INTRODUCTION.

The first three papers of this series (42, 43, 44) were concerned with investigations of the protein metabolism of the embryonic chick.

The fourth paper (45) reported analyses of embryos for non-protein nitrogen in an attempt to gain an idea more accurate than that generally accepted, of the movements of the true protein. With the aid of these figures, a curve was constructed for the intensity of absorption of protein throughout development and this curve was compared with a similar curve for the absorption-intensity of fat. The absorption-intensity of carbohydrate, however, remained to be measured, so a general study of the carbohydrate metabolism of the developing egg was undertaken.

TECHNIQUE.

The most complete study in the literature of chemical embryology dealing with carbohydrate metabolism is that of Sakuragi (48). For this and other reasons, I made some preliminary experiments, using his method in order to see whether it compared favourably with a procedure involving a quite different principle, namely, the method of Hagedorn and Jensen (17). The method of Sakuragi can best be described as Momose's modification (29) of the Kumagawa-Suto modification (25) of the original method of Pavy (46). It consists essentially of reduction under standard conditions of an ammoniacal copper solution by the solution containing the sugar, followed by a back-titration in an atmosphere of ammonia with hydroxylamino hydrochloride which reduces the copper untouched by the glucose.
The Energy-sources in Ontogenesis

The method was tested as follows: two 17th day Black Leghorn embryos were well minced and the tissues made up to 630 c.c. with an end concentration of hydrochloric acid of 5 per cent. They were then boiled for four hours, after which one part of the solution was precipitated with trichloracetic acid and another with acetic acid in order to remove traces of undecomposed protein. The sugar was then estimated in the two samples by the Sakuragi method and also by the Hagedorn-Jensen method. The results were as follows:

<table>
<thead>
<tr>
<th>Solution</th>
<th>mg. glucose %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sakuragi</td>
</tr>
<tr>
<td>A Standard containing 200 mg. % of pure anhydrous glucose</td>
<td>189</td>
</tr>
<tr>
<td>B Embryo hydrolysate (acetic acid)</td>
<td>127</td>
</tr>
<tr>
<td>C Embryo hydrolysate (trichloracetic acid)</td>
<td>112</td>
</tr>
</tbody>
</table>

It was therefore evident that though the Sakuragi method was applicable with moderately good results to pure glucose solutions, it was not so applicable to protein hydrolysates. In such cases it would give double the Hagedorn-Jensen value, if not more.

These observations are in good agreement with the work of Holden\(^\text{(30)}\). He found that when glucose is estimated by copper reduction methods in the presence of amino-acids the results obtained may be 15 per cent. or more too high, even though the amino-acids themselves do not reduce the copper alone. This seems to be due to a coupled reaction of the glucose and the amino-acids. The magnitude of the error discovered by Holden was obtained with added amounts of amino-acids not exceeding double the quantity of glucose present, but the experimental results given above show that in protein hydrolysates the error may not be far from 100 per cent.

The final technique adopted for ascertaining the changes in the total carbohydrate content of the embryo and the rest of the egg throughout development was as follows. The embryos were removed from their membranes and washed quickly in distilled water to free them from amniotic fluid, after which they were thoroughly ground up with or without quartz sand or else passed through a Bolinder mincing machine, depending on their age. The rest of the egg, i.e. the amniotic and allantoic fluids with their membranes, the blastoderm, the yolk and the vitelline membrane, the white, and the chalazae, was similarly treated. Embryos and remainders were then diluted separately to a volume varying from 300 c.c. to 5 litres and it was arranged that the end concentration of the hydrolysate should be 5 per cent. hydrochloric acid by volume. The two hydrolysates were allowed to proceed for four hours exactly and the hydrolysates neutralised with 40 per cent. soda. From the neutral liquid 10, 20 or 25 c.c. was pipetted off and precipitated with 20 per cent. phosphotungstic acid made up in 5 per cent. sulphuric acid. The object of this was to remove uric acid, certain amino-acids, glutathione, traces of undecomposed protein, creatinine, and basic substances, all of which would reduce the ferricyanide in the Hagedorn-Jensen method. Creatine would escape this
treatment and this substance also, as Holmes and Holmes (41) have shown, reduces the ferricyanide. However, the four hours' hydrolysis would convert a considerable part of the original creatine into creatinine, which would be removed, and it must also be remembered that Holmes and Holmes showed that 8 mg. of creatine are only equivalent to 1 mg. of glucose in reducing power.

The phosphotungstic precipitate was filtered off and washed, and the filtrate neutralised with a saturated solution of barium hydroxide. The barium salt was then removed by centrifuging, and the solution brought back to the acid side by the addition of weak sulphuric acid. Further centrifuging removed the barium sulphate, and final neutralisation was effected by weak soda. Evaporation to smaller bulk might or might not be necessary at this stage before the solution was ready for the estimation of the glucose. The Hagedorn-Jensen procedure was exactly followed, care being taken to keep the thiosulphate and ferricyanide solutions in the dark and to standardise the thiosulphate against potassium iodate from time to time. The final back-titration of the iodine liberated by the unreduced ferricyanide was done in quadruplicate.

The eggs used in this research were all of White Leghorn breed laid by pullets on Messrs Chivers' farm at Histon, Cambridgeshire. They were incubated under standard conditions, as adopted in a previous paper (43). A total of 765 eggs were analysed to establish the curve for total carbohydrate in the embryo, and 555 for that in the remainder of the egg. In an experiment in which 500 mg. of pure anhydrous glucose were added to a hydrolysis of 12 eggs, 105.4 per cent. was recovered.

EXPERIMENTAL RESULTS.

These are set forth in Tables I and II, of which the former is concerned with the embryo and the latter with the rest of the egg. In Table I, column 1 gives the age, column 2 the number of embryos used for each determination, column 3 the mg. present in the embryo each day, and column 4 the smoothed curve of column 3. These data are also graphically represented in Fig. 1.

As has been stated above, the eggs used in this research were all provided by pullets. They were smaller than eggs from adult hens and a corresponding difference in their carbohydrate content soon became apparent. As the following figures show, pullet eggs cannot be directly compared with ordinary eggs; to do so a correction is necessary.

<table>
<thead>
<tr>
<th>Nature of hen</th>
<th>Day</th>
<th>Breed</th>
<th>No. of eggs</th>
<th>mg. glucose per egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>0</td>
<td>Plymouth Rock</td>
<td>6</td>
<td>310.5</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>White Leghorn</td>
<td>6</td>
<td>339.0</td>
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<tr>
<td></td>
<td>0</td>
<td></td>
<td>6</td>
<td>354.0</td>
</tr>
<tr>
<td>Pullet</td>
<td>0</td>
<td></td>
<td>12</td>
<td>203.0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>12</td>
<td>212.0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>12</td>
<td>207.0</td>
</tr>
</tbody>
</table>

Average 335.0
Average 207.0
The Energy-sources in Ontogenesis

This relationship seems to hold with a regularity so definite that a correction of 67 per cent. was added on to the figures for the glucose in the pullet eggs, and in Table I this is accomplished in column 5. This is done on the assumption that the weights of embryos from the eggs of pullets are uniformly smaller than those of embryos from the eggs of adult hens. This supposition stands in need of exact confirmation, but it is a commonplace to the poultry farmer that pullet chicks are always smaller than chicks from full-grown hens. In any case the relative shape of the curves will not be affected by these considerations though they affect considerably the absolute values and make comparison possible with other figures in the literature. Murray (36) also worked with pullet eggs and observed an approximation to the weight of the adult hen egg as winter proceeded. Such an approximation to the adult hen sugar level, I have, however, been unable to observe, and

Table I. Total carbohydrate in embryos.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day</strong></td>
<td><strong>No. of embryos</strong></td>
<td><strong>mg. per embryo</strong></td>
<td><strong>Col. 3 smoothed</strong></td>
<td><strong>Pullet correction</strong></td>
<td><strong>Daily increment</strong></td>
<td><strong>Mid-increment</strong></td>
<td><strong>Percentage growth rate</strong></td>
</tr>
<tr>
<td>0</td>
<td>—</td>
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<tr>
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</tr>
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<td>16-40</td>
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</tbody>
</table>

Total 765
the correction of 67 per cent. is made on a sugar basis, so that it is not necessary to assume a difference in weight of the egg and embryo though such a difference must certainly exist.

Table II. Total carbohydrate in yolk + white + membranes.

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of eggs</th>
<th>mg. per egg</th>
<th>Col. 3 smoothed</th>
<th>Pullet correction</th>
<th>Total carbohydrate in the egg</th>
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<tr>
<td></td>
<td></td>
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<td></td>
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<td>Col. 5 + Table 1 col. 5 Sakuragi (Momose-Pavy) Needham (Wood-Oat)</td>
</tr>
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<td>17</td>
<td>42</td>
<td>42</td>
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<tr>
<td>Total</td>
<td>555</td>
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</table>

The rest of Table I is taken up with the calculation of the growth-rate of total carbohydrate in the embryo. Table II and Fig. 2 give the data for the remainder of the egg, and hence the total amount of carbohydrate present in the whole egg.
during its incubation period can be calculated\(^1\). These figures are given in column 6 and beside them are placed two other sets of figures for the total carbohydrate present in the egg. The first lot, shown in column 7, is that of Sakuragi(48) and the second contains some which I obtained in 1923 (see (40)) but did not at the time publish; these are placed in column 8. Sakuragi used his own method and I used that of Wood and Berry(60) (the Wood-Ost method), both of which depend on the reduction of copper. As might be expected from what has been said above under the heading of technique, these copper values are much higher than those obtained by the use of the Hagedorn-Jensen method, but they are nevertheless interesting for they also show a constancy of total carbohydrate in the latter half of incubation. In Fig. 3 they are reduced to the same level and set beside the new curve.

Thus although from the tenth day onwards the total carbohydrate in the remainder steadily falls, the carbohydrate in the whole egg remains constant owing to the growth of the embryo. Before the tenth day the amount of carbohydrate in the embryo is insufficient to affect the curve for carbohydrate in the whole egg to any marked degree.

EXAMINATION OF THE EXPERIMENTAL RESULTS.

(a) The movements of glycogen, free glucose, and ovomucoid.

It will first of all be of interest to compare the total carbohydrate of the remainder with the uncombined glucose there during the first half of development. Figures for the free sugar exist in some number already in the literature and although all are derived from experiments in which copper reduction methods were used, they are yet worthy of credence because total hydrolysis with its production of amino-acids was not involved. Neither creatinine nor glutathione would be present in the protein-free filtrates so that the objections against copper methods discussed above are not so grave in the case of free sugar.

In Table III the values of other observers for the free glucose are summarised together with a note of the estimation-method used in each case. The sets of data are 12 in number, namely, those of Idzumi(22), Sakuragi (48), Pavy (46), Bywaters (8), Satō (49), Tomita (55), Gadaskind (44), Vladimiroff and Schmidtt (57), Pennington (47), Hepburn and St John (19), Kojó (23), and Mörner (30). In some cases investigators only give their results in terms of percentages. In order to reduce them to a common basis, therefore, it has to be assumed that they all worked with normal eggs under approximately the same conditions. The best data for this calculation are those of Carpiaux (5) and von Czadek (11) who state that of the weight of the entire egg at zero hour of development, 10.47 per cent. is accounted for by the shell, 56.07 per cent. by the albumen, and 33.46 per cent. by the yolk. Using Murray's figure (33) for the weight of an egg (average of over 500), namely 57.8 gm., the white will weigh 32.04 gm. and the yolk 19.33 gm. The change in weight

\(^1\) The establishment of this curve on a rigorous statistical basis would require, so Dr R. A. Fisher informs me, analyses of at least one thousand additional embryos.
<table>
<thead>
<tr>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Idzumi</td>
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<td>Pavv</td>
<td>Bywaters</td>
<td>Satō</td>
<td>Tomita</td>
<td>Gadaskin</td>
<td>Vladi- miroff and Schmidtt</td>
<td>Pennington</td>
<td>Hepburn and St John</td>
<td>Kojo</td>
<td>Mörner</td>
<td>Smoothed curve</td>
<td></td>
</tr>
<tr>
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<td>Pavv</td>
<td>Pavv</td>
<td>Schenk-Bertrand</td>
<td>Schenk-Bertrand</td>
<td>Galwialo</td>
<td>Hagedorn-Jensen</td>
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Table III. Disappearance of free carbohydrate.
The Energy-sources in Ontogenesis

during early development due to loss of water by evaporation assuming a constant humidity can be read off on the graph given by Murray (33). The varying water content of yolk and white due to the current of water yolkwards can be obtained from the graph given by Aggazzotti (1).

The free glucose beginning at a maximum of 200 mg. per egg sinks more or less steadily till the tenth day. An interesting point is the difference between the yolk and the white. Pavy and Bywaters only estimated the sugar in the albumen, and their points give a curve on a lower level than the whole egg curve but roughly parallel with it. This must mean that there is not only a current of water yolkwards as Aggazzotti (1), Vladimiroff (56) and others have shown, but also a current of free glucose yolkwards. By the ninth day the albumen has lost all its free sugar, but the yolk has by then lost only half its original amount. This phenomenon may be well observed in the figures of Gadaskin (14) and Vladimiroff and Schmidt (57) as given in their original papers. It is of considerable interest in view of the circumstance that the current of water yolkwards has recently been interpreted by Gray (16) as related to the fact that a typical aquatic egg such as that of the trout, has enough solid in it at the beginning of development to form the embryo but not nearly enough water. Therefore it has to absorb water from the outside, and Gray would regard the albumen of the hen’s egg as partly an arrangement to provide a constant pressure-head of water for the embryo. So it is not without interest to find the water on its way from the albumen to the embryo and its “private pond,” the amniotic fluid, taking a combustible non-electrolyte with it. These considerations fit in well with the conclusions of Bartlemez and Riddle (3) who discuss the origin of the fluid which fills up the subgerminal cavity in the hen and pigeon embryo.

We may now relate the curve for disappearance of free carbohydrate with the curves for other carbohydrate fractions in the hen’s egg. In Fig. 4 it is shown in relation with the total carbohydrate of the non-embryonic part of the egg. The total carbohydrate of the remainder falls from zero hour till the eighth day, rises from then till the eleventh day, and thereafter falls steadily till the end of development. The free glucose also falls till the tenth day but not in the same manner as the total glucose, for at the beginning its fall is slow and thereafter rapid, while the total glucose first falls quickly and slows down as time goes on. The latter part of the curve for free glucose is given in Table IV. The only estimations of free sugar during the last half of incubation are those of Idzumi (22) and Sakuragi (49) and their results are listed in columns 10 and 11 of Table IV. The smoothed curve in column 12 united to the standard curve in column 9 is that used in Fig. 4 to show the free sugar in the whole egg during incubation.

Before returning to Fig. 4 the glycogen in the whole egg and in the embryo must be considered. Glycogen has been estimated in the embryo by Idzumi (22) and Murray (56), and their figures agree together very well. They are shown in Table IV and in Fig. 5 and the corresponding smooth curve in Table IV, column 4. Now that we are in possession of these data it is simple to calculate the non-glycogen sugar in the embryo, and this is accomplished in Table IV, column 8. This last
### Table IV. Movements of glycogen and free sugar.

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Note: Table III has been mentioned but not included in the provided data.
Experiment

Diyi

« Safcurtg (Monvnc.Pjvy)
Nccdlum (Hijiedoin-Jtiaen)
Necdhim (Wood-Osl)

Mgmi per total carbohydrate In remainder

Mgmi total carbohydrate in embryo

Mgmi free carbohydrate in whole egg

Mgmi free carbohydrate in embryo

Mgmi "CKomucoid" in remainder

Mgmi total cyctose in whole egg

Mgmi glycogen in whole egg (Idzumi)
Mgmi glycogen in whole egg (Sakuragi)
Mgmi glycogen in embryo (Murray)
Mgmi glycogen in embryo (Sakuragi)

Mgmi glycogen in remainder

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D
The calculation of Table V give the amount of carbohydrate not present as glycogen or free glucose outside the embryo for every day during development. This is graphically shown in Fig. 4 and numerically in column 6. This calculation

1 For the fate of the yolk unabsorbed at hatching, see Iljin, Materials for the Study of Embryonchemistry, Leningrad, 1913 (in Russian).
The Energy-sources in Ontogenesis

suffers from the fact that no data are available for the free sugar present each day in the embryo. Consequently the non-glycogen sugar is assumed to be all free, which cannot really be the case, but this may be easily amended when the free carbohydrate in the embryo is known. This error does not reach grave dimensions till the last five days of incubation, and as the correction would tend then to increase Table V, column 2, and consequently column 4, it would tend to decrease column 6. We may therefore expect that the descent of the curve of column 6 at the end of development will prove to be more precipitate than it seems to be at present.

This curve, which, for want of a better name, we may call the "ovomucoid" curve, shows some interesting relationships. In the first place, its initial value, namely 133 mg., is in fair agreement with the recent data of Komori (24). Komori prepared ovomucoid from fresh hens' eggs, obtaining from 333 gm. of albumen 4.8 gm. of ovomucoid. The processes were carried out as quantitatively as possible in order to get an idea of the concentration of the substance. This would mean 1400 mg. in 100 gm. of fresh albumen, or 466 mg. in the 33 gm. contained in one egg. Assuming, then, on the basis of the work of Müller (21), Zeller (61) and Seemann (51), that ovomucoid contains 30 per cent. of glucose, we get a result of 140 mg. ovomucoid glucose present at the beginning of development, which agrees very well with the 133 mg. calculated now by difference. This result gives us some confidence in interpreting the changes occurring in this fraction as changes in ovomucoid content.

What are these changes? As can be seen from Fig. 5 the ovomucoid curve falls until the fifth day is reached after which point it rises to a peak on the tenth day, thence to fall steadily till the time of hatching. The initial fall is of great interest in view of Komori's experiments in which he showed that Müller and Masa-yama's (32) egg "diastase" can very efficiently split off the sugar from ovomucoid. By the twentieth day there are at most half-a-dozen mg. of ovomucoid left. All the carbohydrate outside the embryo at that time can be accounted for by glycogen and free glucose. The peak in the ovomucoid curve at the tenth day seems to be responsible largely, but as will later be seen when glycogen is considered, not entirely, for the simultaneous peak in the total carbohydrate. From the fact that this peaked effect is found so marked in the ovomucoid curve the conclusion might be drawn that ovomucoid is a more labile element in the raw materials of the embryo than has hitherto been supposed. The recent work of Anson and Mirsky (4) on another conjugated protein, haemoglobin, has clearly shown the ease with which the prosthetic group can be detached from and reattached to the protein part of the molecule.

Komori (24) gives other figures for the amounts of ovomucoid present during development, but expresses them as gm. percentage of dry weight of albumen, so that although we know the rate at which the albumen is drying up, we cannot calculate his figures in mg. absolute per egg because we do not know the relative weights of yolk and white. Sakuragi's figures for the same fraction are not very valuable because they are few in number and were obtained by the use of some doubtful precipitations prior to total hydrolysis and estimation by copper reduc-
tion. The only previous work on the physiology of ovomucoid is that of Bywaters (8). He found that for the first 15 days of development the ratio of coagulable protein to non-coagulable protein in the egg-white was fairly steady at 6.5 : 0.15, and he therefore concluded that there was no preferential absorption of ovomucoid or ovoalbumin. This does not at first sight agree with the curve shown in Fig. 4. Two hypotheses are open to us: (1) that the curve for ovomucoid glucose calculated in this paper by difference does not at all times of development accurately represent the ovomucoid glucose, or (2) that at varying times in development the amount of glucose combined in the ovomucoid molecule varies considerably. Both these seem possible. If the latter turned out to be true, ovomucoid would have to be considered as a specialised form of glycogen. Bywaters found no change in the amount of sugar in the uncoagulable protein between the first and the eighteenth day; it remained constant at about 27 per cent. and as the ratio of the two, expressed as gm. per 100 gm. egg-white was constant, he considered that the carbohydrate radicle of ovomucoid was not split off before absorption. His methods were, however, not entirely sheltered from criticism for he used the original method of Pavy without modification and only hydrolysed the ovomucoid for 1.5 hours with 5 per cent. hydrochloric acid. Moreover his ratio varied from 1.0 to 2.4 which suggests that the hydrolysis may have been incomplete. It is significant that Bywaters' ovomucoid glucose values are highest between the seventh and thirteenth days though their absolute values are at least 50 per cent. higher than mine. What might be called the "glycogen" interpretation of the behaviour of the ovomucoid fraction receives strong support from Komori's work; for he believes the sugar to be combined with the protein principally as a polysaccharide. Further work on the constitution of ovomucoid and any changes which it undergoes during development, is urgently needed.

In Table V, column 7, there are placed the figures for the inositol content of the whole egg, as determined in a previous paper (39). Owing to the inferior nature of the estimation-method which has to be used for inositol great stress cannot be laid on the absolute values here given, but as will be seen in Fig. 4 there is a striking reciprocal correlation between the total cyclose and the total carbohydrate.

(b) The formation of new carbohydrate from fat.

However the kinematics of the inter-carbohydrate transformations may be supposed to run, it is clear from Fig. 4 that carbohydrate as a whole receives some reinforcement between the eighth and eleventh days. In Table VI the approximate amounts are given, taken from the smoothed curve of Table II, column 5. This increase amounts to about 90 mg., so protein is at once ruled out as a source because during the whole of development only 68 mg. are lost, and of this some at least must be due to true protein catabolism, unless the metabolism of the chick embryo differs entirely from that of all other living organisms. It is true that the peak in protein catabolism occurs at just the same time as the gain of carbohydrate, but as between the eighth and ninth days the egg only loses 1 mg. of protein while it
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Gains 47 mg. of carbohydrate this correlation is probably but a coincidence. We may safely conclude that the protein which is broken down is used for the production of energy and being burnt away does not go to form that extra carbohydrate which appears in the middle of development.

The other possible source is fat, and here the position is a good deal more hopeful. In a foregoing communication (43) I pointed out that between the seventh and the fourteenth days there was a discrepancy between the fat lost as determined by the averaged chemical analyses and that determined by the carbon dioxide output on the supposition that all of it was due to fat, which is not true. More was lost than could be accounted for even on this assumption. If now we suppose

Table VI. Fat \to carbohydrate transference.

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* Reference (43), Table IX, Cols. 5 and 6.

That the estimations were correct and place beside the carbohydrate gained the fat missing each day, as is done in Table VI, column 3, we find that a remarkable correlation exists. Little account need be taken of the fact that the maximum of the carbohydrate gained is reached before the maximum of the fat lost, for the inferiority of methods, the paucity of estimations, and the various conditions of individual workers must all be borne in mind. It is surely striking that nowhere else in development can two such discrepancies be found and that the two are approximately of the same order. As evidence for the possibility of the fat-carbohydrate route \textit{in vivo} the figures described here seem to reinforce strongly the few others at present existing.

1 See Furusawa (1925) (\textit{Proc. Roy. Soc. B, 98}) and Calvocriado (1925) (\textit{Biochem. Zeit. 76, 164}). The latter considers that the liver is the responsible organ: if so, it can, in the embryo, transform fat into carbohydrate before it can store carbohydrate.
The phenomenon of the "foie transitoire"; the glycogenic function of the blastoderm.

The increase of glycogen in the embryo has already been discussed. But we possess also figures for the glycogen in the whole egg owing to the investigations of Idzumi and Sakuragi and as they are quite concordant some confidence may be placed in them. They are shown in Table IV, columns 5, 6 and 7. By subtracting the glycogen in the embryo from the glycogen in the whole egg we obtain that in the remainder, the figures for which are seen in Table V, column 3.

Fig. 5 shows the increase of glycogen in the embryo. By the end of development it has only attained about a quarter of the height of the total sugar in the embryo. It is interesting that Idzumi, Sakuragi, and Shaw all observed a great decrease of embryo glycogen during the process of hatching. They consider that this is related to the vigorous muscular movements which the embryo then makes for the first time, including the lung movements which come into play as the animal lays aside its allantois. As may be seen from Fig. 5 the glycogen is at its highest outside the embryo about the thirteenth day; after that time it rapidly falls and is, indeed, more or less the reciprocal of the glycogen inside. Evidently after the thirteenth day the glycogenic function is shifted from somewhere outside the embryo to somewhere inside.

The conception of a late development of the glycogenic function of the liver is no new one. In 1858 Claude Bernard published his researches on mammalian embryos in which he clearly showed that the glycogenic function later to be undertaken by the liver, was, during the greater part of foetal life, carried out by the placenta. On p. 120 he wrote in a footnote "Dans les oiseaux (poulet) j'ai constaté, avant le développement des cellules glycogènes du foie, l'existence de cellules glycogènes qui se développent dans les parois du sac vitellin."

Immediately following Bernard's paper there is to be found a note by Serres entitled "Des corps glycogéniques dans la membrane ombilicale des oiseaux." Bernard's communication, he said, revealed to him the nature of those little glandular bodies which appear on the surface of the blastoderm during incubation. "On voit ces petits corps glanduleux au microscope dès la vingt-cinquième au trentième heure de l'incubation....Du troisième au cinquième jour leur volume commence à croître." They begin to disappear about the twelfth day, and indeed from histological considerations the liver would not until then be ready to perform a glycogenic function.

The placenta, then, must be regarded as a "foie transitoire." After 1860 the work of Claude Bernard was repeated and confirmed histochemically by Chipman, Driessen, and many others, but not until 1908 was the phenomenon put on a firm chemical basis. Lochhead and Cramer, working with rabbits, estimated the glycogen in the placenta and the foetal liver, and obtained curves very like those shown in Fig. 5. I have calculated from their figures the relations between embryo and exterior in the matter of glycogen and drawn them up in Table VII,
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which shows that the mid-point in the assumption of the glycogenic function by the embryonic liver occurs when 82 per cent. of the total development is achieved in the case of the chick, and 91 per cent. in the case of the rabbit. Mellanby(47) and Schmalhausen(50) have weighed the liver at different ages in the chick, but I cannot find that the assumption of the glycogenic function is accompanied by any sudden or even gradual change in weight. Heaton(58) however has observed a rapid alteration in the liver on the eleventh day. Before that time its cells in tissue-culture grow like epithelial cells (it arises, of course, as a diverticulum of the gut): after that time they grow like fibroblasts. Nor is this only as regards their appearance, for after the eleventh day they are inhibited by yeast extract just as fibroblasts are: but not before. This change occurs regularly and definitely between the

Table VII. The glycogenic function.

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eleventh and twelfth days: *i.e.* just about the time when the curve for glycogen in the embryo inflects and rises sharply (see Fig. 5). Serres illustrated his histological observations in the *Archives du Museum d’Histoire Naturelle* (53) and like effects have since been seen by Wilson (58). The phenomenon of the transitory liver will in part account for the peak in total carbohydrate seen in Fig. 4 but the principal responsibility for this rests on some form of sugar not free and not bound as glycogen, probably ovomucoid. It may also account for the fact previously ascertained that injected glucose is of no value in early development as a protein-sparing agent.

Since both the chick and the rabbit have a transitory liver the practice is probably general. This is an excellent instance of those special functions of embryonic life with which Wintrebert (59) has recently dealt, namely (a) the special protease secreted by some teleosts and anura to dissolve their shell-membranes at hatching, (b) the rhythmic contractions of Selachian myotomes prior to all nervous activity, (c) the ectodermal irritability of amphibians, and (d) the reflex actions in Selachian embryos due to the activity of the Rohan-Beard cells.

(d) The intensity of absorption of carbohydrate by the embryo at different ages.

In the immediately preceding paper of this series (45) the relative intensities with which the embryo absorbs protein and fat during its development were calculated. They were expressed in terms of the number of mg. of protein or fat handed over to 100 gm. of embryo by 100 mg. of protein or fat outside the embryo. Thus, the standards of comparison being kept constant, the curve resulting was a true measure of the intensity of absorption at any given point in time.

Since the amount of total carbohydrate inside and outside the embryo is now known, the same procedure can be applied to carbohydrate. This is done in Table VIII and Fig. 6. The two important questions which this curve can answer are (a) whether there is any relation of simultaneity between the absorption and combustion of carbohydrate, and (b) whether there is any likeness between the absorption curves for protein and carbohydrate, for if so, the conception of rhythmic permeability-changes on the part of the cells of the blastodermal blood-vessels would receive support. All the three curves of Fig. 6 are given as averages of the wet and dry weight data. This, it must be emphasised, is a fictitious entity for it represents what the absorption intensity would be if the embryo was always 50 per cent. drier than it really is. Nevertheless it is convenient and it saves constant reference to wet and dry weight.

The period of predominance of carbohydrate combustion is believed to be in the first week of development and from Fig. 6 it may be seen that the absorption intensity is then at its highest. In this way carbohydrate differs from protein and fat, for with them there is no trace of such a relationship. At present it must

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1 Striking histological confirmation of the curve for glycogen in the embryo has recently been brought forward by Potvin and Aron, *Comptes Rendus Soc. Biol.* (1927), 96, 257.

2 After writing this, I found that Blanchard (Zool. Anz. (1883), 6, 67) confirmed Bernard, working with a Selachian embryo (*Mustelus vulgaris*).
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suffice to record that carbohydrate behaves as regards this in a different manner from fat and protein. During the first six days the absorption of carbohydrate and protein (both "water-soluble substances") is very high, while that of fat is very low. From the sixth to the thirteenth days the intensity of absorption of fat is high and protein and carbohydrate are low. From the thirteenth to the seventeenth days the absorption of fat again drops, and that of protein rises, but carbohydrate does not accompany it; on the contrary it remains very low though moving slowly in an upward direction. At the eighteenth day there is another sharp crossover between protein and fat which is not shared by carbohydrate.

Table VIII. Carbohydrate absorption curve.

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<th>mg. total carbohydrate combusted</th>
<th>Col. 2 + 3 mg. total carbohydrate absorbed</th>
<th>mg. outside the embryo</th>
<th>Col. 4 as percentage of col. 5</th>
<th>Weights of embryo for interdiurnal periods in mg.</th>
<th>Absorption curve</th>
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The behaviour of carbohydrate gives therefore some support to the theory that changing types of permeability in the blastodermal cells are responsible for these effects. In the earlier periods when the absolute amounts of carbohydrate being absorbed are comparable with the absolute amounts of fat and protein being absorbed, the protein-carbohydrate curve does tend to be the reciprocal of the fat curve, but later on the carbohydrate drops out of the relationship and pursues an uneventful course of its own. This would harmonise with the view that at one time the predominant type of permeability in the blastodermal cells would be "water-soluble" and at another time "fat-soluble." For the protein and fat would be expected to show the most marked changes in the latter half of incubation, and
the needs of the embryo for carbohydrate, which are at that time insignificant compared with the fat and protein requirements, could easily be met by absorption through minor channels. Thus on the sixth day the embryo absorbs 5 mg. carbohydrate, 6 of protein, and 2 of fat, while on the sixteenth day it absorbs 11 of carbohydrate, 449 of protein, and 333 of fat. During the period, then, when the absorption of carbohydrate is at all equivalent to that of the other foodstuffs the relations predicted by the theory hold in practice.

SUMMARY.

1. The total carbohydrate in the embryo and in the remainder of the hen’s egg has been quantitatively estimated throughout development. The total carbohydrate in the embryo rises in a regular manner. The total carbohydrate in the remainder of the egg falls from the beginning of development till the seventh day, after which it rises to a peak on the eleventh day before dropping steadily until the time of hatching. The total carbohydrate in the whole egg remains practically constant after the middle of incubation, for then the embryo gains all that the remainder loses.

2. The free glucose in the remainder of the egg falls continuously from the beginning of development till the tenth day, but thenceforward it falls only extremely slowly, if at all. The free glucose in the whole egg suffers a like diminution until the tenth day, after which it rises owing to the accumulation of free sugar in the embryo.

3. There is a current of free glucose yolkwards during the first week of development, flowing in the same direction as the water.

4. The glycogen increases regularly from the beginning of development in the whole egg, but only to any marked extent in the embryo from the eleventh day onward. The extra-embryonic glycogen has a peak about the thirteenth day. The glycogenic function of the embryonic liver is not assumed till the latter part of development; before then it is carried on by the cells of the blastoderm. This closely resembles the mechanism seen in mammalia where the glycogenic function is retained by the placenta till an advanced stage of embryonic life. The peak in the curve for total carbohydrate in the remainder of the egg is partly to be accounted for by the phenomenon of the “foie transitoire.”

5. The ovomucoid glucose (i.e. the total carbohydrate minus the free glucose and the glycogen) falls steadily in the first half of development to reach a minimum about the fifth day, thereafter it rises till the eleventh day only to fall and vanish entirely by the end of incubation.

6. The curve for total cyclose in the developing egg takes a course exactly opposite to that of the total carbohydrate.

7. The gain in total carbohydrate between the seventh and eleventh days coincides remarkably with a loss of fat during the same period, for there is just then a quantity of fat not found in the chemical analyses and not accounted for by the respiration of the embryo, even supposing that all the substance combusted
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is fat. These amounts are of about the same order and the possibility of a transfer of fat to carbohydrate is considered, for the extra carbohydrate certainly cannot be derived from protein.

8. The intensity of absorption of carbohydrate has been calculated in the same manner as that previously used for fat and protein. It is based on analyses of 1320 egg fractions. The curve has a peak at some point prior to the fourth day and descends to a low level by the ninth day, after which it slowly rises. These results afford support for the view already advanced that there are rhythmic permeability-changes in the blastodermal cells.

9. There is a relation of simultaneity between the absorption and combustion of carbohydrate. In this it differs from protein and from fat.

The writer wishes to thank Professor Sir F. G. Hopkins, F.R.S., for his continual interest in this work, and also to record his gratitude to Professor J. T. Wilson, F.R.S., and Mr J. T. Irving for their valuable help. He is, moreover, indebted to the Government Grant Committee of the Royal Society for a grant which partially defrayed the cost of these researches.

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