THE CONVERSION OF FAT TO CARBOHYDRATE IN THE GERMINATING CASTOR BEAN

I. The Respiratory Metabolism

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The possible conversion of fat to carbohydrate in the mammalian body has been one of the most hotly contested problems in physiology. Nobody has yet brought forward evidence so convincing that his opponents on the other side of the argument have been compelled to yield ground. Very much of the so called evidence is worthless. It was with this conviction that the present writer persuaded certain of his pupils to take up the question and attempt to produce evidence which would be completely convincing, one way or the other. Three papers (1–3) already published have, it is believed, produced good evidence supporting the viewpoint of the late Professor Lusk, namely, that this conversion in the mammalian organism is at least extremely difficult and, under the conditions studied, not demonstrable.

From a careful reading of the literature on the germination of the fatty seeds, it appeared that in this instance the evidence, so far as it has been developed, favors the conception that fat is converted to carbohydrate (sugar) for the obvious purpose of increased diffusibility. At all events, this has been the interpretation of botanical physiologists. Since two lines of proof for conversion in the organs of the dog (and cat) had failed so signally under critical examination (2, 3) the writer wondered whether the evidence for the fatty seeds could really be so convincing as it seemed. It was with this attitude of skepticism that the present study was undertaken.

The problem has been approached from three directions: (1) the significance of the respiratory quotient during normal germination;

(2) the changing composition of the seed as shown by combustion; and (3) the nature of the chemical changes. Only the first division of the subject is presented in this paper; the others follow immediately in this issue.

HISTORICAL

A brief sketch of the historical background must suffice, as the subject has been adequately reviewed in several places (4–6). The original observation that in the germination of fatty seeds the volume of oxygen absorbed exceeds considerably that of the carbon dioxide given off, was made by de Saussure (7) and this observation gained special significance through the microchemical work of Sachs (8) confirmed by Schmidt (9) and the chemical analyses of Peters (10) and of Detmer (11) —all demonstrating that during the most active stage of germination fat disappears rapidly and carbohydrate takes its place. It appeared therefore, as Detmer and Godlewski (12) clearly recognized, that oxygen was being used for the production of carbohydrate, an oxygen-rich substance, from fat, an oxygen-poor substance, as well as for combustion of fat. Godlewski found the R.Q. for the period of most rapid absorption of oxygen to be between 0.55 and 0.65. He modifies the equations first proposed by Detmer for the transformation of triolein to starch after hydrolysis to oleic acid and glycerine. Thus:

$$(C_{18}H_{33}O_2)_3\ C_3H_5 + 3H_2O = 3C_{18}H_{34}O_2 + C_3H_8O_3$$

$$C_{18}H_{34}O_2 + 18O_2{}^* = C_6H_{10}O_5 + C_2H_3O_3 + 10CO_2 + 8H_2O$$

$$\frac{10\ CO_2}{18\ O_2} = 0.55 \text{ R.Q. (hypothetical "unknown substance")}$$

* Godlewski uses $17O_2$ which evidently is an error, since his equation does not balance. Ricinolein has a different formula (see Paper III).

The total R.Q. would depend upon how much fat and carbohydrate were being completely oxidized to CO_2 and H_2O at the same time. These equations would result in the formation of 58.3 gm. of starch from 100 gm. fat. Detmer's analyses for the hemp seed show a loss of 15.56 per cent of fat and the neoformation of 8.64 per cent of starch in its place during the first 7 days of germination of the hemp seed. For 15.56 gm. fat Godlewski's equations would yield 9.07 gm. carbohydrate. This agreement makes it highly probable that the destruction of fat actually runs some such course.

Detmer did not find any soluble carbohydrate in his analyses of germinated hemp seeds, but Frankfurt (13) working with sunflower seeds, which have about the same composition, reported increasing quantities of soluble carbohydrates. Green (14) later identified cane sugar in the germinating castor bean in considerable amount, and Rhine (15) reports an abundance of a "non-reducing" sugar both in the castor bean and in the hemp seed of similar stages.

Respiratory quotients as low or lower than those found by Godlewski have been reported for fatty seeds by Gerber (16), Iwanow (17), Harrington (18), and Ermakoff and Iwanoff (19). Gerber argues that if the fats stored in the seed are easily oxidized (highly unsaturated) as in flax seed (linolic acid), the R.Q. of germination will be near that for complete oxidation of fat; but it they are difficultly oxidized (less unsaturated) as in castor bean (ricinoleic acid), the R.Q. will be near that for complete conversion of fat to carbohydrate; *i.e.*, in the neighborhood of 0.3. Sherman (20) has published a table giving respiratory quotients of many seeds both dormant and germinating. Amongst them are several showing that the R.Q. of the endosperm is lower than that of the embryo of the same seed, taken presumably at the same stage of germination. These are for starchy seeds. Sherman gives no similar comparisons for fatty seeds and the present writer has been unable to find any such in the literature. This aspect of the subject is one of considerable significance for the main thesis, as will be shown.

Rhine (15) has developed the fact that the hypocotyl of starchy seeds (pea, wheat, barley, etc.) and that of fatty seeds (cotton, sunflower), taken separately from the rest of the seed in germination, exhibit the same R.Q.; namely, 0.77 on the average. He offers this similarity as proof that fat is transported from the endosperm into the hypocotyl through the cotyledon, not as fat, not even from fatty seeds, but always as sugar, and that any fat found in any part of the young plant is there as the result of synthesis from carbohydrate, as in starchy seeds. "The evidence .... favors the view that all the fat stored in the fatty seed is, as we have known most of it to be, first converted to sugars before being transported."

Terroine and associates (21) have shown by bomb calorimetry that the yield of energy to the young plant expressed as percentage of the energy lost in the process of germination (the resulting coefficient is called the energy yield) is greatest in starchy seeds, next in proteinous seeds, and least in fatty seeds, which are also fairly high in protein.

	Average composition			$\frac{\text{Energy of the young plant} \times 100}{\text{Energy expended}}$
	Protein	Carbohydrate	Fat	
Sorghum	9	84	7	73 (Rice and sorghum)
Lentil	31	62	7	62 (Pea and lentil)
Peanut	16	9	75	54 (Flax and peanut)

If protein is substituted for carbohydrate in the lentil, as compared with sorghum, *i.e.* from 9 to 31 per cent, the energy yield is depressed 11 per cent (73 to 62) but replacing carbohydrate with fat, in peanut as compared with lentil, *i.e.* from 7 to 75 per cent, reduces the energy yield 8 per cent more. In a second paper Terroine (22), using considerably different figures for composition and slightly different for his energy yield, makes out that the first substitution just mentioned,

amounting to a change in 28.7 per cent of the weight of the seed from carbohydrate to protein, results in a loss of efficiency of 10 per cent, which, for 100 per cent substitution, would give a difference of 34.8 per cent in efficiency. Passing from rice (or sorghum) to peanut there would be a reduced total efficiency of 19 per cent (as above), but 4 per cent of this is due to protein change on the basis just given (except that the percentage of protein in peanut now is 21+ per cent) while 15 per cent is due to a change of 64.4 per cent (table above is 66) in weight from carbohydrate to fat. If a change of 64.4 per cent carbohydrate to fat produces a reduced energy yield of 15 per cent, a complete change, or 100 per cent, would produce a lost efficiency of 23 per cent, which agrees with the computation of Zuntz on the assumption that all the carbon of the fat is found in the carbon of the carbohydrate. Godlewski's equation obviously does not give this result.

Malhotra (23) studied the energy changes of some fatty seeds (castor bean, peanut, flax, and hemp) along with other seeds, during the first 8 days of germination, and in the case of the castor bean recorded a total loss of 0.96 cal. per unit weight due to loss of weight and change in chemical composition. He gives no opinion regarding the nature of this change and reports an R.Q. for the 4th day of germination of 0.71.

Ermakoff and Iwanoff (19) investigated the possibility of partial oxidation of unsaturated fatty acids, according to the conception of Warburg (24), in flax seed, but found no change in the iodine number or in the refractive index of the oil found at different stages of germination. They conclude that the low quotients are due entirely to transformation of the fats to carbohydrate.

EXPERIMENTAL

The choice of the castor bean as representative of the oleaginous seeds was made partly because a single specimen is large enough to give an easily measurable rate of oxygen consumption and partly because it was being used in the laboratory as a source of vegetable lipase. A few experiments, given in Table I, were made with flax seed; but they proved difficult to handle, mainly because of their small size, and, for the same reason, the rate of oxygen consumption was so slow as to require many hours for a single determination with as many as three or four seeds at a time.

The so called Warburg method was adopted. The only new feature introduced by Warburg is the modification in the volumetric bottle of Brodie (25) necessary to adapt it to the study of tissue respiration. It happened that the well inside the bottle introduced by Warburg for containing alkali solution, was, in the volumetric bottles used, of just the right dimensions to support a germinating castor bean on its rim (Fig. 1). Barium hydrate or KOH of N/5 concentration was employed in the bottom as absorbent for CO_2 and N/2 HCl in the "sac" or bay off the volumetric bottle, for discharging the CO_2 after the oxygen measurement had been made. In this way both determinations could be accomplished with the same bottle. The methods of manipulation and calculation are well described, in general, by Richardson (26).

A protocol which, as it happened, gave perfect agreement between the respiratory quotients of two beans, is given below. Such perfection was rare. It was not unusual, however, to obtain agreement in oxygen absorption in successive experiments on the same bean within 3 to 6 per cent. The CO_2 was seldom within this range in successive experiments.

Preparatory to germination the beans were treated with 0.8 per cent formaldehyde for 5 minutes, then rinsed at least four times in sterile distilled water. They were then placed on a glass plate between filter papers which had been soaked in the same formaldehyde solution for 15 minutes and then rinsed thoroughly in hot sterile distilled water. The filter papers were covered by an inverted copper water

FIG. 1. A castor bean with well developed hypocotyl inside the respirometer bottle resting on top of the well.

bath, with side tube for admitting air and the glass plate placed away in a dark closet for germination at room temperature, which, at the time these experiments were carried out (July, August, and early September), was on the average about 25°C. The filter papers were moistened occasionally with sterile distilled water. Whenever mould developed on the beans, as happened occasionally, they were discarded. It required in the neighborhood of 3 days to bring the beans to what will be called in these papers the "first stage" of germination; namely, a length of hypocotyl (radicle) not to exceed 10 mm. Actually, of course, the first stage is the stage of swelling before the radicle appears. 2 days more would permit of growth to a length, quite often, of 20 mm. Beans 19 and 20 given in the protocol had been germinating 6 days.

The temperature of the bath in which the manometer bottles were immersed was regulated to 30–31°C. in all the experiments on flax seeds and on single beans

reported here, and practically always remained constant within 0.1° during the period of observation. The control manometers in any case corrected for both thermal and barometric changes. An additional manometer corrected also for any CO_2 contained as carbonate in the barium hydrate solution.

The manometers were attached to a shaking device.

The "stages" of germination adopted in these studies are as follows:

1st	stage,	length	of	hypocotyl	5– 10 mm.
2nd	"	"	"	"	10– 20 "
3rd	"	"	"	"	20– 35 "
4th	"	"	"	"	35– 45 "
5th	"	"	"	"	45– 60 "
6th	"	"	"	"	60– 80 "
7th	"	"	"	"	80–100 "
8th	"	"	"	"	above 100 mm.

(See also Table I of Paper III.)

Protocol

Manometer No.	8	9	10	11	12
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
Volume H_2O (in well)	0.5	0.5	0.5	0.5	0.5
" N/2 HCl (in sac)	1.0	1.0	1.0	1.0	1.0
" N/5 Ba $(OH)_2$ in bottle	1.0	1.0	1.0	1.0	1.0
" of beans		No. 19,0.6	No. 20,0.4		
Volume total		3.1	2.9		
Time of placing bean in bottle, *p.m.*		1:32	1:35:30		
Closing stop-cocks *start* (temperature 30.25°), *p.m.*	1:40:30	1:42	1:43	1:43:20	1:44
Readings O_2 at start, *mm.*	70.0	69.5	70.0	69.0	69.0
While shaking, *end* (temperature 30.25°), *p.m.*	2:08:30	2:08:30	2:10	2:11	2:12
Readings O_2 at end, *mm.*	80.0	−31.5	−35.0	78.0	78.0
HCl dumped at, *p.m.*		2:09	2:10		2:12
While shaking, *final* (temperature 30.25°), *p.m.*	2:13	2:13	2:13	2:13:30	2:14
Readings for CO_2 end, *mm.*	83.0	+25.0	+28.0	80.5	83.5

Calculation Bean 19, Manometer 9

O_2, +69.5 to −31.5, less change for No. 8 control, +91 mm.

91 × 2.99 (vessel constant) = 272.09 c.mm. O_2 in 26.5 min.

CO_2, −31.5 to +25, less change for No. 12 control = 51.0 mm.
51.0 × 3.15 (vessel constant for CO_2) = 160.6 c.mm. CO_2 in 41 min.
272.09 c.mm. O_2 in 26.5 min. = 420.9 c.mm. in 41 min.
160.6/420.9 = 0.381 R.Q.
Bean 20 184.0/481.2 = 0.382 R.Q.
Bean 19, length of hypocotyl 32 mm.
Bean 20, length of hypocotyl 33 mm.

Irwin (27) has raised the question whether high respiratory quotients obtained from animal organisms under the action of ether may not be due to the splitting of carbonates by the action of organic acid formed in the process of metabolism, and has shown that this is not true of plant tissues. If carbonates were formed and retained in the endosperm during germination of fatty seeds a respiratory quotient below that for combustion of fat might be produced and the inference of conversion to carbohydrate would be false. This possibility was controlled by several experiments of which the following is representative. A crushed germinating bean was introduced into the bottom of a manometer bottle and this manometer equilibrated with a control empty bottle. HCl of the usual strength was then dumped from the sac onto the crushed bean and readings taken immediately and after shaking for 6 minutes. Readings were taken as follows:

Manometer No.	10	11
	Crushed bean	Empty
Readings at 5:22 p.m.	73.8	68.0
HCl dumped 5:23 "		
5:27 "	74.0	70.0 correction − 2 mm.
Manometers shaken 5:33 p.m.	76.0	69.0 " − 1 "

Maximum possible effect due to liberation of CO_2 from carbonates therefore would be 2.2 mm., which at the usual level of readings might raise the quotient as much as 0.03, rarely more. Acid dumped onto a detached hypocotyl 23 mm. long, in one instance produced a rise of 6.5 mm., and when dumped onto a bean with attached hypocotyl of 10 mm. length produced a rise of 4 mm. Apparently, therefore, carbonates are stored, or at least CO_2 may be retained, in significant amount in the new growth. However, since no question of a conversion of fat to carbohydrate in any part of the new plant is involved, and as will be seen presently the R.Q. here is always in the range of carbohydrate combustion, or indeed of conversion back to fat (see p. 295) no error of interpretation is produced so far as the main thesis is concerned. However, extreme care was always taken to prevent the acid touching any part of the bean in the process of dumping, and when it was known to occur the result was discarded.

RESULTS

1. Short-Period Experiments

Table I presents typical experiments with germinating flax seeds. The stage at which the seeds were introduced into the well (because

TABLE I

Flax Seed Experiments

Warburg Apparatus

Experiment No.	No. seeds	Stage in	Length of experiment	Stage out	Respiratory exchange		R.Q.
					CO_2	O_2	
				mm.	*c.mm./mg.*	*c.mm./mg.*	
1	3	Tip visible	16 hrs.	24	5.215	9.095	0.570
	3	" "	16 "	27	11.02	14.75	0.750
2	3	1–3 mm.	19 "	12.5	6.65	11.97	0.550
3	2	Tip visible	23 "	9.5	8.194	12.678	0.646
			23 "	15		8.613	0.672
4	2	" "	9 hrs. 40 min.	22	10.79	14.918	0.765
			" "	24	10.98	15.13	0.730
5	2	5–8 mm.	8 hrs.	20	5.72	11.27	0.510
6	4	Tip visible	6.5 "	19.9	5.09	7.41	0.606
	4	" "	6.5 "	23	5.09	7.79	0.653
7	4	" "	7.5 "	20	7.81	12.23	0.640
					Total		
8	3	5 mm.	7.75 "	22	159	241	0.659
	3	"	7.75 "	21	133	273	0.487
Average							0.633

of the gummy substance enveloping these seeds they adhered readily to the inside wall) and the stage which they had reached when final readings were taken, as well as the elapsed time in hours are indicated. Mm. refers to average length of the hypocotyls. When removed from the well the seed coat and gummy substance were removed by gently

pressing out the naked endosperm and the total moist weight was obtained immediately. The CO_2 and O_2 are expressed as c.mm. per mg. of this moist weight. Some of the rather large variations shown are probably due to errors in securing the moist weight, others to injury to the very delicate endosperm and hypocotyl. Dry weights would have been preferable. However, these experiments were purely preliminary and were not followed up for reasons already given. They are shown merely as confirmatory of the low quotients obtainable with a seed which contains only around 37 per cent (Winton) fat and 27 to 30 per cent carbohydrate. In all but two instances the R.Q. is well below the level of fat combustion. Since carbohydrate combustion is not excluded the quotients are doubtless significant of some other use of oxygen than combustion. Gerber's idea regarding the effect of highly unsaturated fatty acids (p. 285) should also be borne in mind.

Table II presents typical experiments with single, entire, germinating castor beans. Because of their large size and very active metabolism, as compared with the flax seeds, these experiments could be completed often in as many minutes as the others required hours. It is difficult to obtain accurate dry weights on seeds containing as much fat as this bean without extraction with fat solvents. The results in this table, therefore, are expressed without reduction to any unit of weight. That it is possible to obtain fair agreement between different beans of approximately the same size and stage of development when expressed in terms of moist or dry weight is seen from Table III.

The respiratory quotients agree rather better than the rates of metabolism. This is true also of successive experiments performed on the same bean on the same day, as may be seen from Table II, particularly for Beans 12 and 16. Other experiments not shown in any of the tables bear out this conclusion, which, otherwise stated, would indicate that the nature of the chemical processes involved is more constant for any given stage than the rate of change.

From Table II it is quite evident that the chemical process responsible for the low quotient proceeds at different rates at different stages of the germination. To give the exact quotient, characteristic of each stage has not been undertaken in this study, for it is not possible to confirm any conclusion reached through respiration experiments on single beans by chemical analyses. That will be undertaken in the third paper of this series.

TABLE II

Castor Bean Experiments

Single Bean in Warburg Apparatus

Variation with Stage

Bean No.	Stage of growth. Length of hypocotyl	Length of experiment	Respiratory exchange		R.Q.
			CO_2	O_2	
	mm.	*min.*	*c.mm., total*	*c.mm., total*	
13	12	69.0	234.9	345.6	0.679
14	12+	46.5	155.8	231.4	0.535
17 (1)	16	30.0	208.2	337.8	0.617
17 (2)	16	32.0	211.4	407.8	0.518
16 (1)	19	32.0	142.6	247.6	0.576
16 (2)	19	32.0	130.0	223.3	0.582
Average					0.585
18	20	15.0	85.4	252.9	0.337
12 (1)	23	34.5	117.3	406.3	0.289
12 (2)	23	23.0	107.8	287.4	0.373
12 (3)	23	22.5	98.0	320.4	0.306
10	24	31.5	146.7	413.8	0.355
11	29	32.5	127.9	496.9	0.257
19	32	41.0	160.6	420.9	0.382
20	33	37.5	184.0	481.2	0.382
Average 20 to 30 mm.					0.323

TABLE III

Respiratory Metabolism of Castor Beans of Similar Stages of Development Per Unit of Weight

Bean No.	Moist weight	Dry weight	CO_2	O_2	R.Q.
	gm.	*mg.*	*c.mm./100 mg. (dry)/min.*	*c.mm./100 mg. (dry)/min.*	
42	0.684	305	1.92	4.9	0.39
43	0.520	262	0.84	3.5	0.24
44	0.449	229	2.13	6.2	0.34
45	0.453	232	2.36	5.6	0.42

The next phase of the subject of special interest is the relation of the respiratory exchange of the embryo to that of the endosperm. If fat were changed to sugar in the endosperm and after diffusion through

the cotyledon into the hypocotyl were there either oxidized as such or converted back to fat, the R.Q. of the hypocotyl would necessarily be considerably higher than that of the endosperm. As noted on page 285 such a difference has been demonstrated for starchy seeds. It should *a fortiori* be true of fatty seeds. Fortunately it was found to be very easy to strip the cotyledons out of the endosperm and to determine their metabolism in the same apparatus as the entire germinating bean, and immediately thereafter. Typical determinations of this kind are given in Table IV. In each case shown the R.Q. of the

TABLE IV

Respiratory Exchange of Whole Germinating Bean Compared with That of Young Plant

No.	Part	Stage	Length of experiment	CO_2	O_2	R.Q.
			min.	*c.mm.*	*c.mm.*	
26	Whole bean	3 branches	16	149.3	490.5	0.304
26	Young plant	3 "	77	305.2	348.8	0.974
27	Whole bean	12+ "	19	131.1	386.0	0.339
27	Young plant	12+ "	72	311.3	315.6	0.986
28	Whole bean	60 mm. 3 branches	23	189.4	500.8	0.378
28	Young plant	60 " 3 "	68	279.8	324.2	0.863
29	Whole bean	30 " 3 "	21	142.9	269.7	0.328
29	Young plant	30 " 3 "	65	260.0	280.0	0.928

germinating bean as a whole was determined, and, as soon as this was finished, the bottles were cleaned and prepared for another observation, the bean meantime being kept in moist filter paper. The cotyledons then were carefully separated from the endosperm and with the hypocotyl attached were placed immediately in the identical bottles in which the parent beans had been observed. Naturally it required a considerably longer time to obtain figures of the same order of magnitude for the two gases from the young plant, because of its much lighter weight. Table IV shows that this time was from three to four times as long.

In every case shown, and in several other determinations not shown, the production of carbon dioxide was more than twice as great in the young plant as in the endosperm, in proportion to oxygen used up. The result was a quotient for the young plant well up in the range of carbohydrate combustion, while that of the entire germinating bean was, as usual for the stage selected, within the range for complete conversion of fat to carbohydrate. Sketches of the cotyledons and hypocotyls of these four beans, in natural size, are shown in Fig. 2. The

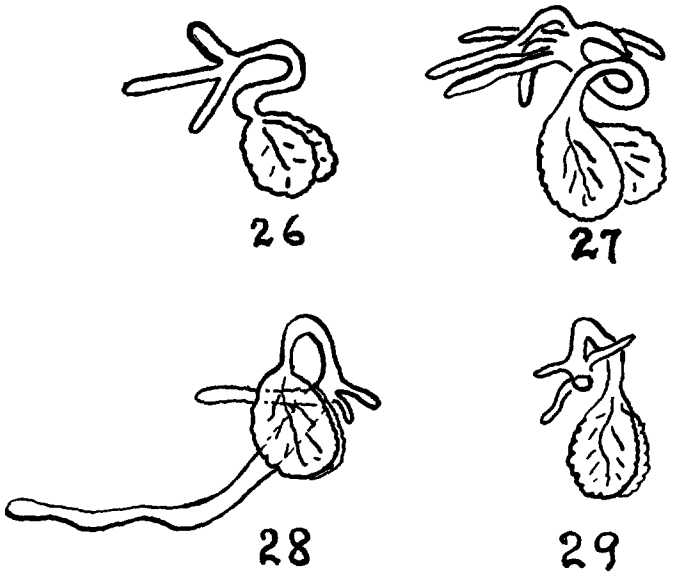

FIG. 2. Cotyledons and hypocotyl (entire young plant) of Beans 26, 27, 28, and 29, metabolism of which is shown in Table IV.

results are strongly indicative of the sequence of chemical changes postulated by Detmer, Godlewski, Iwanow, Rhine, and Ermakoff and Iwanoff. However, physiologists are all too prone to accept an explanation which appears plausible, as already proved, because a *possible chemical reaction* can be written for such a change. Proof in any adequate sense of the word is not given until the *actual chemical changes are shown to occur* at the same time, as the respiratory signs. A number of other possibilities exist. For example, Green (14) has shown that an organic acid which he was not able to identify is formed

in the germination of the castor bean. If this were formed *de novo, i.e.* not merely by hydrolysis of fat, as is suggested by Godlewski's equation, its production would require extra oxygen just as does the formation of starch or sugar from fat. This aspect of the problem will be gone into in the third paper. It is sufficient for the moment to conclude that the respiratory exchanges are consistent in general with the chemical changes postulated.

Two further points should be mentioned, before we leave this division of the subject. One, that the respiratory quotients of the entire germinating bean almost prove too much. They are so low—so much lower than the theoretical for Godlewski's equation (p. 284)—

TABLE V

Respiratory Exchange of Whole Bean, Endosperm, and New Plant

No.	Part	Stage	Length of experiment	CO_2	O_2	R.Q.
			min.	*c.mm.*	*c.mm.*	
32	Whole bean	40 mm. 1 branch	59.5	236.2	612.2	0.386
32	Endosperm	40 " 1 "	52.0	195.9	426.8	0.459
32	New plant	40 " 1 "	72.0	155.8	140.8	1.106
33	Whole bean	45 mm. 4 branches	57.0	292.9	604.5	0.484
33	Endosperm	45 " 4 "	44.5	184.0	331.7	0.555
33	New plant	45 " 4 "	70.0	173.3	192.5	0.90

that they quite exclude that reaction as the only possible one. When we consider that the metabolism of the young plant with its high quotient is included at least in part in these low ones and also that the endosperm itself doubtless is oxidizing some fat or carbohydrate, the wonder is that quotients of the order obtained are possible. Nevertheless it is believed they are correct. Certainly they are consistent and not at all exceptional for these stages. Some further light would be shed on their significance, if it were possible to take the metabolism of the endosperm also separately. This has been done several times and typical results are found in Table V. Two circumstances, however, detract from the value of these experiments for the purpose intended. It is impossible to separate the cotyledons from the endosperm without removing the seed coat. Other experiments, not shown

in the tables, proved that when a germinating whole bean was observed with the seed coat lacking, the respiratory quotient was a little higher for the first 45 minutes than when the coat was in place. The difference was great enough to vitiate the quotients in Table V somewhat. The quotient of the endosperm alone, it was expected, should be lower than that of the germinating whole bean, since the R.Q. of the young plant alone is so much higher. That Table V does not exhibit this relationship is due, certainly to some extent to the fact that the seed coat had to be removed, and, unfortunately, one cannot say to just what extent this circumstance affects the result. Secondly, the observation with the endosperm and that with the cotyledon and hypocotyl (new plant) did not continue long enough to give comparable total figures with the whole bean experiment. The reason is a good one; namely, to avoid postponement of the observation with the separated parts so long as to permit the metabolism to run down; nevertheless it is regrettable. This circumstance makes the quotients a little less reliable both for the endosperm and the young plant. Possibly the very high R.Q. for young Plant 32 is to be explained in this way.

Having just mentioned the possibility that the metabolism of the isolated parts runs down when separated from their natural relationships, some facts in support should be mentioned briefly. Many times successive experiments on the same whole bean have been carried out the same day. Usually, they have agreed quite satisfactorily. With the young plant the agreement is never so good as with the whole seedling. This would be expected, for the plant is not yet old enough to maintain itself even in its natural habitat, much less in a respirometer bottle. The following illustration will suffice.

Young plant	CO_2 in 100 min.	O_2 in 100 min.	R.Q.
	c.mm.	*c.mm.*	
No. 1, 1st experiment.....	275	287	0.96
2nd "	222	281	0.79
No. 2, 1st "	227	237	0.96
2nd "	217	246	0.88

The first experiment in each case lasted 100 minutes, the second 51 minutes; they are calculated, however, to the same basis. In each case the R.Q. is significantly lower in the second experiment.

The second point remaining to be discussed briefly is the relationship of the respiratory exchanges on the basis of unit weight. Several of these experiments also were carried out and two are shown in Table VI. Moist weights were used. The whole germinating bean was weighed minus its seed coat just after the metabolism observation. The endosperm and young plant were weighed immediately after separating them and the endosperm was kept moist until its metabolism could be determined. Thus the three weights were obtained under strictly comparable conditions, and since the fat content of the endosperm is so very much higher than that of the young plant, it is believed the moist basis is just as satisfactory as the dry.

TABLE VI

Whole Seedling, Endosperm, and New Plant Calculated to Unit Time and Weight

No.	Part	Moist weight	CO_2	O_2	R.Q.
		mg.	*c.mm./hr./100 mg.*	*c.mm./hr./100 mg.*	
36	Whole seedling	911	52.6	133.4	0.394
36	Endosperm	575	53.0	140.9	0.378
36	Young plant	144	139.0	180.0	0.776
37	Whole seedling	1047	47.6	104.6	0.454
37	Endosperm	601	53.2	111.1	0.468
37	Young plant	194	141.5	170.5	0.829

It was not surprising to find the metabolism of the young plant much higher than that of the entire bean. In one of the cases (No. 36) shown it is approximately 35 per cent higher on the basis of the oxygen consumption and in the other about 63 per cent. In each case the metabolism of the endosperm is 5 to 7 per cent higher than that of the whole germinating structure, likewise on the basis of the oxygen. This again indicates that the metabolism of the whole bean is somewhat held in check by the seed coat, for the endosperm necessarily was observed devoid of the coat.

Still more interesting are the CO_2 relationships. The endosperm and whole bean give off approximately the same volume per unit of weight, but the young plant gives off 2.6 times as much as either whole bean or endosperm. At first sight this seems like a clear case of temporary storage of CO_2 and its escape from the cotyledon as

soon as it is withdrawn from its housing in the interior of the seed. Experiments have already been referred to (p. 289) which prove that this cannot be true of the hypocotyl. The same sort of experiments were done with the cotyledon. No readings have been preserved, unfortunately, but acid applied to the cotyledon causes the evolution of very little gas.[1] There is no question, therefore, that the large output of CO_2 from the young plant is due to active metabolism. Can any special significance be attached to this fact?

If the statement just made is turned the other way about, it is clear that on the basis of unit weight, the peculiar metabolism which goes on in the endosperm to give very low quotients differs from that of the young plant much more on the basis of CO_2 than on the basis of oxygen. One might have expected that when reduced to unit weight the endosperm would show a greater absorption of oxygen than the young plant, the CO_2 remaining more nearly the same. The statement usually made is that originally reported by de Saussure—the oxygen absorption is much greater than the CO_2 elimination. The indication from these few experiments is rather that CO_2 is produced but held back to form carbohydrate and when the carbohydrate later is broken down the CO_2 is liberated. If the carbohydrate were to be transformed to fat, it would be accomplished by elimination of still more CO_2. Fat is found in the protoplasm of the hypocotyl (Rhine (15)); hence a part of the surplus CO_2 coming from the young plant may be from this reaction. It will require more experiments of the same kind and others of special design to make sure of this as a fact. At present it may be remarked only that Chauveau's (28) conception is favored rather than that of Bleibtreu (29) concerning the nature of the chemical change necessary to produce fat from carbohydrate and *vice versa*. (See, however, the evidence in Paper II.)

2. Long-Period Experiments

The short-period experiments are interesting from many points of view; but with single beans the physiological results cannot be checked

[1] Later check experiments done at our request by Dr. M. Elizabeth Marsh, showed that a maximum of 10–15 c.mm. of gas could be obtained from a single pair of cotyledons. This would lower the R.Q. of the young plant in Tables V and VI about 0.05 if all the CO_2 stored should have been given off in the respiration period, which is very unlikely.

by chemical analyses of the same material. A second type of experiment was designed for this purpose. The chemical analyses will not be reported in this paper but in Paper III. A description of the respiration method may be given at this time, however, and a few typical results merely to confirm for long periods the character of the respiratory quotients reported above for the entire germinating bean in single cases.

Beans were started germinating in the usual way (p. 287) and when they had arrived at some definite stage, *e.g.* our first stage where they had broken the seed coat and put out a little radicle anywhere from 1 to 5 mm. long, a number of these were selected from their healthy appearance and placed in an air-tight bottle of 1 to 4 liters capacity which had been sterilized in a suitable manner. The bottles were provided with a rubber stopper containing two glass tubes closed by means of tight-fitting rubber tubes and screw clamps. The beans were placed on sterile filter paper moistened with freshly sterilized distilled water and the bottles ventilated with filtered outside air. The bottles were then tightly closed and were covered with black paper or tin foil. They were placed in dark cupboards for several days. When by inspection it was found that the beans had made a satisfactory growth, samples of air were drawn from one tubulure; while admitting water or mercury through the other. The samples were analyzed by means of a special Haldane apparatus designed as to the burette and some other features by Dr. Nasset (30).

Table VII shows the results of six such experiments. The first three (Nos. 2, 3, and 5) were first stage, the next two (Nos. 4 and 6) were second stage, and the last enumerated (No. 1) was third stage, when confined. The fourth column gives the number of days of confinement and the fifth the stage of growth reached by the beans when the air was sampled. The last columns give the respiratory exchange in terms of percentage alteration in the composition of the air. Some of these figures suggest, at first sight, that more oxygen was removed than could have been present in the air at the start (20.93 per cent). However, this will not be misleading to anybody familiar with air analysis, for the percentage change is found by subtracting the percentage formed at the end, not from 20.93 but from a figure proportional to the nitrogen remaining at the end. In these cases (Experiments 1, 2, 3, and 6) the oxygen was practically all used up. In these also the CO_2 percentages found at the end were excessively high. It is not certain that the beans were alive in every case. Indeed in No. 2 the odor of

putrefaction was unmistakable, and this condition doubtless is responsible for the exceptional respiratory quotient found. The other quotients are consistent in indicating the formation of fat from carbohydrate.

In such experiments where the amount of material involved is sufficiently large to permit of exact chemical analysis, it will be possible to

TABLE VII

Bean-Bottle Experiments

Experiment No.	No. of beans	Stage in. Average length hypocotyl	Days in	Stage out. Average length hypocotyl	CO_2 gain	O_2 loss	R.Q.
		mm.		*mm.*	*per cent*	*per cent*	
5	24	3	3	13.1	4.63	8.73	0.530
					4.70	8.64	0.544
2	6	5	8	50	15.96	22.15	0.72*
3	6	5	5	35	10.36	21.98	0.471
					10.63	21.96	0.484
6	24	15.6	3	29.2	10.65	21.05	0.506
					10.90	20.86	0.569
4	6	17.5	2	20	9.52	18.59	0.514
					9.13	18.33	0.498
1	5	38	8	89	11.62	21.68	0.538

* Spoiled—putrefaction.

check the respiratory findings by actual composition at the beginning and at the end. Results of this character will be found in Paper III.

SUMMARY AND CONCLUSIONS

1. Respiration studies on single castor beans, made by means of the Brodie-Warburg method, at various times after the start of germination, as well as studies on groups of germinating beans over periods of 3 to 8 days, made by a simple procedure involving analysis of the respired air by the Haldane method, consistently give respiratory quotients from 0.30 to 0.58, indicating the conversion of the oil to carbohydrate.

2. The R.Q. varies with the stage of germination, the lowest point occurring when the new growth (hypocotyl) measures from 20 to 35 mm. in length.

3. The R.Q. of the young plant (cotyledons and hypocotyl), separated from the endosperm and studied in the same apparatus, varies from 0.78 to 1.00. It is invariably high enough to indicate considerable combustion of sugar. The R.Q. of the endosperm alone is low, but usually somewhat higher than that of the entire germinating structure.

4. On the same unit of moist weight the young plant (cotyledons and hypocotyl) produces about 2.6 times as much CO_2 as the endosperm, whereas it absorbs only 1.3 times as much O_2.

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