THE LACTIC ACID CONCENTRATION IN THE MUSCLES OF THE CHICK EMBRYO IN CONDITIONS OF INSULIN HYPOGLYCEMIA

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It has been shown [4] that chick embryos, in early stages of development, have the power to react by hypoglycemia to the injection of extrinsic insulin. This fact served as the basis for a detailed investigation of the action of insulin on the various aspects of carbohydrate metabolism in embryonic tissues [2, 3].

The present research is devoted to the study of the influence of insulin on the lactic acid content in the muscle tissue of the chick embryo.

Lactic acid is an index of the interaction between two processes, namely glycolysis and aerobic metabolism of carbohydrates. The question of how insulin acts on these processes has not yet been finally settled, even in adult organisms. The information given in the literature on the influence of insulin on the lactate concentration in muscle tissue is very contradictory, and is mainly concerned with the postembryonic period [7, 11, 12, 15]. Collazo [11] and Cori [12] explain this contradiction in the findings given in the literature by differences in the dose of insulin administered. These workers consider that the lactic acid concentration in the muscles is unchanged unless hypoglycemic convulsions supervene. With large doses of the hormone, the amount of lactate in the muscles increases, as a result of the convulsions and dyspnea and not of the specific action of insulin. Other writers [9, 14] also consider that insulin has no direct action on the muscle glycolysis. They suggest that the hormone exerts its action through a change in the supply of glucose to the muscle.

We could find only one paper [1] concerned with the influence of insulin on glycolysis in embryonic tissues, and the test object in this case was a culture of fibroblasts. It is difficult to judge its results from the published theses.

EXPERIMENTAL METHODS

Experiments were conducted on chick embryos and also on young chickens of the White Leghorn breed. The muscle tissue (in the early stages of development the muscles of the trunk, in later stages the muscles of the thigh and leg) was frozen with solid carbon dioxide. The lactic acid was estimated in a trichloroacetic extract of the muscles by Barker and Summerson's method [8]. In our experimental conditions the mean square error of this method was ±5-6%. The blood sugar, which was used as a test of the insulin effect, was determined by the Somogyi–Nelson method [17]. Blood was taken from the vitelline artery of the embryos [4] and from the jugular veins of the chickens. Insulin was injected intravenously, by the method described by L. G. Leibson and E. M. Plisetskaya [5], in a dose of 0.43 unit/g weight of the embryo, and to the chickens intraperitoneally in a dose of 0.07 unit/g body weight. Glucose was injected by a similar route in the form of a 40% solution in a volume of 0.1 to 0.5 ml depending on age. Muscle tissue and blood were taken for biochemical analysis 3-4, 6-7 and 17-20 hours after injection of insulin. The results obtained were compared with control figures obtained in embryos and chickens receiving injections of physiological saline.

EXPERIMENTAL RESULTS

Earlier work by M. N. Pertseva [6] has shown that the lactic acid content of the muscles of normal chick embryos is maintained at a constant level throughout almost the whole incubation period (about 20 mg%), and begins

to rise only before hatching, to reach a maximum (100 mg%), and begins to rise only before hatching, to reach a maximum (100 mg%) in the first days of postembryonic life.

We obtained similar values for the lactate content of the muscles in the present investigation in the embryos of the control group.

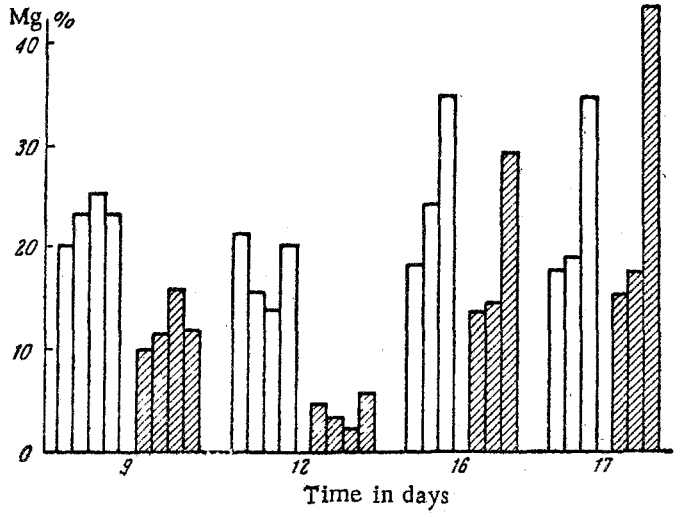

Fig. 1. The effect of insulin on the lactic acid content of the muscles of a developing chick embryo. Unshaded columns – control embryos; shaded columns – embryos receiving insulin.

It will be seen in Fig. 1, that insulin lowers the amount of lactic acid in the muscles of chick embryos. The size of the effect varies with the age of the embryos. In 9-day embryos (the first day of the examination) the lactic acid content fell to almost half its normal level under the influence of insulin. This effect subsequently became stronger, and on the 12th day of incubation it reached its maximum: the lactate content fell to $^1/_4$-$^1/_9$ of its normal level. Later still, on the 15th-16th day, the action of insulin became weaker, and in the 17-day embryos it was detected with difficulty. In the chickens no decrease was observed in the content of lactate in the muscles under the influence of insulin.

TABLE 1. Lactic Acid Content in Muscles and Blood Sugar (in mg %) of Chick Embryos 18-20 Hours after Injection of Insulin

Age (in days)	Control		Insulin	
	sugar	lactic acid	sugar	lactic acid
12	110	20.9	5	5.2
	115	19.7	105	16.2
13	118	18.2	8	6.3
	115	14.2	78	15.3
	118	16.8	115	18.4

Concurrently with estimating the lactic acid in the muscles, we determined the level of the blood sugar in the same animals. The following relationship was discovered between these two indices (Table 1): the lower the blood sugar level, the lower the amount of lactate in the muscles.

It can be seen from Fig. 2, which shows this relationship graphically, that if the blood sugar did not fall below 60-40 mg%, the muscle lactic acid remained within normal limits. If, on the other hand, it fell below this level, the lactic acid fell, and this fall was greater the more marked the hypoglycemia.

It can thus be concluded that the lactic acid concentration in the muscles of 9-16-day embryos, after insulin has been injected, is a function of the blood sugar. The less marked fall in the muscle lactate concentration in the more mature embryos could have been due to the less marked degree of hypoglycemia observed in these embryos 17-18 hours after injection of the hormone. The experiments after a shorter interval of time (6-8 hours), when the hypoglycemia was well marked in the older embryos too, showed, however, the absence of any clearly defined fall in the muscle lactate concentration. The change in the reaction to insulin with age is particularly obvious if the data for embryos are compared with the figures obtained in chickens (Table 2).

TABLE 2. The Effect of the Age of Embryos on the Change in the Muscle Lactic Acid Concentration and the Blood Sugar After Injection if Insulin (after 6-8 hours)

Age (in days)	Control		Insulin	
	sugar	lactic acid	sugar	lactic acid
	(in mg %)			
	Embryos			
13	140	12.6	15	9.6
	130	14.9	20	3.1
	130	10.7	30	17.0
	130	10.4	15	3.5
17	145	28.2	60	14.4
	140	35.2	20	14.0
	115	19.3	15	44.1
	9	18.0	30	17.5
	Chickens			
11	163	46.5	47	53.2
	138	25.4	95	91.5
	178	65.8	110	88.6
	162	30.4	42	49.7
	173	35.0	25	55.1

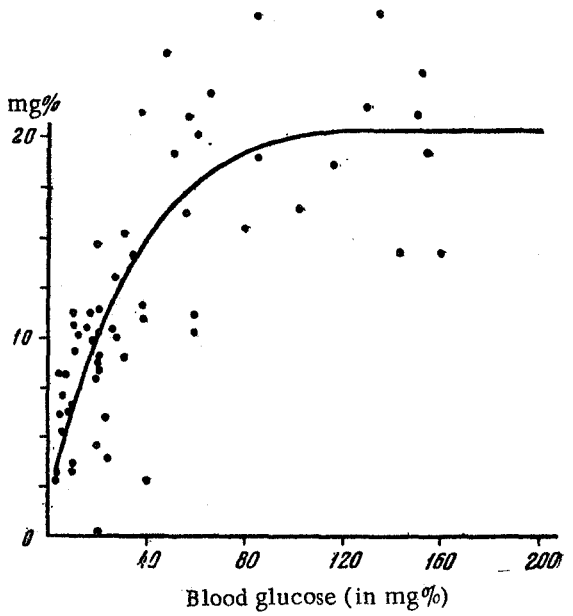

Fig. 2. Relationship between the lactic acid concentration in the muscles and the blood sugar in embryos (9-16 days).

TABLE 3. The Effect of Injection of Insulin and Glucose on the Muscle Lactic Acid Level (in mg %) of 10-Day Embryos (after 17-18 hours)

Physiological saline	Physiological saline + glucose	Insulin	Insulin + glucose
23.2	29.4	9.9	24.5
24.2	25.3	9.3	103.2
20.9	31.7	11.1	18.9
25.9	65.3	–	46.0

TABLE 4. Muscle Lactic Acid Concentration in Day-Old Chicks 3-4 Hours After Injection of Insulin and Glucose

Physiological saline	Physiological saline + glucose	Insulin	Insulin + glucose
89.7	72.5	117.4	110.1
70.	71.4	60.0	115.6
72.4	78.9	91.6	97.1
72.9	116.0	80.5	117.8
61.5	83.6	75.3	113.5

It can be assumed from the results obtained that the fall in the muscle lactate concentration in the less mature embryos during insulin hypoglycemia was due to lack of glucose at the substrate for glycolysis. In order to test this hypothesis we carried out experiments in which both insulin and glucose were injected at the same time. The results of one such experiment are shown in Table 3.

The figures obtained show that the fall in the lactate concentration in the muscles brought about by insulin can be prevented by injection of glucose. In two cases (column 4, Table 3) the lactic acid concentration was within normal limits, and in two others it was actually well above normal. It is important to mention that after injection of glucose into the embryos, their insulin hypoglycemia was present to a less marked degree or was absent. Our findings concur well with the views expressed earlier by Needham and Nowinski [16], that the principal substrate for glycolysis in embryonic muscles is glucose and not glycogen. At the end of the embryonic period the muscles of the embryo, like adult muscles, begin to utilize glycogen. The weak glycogen-utilizing power of the embryonic muscle tissue may probably be explained by its low phosphorylase activity. As But et al. [10] have shown, the phosphorylase activity of the skeletal musculature of the chick embryo in the early stages of development is negligible. Subsequently it increases slightly, but it attains a significant level only after hatching.

In 24-hour chicks not only did insulin not lower the muscle lactate concentration, but in some cases (column 3, Table 4) it actually led to an accumulation of lactate, despite the presence of hypoglycemia.

The increase in the lactic acid concentration in the muscles of the chickens was more marked when insulin and glucose were injected at the same time. It may be assumed that this increase was due to the specific action of insulin on the carbohydrate metabolism of the muscles (on the ability of glucose to penetrate into the cell, and on the hexokinase reaction). In chickens, evidently, insulin stimulates the entry of glucose into the muscles, which accounts for the absence of a fall in the lactate concentration at this age. In other words, in the postembryonic period of development the muscles take part in the development of insulin hypoglycemia, as has been established in the case of adult animals. In embryos, however, it is obvious that the muscle tissue plays no part in lowering the blood sugar.

Although we consider that the main cause of the fall in the lactate concentration in the muscles of embryos under the influence of insulin is a lack of the substrate for glycolysis – glucose, – we must not forget other possible explanations of this fact. Firstly, lactate may disappear on account of an increase in its oxidation. According to Hall's findings [13], however, insulin has no effect on the respiration of normal muscle. Secondly, in our experiments lactic acid may have been utilized for the synthesis of glycogen in the muscles and liver. This hypothesis, however, cannot be accepted because of the absence of changes in the muscle glycogen concentration in embryos younger than 15 days [3] and of the lowered lactate concentration in the blood.

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