CVII. STUDIES ON THE CARBOHYDRATE METABOLISM OF THE CHICK EMBRYO.

By CONSTANTIN DONHOFFER.

From the Medical Clinic of the University, Pécs, Hungary.

(Received April 17th, 1933.)

PART I. THE CARBOHYDRATE CONTENT OF THE EGG.

SINCE the avian egg represents, in a certain sense, a closed system, changes of its carbohydrates during development may lead to the disclosure of some facts concerning the intermediary metabolism of these substances which are not readily revealed under other conditions. As the first step in the investigation of these changes, suitable methods for the differentiation of the various intermediary substances had to be selected, and it is with this problem that the first part of this paper is concerned. Methods used by previous investigators of the problem have recently been critically reviewed by Needham [1927; 1931], but it cannot be admitted that even his procedure is free from possible sources of error. This is particularly the case when data taken from "smoothed curves" and from the published results of other workers are used in calculating total carbohydrate.

The figures given by Needham and others for free sugar vary a great deal. This seems to be mainly due to the presence of a water-soluble substance of considerable reducing power, which is not removable with most protein precipitants. The reducing power of alcoholic extracts represents more closely free sugar, but even these show some non-sugar reduction, depending in amount on the exact method used. The differences are necessarily greater when the estimation involves hydrolysis, in which case only a fraction of the observed reduction may be due to carbohydrates. In order to reduce these sources of error, in the present investigation, fermentation and precipitation with copper-lime were used for determination of the free sugar, although it is realised that even by these methods substances other than carbohydrate may be included.

Methods.

Eggs of various origin and age were macerated for several hours without their shells in 500 cc. of 96 % alcohol and, after removing the alcohol by centrifuging, extraction was repeated twice with 300 and 150 cc. alcohol respectively. The combined extracts were evaporated (under reduced pressure) at 55–65° and, towards the end of evaporation, several 15–25 cc. portions of distilled water were added. After the evaporation of all the alcohol the residue was taken up in a definite volume of water (usually 100 cc.). The resultant emulsion was precipitated with trichloroacetic acid or phosphotungstic acid, the neutralised filtrate being referred to as the "alcohol-soluble fraction."

The alcohol precipitate was extracted for several hours first with 100 cc., and then twice with 50 cc. of boiling distilled water. The combined extracts, containing the water-soluble fraction of the alcohol precipitate along with the water pressed out from the water-insoluble residue, were then analysed in the various ways described later. The residue, freed from water, was weighed and suspended in an equal volume of water to which HCl was added, to make the concentration 5 %, after which it was hydrolysed on a boiling water-bath. Proteins were then precipitated with phosphotungstic acid, and the resultant solution was worked up in the same way as in all other fractions.

In all extracts, reducing power was estimated, after neutralisation to phenol red, by the Hagedorn-Jensen method (total reduction), and this was repeated in the same solutions after fermentation and after precipitation with copper-lime. The difference between total reduction and that observed after fermentation and precipitation with copper-lime is referred to as "fermentable" and "precipitable" reduction respectively.

The excess of phosphotungstic acid was removed by adding concentrated potassium chloride. Filtration was avoided throughout by centrifuging.

Glycogen was estimated by Pflüger's method, with the difference that fermentable reduction alone was finally measured.

Unless otherwise stated reducing power is expressed throughout in mg. glucose per 50 g. of eggs without shell.

Table I shows decisively that the precipitable reduction of the alcohol-soluble fraction is fermentable *in toto*, supporting the assumption that all this

Table I. *Alcohol-soluble fraction.*

Exp.	Total	Reduction (mg. glucose per 50 g. egg)		
		Precipitable	Fermentable	
1	137	126	—	Single eggs of various origin
2	122	106	—	
3	107	94	—	
4	96	96	96	
5	152	129	122	
6	133	122	114	
7	123	108	111	
8	130	95	95	
9	128	121	121	
10	128	111	111	Average of two eggs
11	115	103	107	
12	131	117	118	
13	135	117	114	
14	112	94	96	Fresh Rhode Island eggs
15	109	97	—	
16	106	95	—	
17	102	92	90	
18	130	113	110	
19	124	106	—	
20	120	103	105	
21	129	107	—	
22	131	114	112	

reduction is due to monosaccharides. The figures, which average 108 mg., agree remarkably well in eggs of various origin and laid at different seasons, yet they are considerably lower than those of other workers, which vary between 156 mg. [Pennington *et al.*, 1915] and 225 mg. [Hepburn and St John, 1921]. Only

Müller and Masayama's [1900] are lower (40–97 mg.). This divergence is probably mainly due to methodical errors.

In some of these experiments reducing power was estimated after hydrolysis as well (2.2 % HCl, 1.5–2.5 hours on the boiling water-bath), with the unexpected result, that a regular decrease was observed. The reducing power of glucose does not diminish in similar circumstances, so that the presence of another monosaccharide besides glucose is probable.

Table II. *Alcohol-soluble fraction. Loss in reducing power after hydrolysis. (mg. glucose per 100 mg. originally present).*

Substance	Duration of hydrolysis hours	Reduction loss	
		Precipitable	Fermentable
Whole egg	2.5	20	—
"	2.5	13	16
"	1.5	3	5
"	2.5	24	18
"	2.0	0	0
"	2.0	8	10
2 Whites	2.5	11	13
2 Yolks	2.5	13	8
Whole egg	2.0	9	12
"	2.0	16	—
"	2.0	13	—
"	2.0	13	11

Table III shows that this decrease in reducing power is continuous during a certain period.

Table III. *Alcohol-soluble fraction of egg-white. Loss in reducing power after hydrolysis with 2.2 % HCl.*

Before hydrolysis	Reduction 208 mg./100 cc.
15 mins. at room temperature	202
30 mins. at 100°	196
60 mins. at 100°	192
90 mins. at 100°	186
180 mins. at 100°	173

The water-soluble fraction of the alcohol precipitate. After clearing this fraction of the supernatant fat in a separating funnel, it was hydrolysed with 2.2 % HCl for 2.5 hours in a boiling water-bath, and subsequently the proteins were precipitated with phosphotungstic acid. In other experiments parts of this fraction were precipitated before hydrolysis: (a) with phosphotungstic acid, (b) with trichloroacetic acid. The resulting solutions and the trichloroacetic acid precipitates were then hydrolysed in a similar way. The precipitable reduction of the trichloroacetic acid filtrate was considerable even without hydrolysis, but none of this was fermentable. In Tables IV, V and VI only the figures referring to reduction after hydrolysis are recorded.

Changes in the duration of hydrolysis, in the concentration of the acid or in precipitant have no effect on the precipitable or fermentable reduction, though all of them alter total reduction.

In this way the water-soluble fraction of the alcohol precipitate can be divided into three fractions: (a) not precipitable with phosphotungstic acid, (b) precipitable with phosphotungstic acid but not with trichloroacetic acid,

(c) precipitable with trichloroacetic acid. Table IV shows the behaviour of these fractions. Fraction (b) is the difference of the trichloroacetic acid filtrate and fraction (a).

Table IV. *Reduction precipitable with copper-lime.*

Exp.	Fraction		
	(a)	(b)	(c)
1	17	45	22
2	18	52	20
3	16	42	10
4	18	44	8
5	19	45	20
6	16	57	16
Average	17.3	47.5	16.0

Fraction (a) gives no Molisch test, is not fermentable and is dialysable even before hydrolysis. Fraction (b) is not dialysable before hydrolysis, gives a strong Molisch test and after hydrolysis about 68 % of it is fermentable. Fraction (c) gives also a strong Molisch test and after hydrolysis is fermentable *in toto*.

Table V shows the figures of the total precipitable reduction of the water-soluble fraction of the alcohol precipitate during the months December-March and May-August respectively.

Table V.

Dec.-March	87	77	72	74	96	74			
	93	75	77	83	92	89			
	92	77	82	74	89	81			
May-August	66	52	59	46	45	47	54	53	48
	65	42	53	50	56	60	46	54	55
	62	50	52	44	56	51	46	59	58

The water-insoluble fraction of the alcohol precipitate. This fraction was hydrolysed with 5 % HCl for 5-10 hours and afterwards precipitated with phosphotungstic acid. The fermentable reduction is not affected by these variations in the duration of hydrolysis. Precipitation with copper-lime is not satisfactory in this fraction because of difficulty in reading the end-point of titration. So, besides total reduction only the fermentable reduction is recorded, as shown in Table VI.

Table VI.

Exp.	January-March				July		
	(a)	(b)	(c)	(d)	(e)	(f)	(g)
Total reduction	206	257	230	244	226	268	257
Fermentable reduction	94	101	93	93	86	77	79

Glycogen per egg is shown in Table VII.

Table VII.

Glycogen per egg.										
Exp.	1	2	3	4	5	6	7	8	9	10
Mg.	9.6	9.0	8.2	8.4	7.6	8.0	7.2	11.9	10.0	10.6

The distribution of reducing substances between white and yolk¹ is shown in Table VIII.

Table VIII.

Exp.	Mg. glucose per 100 g. egg		Weight of yolk	Reduction of yolk
	White	Yolk	In % of whole egg	
Alcohol-soluble fraction:				
I	312	162	44.3	28.5
II	342	122	41.5	20.2
III	348	158	39.0	22.4
IV	312	148	37.0	22.5
V	318	152	38.0	22.3
Average	326	148	39.9	23.1
Water-soluble fraction of alcohol precipitate:				
I	296	92	44.3	19.6
II	324	83	41.5	15.4
III	222	58	39.0	14.8
IV	229	75	37.0	16.7
V	245	58	38.0	13.0
Average	263	73	39.9	15.9
VI	0.0	16.0		
VII	0.0	15.2		

The result that the yolk contains about 23 % of the total glucose is in agreement with those of Sato [1916], Pennington *et al.* [1915] and Kojo [1911], though their absolute figures are much higher for both white and yolk, being 440–550 and 180–272 mg. per 100 g. respectively. The ratios given by Sakuragi [1917] and Roger [1908] are somewhat higher (30–31 %), due to a greater reduction of the yolk (300–330 mg. per 100 g.). The probable cause of these higher values has been pointed out previously. Tomita [1921] found a lower ratio for the yolk (15 %) combined with low absolute values in both white (84 mg. per 100 g.) and yolk (15 mg. per 100 g.). Figures for the water-soluble fraction of the alcohol precipitate show that the yolk contributes to this portion an even lower percentage of reduction (15.9 %). Contrary to Sakuragi [1917] no glycogen could be found in the white. In respect of hydrolysis and fermentation the yolk and the white behave in the same way.

DISCUSSION.

Regarding the alcohol-soluble fraction, it is of interest that several investigators have found fructose in the allantoic fluid and foetal blood of various species. This makes the assumption probable that the decrease of reduction observed after hydrolysis may be due to destruction of fructose. The question whether alcohol-soluble higher carbohydrates are present, cannot be answered definitely, since an increase of reducing power due to such could be masked by the presence of the above-mentioned reducing substance sensitive to mineral acids.

Of the three fractions into which the water-soluble fraction of the alcohol precipitate can be subdivided, the nature of fraction (a) (not precipitable with phosphotungstic acid) is questionable, and fraction (b) probably contains all the ovomucoid sugar, the difference between precipitable and fermentable reduction being possibly due to glucosamine hydrochloride. Fraction (c) (precipitable with trichloroacetic acid and fermentable *in toto*) represents protein

¹ All figures represent the mean of two whites or two yolks.

sugar. The same can be said of the fermentable reduction of the water-insoluble fraction of the alcohol precipitate.

Glycogen. Direct estimations show that a part of the glycogen is found in the water-soluble, another part in the water-insoluble fraction of the alcohol precipitate. This fact does not interfere with the figures given above, as the amount of glycogen present is small, but must be taken into consideration in experiments done during incubation, when glycogen is accumulating.

The separation into fractions by solubility and precipitation does not offer information on the chemical nature of the carbohydrates involved; it only differentiates substances containing carbohydrates, though differences in fermentability indicate that the carbohydrate component is not identical in all of them. So the question, whether the carbohydrates of the water-soluble, or water-insoluble fraction of the alcohol precipitate or both of them are identical with Levene and Mori's [1929] polysaccharide, containing a trisaccharide composed of two molecules of mannose and one molecule of glucosamine, or with Pringsheim's [1923] or Lohmann's [1926] amylotriase, cannot be answered. Nevertheless, as the figures of these fractions are fairly constant, the differentiation between them seems useful for observations on the carbohydrate metabolism of the developing egg.

The total carbohydrate content of the egg varies in the present experiments between 272 and 296 mg. These figures are considerably lower than those found by other investigators, which vary between 335 mg. [Needham, 1927] and 507 mg. [Sakuragi, 1917]. The methods applied account for this difference. The figures of Pucher [1928] for total fermentable sugar are the only ones which agree with the present results. He found 265 mg. of fermentable sugar present in the egg, and when one considers only the fermentable part of the water-soluble fraction of the alcohol precipitate in the present experiments exactly the same figures are obtained.

PART II. CHANGES DURING INCUBATION.

Using the methods of analysis described in Part I the present work was started to ascertain (*a*) the source of the glycogen formed during incubation, (*b*) the rôle of gluconeogenesis, and (*c*) the nature of the carbohydrate fraction primarily formed by gluconeogenesis, if such occurs. Results on the carbohydrate metabolism during incubation obtained previously differ widely, and the methods applied involve considerable sources of error which have already been examined.

Rhode Island eggs were hatched at 38.0–40.0° in an incubator, and on various days of incubation the fermentable, and copper-lime precipitable reductions were estimated (*a*) in the alcohol-soluble fraction, (*b*) in the water-soluble fraction of the alcohol precipitate, (*c*) in the water-insoluble fraction of the alcohol precipitate of the whole egg. Glycogen estimations were carried out on separate eggs of the same series. In the more advanced stages of development the embryo was ground up in a mortar containing cold alcohol. All figures are calculated for eggs weighing 50 g. without their shell at the start of incubation.

Alcohol-soluble fraction. The free sugar declines during the first half of incubation but rises again slightly in the last days (Table IX). The minimum was reached somewhat earlier in the series incubated during May–July than in those observed

during January–March. In some cases, during the first days of development, considerable differences were met with, even within the same series; later on, the figures agreed fairly well. The rise observed during the last days, though not very great, is considered important, because it occurred in all series followed up to that stage (IV, V, VI, VII, X).

Table IX. *The fermentable reduction of the alcohol extract (free sugar).*

Days	0	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Season
Series																				
I	100	87	—	—	34	—	—	28	—	—	—	—	—	—	—	—	—	—	—	Jan.–Mar.
	103	100	—	—	45	—	—	32	—	—	—	—	—	—	—	—	—	—	—	
II	110	71	—	—	50	—	—	30	—	—	11	—	—	—	—	—	—	—	—	
	106	—	—	—	56	—	—	35	—	—	13	—	—	—	—	—	—	—	—	
III	108	97	—	—	74	—	—	31	—	—	22	—	—	13	—	—	—	—	—	
	114	93	—	—	44	—	—	32	—	—	17	—	—	11	—	—	—	—	—	
IV	100	90	—	—	45	—	—	25	—	17	—	—	12	—	—	22	—	—	17	
	108	—	—	—	52	—	—	—	—	19	—	—	16	—	—	20	—	—	23	
V	110	104	—	—	—	27	—	—	—	13	—	—	—	11	—	—	—	15	—	
	115	96	—	—	—	30	—	—	—	18	—	—	—	9	—	—	—	18	—	
VI	—	—	—	—	—	—	—	—	—	8	—	—	—	13	—	—	—	17	—	May–July
	—	—	—	—	—	—	—	—	—	9	—	—	—	14	—	—	—	18	—	
VII	106	98	81	—	—	—	21	—	—	—	—	—	—	—	6	—	—	16	—	
	98	—	81	—	—	—	34	—	—	—	—	—	—	—	7	—	—	15	—	
VIII	—	—	—	—	—	—	—	—	6	—	—	16	—	—	—	—	—	—	—	
	—	—	—	—	—	—	—	—	13	—	—	12	—	—	—	—	—	—	—	
	—	—	—	—	—	—	—	—	15	—	—	—	—	—	—	—	—	—	—	
IX	—	—	—	—	—	21	—	—	9	—	—	—	—	—	—	—	—	—	—	
	—	—	—	—	—	36	—	—	11	—	—	—	—	—	—	—	—	—	—	
X	—	—	—	—	51	23	—	10	8	—	—	—	—	—	—	16	—	—	—	
	—	—	—	—	33	22	—	10	8	—	—	—	—	—	—	17	—	—	—	
	—	—	—	—	—	—	—	11	—	—	—	—	—	—	—	—	—	—	—	
Av.	109	92	81	—	48	26	27	24	11	14	15	14	14	12	6.5	19	—	16.5	20	

As shown previously (Part I) the starting level of free sugar is considerably lower than that found by other workers. The divergence of results between the present experiments and those published so far becomes even greater during incubation. Idzumi [1924] states that free sugar falls from 184 to 26 mg. on the 10th day and rises again steadily to 134 mg. on the 20th. Sakuragi [1917] found 200 mg. of free sugar in the fresh egg; this decreased to 19 mg. on the 10th day, subsequently rising to 75–107 mg. on the 13th and 120–162 mg. on the 20th day. Needham's [1927; 1931] smoothed curve starts at 200 mg., reaches its minimum on the 10th day with 33 mg. and rises steadily to 137 mg. on the 20th day. Sagara's [1930] figures show no rise in the second half of incubation; they decline steadily from 173 to 40 mg. on the 10th and to 21 mg. on the 20th day. The fact that Needham uses for his smoothed curves and calculations the data of Idzumi and of Sakuragi for free sugar naturally diminishes their value.

Water-soluble fraction of the alcohol precipitate. Table X shows that the seasonal difference previously noted persists throughout development. Figures were considerably higher in eggs analysed from January to March than in those from May to July. In the same series the values agree fairly well, and they decline in the first half of incubation and rise again in the second. The water-soluble fraction of the alcohol precipitate can be subdivided into three parts

Table X. *The water-soluble fraction of the alcohol precipitate.*

Reduction precipitable with copper-lime.																				
Days Series	0	3	4	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Season	
I	84 89	77 81	— —	97 76	— —	— —	86 82	— —	— —	85 83	— —	— —	— —	— —	— —	— —	— —	— —	} Jan.-Mar.	
II	81 79	80 83	— —	76 83	— —	— —	71 67	— —	— —	67 58	— —	— —	— —	— —	— —	— —	— —	— —		
III	86 78	80 91	— —	68 73	— —	— —	82 70	— —	— —	77 65	— —	— —	91 75	— —	— —	— —	— —	— —		
IV	76 82	88 89	— —	69 65	— —	— —	62 60	— —	67 79	— —	— —	98 110	— —	— —	113 104	— —	— —	98 106		
V	98 88	101 92	— —	— —	61 62	— —	— —	— —	88 87	— —	— —	— —	73 83	— —	— —	— —	— —	— —		
VI	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	82 92	— —	— —	— —	95 100	— —		
VII	— —	— —	— —	— —	— —	— —	— —	— —	98 70	— —	— —	— —	80 77	— —	— —	— —	94 78	— —		
VIII	95 90	— —	99 93	— —	— —	72 64	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —		
Ia	47 57	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	56 61	— —	66 63	69 66	— —		} May-July
IIa	58 50 —	— — —	— — —	— — —	— — —	— — —	— — —	41 49 58	— — —	— — —	53 35 35	— — —	— — —	— — —	— — —	— — —	— — —	— — —		
IIIa	48 54 —	— — —	— — —	— — —	51 46 50	— — —	— — —	44 46 —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —	— — —		
IVa	50 56 49	— — —	56 56 73	47 60 51	— — —	— — —	— — —	45 49 46	37 54 —	— — —	— — —	— — —	— — —	— — —	54 55 —	— — —	— — —	— — —		
Average an.-Mar.	88	86	—	77	65	72	—	77	—	—	—	82	—	—	92	—	—	—		
Average May-July	52	—	—	51	—	—	47	—	—	—	—	—	—	56	—	66	—	—		

(Part I) (a) not precipitable by phosphotungstic acid, (b) precipitable with phosphotungstic, but not with trichloroacetic acid, (c) precipitable with trichloroacetic acid. Fig. 1 shows the relative amounts of fractions *a-c* present during incubation in % of the water-soluble fraction. The initial fall in the latter is, as Fig. 1 shows, not due to fraction (b), consisting mainly of ovomucoid glucose, but to fractions (a) and (c). Fraction (a) contains probably no carbohydrate. The rise observed during the second half of incubation is mainly due to fractions (a) and (b), as (c) falls off again in the last days to reach its lowest level. Leaving fraction (a) out of consideration as probably non-carbohydrate, the observed increase could be accounted for by the accumulation of glycogen, but direct estimations show that only a small fraction of the egg's glycogen is present in the water-soluble fraction. Fermentation shows that the observed increase is only partly fermentable; so the conclusion seems warranted, that a part of the increase is due to the formation of a substance similar to that contained originally in the water-soluble fraction.

Water-insoluble fraction of the alcohol precipitate. Contrary to the case with other fractions, figures obtained here vary widely, so that some difficulty is encountered in calculating averages. Nevertheless, we can state with confidence

Table XI. *The water-insoluble fraction of the alcohol precipitate.*

Days Series	Fermentable reduction.													
	0	4	6	7	8	9	10	11	13	14	16	17	18	19
I	—	—	—	—	—	85	—	—	—	—	—	—	—	44
	—	—	—	—	—	82	—	—	—	—	—	—	—	43
II	94	—	—	—	—	—	—	82	—	55	—	—	—	47
	101	—	—	—	—	—	—	86	—	58	—	—	—	41
III	83	56	—	—	54	—	—	—	—	—	—	45	—	—
	79	54	—	—	57	—	—	—	—	—	—	49	—	—
IV	78	—	—	—	—	—	—	—	—	—	80	—	—	—
	80	—	—	—	—	—	—	—	—	—	79	—	—	—
V	83	—	—	—	—	—	45	—	70	—	—	—	—	—
	78	—	—	—	—	—	45	—	62	—	—	—	—	—
VI	—	—	—	—	—	—	48	—	60	—	—	—	—	—
	—	—	—	51	—	—	39	—	—	—	—	—	—	—
VII	—	—	—	59	—	—	35	—	—	—	—	—	—	—
	—	—	—	64	—	—	—	—	—	—	—	—	—	—
VIII	—	65	47	—	—	—	60	—	—	—	50	—	—	—
	—	61	41	—	—	—	66	—	—	—	56	—	—	—
IX	—	66	40	—	—	—	—	—	—	—	—	—	—	—
	—	—	—	—	41	—	—	—	—	—	—	—	50	—
IX	—	—	—	—	43	—	—	—	—	—	—	—	45	—
	—	—	—	—	33	—	—	—	—	—	—	—	—	—
IX	—	—	—	[8*]	—	—	62	—	—	—	—	—	—	—
	—	—	—	[18]	—	—	63	—	—	—	—	—	—	—
IX	—	—	—	—	—	—	67	—	—	—	—	—	—	—
	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Average	84	60	50		57		58		60		58		45	

* Not included in averages.

Fig. 1.

that this fraction has diminished by the end of incubation, though in many cases a considerable fluctuation may occur during the process. The variability of these results could be explained by a great individual variability or by a very large experimental error. The fact that results obtained in the same series of eggs agree closely rather suggests that such error does not occur, and that this variability is based on some metabolic process. It is obvious that, for certain purposes, only results obtained in the same series can be compared. The significance of these increases (series V, VII, IX of Table XI) will be considered later.

The carbohydrate of this fraction changes during development. At the start of incubation only 4–5 mg. of the fermentable reduction (4–6 %) is due to glycogen; at the end the glycogen represents 80–100 % of the fermentable reduction.

Table XII. *Glycogen.*

(January to March.)														
Days	0	4	6	7	8	9	10	11	14	15	16	17	19	20
Series														
I	9.6	—	—	—	—	17	—	24	30	—	—	42	—	60
	9.0	—	—	—	—	11	—	21	36	—	—	37	—	68
	10.0	—	—	—	—	—	—	—	—	—	—	—	—	—
II	8.2	—	9	10	—	—	19	—	31	—	—	—	—	—
	8.4	—	15	8	—	—	19	—	25	—	—	—	—	—
	—	—	—	—	—	—	15	—	25	—	—	—	—	—
III	10.6	—	—	—	—	—	—	33	—	41	—	—	56	—
	11.9	—	—	—	—	—	—	20	—	42	—	—	58	—
IV	7.2	6.8	—	—	9	—	—	—	—	—	44	—	—	—
	7.6	10.2	—	—	10	—	—	—	—	—	35	—	—	—
	8.0	—	—	—	—	—	—	—	—	—	—	—	—	—
Average	9.0	8.5	10.5		12		18	24	29		40		57	64

Table XII shows the steady accumulation of glycogen throughout incubation. The figures given are considerably higher than those of other workers [Idzumi, 1924; Sakuragi, 1917; Murray, 1926]. A seasonal difference could possibly account for this, as all estimations here considered were done from January to March, and in two experiments done in July, lower figures were found on the 19th day (30–40 mg.).

Table XIII. *Total carbohydrate.*

Without fraction (a) of water-soluble fraction.										
Days	0	3	6–7	8–9	10	11–12	13–14	15	17–18	19–20
Total carbohydrate	261	222	161	146	140	143	144	142	146	137

Table XIII demonstrates that the total carbohydrate content of the egg is practically constant from the 8–9th day to the end of incubation and shows no trace of the accumulation described by Needham [1927], who found, after an initial value of 335 mg., on the 6th day 152, on the 7th 142, on the 8th 145 mg. followed by a subsequent rise to 234 mg. on the 11th day. After the 11th day his total carbohydrate fell to 177 mg. on the 20th. In another of Needham's series (using the Wood-Ost method), the fall is smaller; the respective figures are 253 mg. on the 10th and 235 mg. on the 20th day. Sakuragi's [1917] figures show no rise between the 7th and 11th days but a steady decline from 426 mg. on the 6th to 348 mg. on the 20th day. Sagara's [1930] figures fall steadily from 398 to 94 mg. during incubation.

DISCUSSION.

The source of glycogen. It is evident from the figures included in Table IX that free sugar is not the source of the glycogen. Free sugar reaches the lowest level on the 10th day, when only about 18 mg. of glycogen are present. The major part of the glycogen is formed between the 10th and 20th days (Table XII), in which period free sugar remains unchanged at first and even increases a little during the last days. Neither is glycogen formed to any considerable extent from the reducing substances contained in the water-soluble fraction of the alcohol precipitate, as (1), though the major part of the glycogen is found in the water-insoluble fraction, the reducing power of the water-soluble fraction does not diminish, (2) any glycogen present in this fraction is accounted for by the increase in reduction during the second half of incubation, (3) a formation of glycogen would change the ratio of fermentable reduction and the reduction precipitable with copper-lime; the change actually observed is very limited, (4) in many cases the total fermentable reduction of this fraction does not account for all the glycogen formed. There remains as a source of glycogen the water-insoluble fraction of the alcohol precipitate. The fermentable reduction of this fraction suffices to account for all the glycogen formed, and direct estimations show that in the later stages of development about 80–90 % of the glycogen is contained in this fraction.

It is impossible to decide whether substances which have no reducing power even after hydrolysis do not play some part in the formation of glycogen. But, as a preformed, reducing and fermentable substance accounts for all the glycogen, the supposition of direct glycogen formation from other sources does not seem necessary.

Gluconeogenesis. The evidence brought forward by Needham [1927], according to which about 90 mg. of sugar are formed from fat between the 7th and 11th days, could not be confirmed by the present experiments. The average figures for total carbohydrate (Table XIII) are practically constant from the 8th to 9th days and so furnish no direct evidence for a gluconeogenetic process; yet they would mean that, without the assumption of gluconeogenesis during the second half of incubation, no carbohydrate is consumed at all, a supposition scarcely tenable. Attention was previously directed to the change taking place in the fermentable reduction of the water-insoluble fraction of the alcohol precipitate (Table XI). In some series (V, VII, IX) a considerable increase in this fraction was observed, which was not accompanied by an equivalent decrease in any other fraction. Table XI shows that, within one and the same series of eggs, all the changes were fairly uniform. The fact that this fraction is the main source of glycogen emphasises the importance of these changes. These data seem to afford some evidence that gluconeogenesis is going on during incubation, though on the whole the total carbohydrate remains unchanged, implying that the breaking down and the new formation of carbohydrate are usually in equilibrium.

SUMMARY.

The fermentable and copper-lime-precipitable reductions were estimated in the hen's egg during development:

- (a) In the alcohol-soluble fraction.
- (b) In the water-soluble fraction of the alcohol precipitate.
- (c) In the water-insoluble fraction of the alcohol precipitate.
- (d) Glycogen estimations were carried out on separate eggs of the same series.

The following conclusions are arrived at.

1. Free sugar reaches its lowest level on the 10th day and rises again slightly during the last days of incubation.
2. Reduction in the water-soluble fraction of the alcohol fraction of the alcohol precipitate decreases in the first and increases in the second half of incubation. This increase is probably not due wholly to carbohydrate and is partly accounted for by the accumulation of glycogen.
3. The reducing power of the water-insoluble fraction of the alcohol precipitate decreases on the whole from the start to the end of incubation, though considerable increases may occur in between. This fraction is the principal source of the glycogen formed during incubation.
4. Total carbohydrate diminishes till the 9th day and remains constant later.
5. Though no direct evidence could be obtained from the average figures, the changes observed in the water-insoluble fraction of the alcohol precipitate combined with other considerations warrant the supposition of gluconeogenesis.

The author owes a debt of gratitude to Mr Noltie of the Physiology Department, University of Aberdeen, who assisted him in writing this paper.

REFERENCES.

- Hepburn and St John (1921). *J. Biol. Chem.* **46**, 48.
 Idzumi (1924). Mitteilung. med. Fakultät, Tokio, **32**, 197 (cited from Needham).
 Kojo (1911). *Z. physiol. Chem.* **75**, 1.
 Levene and Mori (1929). *J. Biol. Chem.* **84**, 49.
 Lohmann (1926). *Biochem. Z.* **178**, 444.
 Müller and Masayama (1900). *Z. Biol.* **39**, 547.
 Murray (1926). *J. Gen. Physiol.* **9**, 405.
 Needham (1927). *Brit. J. Exp. Biol.* **5**, 26.
 — (1931). Chemical embryology. (Cambridge University Press.)
 Pennington, Hendrickson, Conally and Hendrix (1915). *J. Biol. Chem.* **20**, 21.
 Pringsheim (1923). Die Polysaccharide. (Julius Springer, Berlin.)
 Pucher (1928). *Proc. Soc. Exp. Biol. Med.* **25**, 72.
 Roger (1908). *J. Physiol. Path. Gen.* **10**, 797.
 Sagara (1930). *J. Biochem. (Japan)*, **11**, 433.
 Sakuragi (1917). *J. Tokio Med. Soc.* (in Japanese), **31**, 1 (cited from Needham).
 Sato (1916). *Acta Schol. Med. Univ., Kyoto*, **1**, 375 (cited from Needham).
 Tomita (1921). *Biochem. Z.* **116**, 22.