DEVELOPMENT OF METABOLIC DISTURBANCES AFTER RAPID INACTIVATION OF INSULIN

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Diabetes mellitus was produced in rats by injection of an anti-insulin guinea pig serum. The blood levels of glucose and free fatty acids rose 7 min after termination of the action of insulin. The increase in the fatty acid concentration was much greater after 7 min and, in particular, after 15 min than the increase in the blood sugar. The glycogen content in the liver and muscles was reduced after 30 min.

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It is generally accepted [1] that in the absence of insulin the tissue permeability to glucose is initially lowered and glycogenolysis is stimulated, but later, as the glycogen content in the liver falls and the utilization of glucose in the tissues decreases, lipids are mobilized and hyperlipemia develops. This sequence of events is deduced from investigations on animals in which diabetes was produced by depancreatization or alloxan. In these cases, however, the diabetes develops after many hours. It is difficult, therefore, to determine when the mobilization of lipids begins.

If diabetes is produced by injection of antibodies against insulin [6], this problem can be studied because the diabetes develops very rapidly and is uncomplicated by side-effects.

This form of diabetes was produced in order to determine the time of appearance and intensity of development of disturbances of carbohydrate and lipid metabolism after termination of insulin action.

EXPERIMENTAL METHOD

An anti-insulin serum (AIS) was produced in guinea pigs weighing 200-300 g by injection of insulin together with Freund's adjuvant [5]. The course of immunization consisted of four injections, each of 20 units, at monthly intervals. Blood was taken from the heart or after decapitation 10 days after the last injection of insulin. The titer of insulin antibodies was determined by a modified [7] passive hemagglutination reaction [3] with human Group O erythrocytes. Diabetes was produced in female albino rats weighing 190-310 g, kept on a normal diet throughout the experiment to prevent lipid mobilization. One group of rats received an intraperitoneal injection of 3 ml of serum from normal guinea pigs, while the other group received AIS (titer 1:20 480).

The blood sugar was determined by the Hagedorn–Jensen method, free fatty acids (FFA) by Dole's method [4], glycogen in the liver and muscles by Pflüger's method, sugar in the urine by the "Glucotest" method, and acetone by means of sodium nitroprusside. The blood sugar curve was first investigated before and 7, 15, and 30 min and 1, 3, 5, and 7 h after injection of the serum. After the time taken for the blood sugar curve to reach a maximum had been established, the FFA concentration was also determined at these intervals.

Altogether 20 guinea pigs and 99 rats were used in the experiments. The results obtained were subjected to statistical analysis by the Student–Fisher method [2].

EXPERIMENTAL RESULTS

The blood sugar showed a significant increase 15 min after AIS injection and continued to rise until 3 h and, in some rats, until 5 h thereafter. In most animals the blood sugar by this time had begun to fall to a varied degree, but nevertheless it was still considerably above normal.

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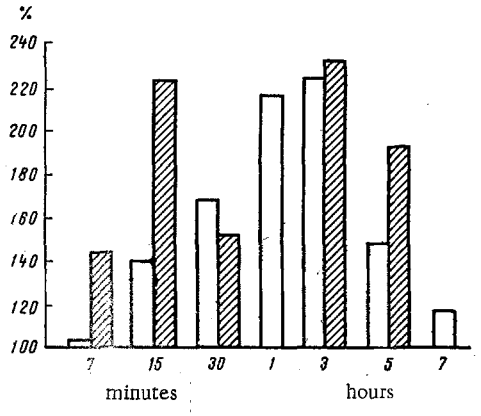

Fig. 1. Blood sugar (unshaded columns) and free fatty acids (shaded columns) in rats after injection of anti-insulin serum (in % of control values).

The FFA concentration was increased 7 min after injection of AIS, and after 15 min it was more than doubled. After 30 min it had started to fall but was still higher than in the controls. After 3 h the FFA level had increased further to its maximum, being somewhat reduced after 5 h (Fig. 1).

From the point of view of the time of appearance of hyperglycemia and the increase in FFA concentration, the results of individual experiments are interesting. AIS produced a significant increase in the blood sugar in 6 of 9 rats after 7 min, and in the same number of animals it increase the FFA concentration to values (mean 828 μeq/liter) considerably higher than the control maxima (468 μeq/liter; $P < 0.05$). AIS did not raise the FFA level in only 3 rats. The blood sugar was increased 15 min after injection of AIS in 7 of 9 rats, and the FFA concentration in 8 animals was significantly higher (mean 1260 μeq/liter) than the control maxima (323 μeq/liter). An increase in FFA concentration in the experimental rats accompanying the increase in blood sugar was observed after 7 min in 4 of 6 animals, and after 15 min in 6 of 7 animals. An increase in FFA in the absence of an increase, or even in the presence of a decrease of the blood sugar was found in 2 rats after 7 and 15 min.

The glycogen content in the liver and muscles was essentially unchanged 7 and 15 min after injection of AIS. It was reduced only after 30 min.

The equally rapid increase in blood sugar and FFA concentration after termination of the action of insulin can be explained by the rapid decrease in the intensity of glucose transfer into the tissues. In adipose tissue under these circumstances the lipase activity is increased and the liberation of FFA into the blood stream is stimulated. This may perhaps potentiate the action of anti-insulin hormones. The more marked and, in some rats, the earlier increase in FFA than in blood sugar, which the last investigation showed to persist for a long period even after restoration of the normal blood sugar, provides evidence that mobilization of lipids in the absence of insulin may take place independently of the decreased rate of transfer of glucose into adipose tissue.

LITERATURE CITED

1. S. G. Genes, Diabetes Mellitus [in Russian], Moscow (1963).
2. N. Bailey, Statistical Methods in Biology [Russian translation], Moscow (1962).
3. S. V. Boyden, J. Exp. Med., 93, 107 (1951).
4. V. P. Dole, J. Clin. Invest., 35, 150 (1956).
5. J. Freund and K. McDermott, Proc. Soc. Exp. Biol. (N. Y.), 49, 548 (1942).
6. P. J. Moloney and M. Coval, Biochem. J., 59, 179 (1955).
7. A. B. Stavitsky, J. Immunol., 72, 360 (1954).