Further Observations on the Germination of the Seeds of the Castor Oil Plant (Ricinus communis).

By J. Reynolds Green, Sc.D., F.R.S., Professor of Botany to the Pharmaceutical Society of Great Britain, and Henry Jackson, M.A., Fellow and Tutor of Downing College, Cambridge.*

(Received March 22,—Read May 18, 1905.)

About 15 years ago one of the authors carried out a series of researches on the germination of the seeds of the castor oil plant (*Ricinus communis*),† and endeavoured to ascertain the course of the decomposition and utilisation of the reserve materials which are present in the seed. As the results of this investigation formed the starting point of the present series of researches, it will be well at the outset to restate the conclusions which were then arrived at.

The larger part of the reserve materials of the seeds of Ricinus, which are laid up in the cells of the endosperm, consists of the well-known castor oil. The amount varies in different seeds, but it ranges from as little as 50 per cent. to upwards of 80 per cent. There is a considerable amount of proteid matter in the cells, most of which is found in the so-called aleurone grains. These have a somewhat intricate structure; an ovoid mass of phytoglobulin, soluble in 10 per cent. solution of common salt, surrounds a proteid crystal, soluble in saturated solution of the same salt. In the grain by the side of the crystal there is a rounded aggregation of mineral matter, the so-called globoid, long considered to be a double phosphate of calcium and magnesium, but probably a more complex body containing its phosphorus in some form of organic combination. According to Vines‡ the proteids of the grain are an albumose and a globulin; in the opinion of Osborne and Harris§ this is not the case, only a globulin being present, probably identical with the *edestin* of the hemp seed. There are other substances present in small amount, but in very trifling proportions when compared with the oil and the proteids.

* The present series of experiments was commenced by me in collaboration with Mr. W. T. N. Spivey, of Trinity College, Cambridge. After his lamented death in 1901, Mr. Jackson took his place.—J. R. G.

† Green, 'Roy. Soc. Proc.,' vol. 48 (1890), p. 370.

‡ Vines, "Proteid Substances in Seeds," 'Journ. of Physiol.,' vol. 3 (1880), p. 91.

§ Osborne and Harris, "Nitrogen in Protein Bodies," 'Amer. Chem. Journ.,' vol. 25 (1903), p. 335.

The conclusions arrived at in 1888 with reference to the changes set up during germination were the following :—

"1. The reserve materials in the endosperm of *Ricinus communis* consist chiefly of oil and proteid matters, the latter being a mixture of globulin and albumose.

"2. The changes during germination are partly due to enzyme action, there being three enzymes present in the germinating seed: one is a protease resembling trypsin, the second splits the oil into fatty acid and glycerine, the third is a rennet enzyme.

"3. At least two of these, and therefore presumably all of them, are in a zymogen condition in the resting seed, and become active in consequence of the metabolic activity set up in the cells by the conditions leading to germination, especially moisture and warmth.

"4. The changes caused by the enzymes are followed by others, due to the metabolism of the cells, these being processes of oxidation.

"5. The embryo exercises some influence on the latter, setting up as it develops a stimulus probably of a physiological description.

"6. The result of these various processes is to bring about the following decompositions :—

"The proteids are by the enzyme converted into peptone, and later into asparagin.

"The oil is split by the glyceride enzyme into fatty acid and glycerine; the latter gives rise to sugar, and the former to a vegetable acid which is soluble in water and in ether, is crystalline, and has the power of dialysis.

"7. Absorption in all cases takes place by dialysis.

"8. The appearance of starch and of oil in the embryo or the young plant is due to a secondary formation, and not to a translocation of either."

Formation of Lecithin.

The advances in our knowledge of the metabolic processes of plants that have been made during the interval that has elapsed since the publication of this paper, and the new methods of experiment that have been introduced, suggested that the work which was admittedly incomplete and tentative should be taken up again. There remained especially the question of the meaning of the reserve supplies of phosphorus and the part which they take in the general metabolism accompanying germination. The aggregates of phosphates referred to as the globoids of the aleurone grain undergo a change during the process, by virtue of which they slowly pass into solution.

As this change supervenes upon the development of an acid reaction in the seeds, it seems not unlikely that it may be caused by the action of the organic acid which is formed in the cells of the endosperm almost as soon as germination begins.

On resuming the work a more careful examination of the oily contents of the endosperm cells led to the discovery that they contained, mixed with the oil, a certain quantity of a substance, the decomposition products of which pointed to its being a lecithin (a peculiar fatty body containing phosphorus). The resting seeds were pounded in a mortar till they formed a homogeneous paste. This was extracted for some hours with ether in a Soxhlet's apparatus, and was afterwards twice extracted further on a water-bath with absolute alcohol, the flask being fitted with a reflux condenser. The alcoholic and ethereal extracts were mixed and evaporated to dryness at a gentle heat on a water-bath, and the fatty residue fused with dry carbonate and nitrate of potassium until all trace of free carbon had disappeared. After cooling, the fused residue was dissolved in water, and the addition of ammonium molybdate and nitric acid produced a yellow precipitate, indicating the presence of phosphorus. The quantity of the latter was ascertained by converting it into magnesium pyrophosphate and weighing.

A little of the oil was then hydrolysed by boiling with baryta, when there separated out a flocculent precipitate of a barium salt, which, after washing and drying, was found to contain no phosphorus. This barium salt had the characteristic soapy appearance of the stearates. It was found possible to identify cholin in the endosperm of the germinating seeds, as will be more fully shown a little later (p. 74).

The only way of estimating the amount of the lecithin present in the alcohol-ether extracts of the endosperm was to determine accurately the phosphorus as magnesium pyrophosphate. Great precautions were taken to ensure the repeated use of very dry ether so as to exclude the possibility of extracting any inorganic phosphates. A little of the oily residue from the extraction was incinerated in a platinum dish, and it was found to leave no inorganic ash. We therefore assumed that all the phosphorus extracted as described was originally present in the complex lecithin form. Taking the formula usually given for lecithin ($C_{44}H_{90}NPO_4$), we calculated the amount of the latter that would be present. Its average amount was equal to 0·236 per cent. of the dry weight of the seeds.

Several series of experiments were made to investigate the changes in the fatty constituents of the endosperm during germination. The action of the fat-splitting enzyme known now as *lipase* was confirmed, and the early

stages of germination were found to be as set out in the former paper. Consequently, examinations of the contents of the seeds were made at certain stages of the germination, and before the process had begun. The stages were the following:—(1) The seed at the time of the cracking of the testa, usually after 24 to 48 hours in the soil; (2) the seed with the radicle protruding for a length of 1 to 2 cm., usually about three days after sowing; and finally, (3) seeds whose lateral root system had become fairly well developed. The times at which these stages were reached varied with the samples of seeds used, and the temperatures at which the germination took place.

The results of a typical experiment are stated in the subjoined table:—

Table A.

Degree of development.	Dry weight of seeds used.	Oil in seeds.		Fatty acid in seeds.		Lecithin per cent. of weight of seeds.
		Actual weight.	Percentage of weight of seeds.	Actual weight.	Percentage of weight of seeds.	
	grammes.	grammes.		grammes.		
Resting seeds	4·48	3·7115	82·8	0·1	2·2	0·236
Seeds just cracking testa	4·47	3·016	67·5	0·204	4·6	0·17
Radicle protruding 1—2 cm.	4·17	2·19	52·5	0·5	11·9	0·475
Lateral roots spreading. Root system established	3·34	0·789	23·6	0·565	16·89	0·873

It will be seen that the amount of lecithin diminished during the early stages of germination, the reserve supply becoming almost exhausted. After the young seedling had begun to develop, however, there was a gradual increase in the amount. This increase was maintained during the later stages and was fairly constant till the endosperm was used up. There was clearly a consumption of the oil throughout.

The amount of lecithin, though small, varied somewhat in different experiments. In one series it was in much larger proportion than in that quoted. The residue soluble in alcohol and ether amounted to 0·9 per cent. of the weight of the resting seeds, and in the later stages of germination the amount present rose to approximately 2 per cent. This quantity, however, in our experiments was exceptional.

These experiments suggest that in the utilisation of the fatty reserves lecithin certainly plays a part and, possibly, a predominant part.

Lecithin has been shown by Overton * to be a normal constituent of living cells, and to exercise considerable influence on the transport of various materials across the limiting layers of the protoplasm. It has no doubt also a certain, though at present undetermined, nutritive value.

The composition of lecithin is indicated by the change which it undergoes on hydrolysis, when it is decomposed into *stearic* (or *palmitic* or *oleic*) *acid*, *glycero-phosphoric acid* and *cholin.*

$$\underset{\text{Lecithin.}}{C_{44}H_{90}NPO_9} + 3H_2O = \underset{\text{Stearic acid.}}{2C_{18}H_{36}O_2} + \underset{\text{Glycero-phosphoric acid.}}{C_3H_9PO_6} + \underset{\text{Cholin.}}{C_5H_{15}NO_2}.$$

From this, its constitution has been represented as

$$C_3H_5\begin{cases} OCOC_{17}H_{35} \\ OCOC_{17}H_{35} \\ O-PO(HO)O(CH_2)_2N(CH_3)_3OH \end{cases}$$

Only a trace of it exists in the resting seed; as it increases during germination and the quantity remains fairly constant during the whole period of absorption of the fatty reserves by the seedling, we have evidence of a formation of it during the germinative processes. The endosperm contains such substances as may yield the several groups necessary for its formation. The decomposition of the oil by the enzyme *lipase* can furnish the fatty component, belonging to the oleic group, and at the same time the glycerine of the glycero-phosphoric acid. The phosphorus of the latter is at hand in the shape of the phosphatic globoids whose solution has already been alluded to. The nitrogenous body cholin may be looked for among the products of the decomposition of the proteids of the aleurone grains.

Examination of the contents of the endosperms during germination ultimately established the presence of all these constituents. The fatty acids and the glycerine were identified in 1888, and the methods of detection and estimation were quoted in the former paper. A careful examination of the phosphates of the globoids, taken for purposes of comparison from seeds at the respective stages of germination quoted in Table A (p. 72) showed that their solution proceeded side by side with that of the oil.

No change in them could be observed under the microscope till the testa was cracking, and the time of its inception varied a good deal. In the early stages, prior to such cracking, no reaction for phosphorus could be obtained from a watery extract. The quantity of phosphorus present in the resting seed was 0·205 per cent. of the dry weight; this diminished in Stages 2, 3, and 4 of Table A to 0·16, 0·14, and 0·11 per cent. The globoids are decom-

* Overton, 'Pringsheim's Jahrb.,' vol. 39 (1900).

posed gradually but fairly rapidly during the germination, and in the later stages contribute to the acidity of the cell-sap, which contains phosphoric acid.

Search was made in a mass of endosperms for cholin. The germinating seeds were ground up in a mortar and allowed to stand for some days under alcohol which was nearly absolute. This was decanted and evaporated to dryness, the residue being again extracted with absolute alcohol and subsequently by a mixture of alcohol and ether. These extracts were mixed and evaporated to dryness, leaving a final residue, soluble in water. When a strong aqueous solution of this was boiled, decomposition took place, and a gas was evolved which possessed the well-known ammoniacal and fishy odour characteristic of tri-methylamine. The decomposition can be represented by the following equation:—

$$CH_2(OH)CH_2N(CH_3)_3OH = CH_2(OH)CH_2OH + N(CH_3)_3.$$

When to some of an aqueous solution of the residue from the alcoholic and ethereal extracts a little platinic chloride was added, after standing for some time the characteristic yellow octahedral crystals of the compound which cholin forms with platinic chloride separated out. These were soluble in 15 per cent. alcohol, and on combustion yielded a residue of metallic platinum. We have thus all the constituents of lecithin present in the germinating seeds.

It was difficult to apply the ordinary tests for lecithin when so large a quantity of oil was present. Towards the close of the germination, however, conditions were more favourable, the lecithin being present in relatively large proportion.

The existence of a proteolytic enzyme of a tryptic nature in the germinating seeds was shown in the former paper. Among the products of its action a considerable quantity of crystalline amino-bodies were detected, though not sufficient for a complete analysis. They separated out from the concentrated alcoholic extracts, after removal of the sugars, in quantities that enabled their amino-nature to be proved. The power of the enzyme to produce these *in vitro* has already been noted.* We have found the cholin also to be due to the action of this enzyme. 150 cc. of an extract of the endosperms of a quantity of germinating seeds was prepared by steeping them for several hours in water containing 0·2 per cent. of formaldehyde as an antiseptic. It was then strained through muslin and filtered till it appeared as a clear straw-coloured liquid. This was divided into two, and half of it boiled to destroy the protease. A quantity of globulin was

* Green, *loc. cit.*, p. 377.

prepared from a further quantity of the same germinating seeds by extracting them with a 10 per cent. solution of common salt, and precipitating the proteid by addition of alcohol. The precipitate was rapidly collected on a filter, washed and suspended in a little water.

The 75 c.c. of the extract that had not been boiled was put into a beaker and 5 c.c. of the suspended globulin added; a similar preparation was made of the 75 c.c. that had been boiled. Both were kept in an incubator at 40° C. for a week. At the end of that time digestion was complete in the unboiled preparation, the globulin having disappeared, leaving a morbid solution. Both were perfectly free from bacteria, the formaldehyde acting extremely efficiently as an antiseptic. The two digestions were then filtered and the filtrates evaporated to dryness. The residues were extracted successively with absolute alcohol and with a mixture of absolute alcohol and ether, each extraction being continued for two days. The first alcoholic extract was evaporated to dryness and the residue again extracted with ether. The two ethereal extracts were subsequently mixed and evaporated to dryness and the residue taken up with a little water. There was considerably more of this residue in the digestion carried out by the unboiled extract of the seeds than in that associated with the other. To each a little platinic chloride was added in watch-glasses, and they were set aside. After 24 hours, in both cases minute crystals had settled to the bottom of the liquid, which were soluble in alcohol of 15 per cent. concentration. From this solution the characteristic yellow octahedra slowly settled out, and these gave the same reactions as those prepared from the extracts of the endosperms. The amount obtained from the digestion by the unboiled extract was much greater than that from the boiled one, though the latter yielded some, attributable no doubt to a certain quantity present in the 75 c.c. of the original extract of the seeds employed.

The experiment shows, therefore, that the cholin of the lecithin can be prepared from the proteids of the seeds by an enzyme which is developed during germination, and is presumably the enzyme already described as a trypsin.

The similarity of this enzyme to the trypsin of the pancreas is borne out by the occurrence of tryptophane among the products of its activity both in the plant and *in vitro* in the laboratory.

The contribution of material for the synthesis of lecithin does not seem, however, to be the only result of the decomposition of the fat. There is not sufficient phosphorus in the resting seeds to enter into the composition of as much lecithin as the fat would produce. It is, of course, possible that the lecithin may be decomposed during consumption and part of its phosphorus

set free to combine again, but even then the quantities do not seem to be proportional. Another fate must attend a considerable quantity of the fat. To this point we shall return later.

These results suggest that the utilisation of the oily reserves is a much more complicated process than was supposed. The enquiry took from this point a wider range, and soon involved the abandonment of the idea that the separate reserves undergo independent changes during germination.

THE SUGARS OF RICINUS.

A more complete study of the sugar was next undertaken. Du Sablon showed, in 1895* that it is a mixture of at least two sugars, one of which has not the power of reducing Fehling's solution. In our experiments, a large number of seeds having been germinated, the endosperms were separated from the embryos and ground to a paste in a mortar. The mass was then extracted with large quantities of water, by keeping it for some hours in a steriliser at 100° C., removing the water at intervals till the extract showed that all the sugar had been dissolved. The extracts were mixed and concentrated to about one-tenth of their volume. Addition of normal acetate of lead separated from this extract the acids present, together with the bulk of the proteids and certain other constituents. These were filtered off, and the sugars were precipitated from the filtrate by adding basic lead acetate and ammonia. The precipitate was separated by filtration and suspended in water, and the lead removed by a stream of sulphuretted hydrogen. The solution so obtained was concentrated, and the process repeated, the final solution being then concentrated to a thick syrup, which showed the presence of two constituents possessing different solubilities in alcohol. By a repetition of concentration and extraction, the syrup was ultimately separated into two parts, one of which reduced Fehling's solution, while the other did not. Unfortunately the separation did not involve the complete isolation of the two sugars, as the reducing power of the first fraction was always increased after boiling with dilute mineral acid. The increase was not constant in different preparations, a fact which pointed to incomplete separation rather than to the reducing sugar being of the maltose type.

The second fraction of the syrup was, however, free from the reducing sugar. Treated with invertase or with a dilute mineral acid it speedily reduced Fehling's fluid. A quantity of it was concentrated nearly to dryness and with some difficulty dissolved in alcohol. Addition of ether to a little of the solution caused precipitation of the sugar. To the great bulk of the

* Du Sablon, " Sur la Germination des Graines Oléagineuses," 'Rev. Gén. de Bot.,' 1895, p. 145.

solution, therefore, a little ether was added, drop by drop, till a faint turbidity was apparent.

After standing in this condition for some days, a crop of aggregates of crystals separated out. When dissolved in water they were found to have a specific rotatory power of about $\alpha_D = +66$. After inversion with a dilute mineral acid the specific rotatory power became about $\alpha_D = -18$. The solution of the crystals gave no crystalline osazone on warming with phenylhydrazine acetate.

These reactions are fairly conclusive that the non-reducing sugar is cane-sugar.

The reducing sugar was refractory and no method succeeded in rendering it crystalline. It was also found impossible to separate it completely from the cane-sugar, so that its specific reducing power could not be obtained. Readings with the polarimeter were unsatisfactory on account of its proving impossible to free its solutions from a yellow colouration. When the latter were warmed with phenylhydrazine acetate they yielded a quantity of a pale yellow osazone which analysis proved to be the osazone of a hexose. After several recrystallisations from alcohol and from ethyl acetate the crystals were found to have a constant melting point at 204° C. This is consistent with the view that it is invert sugar produced from the cane-sugar with which it is associated. It negatives the hypothesis put forward in the former paper that it is derived from the glycerine of the fat, for this sugar (glycerose), now much more completely investigated, is known to yield an osazone melting at 130° C. to 131° C.*

The occurrence of two sugars exhibiting the characters just described suggested a search for invertase among the constituents of the endosperm. A good number of well germinated seeds were selected, having most of the endosperm absorbed; the embryos were well developed, their root system considerably branched. The endosperms were removed and ground up into a paste, which when strained through muslin yielded 95 c.c. of an acid sap. This was carefully neutralised and a little antiseptic added. It contained a quantity of both reducing and non-reducing sugar, 10 c.c. of the sap reducing 0·2 gramme of cupric oxide. Tubes were prepared containing respectively 10 c.c. of the neutralised juice with 10 c.c. of a solution of the non-reducing sugar from the seeds, and 10 c.c. boiled juice with the same quantity of the sugar solution, and they were digested in a water-bath at 40° C. for several hours. On titration the weight of cupric oxide reduced by the digestion containing unboiled juice was 0·31 gramme while the other

* Fischer and Tafel, 'Ber. d. deut. Chem. Ges.,' vol. 20, p. 1088; Fenton and Jackson, 'Trans. Chem. Soc.,' 1899.

gave the same weight as the original juice, 0·2 gramme. The treatment with the juice had increased the original reducing power 50 per cent., showing the presence of invertase.

Further experiments upon the same point showed that invertase appears in the endosperms at a very early period of germination, usually after a few hours; it is well established in 48 hours, and increases in amount up to the stage at which a good root system has been established. In a series of experiments upon its development during the germination three stages were compared: (1) The seeds had the radicle protruding about 0·3 inch; (2) The roots were 1 inch long and the secondary rootlets were just cracking the primary root; (3) There was a good root system and the endosperms were about half consumed. Extracts were made of all these and 2 c.c. of each allowed to act on 20 c.c. of a 1 per cent. solution of cane-sugar, at 40° C. for 24 hours. They were then titrated with Fehling's fluid, when the weights of cupric oxide obtained were:—

(1) 0·003 gramme; (2) 0·006 gramme; (3) 0·007 gramme.

These experiments lead us to the conclusion that the sugars of the endosperms may be put down as cane-sugar and invert sugar.

The relative quantities of these two sugars during the progress of germination have been ascertained and are given in Table B. Experiments on this point have been published by Du Sablon in the paper already referred to. He states that he found non-reducing sugar to be slightly in excess of reducing sugar in the resting seed and to increase more rapidly than the latter till the radicle is about 1·5 to 2 inches long, when the reducing sugar becomes equal in amount and, later on, preponderates considerably.

Our experiments were carried out in the following manner:—A number of seeds were germinated in sawdust in an incubator kept at a temperature of 22° C. In each experiment three were taken, peeled, and ground up to a smooth paste in an agate mortar. The paste was then boiled with a sufficient quantity of water for an hour, the extract strained off, filtered, and divided into two. Half was warmed to 40° C. with 1 c.c. of a solution of invertase prepared from yeast, and kept at that temperature for 24 hours. The invertase solution was ascertained to be free from sugar or other substance capable of reducing Fehling's fluid. The two halves of the extract were then titrated side by side, and the weight of the cupric oxide taken in each case. From these weights the quantities of the two sugars were computed in the usual way.

Table B.

Time of germination in hours.	Condition of seeds when ground up.	Invert sugar in milligrammes.	Cane-sugar in milligrammes.
0	Resting seeds	1·1	10·7
45	Caruncle swollen	2·7	5·17
69	Little further external change...	2·3	0
117	Root about 0·75 inch long	6·7	19·4
168	Root 1·5 inch long	5·2	10·5
216	Roots branching	19·5	35·7
240	Endosperms cracking	29·01	35·8
312	Good root system	40·8	52·6

A comparison of this Table with Table A suggests that the course of events in which the sugars are involved proceeds upon much the same lines as that connected with the lecithin. The cane-sugar is present in greater quantity in the resting seeds, it gives place to invert sugar under the influence of the invertase during the early period of germination, and subsequently increases in amount and remains slightly in excess of the invert sugar during the later stages when absorption is more active. This suggests that cane-sugar is the actual reserve, and that the invert sugar represents the form which has the greater nutritive value.

In accounting for the increase in the quantity of cane-sugar which marks the progress of germination, it is necessary to call attention to a fact noticed for the first time a few years ago by Mr. Biffen in the Cambridge Botanical Laboratory. Emphasis has already been laid upon the fact that a very vigorous metabolism in the endosperm cells is an accompaniment of germination. This was commented on by Van Tieghem * in 1877, when he found that endosperms deprived of their embryos were capable of swelling and apparently starting a kind of development. In the former paper on this subject one of us described experiments † confirmatory of Van Tieghem's views. Biffen has found that a considerable increase of the protoplasm of these endosperm cells is a marked feature of the early stages of germination. The exact time at which it occurs varies somewhat, but it corresponds fairly closely with the recommencing formation of cane-sugar. The coincident occurrence of these two events points to a growth of the protoplasm of the endosperm cells at the expense of the initial reserves, which we have seen are undergoing conversion changes at and before this time, and a subsequent construction of further carbohydrate reserves by

* Van Tieghem, "Sur la Digestion d'Albumen," 'Comptes Rendus,' vol. 84, p. 578.

† Green, *loc. cit.*, p. 389.

the protoplasm in the endosperm for the nutrition of the outlying embryo as its growth continues. Apart from such secretion the endosperm contains no carbohydrate material, while the latter seems to be essential for the maintenance of merismatic tissue. The fact that this carbohydrate substance is cane-sugar coincides with the observation of Brown and Morris* that cane-sugar is always present in the growing embryo of the barley-grain. It appears to be a form of carbohydrate very suitable for serving as a temporary reserve material, more easily utilisable than starch, and therefore formed where the deposit of the reserve will be of very short duration, as in the case of the embryo, and in that of the foliage-leaf, where Brown and Morris found it at a very early period of the photosynthetic construction. Indeed, from the results of analyses of the mixed sugars then present they suggested that it might even be the first sugar formed.†

It may again be noted that in the case of Ricinus its formation is accompanied or speedily followed by the secretion of invertase. The enzyme is not present in the resting seeds, but develops in the endosperms after exposure to a temperature of 25° C. in moist earth or sawdust for 48 hours or less, though germinative changes are not visible so soon in the external appearance of the seeds. The amount of the enzyme increases continuously all the time of germination, and the invert sugar increases coincidently. The protoplasm appears to keep up a secretion of cane-sugar and the invertase seems to keep working on the latter, so as to supply invert sugar at once to the protoplasm of the cells and to the young absorbing embryo.

It will be seen from what has been said that we do not associate the formation of this carbohydrate material during the germination *directly* with the diminution in quantity of the oil which is taking place at the same time. Our experiments lend no support to the views of Sachs that the oil was directly transformed with either sugar or starch. The two processes are features of a new metabolism set up in the cells as germination becomes established. To this point we shall return later.

The Acids of the Germinating Seeds.

The question of the nature of the acid to which the reaction of the germinating seed is due remains to be dealt with. Evidence of acidity can

* Brown and Morris, "Researches on the Germination of some of the Gramineæ," 'Journ. Chem. Soc.,' vol. 57 (1890), p. 518.

† Brown and Morris, "A Contribution to the Chemistry and Physiology of Foliage Leaves," 'Journ. Chem. Soc.,' May, 1893, p. 673.

be obtained after a seed has been exposed to warmth and moisture for 24 hours, and it becomes more and more intense for six or seven days.

While the reaction to litmus paper becomes very prominent, only very small quantities of acid can be obtained from the seed. The expressed juice of a parcel of germinating seeds was titrated with decinormal potash solution, and 10 c.c. of it neutralised only 4 c.c. of the alkaline solution. We made several attempts to prepare it in quantity by experimenting upon about a thousand seeds at once. They were germinated for a week, and the endosperms separated from the embryos, ground and boiled in water in a steriliser for several hours. After straining and filtering part of the extract was distilled by the aid of steam. The distillate was practically neutral in reaction, the merest trace of acidity coming over. The acid in the remainder, after removal of uncoagulable proteid, was precipitated by normal lead acetate, and the lead salt filtered off, suspended in water and treated with a stream of sulphuretted hydrogen till the lead was all converted into sulphide. The filtrate from the latter was concentrated to a small bulk, and the precipitation and subsequent treatment repeated. The final filtrate was concentrated to a small bulk *in vacuo* over sulphuric acid.

The acid residue, somewhat syrupy in consistence, was then washed repeatedly with dry ether, which dissolved a certain quantity, leaving behind, however, a good deal of acid which was soluble in water only. The bulk of the latter was ascertained to be phosphoric acid. The solution in ether was concentrated *in vacuo* and formed a syrupy residue. We found it impossible to crystallise this acid or to obtain a crystallisable salt. Many attempts were made to effect crystallisation, but in only one case was any success obtained, and then only a few crystals on the surface of the syrup were formed. Unfortunately, therefore, the nature of the acid has not been ascertained.

After looking for the source of this organic acid we again find reason to attribute it to the oil. We have already pointed out (p. 75) that the amount of lecithin formed is not sufficient to account for the disappearance of the whole of the oil of the seed, but that another fate awaits a considerable quantity. It was suggested in the former paper* that the acid of the germinating seed was derived from the oil by certain processes of oxidation, and served as the means of its utilisation. It is extremely unlikely that this acid is directly or indirectly connected with the sugars. We think we have here the explanation of the gradual diminution of the oil in the early stages of germination, and of the development of the coincident acidity. The acid reaction of the endosperm sets in before any change can be detected in the

* Green, *loc. cit.*, p. 385.

globoids of the aleurone grains and before any reaction for phosphoric acid is obtainable. The probability of an oxidation of the oil taking place in the early stages of germination has already been pointed out. This is now rendered still more probable by the discovery of an oxidase in the germinating seeds. On mixing a strained and filtered extract of the endosperms with a solution of hydroquinone, the colour of the latter speedily becomes pink and, later, red. The extract gives instantaneously a blue colour with an emulsion of guaiacum, and slowly turns a solution of pyrogallol purple. Boiling the extract destroys the power of setting up these changes. The oxidase adheres very tenaciously to the tissue of the endosperm, and it is very difficult to extract it completely.

Though the oxidase can be extracted and the extract found to act on such easily oxidisable bodies as those mentioned, no attempt has succeeded in making it oxidise ricinoleic acid outside the plant. This may, however, be due to non-attainment of the conditions which exist in the cells of the endosperms. Though its appearance is suggestive, it has not been proved that it plays a part in the oxidative processes of the fats, if the latter take place. The probability of such oxidative processes is considerable, for, in addition to the considerations just put forward, it should be remembered that one of us has shown that the formation of the acid is dependent upon the access of oxygen. In seeds germinated in its absence, though part of the oil was transformed, no acid soluble in water was formed.*

The problem is complicated by the fact that the distribution of the lipase, invertase, and oxidase of the germinating seed is practically the same.

Nutrition of the Embryo.

The sequence of changes which has, so far, been described, suggests a modification of the views now current as to the mode of utilisation of reserve materials in albuminous seeds. It has been commonly held that the efforts of the parent plant ceases with the deposition of reserve food in or near the embryo, in such a condition as to be easily used. Possibly, also, certainly in some cases, the parent is responsible for the provision of an enzyme to effect the change of the reserve food into a suitable condition for absorption. The utilisation is, however, attributed more or less fully to the embryo. In many cases the latter secretes the enzymes itself, and in others it is the active agent in absorption. The metabolic changes in the endosperm attributable to the parent are held to be more or less independent of each other, and to consist of the enzyme actions only, each enzyme fitting its appropriate food for absorption.

* Green, *loc. cit.*, p. 389.

This, as we have shown, is far from being the case with *Ricinus*. Here we have a series of most complex changes set up by the parent in the endosperm, accompanied by a renewed growth and revived secretory activity of the parent itself. The various constituents are made to act upon each other under the influence of the protoplasm of the endosperm cells, the latter showing a great increase in the amount of their protoplasm, while the protoplasm initiates a complex metabolism comparable in intensity with any which can be marked in the adult plant. It feeds itself, having prepared the food from the reserves; it secretes new products, which were represented but sparingly in the original cell-contents, thus preparing a new and completely representative food supply which it places at the disposal of the embryo. At the same time, however, the latter plays a considerable part in the scheme of nutrition, besides carrying out the processes of absorption.

A study of the distribution of the enzymes of the seed shows us that the preparation of food is not all carried out by the parent. The lipase was stated in the earlier paper* to originate in the endosperm cells and to continue to be developed there during the whole course of the germination. The invertase and the oxidase appear to have a distribution similar to that of the lipase. The trypsin, however, originates in the embryo.

In the course of the researches made by Mr. Biffen, which have already been referred to, he found that the epidermis of the young cotyledons contained cells, occurring at short intervals, which stained quite differently from the rest, and were full of granular contents. We prepared a large number of cotyledons from seeds in course of germination, taking them at an early stage when it was just possible to separate them cleanly from the endosperm. They were then washed carefully in warm distilled water till all organic matter was removed from their surfaces. Each cotyledon was then cut in half along the mid-rib. One set of halves was dipped for a moment in boiling water. The two sets were put into a solution of the globulin of the seeds prepared by dissolving it from the seed in 10-per-cent. solution of common salt and precipitating it by strong alcohol. The tubes containing them were put for a few hours into an incubator at 30° C. At

* A curious misstatement of what I said on this point in my earlier paper has been made by Connstein, Hoyer, and Wartenburg ('Ber. d. d. Chem. Ges.,' vol. 35 (1902), p. 3988), and recently repeated by Vierling ('Journ. Suisse de Chim. et Pharm.,' vol. 42, (1904), p. 391). I am represented as saying that the action of the lipase is stopped by the liberation of the acids in the endosperm. My paper contains no such statement. What I said was that if the enzyme was set to work *in vitro* in the presence of *dilute hydrochloric acid* it was rapidly destroyed. Reference to my paper will show that I regarded the organic acids formed in the endosperm helpful and not deleterious.—J. R. G.

the end of this time the uninjured epidermis had produced such a change in the globulin that the solution gave a vivid reaction for tryptophane on addition of a little chlorine water. The contents of the other tube were unchanged. The presence of trypsin in the cotyledonary epidermis was consequently proved. An extract of the cotyledons gave the same results. Taking these experiments in conjunction with Mr. Biffen's observations, there can be little or no doubt that the special cells alluded to secrete the trypsin.

These observations throw a light upon certain phenomena already alluded to, which were first recorded by Van Tieghem,* and subsequently corroborated by one of us.† Van Tieghem dissected the embryos out of seeds of Ricinus and exposed the endosperms on damp moss for some weeks to a temperature of 25 to 30° C. After several days of this exposure he found them growing considerably, and at the end of a month they had doubled their dimensions. The change was caused by the enlargement and partial separation of the constituent cells. In the interior of the cells he found the aleurone grains to be gradually dissolving, and the oily matter to be slowly diminishing. In the confirmatory experiments made by one of us the changes were found to be much more rapid when pieces of the cotyledons were left in contact with the endosperms than when the embryo was entirely removed. No satisfactory explanation of these phenomena was forthcoming at the time that they were observed, but the discovery that the tryptic enzyme is secreted by the cotyledons affords one. That a very slow germination takes place in the complete absence of the cotyledons may be explained by a small exudation of the enzyme from the latter before their removal or by the endosperm-cells themselves secreting a small quantity of it when the growth of the protoplasm is resumed during the early stages. The diffusion of the trypsin from the cotyledons into the tissue of the endosperm is exactly paralleled by the diffusion of diastase from the scutellum of the barley grain, described by Brown and Morris.‡

Conclusions.

The germination of the seed of Ricinus is shown by the experiments now recorded to be associated with a remarkable activity of the cells of the endosperm, which spring into renewed life and set up a very complex

* Van Tieghem, "Sur la Digestion d'Albumen," 'Comptes Rendus,' vol. 84 (1877), p. 578.

† *Loc. cit.*, p. 389.

‡ Brown and Morris, "On the Germination of some of the Gramineæ," 'Journ. Chem. Soc.,' vol. 57 (1890), p. 495.

metabolism. Their protoplasm grows and takes a prominent part in these metabolic changes, secreting enzymes, and setting up various chemical changes in the cells partly by means of the latter and partly independently of them. In this renewed activity the embryo also takes a share by contributing to the enzyme-formation. The result is the production of a great variety of nutritive material, partly the direct product of enzyme-action, partly produced by the secretory activity of the protoplasm and partly by the interaction of the products of the first two agents. Two varieties of sugar, lecithin, fatty acids, and the products of their oxidation, proteids, and the products of their digestion, including various crystalline nitrogenous bodies, amino- and amido-compounds at least are present. In this mass of nutritive material the embryo is plunged, and by the delicate epidermis of its cotyledons it absorbs, probably selectively, what it needs for its own growth. It is not easy to follow the process of absorption in detail, on account of the metabolism accompanying growth, which is very speedily set up in the cells of the embryo.

Analyses of the cotyledons show them to contain a varying quantity of lecithin, amounting in some cases to 1·36 per cent. of their dry weight. Both the sugars can be detected in them, the relative amounts, however, varying, but cane-sugar being usually present in largest quantity.

The reaction of the sap is acid, traces of phosphoric acid being mixed with an organic acid whose nature has not been ascertained. In fact, the transport of the nutritive substances to the embryo seems to be much the same in character as their transport in the tissue of the endosperm. Probably in both cases the presence of protoplasmic threads in the various cell-walls plays an important part in the matter; it seems at any rate probable that this agency is necessary to explain the transport of lecithin to the embryo. A very small quantity of lecithin can be dissolved in water or exist as a fine emulsion. It is improbable, however, that it can be transmitted through the cell-walls by dialysis alone. Dialysis no doubt plays a large part in the absorptive processes, especially where the crystalline substances are concerned.

The renewed metabolism in the endosperm-cells thus furnishes a mass of nutritive material on which both the endosperm-cells and the young embryo feed, and there seems to be no particular difference in the manner in which they are severally nourished.