THE CONVERSION OF FAT TO CARBOHYDRATE IN THE GERMINATING CASTOR BEAN

III. The Chemical Analysis, and Correlation with Respiratory Exchange

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In considering the conversion of fat to carbohydrate in the germinating castor bean, two phases of the problem have been discussed in the preceding papers. The results of chemical analyses made on the beans at various stages of their germination will be presented here. The changes in the fat stores of seeds during germination have been studied by numerous investigators who used many different seeds. Hellriegel (1), Leclerc du Sablon (2), Green (3), Green and Jackson (4), Maquenne (5), Deleano (6), Miller (7–8), Mazé (9) and many others have investigated the transformation of oil into starch or sugar during germination. For a more detailed bibliography covering this phase of plant chemistry, the reader is referred to the book by Miller (10) and the articles by Deleano (6) and von Fürth (11). All of the above investigators observed that sugar was formed in increasing amounts as the oil disappeared in germinating fatty seeds, and several noted that the sugar increased up to a certain point and then decreased along with the oil.

The increase in the carbohydrate content in the seedlings of oily seeds necessarily raises the question as to its orgin. Miller (7) working with sunflower seeds found that changes in the protein reserve could not account for the carbohydrate formed. We have observed that there is a slight increase in amino nitrogen in castor beans during their germination, but this increase is exceedingly small, less than 1 per cent. As there is an exceptionally small amount of preformed sugar in ungerminated castor beans it seems likely that the oil must serve as the source of carbohydrate. Miller (10) notes that no one has found free glycerol in germinating seeds or seedlings. However, even if all of the glycerol from the fat which disappeared were utilized for building of carbohydrate, it would not be

sufficient to account for that formed. A simple calculation will be presented below in proof of this. Thus it seems evident that the fatty acid fraction must be utilized in carbohydrate production. This statement is substantiated by the investigations of Leclerc du Sablon (12), Maquenne (5), and Ivanow (13) but von Fürth (11) does not agree with their conclusions, or those of Green (3) whose work will be mentioned later. Von Fürth worked with seedlings with a root length (hypocotyl?) of about 40 mm. We will show later that marked changes in the oil and sugar content did not take place until the hypocotyls were over 35–40 mm. in length. Furthermore von Fürth (11) felt that Müntz (14) had no right to conclude that the fatty acids of germinating seeds were converted into oxy-acids since the acetyl value of the oil extracted by von Fürth did not rise during germination. Miller (8) also found no change in the acetyl value of the oil obtained on extraction of germinated seeds. Green (3) isolated a crystalline acid of unknown structure from germinating castor seeds and believed that this might be an intermediary product in the conversion of the oil to sugar. Von Fürth (11) was unable to confirm Green's work, and expressed the belief that if volatile acids, alcohol, acetone, or aldehydes were intermediates between fat and carbohydrate, the amount present at any given time might well be so small as to be undetectable. Deleano (6) realized that many different organic acids could be formed during germination, but he was able to isolate only two, lactic and acetic. Recently Pirschle (15) reported having found considerable amounts of acetaldehyde in germinating fatty seeds, and he believed that this might be the possible intermediate in the process under discussion.

The question as to whether fat is converted directly to starch, glucose, or dextrins (16, 17) or whether numerous substances are formed as intermediates, cannot be discussed profitably at present.

EXPERIMENTAL

Sound castor beans (Brazil seeds) of different sizes and colors were germinated in various ways to determine the best means of procuring successful germination without mould. The most successful method proved to be the following: The selected beans were dipped momentarily into a 1:1000 mercuric chloride solution to destroy mould spores and then introduced into sterilized moist chambers, being placed upon filter paper over sterile cotton, and on top of them was laid another layer of filter paper and cotton. The dish and cotton were sterilized in an oven at 100°C. for 24 hours. The filter papers were moistened with sterile distilled water and the dishes set away in a dark place at room temperature. Water was added as required. Success in preventing mould depends not so much on securing perfect sterility as on prevention of too much moisture in contact with the beans.

The rate of growth not being entirely uniform, the time required for the hypocotyl to reach the length desired was variable. A length of 100 mm. was attained on the average in about 2 weeks, and usually no side branches appeared until the hypocotyl was approximately 35 mm. long. The length of the hypocotyl was selected as a measure of the degree of development rather than the days of

germination, in accordance with the experience of Leclerc du Sablon (2), and Deleano (6).

The seedlings were removed from the moist chambers when the hypocotyls reached the desired lengths, the seed coat and sheath pulled off, and the germinating structure dried for about 24 hours at 90°C. Some investigators (6, 18) have recommended drying for a longer time at lower temperatures to prevent changes in the composition of the oil, but soluble carbohydrates might be lost by this slow drying due to respiration of the seed or fermentation brought about by moulds and bacteria. Von Fürth (11) too, has shown that the iodine number of the oil extracted from seedlings dried at 90° for some time has approximately the same value as that of oil extracted from ungerminated seeds. When the plant material was practically dry it was prepared for analysis by grinding into a fine paste or powder, depending upon the amount of oil present.

In a second series of experiments castor beans were dipped in 0.8 to 2 per cent formaldehyde, rinsed in sterile distilled water, and allowed to germinate in the dark. When the hypocotyls were a few millimeters long (see Nos. 7 and 8, Table IV) these seedlings were placed on moist cotton at the bottom of 3 to 8 liter bottles and these were then closed tightly to prevent the loss or entrance of air. The rubber stopper was provided with two glass tubes closed externally by means of rubber tubing and screw clamps, also with a small manometer filled with mercury for recording pressure change in the bottle. The bottles and seedlings were set away in a dark cupboard and after a period of time—the number of days varied—the bottles were removed, a portion of the contained gas being transferred to sampling tubes and analyzed in a Haldane gas analyzer. Ungerminated seeds at times were confined in the same manner. The chief difficulty in these experiments lay in the fact that not all of the seeds germinated or continued to grow. After the gas samples were taken, all the beans or seedlings were removed, dried, ground, and analyzed as set forth in the following paragraphs, analyses being made for water, ash, ether extract, protein, crude fiber, total reducing matter as invert sugar, and in the later experiments glucose.

Moisture was determined by placing samples in shallow evaporating dishes and drying to constant weight at 100°C. These same samples were then ashed in a muffle at dull red heat until the ash assumed a grayish white cast. For ether extract samples of the dried material were weighed by difference into double thickness extraction thimbles and extracted with dry ethyl ether in Soxhlet extractors for 16 hours. In several analyses, samples were extracted with petrolic ether, and the percentage of fat obtained corresponded almost exactly with that obtained with ethyl ether. In one analysis the sample was extracted with both ethyl and petrolic ethers and in the ether extract nitrogen was determined. This amounted to approximately 0.1 per cent, showing that very little nitrogenous matter was removed during two 16 hour extraction periods.

Nitrogen was determined by the Kjeldahl method, the factor 6.25 being used to convert nitrogen to protein. To determine reducing matter a weighed sample of

the material was boiled with water for 30 minutes and filtered into a 250 cc. volumetric flask, the residue being washed with hot water. The contents of the flask were cooled and made to 250 cc. 50 cc. portions were removed and put in each of two beakers. To the contents of one beaker, 1 cc. of concentrated HCl was added and the solution heated on a water bath for 15 minutes to hydrolyze the sugar. This was then neutralized with strong NaOH and a drop of HCl added to discharge the pink color of phenolphthalein. The total reducing matter was then determined by the Bertrand method. The reducing substances in the unhydrolyzed 50 cc. portion were determined directly by Bertrand's method. The cubic centimeters of $KMnO_4$ used here were subtracted from the cubic centimeters used for the total reducing matter and the difference calculated as glucose. Zinc sulfate added to the original solution or to the solution after hydrolysis had no effect upon the final results. Repeated boiling of the fat-rich samples failed to yield any additional quantity of sugar. Crude fiber was determined according to the method of the Association of Official Agricultural Chemists (19).

DISCUSSION OF RESULTS

The results of the chemical analysis of beans having hypocotyls from 5 to 100 mm. are presented in Table I. All values are averages of several analyses of beans which had been germinated at different times during the year. The percentage of fat shows a steady decline, the most rapid fall occurring during the period when the hypocotyls are between 35 and 45 mm. in length. Deleano (6) also observed the fact that chemical changes were at a maximum during this stage of germination. As the fat decreases, we notice a marked rise in the reducing matter calculated as invert sugar. There is very little reducing matter in the unhydrolyzed water extracts until the length of the hypocotyl is 35 mm. or more. The crude fiber shows a rather irregular series of values, but there is a definite tendency toward a rise as one would expect. Certainly the accuracy of the method is not of the highest order. Some of the variations may have been due to irregularity in the development of the root system, for some lots of seedlings had far more roots than others. The nitrogen content of the germinated beans was fairly constant, although there is a noticeable rise as germination progresses. This apparent increase in nitrogen content is set forth more clearly in the results shown in Table II. The amino nitrogen content is small, usually less than 1 per cent until the later stages of germination are reached. Loss of a volatile substance during drying would explain this rise in the amount of nitrogen

present. The percentage of ash in the seedlings at various times during their growth is fairly constant. There is a gradual increase in the undetermined matter the longer the period of germination.

TABLE I

Chemical Composition of Germinating Castor Beans

Stage	Length hypocotyl	Ether extract	Total reducing matter as invert sugar	Glucose	Crude fiber	N × 6.25	Ash	Total	No. of lots of beans analyzed
	mm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
0	Ungerminated	67.85	1.18		1.87	25.72	2.61	99.01	5
1	5–10	63.74	1.62		2.62	26.32	2.63	96.93	2
2	10–20	61.55	6.02		3.23	24.26	2.57	97.64	3
3	20–35	57.40	6.88		2.37	25.86	2.48	98.62	4
4	35–45	45.30	15.68		4.02	26.51	2.77	93.33	3
5	45–60	38.03	17.26		7.01	27.48	2.43	91.69	2
6	60–80	32.58	25.84	4.47	4.08	26.84	2.86	92.19	3
7	80–100	25.28	26.63	6.32	5.01	27.26	3.04	87.21	2
8	Above 100								

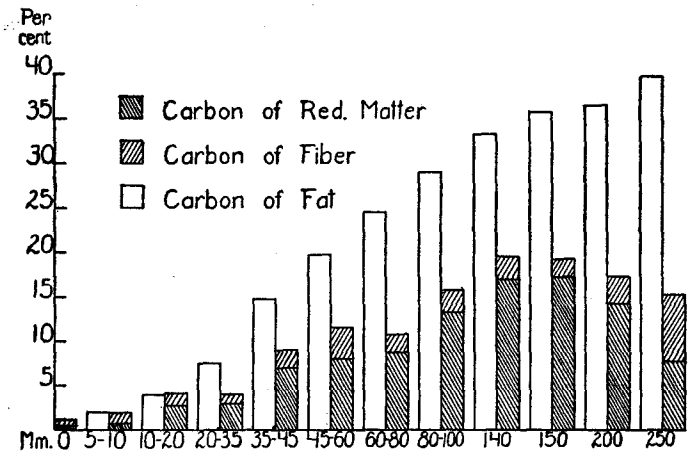

CHART 1. Carbon of fat disappearing and of carbohydrate appearing—in percentage.

In Chart I the percentage of carbon of the fat disappearing and of the reducing matter and crude fiber appearing has been calculated using the data in Table I. The fat carbon was calculated from the

content in ricinolein, the carbon in the reducing matter from the content in sucrose, and in that of fiber, from the formula $C_6H_{10}O_5$. Due to the fact that we were unable to obtain 100 per cent of the constituents present in the various lots of castor beans, the fat, reducing matter, and fiber were converted to terms of 100 per cent to permit a satisfactory comparison of the results. Always the fat disappearing was determined by subtracting the percentage of fat present at each stage of germination from that present in the ungerminated seeds, corrected values, of course, being substituted for those shown in Table I. From the data in this chart it is seen that in the first two stages of germination the carbon of the fat disappearing and of the reducing matter and crude fiber appearing practically balance each other. Growth at

TABLE II

Chemical Composition of Castor Beans in More Advanced Stages of Germination

Length hypocotyl and roots	Ether extract	Total reducing matter as invert sugar	Glucose	Crude fiber	N × 6.25	Ash	Total
mm.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
100–120	34.73	23.21	5.46	3.77	27.52	2.81	92.04
80–140	22.53	39.39	—	5.47	27.32	2.79	97.50
100–150	17.36	35.68	18.45	4.30	27.11	3.02	87.47
150–200	15.21	26.93	10.55	5.20	29.32	2.88	79.54
175–250	11.97	14.28	10.75	12.89	34.68	4.42	78.24

this stage is slow, so that respiration is not as great here (see Paper I) as at some of the later periods in the development of the seedling. After these stages just mentioned, the carbon of the fat disappearing is always much greater than the carbon of the reducing substances and fiber. Much of the remaining carbon can be accounted for as that of the respiration, as will be pointed out in the discussion of the results in Tables III and IV.

In Table II, the results of the analyses on seedlings, which had been permitted to germinate for a longer period than those shown in Table I, are given. These beans were kept in the dark constantly. The percentage of fat in the latest stages has now fallen to a low value. The total reducing matter of the beans with hypocotyls 80 to 140 mm.

in length calculated as invert sugar reaches a peak at approximately 40 per cent, then falls along with the fat. The reducing matter obtained prior to hydrolysis and calculated as glucose also reaches a maximum at 100 to 150 mm. length of hypocotyl and then decreases in amount. The crude fiber increases materially as would be expected.

TABLE III

Composition of Castor Beans at Beginning and End of Respiration Period

Lot No.		Ash	Ether extract	Reducing substance as invert sugar	N × 6.25	Crude fiber	Total	Undetermined
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
7	Beginning	2.31	68.48	1.21	26.75	1.08	99.83	0.17
	End	2.40	63.46	2.12	24.63	2.28	94.89	5.11
	Difference	+0.09	−5.02	+0.91	−2.12	+1.20		+4.94
8	Beginning	2.58	60.27	3.78	26.77	5.16	98.56	1.44
	End	2.61	56.00	6.38	24.68	2.97	92.65	7.35
	Difference	+0.03	−4.27	+2.60	−2.09	−2.19		+5.91
11	Beginning	2.61	67.85	1.18	25.72	1.87	99.01	0.99
	End	2.58	62.93	2.38	27.99	1.23	97.11	2.89
	Difference	−0.03	−4.92	+1.20	+2.27	−0.64		+1.90
12	Beginning	2.61	67.85	1.18	25.72	1.87	99.01	0.99
	End	2.67	63.94	2.82	29.62	1.35	100.40	
	Difference	+0.06	−3.91	+1.64	+3.90	−0.52		
13	Beginning	2.61	67.85	1.18	25.72	1.87	99.01	0.99
	End	2.38	67.72	2.60	23.63	1.24	97.17	2.83
	Difference	−0.23	−0.13	+1.42	−2.09	−0.63		+1.84
17	Beginning	2.59	57.34	10.71	24.51	1.72	96.87	3.13
	End	2.68	44.73	18.86	26.32	2.62	95.21	4.79
	Difference	+0.09	−12.61	+8.15	+1.81	+0.90		+1.66

The significance of the values given for protein is questionable as there is no certainty that the factor 6.25 is correct at all stages of the development. The ash in these beans also remains fairly constant and agrees with that of the younger stage. There is a still greater increase in the undetermined fraction.

The results as shown in Table III were obtained by analyzing beans

which had been confined for various lengths of time in closed bottles as previously described. The purpose of this part of the study was to compare directly the nature of the respiratory exchange with the nature of the chemical changes as shown by analysis. It was the hope indeed to make a complete carbon and oxygen balance for the time during which the beans were confined by taking account of the composition at the beginning and end of the respiration period and the respiratory exchange itself. This hope was frustrated in part by the independent discovery of an undeterminable (by ordinary methods of proximate analysis) remainder which increased with age, in part by failure to secure 100 per cent germination or growth of the beans confined as described, and in part by the inexactness of the crude fiber method of the A. O. A. C. as applied to the material of castor beans. Nevertheless it is instructive to lay the chemical analysis side by side with the respiration figures *on the same beans*.

It is evident that the values given for the "End" in Table III cannot be put down as describing the composition of any given stage. They represent rather the mixed composition of beans which had grown unequally and some not at all. They are none the less correct analyses within the limits of the methods used.

By comparing the actual composition at the end with the average analyses of corresponding stages when the beans were confined to the respiration bottles, a difference in content is obtained which, combined with a certain amount of combustion, should account for the respiratory exchange. This obviously cannot be expected to give a perfect accounting until the nature of the undetermined material is found.

Table IV gives the respiratory exchange as found in the same lots of beans as shown in Table III. The bottles in all cases were filled with outdoor air by suction and filtration through a cotton plug, placed in the inlet tube, immediately after the beans were introduced. Hence the composition of the air at the end compared with the composition of outdoor air gave the respiratory exchange of the beans.

The respiratory quotients in all cases were between 0.48 and 0.58 and were therefore in good agreement with those shown in Table VII of Paper I, and obtained by the same method.

The changes in composition (Table III) which are characteristic in all of these cases are similar in kind, though not in amount, to those

shown in Tables I and II; namely, a decrease in the percentage of ether extract, an increase in the percentage of reducing substance, and an increase of the undetermined residue. The changes in ash are negligible; those in the protein (N × 6.25) are variable, some showing an increase, others a decrease. The changes in crude fiber, which were variable in Tables I and II, though usually increasing with age, are in these special lots exceedingly variable. In four of the six lots it decreased, which we can only attribute to the fact that some of the beans, as previously mentioned, failed to germinate or to grow normally.[1]

TABLE IV

Representative Respiratory Metabolism of Several Lots of Castor Beans Whose Final Composition is Given in Table III

Experiment No.	No. of days in bottle	No. of beans	Stage in mm. length of hypocotyl		CO_2 produced	O_2 absorbed	R.Q.
			At beginning	At end			
					cc.	*cc.*	
7	3	24	1–5	Average 13.1	208	386	0.53
8	3	24	Average 15.6	" 29	326	628	0.52
11	5	21	Ungerminated	Ungerminated to 30	322	552	0.58
12	7	15	"	" " 40	302	524	0.58
13	5	20	"	" " 45	270	462	0.58
17	3	30	20–35	45–90	676	1419	0.476

The O_2 content of the bottles at the end of the respiration period in all the lots showing a decrease of crude fiber was quite low (from 0.12 per cent in No. 11 to 3.8 per cent in No. 13) but there is no parallelism between this final percentage and the decrease in crude fiber. In Lots 7 and 17, where the crude fiber increased, the percentage of O_2 at the end was relatively high (8.7 per cent in No. 7 and 5.4 per cent in No. 17).

[1] In some other lots which had to be discarded beans were found which not only failed to germinate but degenerated, and became quite soft. It is possible that some of the ungerminated beans in the lots analyzed (Nos. 11, 12, and 13) also degenerated slightly, though they were not perceptibly softened. Softening might be due to a fermentation of the cellulose contained. The fiber therefore would escape the method of straining through fine linen used by the Association of Official Agricultural Chemists. This is only surmise.

Notwithstanding the very low final oxygens in the bottles where this occurred, most of the new growths were perfectly normal in appearance when the beans were removed and there was no odor of spoilage. Exceptionally the end of a rootlet or the point of junction of the rootlet with the hypocotyl would be darkened. It is not believed that these slight changes could alter the chemical composition or the respiratory activity.

Table V presents the results of an attempt to balance the carbon of the fat and protein (where this decreased) disappearing against the carbon of the determined amounts of invert sugar (all of the sugar being

TABLE V

Trial C Balance

Experiment No.	Lost		Gained			Excess of C loss over gain	Undetermined substance	Per cent C in undetermined substance
	C of fat	C of protein	C of invert sugar	C of crude fiber	C of respiration			
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
7	0.2225	0.0657	0.0305	0.0219	0.1117	0.1241	0.2833	43 per cent (cellulose 44.4 per cent)
8	0.1813	0.0606	0.0600	−0.0533	0.1751	0.0601	0.3241	18.5 per cent
11	0.1778	—	0.0236	−0.0132	0.1730	−0.0056	0.0887	Too much CO_2 (from fermentation?)
12	0.0980	—	0.0223	−0.0075	0.1623	−0.0790	—	" "
13	0.0039	0.0414	0.0232	−0.0108	0.1451	−0.1132	0.0713	" "
17	0.897	—	0.3156	0.0268	0.3633	0.1813	0.1527	(No fermentation possible)

so expressed) and crude fiber formed and the C of CO_2 produced. To be perfectly fair to the experiment, the C of crude fiber has been deducted where the analysis shows a decrease. The attempt is not very successful and for this reason the balance is called a "trial balance." It is instructive only as showing the points at which the technique must be improved in future studies of this character. In Experiment 7 (first of the table), the carbon of fat and protein disappearing exceeds the carbon of sugar and crude fiber formed and of CO_2 produced, by a substantial amount. The undetermined substance indicated by the analysis as having been formed comes to 0.2833 gm., and, dividing the excess carbon by this weight, an indicated percentage

for the undetermined substance of 43 is obtained, which suggests a substance of about the composition of cellulose (44.4 per cent). In Experiment 8 it is possible also to calculate an excess by deducting the C of crude fiber, which decreased, instead of adding it to the right hand side of the balance; but the indicated percentage of carbon for the undetermined matter is only 18.5, which does not agree with the C content of any possible intermediary substance. Without deduction of the C of crude fiber the carbons of the two sides almost balance; but this leaves none for the undetermined residue. The next three experiments all show an excess on the side of C compounds formed, which we can only attribute to production of too much CO_2 by fermentation in the beans which failed to germinate, or having germinated, failed to grow. Experiment 17 gives a poor balance on this basis, but as will be seen below, it agrees with No. 7 on the basis of the ratio of C to O_2.

To account for the R.Q. found in Experiment 7 we may proceed as follows: We may imagine cane sugar to be formed from the ricinoleic acid by the reaction $2C_{18}H_{34}O_3 + 14\,O_2 = 3\,C_{12}H_{22}O_{11} + H_2O$. The amount of C found in the invert sugar was 0.0305 gm. (Table V). This would require (448:432::x:0.0305) 0.0316 gm. O_2. Likewise we may assume that crude fiber (cellulose) is formed by the reaction $C_{18}H_{34}O_3 + 7\,O_2 = 3\,C_6H_{10}O_5 + 2\,H_2O$. The amount of C in the fiber formed was 0.0219 gm. This would require 0.0227 gm. O_2. The C of the respiration found was 0.1117 gm. If this all came from the fatty acid by the reaction $3\,C_{18}H_{34}O_3 + 75\,O_2 = 54\,CO_2 + 51\,H_2O$ it would require 0.414 gm. O_2 and would produce 0.4095 gm. CO_2, which itself would give the R.Q. $0.4095/0.414 \times 8/11 = 0.719$. Adding the oxygen necessary for formation of the sugar and cellulose we get a total of $(0.414 + 0.0316 + 0.0227 = 0.4683$ gm. and this would give an R.Q. of $0.4095/0.4683 \times 8/11 = 0.636$. Since the R.Q. found was 0.53, we may be certain either that some sugar was oxidized to give the CO_2 (C) found in the air or that oxygen was used also for production of the undetermined substance. There remains of the C of fat which disappeared $0.2225 - (0.1117 + 0.0305 + 0.0219) = 0.0584$ gm. and to give an R.Q. of 0.53 with the CO_2 found we require in addition to the O_2 already accounted for 0.0936 gm. Therefore it is not unreasonable to suppose this amount of oxygen has combined with

0.0584 gm. C to produce the undetermined substance, which is somewhat suggestive of arabonic acid, an oxidation product of arabinose. It is interesting to find that if sugar and cellulose formation and combustion of fat were to take place in the proportions of the reactions postulated above, *i.e.* 2 mols of $C_{18}H_{34}O_3$ to cane sugar, 1 mol to cellulose, and 3 mols for combustion, we should have 54 mols CO_2 produced and 96 O_2 absorbed which would give an R.Q. of 0.562.

If C from protein in slight amount were used to produce either cane sugar or cellulose as assumed in the trial carbon balance of Table V, the argument would not be altered and the R.Q. would not be greatly affected. If all the glycerol of the ricinolein were transformed to sugar instead of being oxidized the quotient would be diminished as indicated below.

If the entire fat molecule were oxidized, we should get:

$$C_3H_5(C_{18}H_{33}O_3)_3 + 78.5\ O_2 = 57\ CO_2 + 52\ H_2O$$

$$\frac{57\ CO_2}{78.5\ O_2} = 0.726\ \text{R.Q.}$$

But if the fat were first hydrolyzed, the fatty acid only oxidized, and the glycerol converted to glucose, we should have:

$$3\ (C_{18}H_{34}O_2) + 75\ O_2 = 54\ CO_2 + 51\ H_2O$$

$$2\ C_3H_8O_3 + O_2 = C_6H_{12}O_6 + 2\ H_2O$$

$$\frac{54\ CO_2}{75\ O_2 + 0.5\ O_2} = 0.715\ \text{R.Q.}$$

In the case of Experiment 17 we get the following carbon balance

C of fat disappearing		0.897 gm.
C " sugar formed	0.3156 gm.	
C " fiber "	0.0268 "	
C " respiration	0.3633 "	0.7057
Excess C		0.1913 gm.

Computing the O_2 necessary for formation of sugar by the reaction

$$\underset{\text{ricinoleic acid}}{2C_{18}H_{34}O_3} + 14\ O_2 = \underset{\text{cane sugar}}{3C_{12}H_{22}O_{11}} + H_2O$$

as before, we get 0.3273 gm. and for formation of crude fiber by the reaction

$$C_{18}H_{34}O_3 + 7O_2 = 3\ C_6H_{10}O_5 + 2\ H_2O$$

we get 0.0278 gm.

The oxygen necessary to form the CO_2 of the respiration (1.332 gm.) by regular combustion of the ricinolein would be 1.334 gm. with the regular R.Q. of 0.726. If we add the oxygen necessary to form the sugar and cellulose (crude fiber) found (see above) we get a total of 1.689 gm. O_2 which with the CO_2 found in the respiration bottle would give an R.Q. of $1.332/1.689 \times 8/11 = 0.574$. The actual R.Q., however, was 0.476 and with 1.332 gm. CO_2 this would require a total of 2.035 gm.[2] O_2 or a surplus of 0.346 gm. which presumably has combined with the excess carbon of 0.1913 gm. to form the undetermined substance. The proportions are again suggestive of an oxidation product of pentose.

The evidence adduced from these calculations and correlations is not at all conclusive for the chemical nature of the undetermined residue. It is presented merely as indicative of a direction in which to apply further chemical search.

Returning to the analytical results we call attention to the fairly close agreement with the analyses of Maquenne (5) and Deleano (6) also on the castor bean, and especially to their original discovery of the increasing percentage of undeterminable residue which we have confirmed. The significance of this residue in the problem of conversion of fat to carbohydrate they apparently did not appreciate. We have not been able to confirm the presence of lactic acid by Uffelmann's reagent or the thiophene reaction. No starch has been found, except in the later stages studied and then only in the hypocotyl and rootlets.

The chemistry of the germinating fatty seed is far from complete and until much more is known about it the details of the transformation of fat to carbohydrate cannot be filled in. Of the occurrence of this transformation in the castor bean, however, we no longer entertain any doubt.

SUMMARY AND CONCLUSIONS

1. Analyses for fat (ether extract), protein ($N \times 6.25$), sugar including glucose, crude fiber, and ash have been made on all stages of the germinating castor bean up to 250 mm. length of hypocotyl and root system.

[2] This is very close to the total actually found by analysis of the air.

2. There is a continual decrease in the amount of fat present in the whole germinating seedling, and a continual increase in the amount of sugar up to about 40 per cent (dry weight) at a hypocotyl length of 80 to 140 mm., after which it decreases as crude fiber (cellulose) increases. The most rapid decrease in fat content coincides roughly with the most rapid increase of sugar.

3. The carbon balance between fat loss and carbohydrate (including fiber) gain is not at all close, except at the very beginning of growth. An undetermined residue occurs, which increases steadily along with the total carbohydrate and accounts for more and more of the carbon.

4. The protein content which in the ungerminated bean is about 26 per cent, at first falls a little and then rather steadily increases to reach nearly 35 per cent (dry) at the last stages studied. The most plausible explanation of this is the occurrence of more and more volatile substance which is lost in drying.

5. The ash increases irregularly but in the end shows about the same ratio of increase as the protein.

6. Respiration studies on several lots of these beans at different stages of germination exhibited the same low respiratory quotients as reported in Paper I. Comparing their composition at the end of the respiration period with that of corresponding stages when the period began, the chemical change can be compared with the respiratory exchange.

7. A trial balance of all the carbon changes including the respiratory carbon and protein carbon is not very satisfactory, because of our ignorance of the undetermined residue.

8. The respiratory quotient found can be accounted for quite satisfactorily on the assumption that two out of six molecules of ricinoleic acid are converted to cane sugar, one to cellulose, and three are oxidized.

9. The oxygen needed to produce the quantities of known carbohydrate found, added to that used for combustion, and the total subtracted from the observed loss from the respired air yields in two experiments a quantity which, combined with the excess carbon, suggests that the undetermined substance may be an oxidation product of pentose.

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