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Carbohydrate Metabolism of the Chick Embryo.

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These investigations are a continuation of previous studies[1] and have been made with the primary aim of further elucidating certain changes occurring in the physiology of the chick embryo centering around the 12th day of incubation. At this time a secondary peak in metabolic rate occurs,[2, 3] associated with an approach of the respiratory quotient toward unity.[4, 5] Liver glycogen, first demonstrable in considerable amounts on the 7th day of development and increasing steadily until the 9th day, is reduced to a new minimum on the 12th day.[6, 7] Further, no significant change in the blood sugar level occurs at this time.[8] It is during this same period that the endocrine glands associated with carbohydrate metabolism first give evidence of active functioning. Between the 10th and 13th days of development the thyroid,[9] the medulla of the suprarenal[10] and the islets of Langerhans[11, 12] first give evidence of activity. That this onset of function in the endocrine glands was concerned in some way in these changes seemed almost certain. For this reason, extracts of the endocrine glands known to be concerned with carbohydrate metabolism in the adult mammal were injected individually into chick embryos of various ages and the resultant effects on liver glycogen and the blood sugar level were determined.

Methods. The various extracts used were injected under the chorio-allantois through the shell membrane lining the air sac. Adrenalin was diluted with distilled water immediately before injection, while thy-

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roxin was injected in alkaline aqueous solution. Crude anterior lobe extract (Parke, Davis & Co.) and the suprarenal cortical extract, eschatin (Parke, Davis & Co.), were injected in the original concentrations. Control injections of phenol, NaOH and distilled water were made.

Blood sugar determinations were made by the Jeghers and Myers' modification[13] of Folin's micro method.[14] Roughly quantitative estimates of liver glycogen were made from histological sections stained with Best's carmine. Tissues were fixed in absolute alcohol and embedded in celloidin. Blood for the blood sugar determinations was obtained by inserting a glass cannula into one of the larger vitelline veins. The embryos used were of the White Leghorn strain.

Results. The control injections mentioned above had no demonstrable effects on liver glocogen. Table I summarizes the results obtained after the injection of adrenalin and thyroxin. Adrenalin injected in approximately physiological concentrations (0.1 cc of

TABLE I.
Liver Glycogen—Adrenalin and Thyroxin Injections. 8-day Embryos.

Subst. inj.	Amt injected in 0.1 cc	Time, hr	No. of embryos	Liver glycogen
Contr.	—	—	15	12+ 3+—
Adren.	1/100,000	2	9	8+ 1+—
"	1/50,000	2	9	6+ 3+—
"	1/4,000	2	3	2+— 1 trace
"	1/2,000	2	3	1+— 2 trace
Thyr.	1/800 mg	24	3	2+ 1+—
"	1/400 "	24	3	2+ 1+—
"	1/160 "	24	3	1+ 2+—
"	1/400 "	48	10	2+ 4+— 4—
"	1/625 "	72	2	2—
"	1/800 "	72	7	7—
"	1/1000 mg	72	8	3+— 5—

TABLE II.
Liver Glycogen and Blood Sugar—Eschatin, Anterior Pituitary and Insulin Injections. 8-day Embryos.

Subst. inj.	Amt. inj. 0.1 cc	Time, hrs	No. of embryos	Blood sugar Range	Mg% Avg	Liver glyc. %+
Contr.	—	—	11	94.0-137.5	112.2	100.0
Esch.		8-16	14	94.0-154.0	128.6	7.7
Ant. Pit.		6-16	25	105.0-196.0	145.2	100.0
Insulin	4U	4	3		102.3	"
"	"	8	2		76.1	"
"	"	12	3		86.8	"
"	"	16	3		67.7	"
"	"	20	3		62.2	"
"	"	24	3		62.8	"

[13] Jeghers, N. J., and Myers, V. C., *J. Lab. and Clin. Med.*, 1930, **15**, 982
[14] Folin, O., *J. Biol. Chem.*, 1928, **77**, 421.

1:100,000 and 1:50,000) induces no detectable changes in the liver glycogen of 8-day embryos, although the higher concentrations of 1:4,000 and 1:2,000 do cause some glycolysis. With these high concentrations necrosis was evident. Relatively large amounts of thyroxin allowed to act for 24 hours have no effect on liver glycogen, while one gamma of thyroxin induces considerable glycolysis if allowed to act for 72 hours.

Table II summarizes the results dealing with the action of eschatin, crude anterior lobe extract and insulin on both the blood sugar level and liver glycogen of 8-day embryos. The results indicate that eschatin induces a distinct rise in the blood sugar level and a decrease in liver glycogen. The study of sections indicates some decrease in skeletal muscle glycogen also with no perceptible change in cardiac muscle or yolk sac glycogen. The injection of the anterior pituitary extract causes a marked rise in the blood sugar level together with an increase in skeletal muscle and liver glycogen. There was no obvious change in cardiac muscle or yolk sac glycogen in these cases. During the first 16 hours after the injection of insulin an almost straight-line fall in the blood sugar level occurred with an apparently associated increase in liver glycogen. The livers of embryos exposed to the action of insulin for 8 hours or more consistently showed amounts of glycogen well above the normal. It should be mentioned that, while the injection of insulin in the amounts used is invariably fatal at this time, embryos receiving either anterior pituitary extract or eschatin frequently survive and hatch.

In a previous communication[1] we concluded from the study of a smaller series that no change in liver glycogen occurred after anterior pituitary injections. The study of this larger series indicates that a definite increase in liver glycogen occurs as well as a rise in the blood sugar level.

Experiments were made to test the possibility that the low glycogen stores present in the livers of 12-day embryos might be related to the small amounts of free sugar available at this time.[15] Table III summarizes these results. It shows that raising the blood sugar above the

TABLE III.
Liver Glycogen and Blood Sugar—Glucose and Anterior Pituitary Injections. 12-day Embryos.

Subst. inj.	Amt inj.	No. of embryos	Blood sugar Range	Mg% Avg	Liver glyc. %+
Contr.	—	26	94.3-129.0	112.6	37.7
Gluc.	300 mg	21	93.0-165.0	121.2	29.4
Ant. Pit.	0.4 cc	(30)	168.0-175.0	172.0	86.2

15 Needham, J., *Brit. J. Exp. Biol.*, 1926, **5**, 6.

normal level by the injection of glucose in 100 mg amounts on the 10th, 11th and 12th days does not increase the percentage of livers containing glycogen. The injection of anterior pituitary extract, on the other hand, increases liver glycogen and causes a rise in the blood sugar level just as it does on the 8th day of development. It should be mentioned that, although 30 livers were examined for glycogen in this group, blood sugar determinations were made in only 4 cases. However, these 4 had blood sugar levels above any found in either the controls or in the glucose group.

A series of 12 embryos not included in Table III were injected with 4 units of insulin on the 11th day. Examination of the livers from these embryos sacrificed on the 12th day showed that 9 contained considerable amounts of glycogen, while 3 contained only traces. This suggests that insulin is also capable of increasing the glycogen content of the livers of 12-day embryos. No blood sugar determinations were made on this group.

Summary. 1. Thyroxin and suprarenal cortical extract induce glycogenolysis of the glycogen normally present in the livers of 8-day embryos. 2. The injection of adrenalin in approximately physiological concentration appears to have no effect on liver glycogen. 3. Increased liver and skeletal muscle glycogen and a rise in the blood sugar level have been noted after the injection of crude anterior pituitary extract, suggesting a stimulation of gluconeogenesis. 4. Liver glycogen is increased in 8-day embryos by the injection of insulin and this increase is to be correlated with a rapid fall in the blood sugar level. 5. Injection of glucose fails to interrupt the normal disappearance of glycogen from the livers of 12-day embryos. 6. Both insulin and anterior pituitary extract cause an increase in the liver glycogen of 12-day embryos. The anterior pituitary extract also causes a rise in the blood sugar level at this time.