CVIII. THE ABSENCE OF EFFECT OF INSULIN ON THE HEAT PRODUCTION IN ISOLATED FROG'S MUSCLE.

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(Received October 9th, 1923.)

WE have carried out a few experiments on the effects of insulin on the heat production of muscle, and although the results so far are merely of a negative character, it seemed to us to be worth while to put them on record since they help at least to restrict the possible methods by which insulin may act upon the system.

For the first four experiments, the insulin used was of the "crude" type, very kindly supplied to us by Messrs Smith and Winter and prepared according to Dudley's method [1923] at the Biochemical Laboratory, Cambridge. Its strength may be taken as being about 5 mg. to the "unit." For the last two experiments, the insulin used was of the "A-B" Brand supplied by the British Drug Co. Its strength is 0.1 cc. to the "unit."

The muscles employed were the sartorius of *Rana temp.*, these being mounted on a thermopile, and the heat production after stimulation being observed by recording photographically the curves of deflection of the galvanometer in series with the thermopile, as fully explained elsewhere [1920, 1922]. The temperature was 14° to 15°. In the present series of experiments, the better to maintain their condition, the muscles were always kept in a salt solution in the thermopile chamber. In this case the actual heat production cannot be calculated from the observed curves, since there is no possibility of making control observations under the same conditions. It is possible, however, by first taking the curves when the muscle is in ordinary Ringer's solution, and then taking another set of curves after insulin has been added, to make a fairly correct estimate of any *change* in the heat production which may be produced by the insulin.

The strength of the insulin in the solution around the muscle was varied between wide limits, but even the weakest solution used would be considered strong if injected into a small animal to make the same concentration in its body.

The chamber containing the muscle was 40 cc. in volume, of which the muscle occupied 0.25 cc.

In Exp. 1 there was 1 mg. of freshly prepared crude insulin in the chamber. In Exp. 2 there were 4 mg. of crude insulin, which had been put into solution on the previous day. In the interval the preparation had been kept on ice.

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In Exp. 3 there were 4 mg. of freshly prepared crude insulin. In Exp. 4 there were 16 mg. of crude insulin which had been put into solution on the previous day and kept in the interval on ice. In Exp. 5 there was 1 cc. and in Exp. 6 there was 0.5 cc. of insulin "A-B" Brand. The first readings after adding the insulin were taken about an hour after the insulin had been given, and the readings were continued on muscles continuously subjected to the insulin solution for several hours.

RESULTS.

A. *Initial Heat.* Even with the strongest solution of insulin employed the condition of the muscle was apparently unaffected, the initial heat production being practically the same after the muscle had been for five hours in the solution with insulin as it was in the Ringer's solution before insulin was added. Presumably, therefore, the initial breakdown of glycogen or of lactacidogen into lactic acid is completely unaffected by the presence of insulin.

B. *Recovery Heat.* On comparison of the long continued galvanometer curve for about ten minutes, to see if there were any change in the recovery heat production, a distinct difference was at first detected, showing apparently after insulin had been given that the recovery heat in its earlier stages was less and its later stages more than before insulin. Control experiments, however, were subsequently made on two or three occasions in which long-continued curves of galvanometer deflection were compared (*a*) for a muscle before, and (*b*) for a muscle after being kept in Ringer's solution for several hours. In every case a difference was found between the late and the early curves exactly as between those before and after insulin. This change is presumably due to small alterations in reaction or in the physico-chemical condition of the muscle produced by keeping it in Ringer's solution. There seems to be little doubt, therefore, that even a very strong solution of insulin surrounding an isolated frog's muscle has no noticeable effect upon the oxidative breakdowns involved in the recovery process.

In order to ascertain whether the insulin used has any effect on the frog, six injection experiments with insulin "A-B" Brand were carried out. In two of them 0.25 cc. and in four 0.5 cc. were injected into the dorsal lymph sac. About 24 hours after the injection the frogs were found very weak, relaxed, passive and pale, and in two cases were found dead within 48 hours. By the injection of 0.5 cc. of 4 % glucose solution, the suffering frogs seemed to recover temporarily. By repeated injections of glucose, two frogs recovered completely, while one lived for five days without showing any sign of convulsions. Only one frog (which had been injected with 0.25 cc.) showed proper convulsions. After six days this frog fell into strong convulsions (just like the strychnine convulsions) which ceased promptly on the administration of glucose. On the next day, the convulsions were again produced by touching the frog and were again stopped by glucose. Two days after the frog was found dead.

SUMMARY.

Experiments have been performed on the sartorius muscle of *Rana temp.* comparing (a) the magnitude of the initial anaerobic heat production, and (b) the magnitude and course of the oxidative recovery heat production before and after subjection to a high concentration of insulin. No change whatever has been found in either case. The injection of insulin, on the other hand, into the intact frog was shown to produce a well-defined effect.

It is clear, therefore, that the action of insulin does not reside in any effect on the muscle, either on its sudden initial anaerobic breakdowns or its slower oxidative processes of recovery.

REFERENCES.

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