XXXI. LIPOID METABOLISM IN THE MUNG BEAN DURING GERMINATION.

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FAT in the seed serves presumably as a reserve food supply for the development of the young seedling. That this is especially true in the case of oily seeds has been demonstrated by many investigators. Peters [1861] early discovered that fat is transformed into carbohydrate in the germination of the pumpkin seed. Miller [1910; 1912] has shown that a successive decrease in the percentage of fat (ether extract) is accompanied by increase in carbohydrate in the development of the sunflower seedling. Recently Pierce *et al.* [1933] by studying the chemical changes and Murlin [1933] by observing the R.Q. have shown that in the castor bean fat is consumed and carbohydrate formed in the course of germination.

The lipoids in starchy seeds however have received less attention. Le Clerc and Breazeale [1911] find that the crude fat (ether extract) of wheat seedlings increases during germination. Jordan and Chibnall [1933] have studied the transfer and transformation of phosphatides and glyceride fatty acids in the germination and growth of the runner bean. Malhotra [1933] has reported the changes in energy content and reserve constituents which take place in the development of the corn seedling. In the present investigation, we have undertaken to study the changes in different reserve food constituents taking place during the germination of a typical starchy seed, *viz.* the mung bean. This has included a determination of the distribution of the lipins at each stage, and also a determination of the solid and liquid fatty acids at different stages of germination.

EXPERIMENTAL.

Preparation of material for extraction.

50 kg. of mung bean (*Phaseolus aureus*) were purchased locally. The beans were free from worms and insects.

Exactly 1 kg. was employed for each germination experiment. The beans after weighing were thoroughly washed with tap water, followed by three washings with distilled water. The washed beans were then soaked in warm distilled water at 30° for about 16 hours. During this process the beans swelled, and in many cases the seed coats were broken. The germinating sprouts were placed in metal trays in the laboratory and covered with thick wet cloth; the temperature ranged from 20 to 23°. The sprouts were washed twice daily with distilled water.

When the sprouts were 5 days old, exactly $\frac{1}{3}$ was weighed out and heated at 100° in an electric oven for 1 hour, to inactivate the enzymes. The heated sprouts were then immediately separated into cotyledons (the endosperm), germinating portion (the embryo axis), and coats (the skin). The materials in each case were then dried at a temperature of 30–35°, ground to pass a 20-mesh sieve and preserved for later extraction.

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After 10 days, the second lot was collected by weighing out exactly $\frac{1}{3}$ of the remainder; this was treated in the same manner as above. The third portion, the fraction remaining, was allowed to germinate for a total of 15 days. In this way, each lot represented exactly $\frac{1}{3}$ of the original ungerminated seeds by weight, and consequently each lot contained approximately the same number of individual beans.

The above procedure was carried through five times, and the corresponding fractions of each lot were united. It was assumed that the seed coat did not alter in composition during the germination. All the seed coats therefore were united into one single sample, weighing 510 g.

Distribution of reserve constituents during germination.

An additional 900 g. of the mung bean were sprouted, treated as above and submitted to proximate chemical analysis for all constituents. In this way, material equivalent to 300 g. of the original mung bean for each of the 5-, 10- and 15-day germination periods was obtained for analysis and is so recorded in Table I, in which the results are all stated in terms of air dried material. The

Table I. *Distribution of reserve materials in cotyledon, germinating portion and coat at various intervals during the germination of mung bean.*

(Figures in parentheses indicate totals for entire seedling at each interval exclusive of the coat.)

	Dried weight g.	Nitrogen g.	Ether extract g.	Alcoholic extract g.	Ash g.
Original seed	900.0	32.2	8.91	36.9	24.9
5 days' seedling: Cotyledon	185.5	7.27	1.95	9.55	4.29
Germinating portion	33.0 (218.5)	1.65 (8.92)	1.07 (3.02)	12.04 (21.59)	2.12 (6.41)
10 days' seedling: Cotyledon	114.0	5.35	1.63	10.46	3.24
Germinating portion	81.5 (195.5)	4.42 (9.77)	2.49 (4.12)	34.63 (45.09)	3.90 (7.14)
15 days' seedling: Cotyledon	50.0	2.71	1.39	8.05	1.91
Germinating portion	115.5 (165.5)	6.84 (9.55)	3.43 (4.82)	43.80 (51.85)	5.08 (6.99)
Coat	90.5	1.59	0.63	3.88	1.96
Ungerminated seed	25.0	0.96	0.25	1.03	0.75
Total recovered	695.0	30.79	12.84	123.44	23.25

nitrogen content was determined by the usual Kjeldahl method. The extraction with ether was carried out in a Soxhlet apparatus for 24 hours, the extract being dried at 100° to constant weight, and this was followed immediately by extraction with alcohol in the same apparatus. The treatment with alcohol was continued till the extraction of the coloured substances in the sprout material was complete; this required from 30 to 72 hours. The ash was determined by igniting weighed samples to constant weight at a temperature of 400–500° in an electric furnace. The results in Table I have been recalculated to a percentage basis in Table II.

It will be noted from Table I that during germination there is a gradual transfer from the cotyledon to the germinating portion of all the constituents determined in the analysis. Under the conditions of growth imposed (absence of sunlight), the total amount of inorganic matter and of nitrogen in the plant as a whole remain practically constant for the entire 15-day period. The ether-soluble and alcohol-soluble fractions both increase in amount from stage to

Table II. *Proximate analysis of cotyledon, germinating portion and coat, at various intervals during the germination of mung bean.*

(In percentage of air-dried material.)

	Dried weight %	Nitrogen %	Ether extract %	Alcoholic extract %	Ash %
Original seed	100.0	3.54	0.99	4.10	2.77
5 days' seedling: Cotyledon	61.7	3.95	1.05	5.14	2.31
Germinating portion	11.0	5.00	3.25	36.50	6.48
10 days' seedling: Cotyledon	38.0	4.68	1.42	9.16	2.84
Germinating portion	27.2	5.43	3.06	42.51	4.82
15 days' seedling: Cotyledon	16.7	5.41	2.78	16.30	3.82
Germinating portion	38.5	5.77	2.97	37.92	4.44
Coat	10.1	1.77	0.70	4.28	2.17

stage; this seems to indicate a conversion of starch into fat and into soluble sugar. The ungerminated seeds comprised about 3 % of the original seeds, and their constituents are assumed to be the same as those of the original seed.

Analysis of the ether extract (lipoid fraction).

The method of analysis followed was essentially that of Chibnall and Sahai [1931], except that dried material only was used in the ether extractions as recommended by Jordan and Chibnall [1933]. The material was in each case first powdered and extracted with ether in the Soxhlet apparatus for 24 hours; the extracted material was dried in a vacuum oven at 40°, and the material thus obtained was reported as total ether extract. The extract was then redissolved in 1 vol. of ether (50 ml. ether for each 10 g. of the extract), and 3 vols. of acetone were added. A white precipitate of phosphatides and crude hydrocarbons was always obtained. This treatment was repeated using minimum volumes of ether and acetone. The amount of precipitate obtained was so small that it did not permit of further separation by hot acetone into phosphatides, waxes *etc.*

The extract obtained by the ether-acetone treatment, containing the glyceride fats, sterols *etc.* was evaporated nearly to dryness, dissolved in ether and diluted exactly to 100 ml. A 1 or 2 ml. portion of the ethereal solution was taken for sterol determination by the digitonin method of Jowett and Lawson [1931]; 2 ml. were used for the determination of iodine value by the Hanus method. The remainder of the solution was again evaporated to dryness and the total free fatty acids obtained from this residue by the usual saponification method, which was briefly as follows: the fats were saponified by refluxing for 2 hours with 50 ml. of 5 % KOH in alcohol, the alcohol evaporated and the soaps decomposed by adding 200 ml. of 3 % HCl and boiling for 10 min. The free fatty acids were separated in a separating funnel, and the small portion of free fatty acid remaining in solution was extracted with ether and combined with the main portion. The fatty acids were finally washed with distilled water until free from chloride.

The results of the analysis of the ether extract are shown in Table III. An increase in total fatty acids and in the sterols is noted in successive stages. The phosphatide fraction however shows a slight decrease. It may be noted that the figures for ether extract reported in Table I differ somewhat from those in Table III. This difference is essentially due to the temperature at which the

Table III. *Analysis of ether extract of mung bean seeds and germinating sprouts.*

(Expressed in g. per kg. of the original seed.)

	Dried weight	Ether extract	Phosphatides and crude hydrocarbons (including waxes)	Total fatty acids *	Sterols	I.V. of ether extract*
Original seed	1000.0	14.71	3.21	9.50	0.63	74.2
5 days' seedling:						
Cotyledon	603.2	6.15	0.23	4.52	0.44	93.6
Germinating portion	149.0	4.61	0.07	3.32	0.37	81.1
10 days' seedling:						
Cotyledon	442.0	6.21	0.12	4.50	0.48	85.0
Germinating portion	261.8	7.97	0.08	5.34	0.53	61.4
15 days' seedling:						
Cotyledon	272.1	6.22	0.14	4.28	0.52	87.7
Germinating portion	347.6	9.14	0.09	6.40	0.56	63.7
Coat	102.0	0.59	0.07	0.24	0.07	201.5

* The iodine value was determined on the ether extract after the phosphatides and crude hydrocarbons were removed.

material was dried; in the former case the extract was dried at 100° and in the latter case in a vacuum oven at 40°. The free fatty acids were submitted to further analysis as follows.

Determination of solid (saturated) and liquid (unsaturated) fatty acids in the total free fatty acids.

Twitchell's [1921] lead acetate method was used in the separation of liquid and solid soaps. Each soap fraction after being separated was decomposed into fatty acids by boiling in 3 % HCl and then separated from the aqueous solution by means of a separating funnel. The material was finally washed with water until free from acid (free from chloride), and dried to constant weight in an electric oven at 40°, under reduced pressure in an atmosphere of carbon dioxide. This determination was not performed on the seed coat fraction, since the total free fatty acid obtained was insufficient. The results are shown in Table IV.

Table IV. *Solid and liquid fatty acids in mung bean seeds and germinating sprouts.*

(Expressed in g. per kg. of the original seed.)

	Total fatty acids	Solid fatty acids	Liquid fatty acids	I.V. of solid fatty acids	I.V. of liquid fatty acids
Original seed	9.50	4.38	4.97	41.2	96.2
5 days' seedling: Cotyledon	4.52	2.19	2.31	56.5	152.3
Germinating portion	3.32	1.73	1.56	61.0	127.3
10 days' seedling: Cotyledon	4.50	2.34	2.14	55.8	143.7
Germinating portion	5.34	2.44	2.72	57.6	116.2
15 days' seedling: Cotyledon	4.28	2.21	2.05	58.4	151.7
Germinating portion	6.40	2.95	3.02	57.0	112.8
Coat	0.24	—	—	—	—

RESULTS AND DISCUSSION.

We have followed the method of Jordan and Chibnall [1933] in expressing results on a weight basis taking the original seed as a unit (Table I). This arrangement emphasises the significant changes taking place during the process of germination more effectively than if expressed on a percentage basis (Table II). 100 mung bean seeds weighed 3.5 g.; this means that the figures for the analysis in Tables I and II, based on 900 g. of mung bean seeds, represent a total of 26,000 individual plants.

In the whole germinating plant, as shown in Table I, there is no change in the nitrogen and ash contents in the three stages of germination. It is significant that these constituents are carefully conserved by the plant, so that during a period of 15 days, very little was lost, even though the sprouts were washed with an abundance of water twice a day during the entire germinating period. The total dry weight of the plant decreased while the amounts of ether extract and alcoholic extract increased. This is the usual situation when carbohydrates are consumed in respiration and starch and proteins transformed into soluble amino-acids and soluble sugars. The distribution of constituents between cotyledon and germinating portion in succeeding stages demonstrates how the cotyledon functions as a storage organ for the germination of the plant. Le Clerc and Breazeale [1911] have also shown in the germination of another typical starchy seed, wheat, that the ether extract of the whole seedling is increased and the nitrogen, fibre and other constituents translocated from the seed residue into the axis.

Beumer [1933] found that sterols were synthesised during the germination of beans kept in the dark, but that they were utilised when the germination was carried out under sunlight. Our figures for the sterol fraction in Table III show a gradual increase at successive stages, which would indicate that sterols are synthesised both in cotyledon and germinating portion, when the beans are sprouted in the dark. Our observation that the phosphatide fraction is utilised by the plant during germination accords with the results of Jordan and Chibnall [1933] in experiments on the germinating runner bean.

Whilst the fat fraction in the starchy seed does not function as an energy store, there are changes in the fats and fatty acids which are significant. In the first place it is noted that the fat, whether estimated as ether extract or as total fatty acids, decreases very considerably during the first 5 days' germination, but this is followed by successive increases in the later stages of germination. It appears that in the early stages the fat fraction either serves in part as an energy reserve or takes part in a sudden tissue breakdown which accompanies the growth impulse. It is evident however that for the most part fat exists in the starchy seed as *l'élément constant* [Terroine, 1919] in contrast to *l'élément variable*. It is noted that in the cotyledon the amount of fat, evidently tissue fat, remains practically constant (Table IV), whilst an increase of fat, evidently the synthesis and the building up of tissue fat, takes place in the germinating portion. In the germination of fatty seeds, Miller [1910; 1912] has shown a similar decrease in the ether extract for the first 5 days of germination in the hypocotyl and root portion, which is followed by increases in the later periods. The i.v. determinations give further evidence of this apparent reversal from fat breakdown to fat synthesis after the first stage in germination, and, as seen in Tables III and IV, the greatest change in the i.v. takes place in the germinating portion.

Table IV shows that the transfer of liquid (unsaturated) fats from the cotyledon into the germinating portion, or their synthesis, is more rapid than that of solid (saturated) fats. The iodine values indicate a rapid increase in the degree of saturation of the liquid fat; in other words the process of germination appears to be a reducing process. The increase in degree of saturation during germination, observed in these experiments on a starchy seed, accords with the observations of Miller [1910; 1912] and of Komatsu and Okada [1933] on oily seeds (sunflower and soy bean).

The analysis of the seed coat, shown in Table III, indicates that the fatty constituents in this portion of the plant are small in amount. It is evident however that the phosphatides and crude hydrocarbon fraction of the ether extract represents a relatively more important part of the seed coat than of the other portions of the plant. A portion of this fraction was treated with hot acetone and found to be completely soluble; that is, the mixture most probably consists of waxes. These waxes apparently serve an important part in the protecting mechanism of the seed coat. It is of note that the fatty substances of the seed coat show a very high i.v.

SUMMARY.

The mung bean has been studied as an example of a starchy seed and changes in the lipoids have been examined for three 5-day intervals during germination in the absence of sunlight.

The amounts of the ethereal and alcoholic extracts of the whole mung bean sprout increase during the successive stages, whilst the total nitrogen and ash contents remain constant. At the same time, the principal constituents of the cotyledon are gradually transferred to the germinating portion of the plant.

There is an increase in the sterol fraction and a decrease in crude phosphatides during germination. The total fatty acids decrease in the early stages and increase again in the later stages. The fatty acid content of the growing portion of the plant shows a pronounced increase whilst that of the cotyledon remains practically constant. The degree of saturation of the fatty acid in the sprouting portion also shows a decided increase, as indicated by decreasing i.v.

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