the results.

An extract of animal pancreas is a very good solvent for starch, but since this possesses slight proteohydrolytic power in feebly acid solutions, there is a danger of solution of the protoplasmic matrix along with the starch; and, moreover, when "liquor pancreaticus" (Benger) is used, there is considerable precipitation at 40—46° C.

No such objections, however, apply to the use of diluted and filtered mixed† human saliva. With the addition of a little thymol, to prevent putrefaction, this agent may be allowed to act on the very thinnest sections of seeds at a temperature of 46° C. (the optimum temperature for ptyalin) for many hours, without any change in the sections other than the dissolution of the starch. The starch-granules dissolve very completely, leaving sharply marked lacunæ in the protoplasm, which can then be stained in any desired manner.

In staining, we have used a mixture of iodine-green and fuchsine. With this reagent the nucleus is stained green, and is strongly contrasted with the cytoplasm which takes up the red stain.

* Tangl also observed the continuity of the protoplasm in the "aleurone-layer," a continuity effected by means of fine threads passing through pores in the thick walls of contiguous cells. Walter Gardiner ('Roy. Soc. Proc.,' vol. 52, 1897, p. 100) has confirmed this, and informs us that he has also proved the existence of continuity in the cytoplasm of the amyliferous cells.

† By this is meant ordinary human saliva, consisting of the mixed secretions of the three sets of salivary glands.

When sections of the starch-bearing portions of the mature endosperm are thus treated, it is seen that the nucleus is either extremely deformed, or, indeed, in many cases even completely disintegrated. That these appearances are not in any way due to the treatment to which the sections have been subjected is clearly shown by an examination of sections made from the endosperms of barley, taken from the fields at different stages of development, when starch is still being actively deposited within the cells. In the early stages of development the saliva-treatment gives sections in which normal and well-defined nuclei exist, but as the grain approaches maturity there is a corresponding senescence of the nucleus, resulting in the appearances just described.

It is interesting to trace the progress of this nuclear senescence, which first commences in the more deeply seated and older cells of the endosperm, gradually extending towards the periphery as the period of maturation approaches.

Just before complete ripening, the only well-formed nuclei which can be recognised are those of the last row of starch-bearing cells immediately under the "aleurone-layer." Ultimately, unless some unfavourable circumstances arise to prevent complete maturation, these nuclei to a great extent share the fate of those of the more deeply seated cells, but they are generally deformed to a less degree.

We shall at a future time have more to say on this question as regards other seeds and its connection with the particular nature of the reserve products. The point to which we now particularly wish to draw attention is that the cytological observations indicate the existence of a very marked difference between the nuclei of the "aleurone-cells" and those of the amyliferous cells. There can be no doubt about the functionating power of the former, whereas it seems difficult to admit that the starch-bearing cells can exercise their full powers as living units after complete maturation, although the destruction of their nuclei may not preclude *all* possibilities in this direction.

Nothing short of actual trial, however, can determine whether the starch-containing cells of the endosperm retain sufficient vitality to have any action on their own cell-membrane or cell-contents, and, with this object in view, we have conducted a number of experiments on large fragments of endosperm deprived completely of their adherent "aleurone-layer" after being steeped for twenty-four hours, and placed under the usual favourable conditions for the rapid outward diffusion of any products of change. For purposes of comparison we also employed other similar fragments which had been treated with chloroform-water for a sufficiently long period to effectually destroy any residual vitality, the chloroform being removed

in the same manner as described previously when treating of the intact endosperm.

The results were in no sense doubtful. No visible changes of any kind took place until micro-organisms had established themselves, when dissolution of the cell-membrane commenced. Moreover, there was the strictest possible parallelism, at all stages, between the "dead" and the "living" endospermous fragments, using these terms to express the state, at the commencement of the experiments, of those fragments which had or had not been previously put under conditions for extinguishing any residual vitality which their cells possessed. In this respect our later experiments have fully borne out the statement of one of us in 1890* that the *starch-containing portions of the endosperm* are unable to originate any visible changes in the reserves which they contain.

Thus we must conclude that it is to the influence of the "aleurone-layer," and the "aleurone-layer" only, that we must look for those well-marked changes which undoubtedly take place in the endosperm when this is separated from its embryo and placed under favourable conditions.

This is a conclusion differing materially from that of the 1890 paper referred to above, which concludes with the following passage: "As far as the evidence goes at present, we are certainly not justified even in suspecting that the cells of the 'aleurone-layer' are glandular in the same sense as are the epithelial cells of the scutellum, and until evidence of a far more convincing nature is forthcoming we must adhere to the opinion that the diastase" (and, we might have added, the cytase also) "accumulated in the germinating seeds of the Grasses owes its origin exclusively to the secretory glandular cells forming this scutellar epithelium, and that the aleurone-cells belong solely to the reserve-system of the seed."

This opinion was justified by the known facts of seven years ago, but certainly requires modification in the light of our more recent experiments. It seems, in fact, quite impossible to understand the results of these later experiments, if we deny the power of the "aleurone-layer" to produce a considerable amount of cytohydrolytic action on the cell-membrane, and even a certain amount of action on the starch itself. The relative share in the modification of the endosperm-reserves which falls to the scutellum and the "aleurone-layer" respectively in normal germination we shall consider presently, but it is in the first place necessary to criticise an important experiment of the 1890 paper, which at the time seemed absolutely conclusive against the view that the "aleurone-layer" has any power of modifying the endosperm-contents. Whilst investigating the best conditions for the development of excised

* Brown and Morris, *loc. cit.*

embryos on artificial nutrients, it was found, as we have already stated, that it is possible to "graft" the embryo from one grain on to the endosperm of another, and to obtain such close apposition of the two surfaces, by means of binding with a loop of thin silver or platinum wire, that the "graft" develops into a young plant almost as readily as if it were still nursed by its own endosperm. This fact afforded an opportunity of more closely studying the relative parts played by the embryo and endosperm in producing the initial changes in the reserve materials; for it is evident that if a degermed endosperm is subjected to some process which will with certainty kill its tissue, and a living embryo "grafted" on this endosperm will bring about in the reserve substances of the latter all the changes incidental to normal germination, then the whole idea of residual vitality in the endosperm-cells being a necessary condition of germination would become superfluous. Experiments in this direction were in the first place made by treating grains of barley with chloroform-vapour for twenty-four hours, a course of treatment which we now know must have been insufficient to have killed the resting protoplasts; it is, therefore, not to be wondered at that embryos "grafted" on endosperms so treated should have grown perfectly. In a further set of experiments, also described in the 1890 paper, grains of barley were soaked in absolute alcohol for six months, and after drying off the alcohol, soaking well in water, and degerming, fresh embryos applied to the endosperms were found to produce in them all the ordinary visible signs which accompany germination. This experiment was deemed to conclusively prove that the degradation of the reserve products is conditioned by the embryo itself, and that the endosperm-cells do not take part in it.

We have now, however, every reason to believe that the "aleurone-layer" was *not* killed by this drastic treatment with alcohol, for we have found that these cells are much more resistant to injurious influences than the tissue of the embryo itself, and we have seen cases in which even the embryo will sprout after the grain has been immersed in alcohol for about four months.*

We have recently found, in repeating and varying these experiments, that when the grain is immersed in a dry state in chloroform-water (*i.e.*, a saturated aqueous solution) a few hours suffice to

* Giglioli ('Nature,' vol. 52, 1895, p. 544) found that seeds of *Medicago sativa* retained their vitality after submersion in absolute alcohol for more than *sixteen years*. Ewart ('Liverpool Biolog. Soc. Trans.,' vol. 8, 1894, p. 207) also states that the resistance of seeds to absolute alcohol is very considerable, and that those of *Hordeum*, although killed very quickly by alcohol of 50 per cent., require submersion in *absolute alcohol* for seven weeks before all germinating power is lost. It will be seen, from what has been said above in the text, that the embryos of some grains of *Hordeum* may be made to grow after a much longer submersion than this.

destroy the vitality of the germ, but that at least twenty-four hours' immersion is required to permanently destroy the vitality of the "aleurone-layer," and that if this is not perfectly effected, subsequent "grafting" experiments may suggest entirely erroneous conclusions.

In the following remarks we shall refer to those endosperms which have been thus treated with chloroform-water as "dead," whilst those which have been merely soaked in water after degermation we shall regard as "living."

When "graftings" of embryos are made on living and dead endosperms respectively, and these are placed under favourable conditions for germination, very strongly marked differences are observable within a few days, both in the rapidity of growth and general appearance of the two sets of embryos, and in the nature and extent of the concurrent changes in the endosperms.

On the "living" endosperm the axial organs of the young plant develop freely, healthy rootlets are protruded, and the freely growing plumula has all the appearances of turgidity and firmness incidental to good nutrition. Simultaneously with this development cytohydrolysis commences under the "aleurone-layer," and, whilst attacking the "depleted layer," progresses peripherally and distally along the usual path in the endosperm. At the same time a distinct and sometimes considerable amount of starch-erosion is noticeable in the amyliferous cells immediately in contact with the "aleurone-layer" of the proximal end of the grain, but this is entirely of a "sub-aleuronic" type (see *antea*), whilst the starch-erosion which has taken place immediately under the scutellum of the "grafted" embryo is wholly of the "pitted" or "sub-scutellar" type.

The phenomena presented by the "grafting" on the "dead" endosperms are, on the other hand, of a very different character. Here the embryo is evidently under much less favourable conditions for healthy growth, since the young plant is much smaller, the tissues of its axial organs are flaccid, and there is very poor root-development. At the same time it is also clear that the embryo is deriving *some* nutriment from the dead endosperm and is increasing in weight, a fact which can readily be proved by a comparison with the development of excised embryos in water-culture on a porous tile.

The internal changes which the dead endosperm itself undergoes when in contact with the living embryo are very instructive, and a careful study of them enables us, with certainty, to distinguish and delimit the autonomous changes of the endosperm from those induced by the embryo itself. Even after eight or ten days the dead endosperms under these conditions exhibit no softening or cytohydrolysis of the tissues immediately underlying the "aleurone-layer," and this layer remains firmly attached to the subjacent amyliferous cells.

We can in this case only detect a small amount of disintegration immediately under the "grafted" embryo, and even after eight or ten days this is not found to proceed for more than 0·5 to 1 mm. from the "depleted layer." There is a partial but very incomplete cytohydrolysis of the cell-membranes constituting the "depleted layer," and a similar imperfect action can be traced microscopically in the amyliferous cells as far as the disintegration has proceeded. The starch-grains of the amyliferous cells immediately underlying the "depleted layer" show unmistakable signs of attack by normal subscutellar "pitting" without any admixture of that particular form of erosion which is characteristic of the action of the "aleurone-layer."

There can be no doubt that we here have further proof that the embryo, by means of the secretion of enzymes from its scutellar epithelium, is able to attack starch, and to assimilate the products of its hydrolysis. Abundant proof of this fact was brought forward in the 1890 paper, in which were described many experiments on the artificial nutrition of excised embryos, and this fact has been amply confirmed by Grüss in a series of very careful experiments he has recently described.*

It will be remembered that when embryos were cultivated on gelatine in which starch-granules had been suspended, it was found that a secretion of diastatic enzyme took place from the epithelial cells of the scutellum, which manifested itself by erosion of the starch, and that this erosion gradually extended to a relatively considerable depth in the gelatine medium. That this action does not proceed with the same rapidity in "dead" endosperms, on which embryos have been grafted, is due to a great extent to the fact that in this latter case the starch-grains are locked up in cell-membranes which retard the diffusion of the highly colloidal diastase. Until these cell-membranes are broken down we have not the most favourable conditions for a rapid formation of soluble nutriment from the reserve materials, especially as the amyliferous cells appear to be devoid of any power of initiating such changes autonomously. One of us was originally of the opinion† that the necessary cytohydrolytic function resided in the embryo itself, and that it was manifested by the same epithelial cells as those which produce a very active form of diastase, but our more recent experiments have clearly shown that this power of the embryo was much overrated, and that the greater part of the cytohydrolytic process preliminary to the amylohydrolytic is due to the cells of the "aleurone-layer," the treatment to which the grain was subjected in 1890 not having been sufficient to completely destroy the vitality of these cells.

This layer is the only part of the endosperm which can be recog-

* 'Pringsheim's Jahrb.,' vol. 30, 1897, p. 645.

† *Loc. cit.*, 1890.

nised as taking part in the preparation of the food-material for the embryo, since no evidence can be obtained of any changes being initiated by the starch-containing cells themselves; in fact, the highly disintegrated appearance of the nuclei of these cells would in itself suggest they had ceased to function.

If we were to limit ourselves to the observations on degermed endosperms in water-culture, we should conclude that the diastatic function of the "aleurone-layer" is very small indeed, and this is also apparently confirmed by the impossibility of demonstrating the existence of such a function in the "aleurone-layer" when we employ the methods which have been so successful in this direction in the case of preparations of the scutellum.

When the integuments with the "aleurone-layer" attached are perfectly freed from the starch-containing cells, and are placed face downwards on starch-gelatine, we have never been able to obtain any evidence of action on the starch, and even when the preparation was made so as to include a layer of the amyliferous cells, which were kept moist on gelatine, no influence was exerted on the contained starch. Under these circumstances, however, there is an entire absence of cytohydrolytic as well as of amylohydrolytic action, and since the former is so well marked in degermed endosperms in water-culture, we can only conclude that the separated "aleurone-layer" for some reason or other will not exercise its normal function in the same manner as the scutellar epithelium placed under similar conditions.

It is also to be remembered that in those cases where the endosperm is in actual contact with the embryo, either as in natural germination, or as in the "grafting" experiments, the special changes induced by the "aleuronic" layer proceed much more rapidly than in isolated endosperms in water-culture, and this accelerated action is much more evident in the case of the diastatic than of the cytohydrolytic action.

It would appear, therefore, that although one of the principal functions of the "aleurone-cells" is to break down the cell-membrane of the amyliferous endosperm, these cells also share with the scutellum the power of eroding starch-granules.

Owing to the different method of attack on the starch, it now becomes possible, for the first time, to discriminate one form of action from the other, but it is very difficult to apportion the part played by scutellum and "aleurone-layer" respectively, for the amount of action of either depends not only on the enzymic intensity for equal areas of the two tissues, but also on the total areas facing the endosperm-contents in each case.

Since the total area of the "aleurone-layer" is considerably greater than that of the scutellar epithelium, the influence of the

former may be as great or even greater than that of the scutellum in the early stages of germination, even if its specific enzymic intensity is very much less.

There is another probable function of the "aleurone-layer" which may indirectly be of great value to the seed. These cells, which undoubtedly contain living elements, constitute the outermost peripheral layer of an otherwise *dead* endosperm, which, were it not for this protective sheathing of living cells, would be much more liable to the attacks of any of the micro-organisms of the soil which succeeded in penetrating the seed-envelopes. It is a noteworthy fact that the "aleuronic" cells are much more fully developed over those parts of the seed which may be regarded as devoid of life, and become very much more attenuated where they come into proximity with the embryo whose cells, owing to their activity, do not require an equal amount of protection. In the case of barley the threefold layer of "aleurone-cells" lying within the pericarp and testa constitutes a triple line of defence, which must be of some value in protecting the amyliferous cells against the hordes of external organisms when the grain is placed under the natural conditions suitable for germination.

We must express our great thanks to Mr. W. T. Thiselton Dyer and Dr. D. H. Scott for the opportunities they have afforded us for carrying out this research at the Jodrell Laboratory.

Addendum.

Since writing the above we have for the first time seen the full and expanded account which Puriewitsch has given of his work in Pringsheim's 'Jahrbuch,' vol. 31, 1897, p. 1.

His observations on the self-depletion of the endosperm of the *Gramineæ* take account only of the erosion and dissolution of the reserve starch. He does not call attention to the equally important and necessary antecedent phenomena of cytohydrolysis, which admit of a determination of the "aleuronic" or peripheral origin of the autonomous changes and their mode of progression in the endosperm. Puriewitsch, in fact, regards every cell of the endosperm as capable of functioning as a depletive agent, whereas our own work points strongly to the conclusion that when an endosperm is deprived of its embryo the subsequent chemical changes within it are initiated by the "aleurone-layer" only.

It is correctly stated that such action commences near the scutellar surface, and extends peripherally under the "aleurone-layer"; but the author explains this by the observations of Brown and Morris, and Grüss,* that the proximal half of the endosperm contains more

* The recent experiments of Grüss in this direction were made on maize, not on barley.

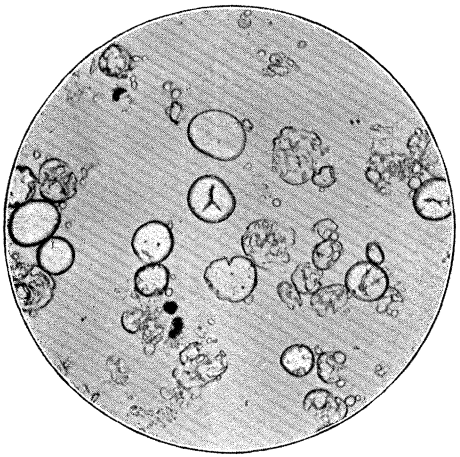

FIG. 1.

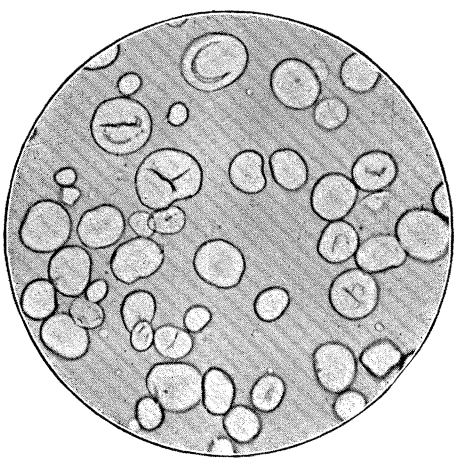

FIG. 2.

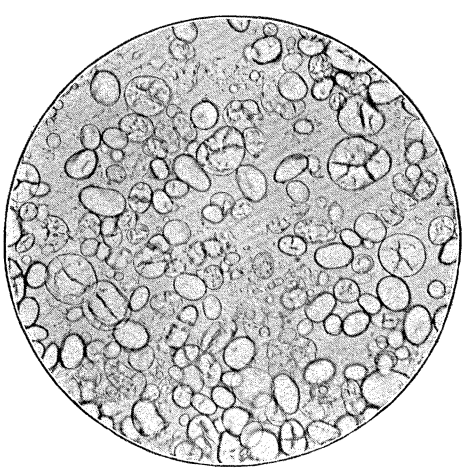

FIG. 3.

diastase than the distal. This cannot be the true explanation, since we find that the pre-existent enzymes of the endosperm practically play no part in the self-depletion.

DESCRIPTION OF PLATE 1.

FIG. 1.—Examples of "sub-scutellar" starch-erosion, showing incipient and advanced forms. Two granules, the one in the centre of the field, the other on the right, show incipient "sub-aleuronic" erosion.

FIG. 2.—Examples of "sub-aleuronic" starch-erosion in incipient stages.

FIG. 3.—Examples of "sub-aleuronic" starch-erosion in more advanced stages.

(For the production of these photographs we are indebted to Mr. Albert Norman.)

March 10, 1898.

Sir JOHN EVANS, K.C.B., D.C.L., LL.D., Treasurer, in the Chair.

The following Papers were read:—

I. "On the Rotation of Plane of Polarisation of Electric Waves by a Twisted Structure." By Professor J. C. BOSE. Communicated by LORD RAYLEIGH, F.R.S.

II. "On the Production of a "Dark Cross" in the Field of Electromagnetic Radiation." By Professor J. C. BOSE. Communicated by LORD RAYLEIGH, F.R.S.

III. "An Extension of Maxwell's Electro-magnetic Theory of Light to include Dispersion, Metallic Reflection, and allied Phenomena." By EDWIN EDSER, A.R.C.S. Communicated by Captain ABNEY, F.R.S.

IV. "On the Relative Retardation between the Components of a Stream of Light produced by the Passage of the Stream through a Crystalline Plate cut in any Direction with respect to the Faces of the Crystal." By JAMES WALKER, M.A. Communicated by Professor CLIFTON, F.R.S.

V. "On the Relation between the Diurnal Range of Magnetic Declination and Horizontal Force and the Period of Solar Spot Frequency." By W. ELLIS, F.R.S.

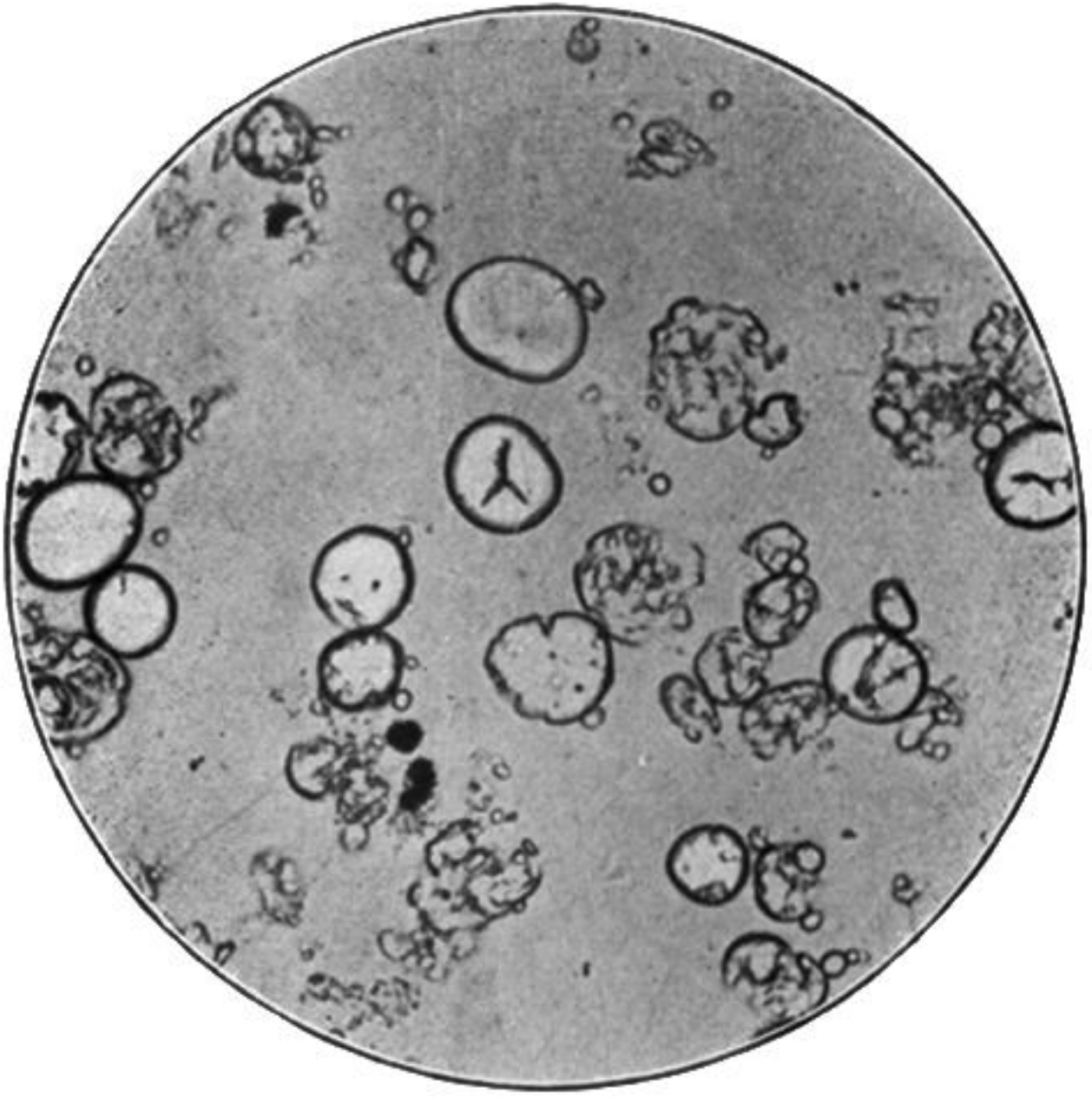

FIG. 1.

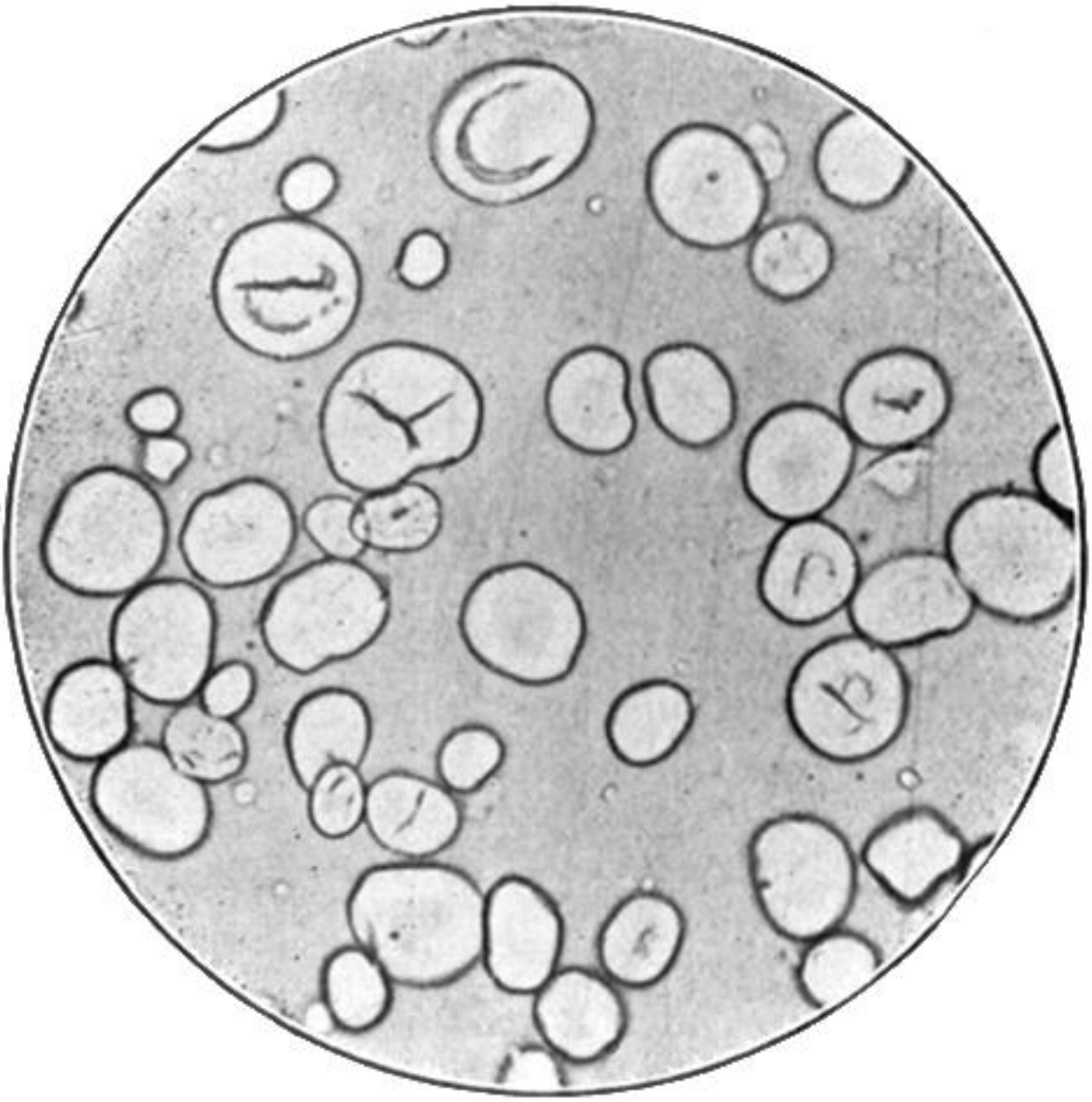

FIG. 2.

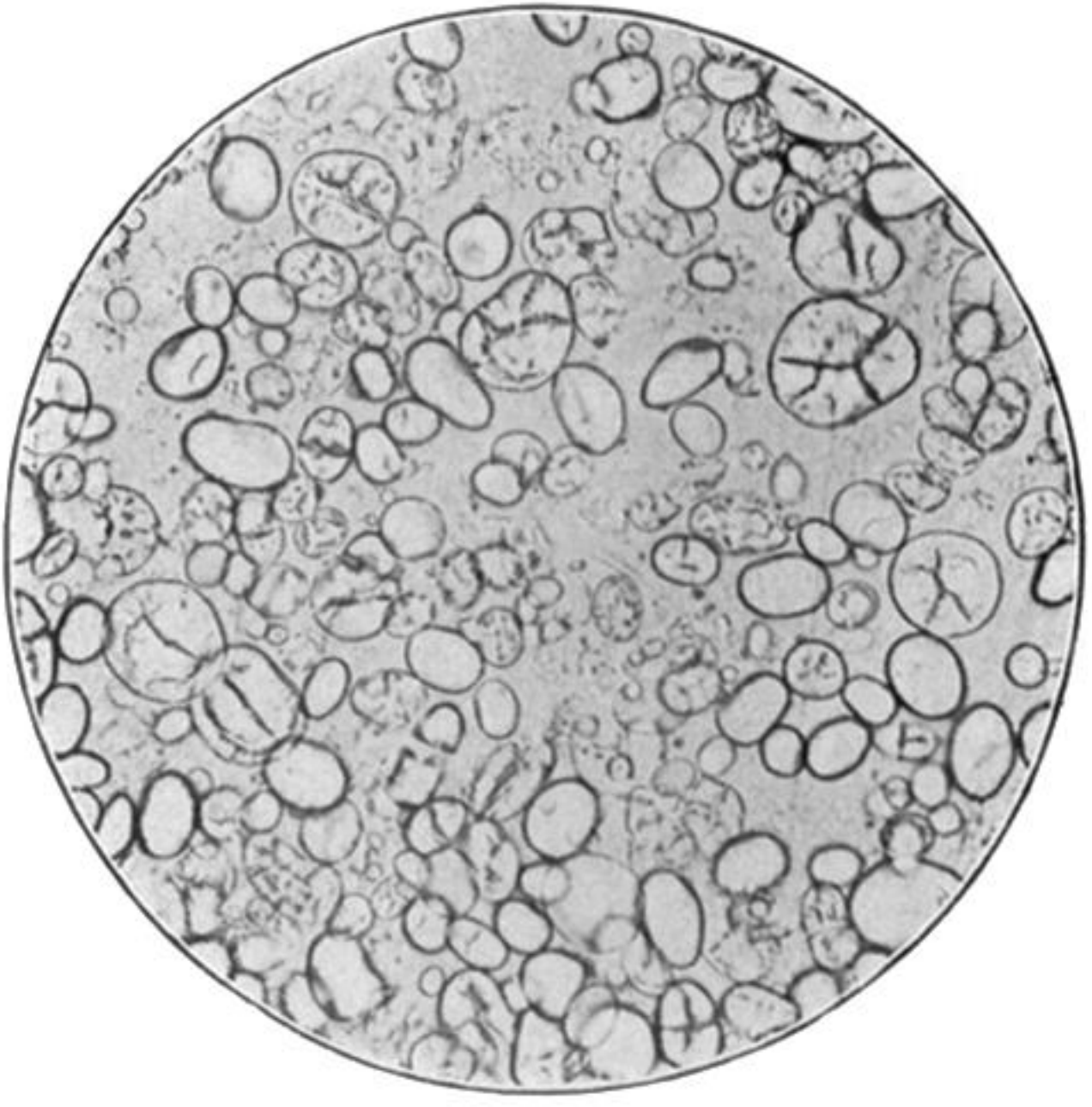

FIG. 3.