EVIDENCE OF THE PRESENCE OF INSULIN IN URINE

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The question of the presence of insulin in urine has not yet been definitely settled. Experiments involving the use of I^{131}-labeled insulin have shown that it passes into the glomerular filtrate, and undergoes reabsorption in the tubules, and that protein-bound iodine, precipitable by trichloroacetic acid, could not be detected in the urine [9]. At the same time, the clearance of iodine following administration of I^{131}-labeled insulin was greater than when labeled iodide was given [2], which suggests that deiodination of labeled insulin may have taken place in the kidneys, and it hence follows that evidence supporting the view that insulin is not excreted in the urine, based on experiments involving the use of labeled insulin, cannot be regarded as being althogether conclusive. It should also be remembered that a substance was extracted from a large volume of urine, which, when administered to mice, caused convulsions [8]; its amount was equivalent to 0.1 - 0.25 insulin units per diem.

We showed, in a recent publication, that, using highly porous sulfonic acid cationites in the hydrogen form, it was possible to isolate fractions of urine which had properties resembling those of insulin [6]. The further study of these fractions is described in the present communication.

EXPERIMENTAL METHODS

Urine was treated with the resins SDV-2 and SDV-3, according to the procedure described in [6], using for the hypoglycemia test on mice a 24-h collection of urine which had been dialyzed for 24 h; for other experiments we used urine which had been passed during the preceding 15 min, and had had no preliminary treatment. Unless otherwise stated, insulin activity was assessed from the magnitude of glucose uptake by the epididymal fat pad of rats [1, 7]; weighed amounts of tissue taken from three animals were usually taken for each test. In the test, one portion of fat was incubated with buffer solution containing glucose, while a second portion was incubated with the same solution, containing glucose and urine fraction. The difference between the glucose contents of the two solutions after incubation was taken as being the excess uptake of glucose due to the urine fraction, i.e., as being the insulin effect, expressed as milligrams of glucose taken up by 1 g of fat during 3 h of incubation. Glucose was determined by the Somogyi-Nelson method, as modified by Frank and Kirberger [5]. Under our experiment conditions (350-400 mg-% glucose in the medium, with 250-300 mg of fat, etc), differences in glucose uptake exceeding 0.32-0.40 mg/g during 3 h of incubation could be regarded as being significant. The same value was taken for assessing the significance of the differences found in experiments on the activity of fractions of urine taken from the same person at different times.

EXPERIMENTAL RESULTS

We performed 4 series of experiments, for the study of the properties of the urine fraction.

1. The urine fraction was treated in various ways, neutralized, passed through an ion exchanger column, and the eluate from the column was tested by measuring its effect on glucose uptake by rat epididymal fat pad (Table 1).

2. The activity of the preparations was assessed from their effect on glucose uptake by rat epididymal fat [1, 7] and by rat diaphragm [10], and on respiration of rat epididymal fat tissue [3]. In the third of these tests, any increase in evolution of gas was taken as being significant, since, in the absence of insulin, the given method always shows uptake of gas, and never its evolution (Table 2).

3. In these experiments extractives of 24-h urine collections were concentrated by passing through ion-exchange columns, from which they were eluted, and 0.5-ml portions of eluate were injected subcutaneously into mice. Fifteen of 38 mice showed convulsions, which were relieved after a few minutes by administration of 0.5 ml of 15% glucose.

TABLE 1. Effects of Various Treatments on the Insulin Activity of Urine Fractions

Nature of treatment	Duration of treatment (hr)	Number of rats used in the experiment	Insulin activity (as mg/g in 3 hr)	
			before treatment	after treatment
Sodium 2,3-dimercaptopropanesulfonate 10 mg/ml	24	12	+1.45	-0.40
Boiling water bath	1/2	8	+0.81	+0.18
0.2 N Sodium hydroxide	24	11	+1.18	+0.09
0.2 M Sulfuric acid at 100°	1/4	10	+1.18	+1.02

TABLE 2. Effect of Urine Fraction on Rat Diaphragm and Epididymal Fat

Effect examined	Number of rats	Insulin effect of urine fraction
Uptake of glucose by a half-diaphragm (mg/g per hr)	8	+1.53
Uptake of glucose by epididymal fat (mg/g in 3 hr)	41	+1.19
Net respiration of epididymal fat (as percentage of maximum evolution of gas in presence of 0.1 units per ml of insulin)	11	56%

4. We examined the insulin activity of the urine of eight persons not suffering from any derangement of carbohydrate metabolism. Urine was collected over a 1-hr period from fasting individuals, and over the same time after intravenous injection of 16 g of glucose (40 ml of 40% solution). Usually a quarter of the hour's output of urine was taken for chromatographic separation, and this amount thus reprsented the amount excreted in 15 min.

The first series of experiments showed that the insulin activity of the urine extracts was not abolished by treatment with 0.02 M sulfuric acid for 15 min at 100°; but was abolished by heating on a water bath for 90 min, and by treatment with alkali or with sodium dimercaptopropanesulfonate (as the preparation Inithiol, which contains free sulfhydryl groups). These factors affected the activity of crystalline insulin in exactly the same way. The results of the second series of experiments showed that the urinary fractions caused enhanced uptake of glucose by rat diaphragm and epididymal fat, and activated respiration of rat epididymal fat pad, i. e., their activity was the same as that of insulin. The third series of experiments, in which concentrated extracts were used, showed that they caused typical hypoglycemic convulsions when administered to mice. Finally, the fourth series of experiments showed that the insulin activity of the urine was raised considerably following intravenous injection of glucose into 8 individuals.

The only known substance capable of giving all the specified effects is insulin. No other hormone, in the amount in which it is excreted in the urine during a 15-minute period, is known to have these effects on uptake of glucose by rat diaphragm and epididymal fat, and on respiration of the latter [11]. We would add that we used bicarbonate buffer, in which catecholamines exert no action on muscle tissue. In some respects, including its ability to cause hypoglycemia, the "fat-mobilizing factor", which has been isolated from urine and from the hypophysis, resembles our urinary preparation, but it differs from it in being excreted in fasting urine, and disappearing from the urine following administration of carbohydrates [4]. In our experiments administration of glucose caused a considerable rise in urinary excretion of insulin-like factor. The fat-mobilizing factor could not therefore have significantly affected the results of our experiments. Moreover, we found considerably heightened excretion of insulin in the urine of two patients suffering from islet tissue tumors. This lends further support to our contention that the factor isolated by us from urine by means of ion-exchange chromatography contains insulin.

TABLE 3. Change in Insulin Activity of Urine Following Administration of Glucose

Subject	Insulin activity of urine assessed from glucose uptake by isolated rat epididymal fat (mg/g in 3 hr)	
	after injection of glucose	fasting
L-n	+0.86	+1.51
G-a	+1.05	+2.94
B-ya	+0.95	+2.25
V-a	+4.28	+5.19
K-a	+0.35	+1.08
L-a	+1.57	+3.43
G-na	+0.52	+0.97
Sh-f	+0.44	+0.89

The present paper is not concerned with the quantitative aspects of insulin excretion, which must undoubtedly be small, amounting to 0.1-0.2 units per diem, according to our preliminary findings, which are in accord with those of Mirsky [8]. However, the greatest interest attaches to the study of the dynamics of insulin excretion under various conditions, and in response to different factors. If any regular changes are found in diurnal urinary excretion of insulin, assay of its content in urine, parallel with assay of the insulin activity of blood, may contribute significantly to the elucidation of islet function.

SUMMARY

A fraction of urine isolated by means of ion-exchange chromatography caused enhanced uptake of glucose by rat diaphragm and epididymal fat pad, and intensified respiration of the latter. Concentrated solutions of the fraction induced hypoglycemic convulsions in mice. The yield of activity in the urine rose following administration of glucose to healthy individuals. The activity was not abolished by treatment with acids, but was abolished by alkali treatment, and by heating at 100°, as well as by thiols. On these criteria, it is concluded that the urinary fraction examined contained insulin.

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