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Experimental hypoglycemia and hyperglycemia in the chick embryo.

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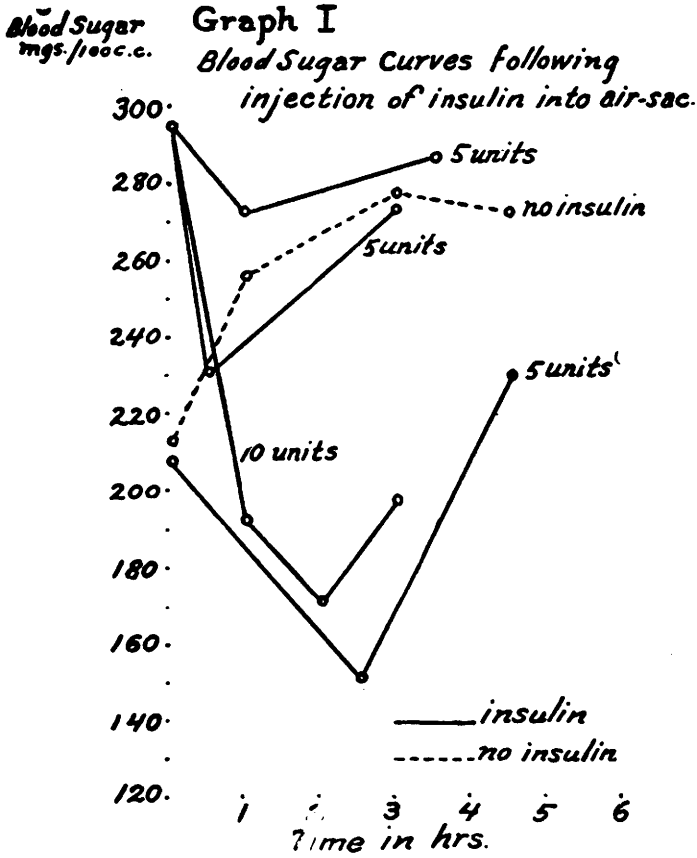
It has been found possible to produce hypoglycemia and hyperglycemia in the chick embryo. These experiments were carried out of 14 to 16 day embryos of the single combed white Leghorn variety. Immediately after the normal blood samples were taken, the injection was made into the air-sac from which absorption takes place into the blood stream of the respiratory capillary vascular network of the chorio-allantois.

The normal blood sugar of the chick embryo at this stage of incubation varies between 209 and 296 mg. per 100 cc. It appears that the glucose reaches its normally high level in birds at an early stage of development. The graph shows the effect of insulin upon the blood sugar. Riddle[1] has shown that adult birds survive thirty times the proportional amount of insulin required to kill a one kilogram rabbit. It is interesting to note that this resistance probably is established very early in the embryo. An important factor in this resistance is suggested by the hyperglycemia which is known to follow hemorrhage in the adult mammalian. That this occurs also in the chick embryo is shown by the dotted curve in the graph, obtained from an experiment in which no insulin was given. In an adult animal with a large volume of blood, the increase of sugar following blood sampling would be negligible, but in the case of the embryo with its comparatively small volume of blood, the taking of one-tenth cc. samples is a severe strain upon the embryonic physiological processes. The result is a marked hyperglycemia as shown, which would neutralize to some extent the hypoglycemic effects of insulin.

Very marked hyperglycemia may be produced by injecting a glucose solution into the air-sac as shown in Table 1. In the first two experiments of the series, the rapid clotting of the blood

[1] Riddle, Oscar, PROC. SOC. EXP. BIOL. AND MED., 1923, xx, 244.



failed to occur after the taking of the second samples. In the last experiment the glucose injected was decreased to 100 mg. in one-half cc. of distilled water. At the end of the first hour gross inspection showed that it had been completely absorbed from the air-sac. The blood sugar had increased from 221 to 859 mg. per 100 cc. By the fourth hour the sugar level had fallen to practically the normal, 226 mg. per 100 cc.

The blood sugar curves in the graph were determined by the usual Hagedorn Jensen volume method[2] while the hyperglycemia data in Table I was obtained by a modified technique as follows: The Hagedorn Jensen procedure is standardized in milligrams of glucose per one cc. of the potassium ferrocyanide solution. In preparation for the blood sample, several one inch length are cut

[2] Hagedorn and Jensen, *Biochem. Ztschr.*, 1923, cxxxv, 46.

from a five millimeter glass tube and are thoroughly cleansed and dried. Using fine forceps, a piece of sterile absorbent cotton just large enough to fit snugly is folded and pushed into one end of a tube so that none of the cotton fibers extend beyond either of the tube ends. The folding of the cotton as it is inserted at one end of the glass tube presents towards the other end the smooth rounded end of the cotton plug. These cotton tubes are placed in weighing tubes and set aside in a desicator for safe keeping.

To secure a sample of blood, the weight of a weighing tube with its cotton tube is carefully determined. The cotton tube is removed, and the rounded end of the cotton pushed out about one millimeter. The egg is candled and the allantoic vein at its bifurcation just below the air-sac is marked on the shell. About a square centimeter of shell is removed from over this area, and the vein exposed by turning back the shell membranes. The embryonic membranes are dried with sterile absorbent cotton, and the vein is punctured by means of a very sharp dissecting needle, using care not to puncture into the allantoic space or to produce an extensive wound. The cotton as previously prepared is brought into contact with the blood as it flows from the wound. All of the constituents of the blood are rapidly absorbed and the sample secured within a few seconds. The cotton is immediately pulled back into its glass protector and returned to its weighing tube. Several samples may be taken. The egg is sealed by Clark's method[3] and returned to the incubator. The weighing tube is weighed as before, and the weight of the blood sample determined by subtraction.

Following the usual Hagedorn procedure for precipitation of proteins, the cotton blood sample is pushed into the test tube by means of a glass stirring-rod and its glass protector allowed to follow. The contents of the test tube are thoroughly stirred, and then subjected to the usual water bath for five minutes. The rest of the steps are the usual procedure.

The blood sugar in mg. per 100 cc. of blood may be calculated by the following formula:

$$\frac{1120}{\text{Blood sample in mg.}} \times \text{glucose in mg.} \times 100 = \text{mg. glucose per 100 cc. of blood.}$$

1.120 grams = average weight of 1 cc. of blood from newly hatched chick.

[3] Clark, Eliot R., *Science*, 1920, li, 1319.

But the specific gravity of the chick's blood is not known for the incubation period, so that glucose should be expressed in milligrams per so many grams of blood pending such investigation.

This technique avoids the use of anticoagulants as the blood does not clot in the cotton, and it also makes unnecessary the use of oil to prevent evaporation. These advantages permit the drawing of the smallest possible samples of blood.

By means of such technique it is proposed to determine the normal blood sugar curve for the developing chick.

TABLE 1.

Hyperglycemia in chick embryo resulting from injection of glucose into air-sac. Blood sugar in mg. per 1.120 grams of blood × 100.

Age in days	Amount of glucose injected	Volume of fluid injected	Time in hours	Blood sugar	Remarks
15	400 mg.	2 cc.	0	202	Normal.
			1	441	Absorption incomplete.
			2		Death from loss of blood.
16	200 mg.	1 cc.	0	202	Normal.
			1	347	Absorption incomplete.
			2		Death from loss of blood.
16	100 mg.	½ cc.	0	221	Normal.
			1	859	Complete absorption.
			2	804	
			3	801	
			4	226	Death resulted.

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The nature of insoluble urease.

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Soluble urease is prepared by the authors from the jack bean by the use of two principles: (1) repeated precipitation from slightly acid 30 per cent alcohol by cooling and centrifuging; (2) removal of the two globulins, concanavalin A and B, by al-