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Lipoatrophic Diabetes: An Improved Procedure for the Isolation and Purification of a Diabetogenic Polypeptide from Urine

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An improved procedure is outlined for the isolation of a diabetogenic polypeptide from urine of patients with lipoatrophic diabetes. The compound provokes insulin resistance and temporary loss of glucose tolerance when administered to dogs and humans. It is now free of a toxic contaminant which, by the previous method, had to be removed by more drastic treatment of the compound than was deemed suitable. Its physicochemical properties closely resemble those of a diabetogenic polypeptide isolated from the adenohypophyses of beef, sheep, and hog described previously from this laboratory. (Metabolism 18: No. 7, July, 545–555, 1969)

IN A PREVIOUS communication we described a method for isolating a diabetogenic polypeptide from the urine of patients with lipoatrophic diabetes.[1] This substance, designated as fraction 1, was shown to provoke insulin antagonism and loss of glucose tolerance when administered to dogs and men. Isolation of the compound and demonstration of its diabetogenic activities have now been confirmed.[2] However, interpretation of the results was somewhat clouded by the occurrance in man of mild toxic effects which included myalgia, cephalalgia and pyrexia. Further purification of the material was initially achieved by somewhat drastic treatment of the material with 0.1N hydrochloric acid at a temperature of 100° C. From this solution an

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active polypeptide (isoelectric point approximately 4.7) was isolated which gave no evidence of toxicity in man. This hydrolytic product was designated as Fraction III C2; and it was this fraction that was employed in most of our early anti-insulin studies in humans.[1]

The present report describes an improved procedure for isolation of the original diabetogenic polypeptide (fraction 1) which eliminates toxic contaminants without requiring hydrolysis. In physicochemical properties the substance resembles closely the diabetogenic polypeptide designated as fraction PI isolated from beef, sheep, and hog pituitary glands as recently reported from this laboratory.[3-5] The isoelectric point of the polypeptide prepared by the present procedure is approximately pH 4.1 which is the same as that of the diabetogenic peptide obtained from the adenohypophyses of three species mentioned.[5] It appears reasonable to assume that the urinary peptide had its origin in the pituitary gland.[4]

Materials and Methods

Urine samples were collected and preserved as previously described.[1] For the present studies, urine specimens from two patients (M.D. and T.R.) with lipoatrophic diabetes were employed. Both subjects had moderate proteinuria.

The oxycellulose powder (17%-21% COOH, Eastman Chemical Products, Inc., Kingsport, Tenn.) was kept refrigerated and before use was washed successively with distilled water, 0.1N HCl, and distilled water.

Methods and procedures for glucose and insulin tolerance tests were carried out in the same manner as described in previous reports.[1,4,5] Two healthy male volunteers without a family history of diabetes served as subjects. They were maintained on a constant high carbohydrate diet throughout the testing period. Solutions of the peptide were prepared for parenteral administration as previously described.[1]

Results

Figure 1 outlines in detail the new procedure employed for isolation of the active polypeptide from urine.

Table 1 shows the elemental composition of the compound obtained from the urine of patients M.D. and T.R. Each patient excreted approximately 12 mg per day of the isolated peptide.

Fraction 1 extracted from the urine of patient M.D. was tested for diabetogenicity on six normal dogs and two humans. Figures 2–8 illustrate the diminished glucose tolerance induced in the dogs. The amount of the compound injected varied from 1–4 mg. per kilogram body weight. In Dog P (Fig. 2) and Dog R (Fig. 3) a significant decrease of glucose tolerance was evident after a single injection of 1 mg. per kilogram body weight. In Dog O abnormal glucose tolerance was still evident 34 hours after administration of the compound (Fig. 8).

Insulin resistance was demonstrated on two normal dogs (Dog N and Dog O). A single dose of 2 mg. per kilogram body weight was administered to Dog N. In Dog O, a second dose of 4 mg. per kilogram body weight was administered 24 hours after the first injection of 3 mg. per kilogram body weight. Insulin tolerance tests were performed 10 hours after injection. These results are shown in Figs. 9 and 10. Both animals exhibited marked resistance

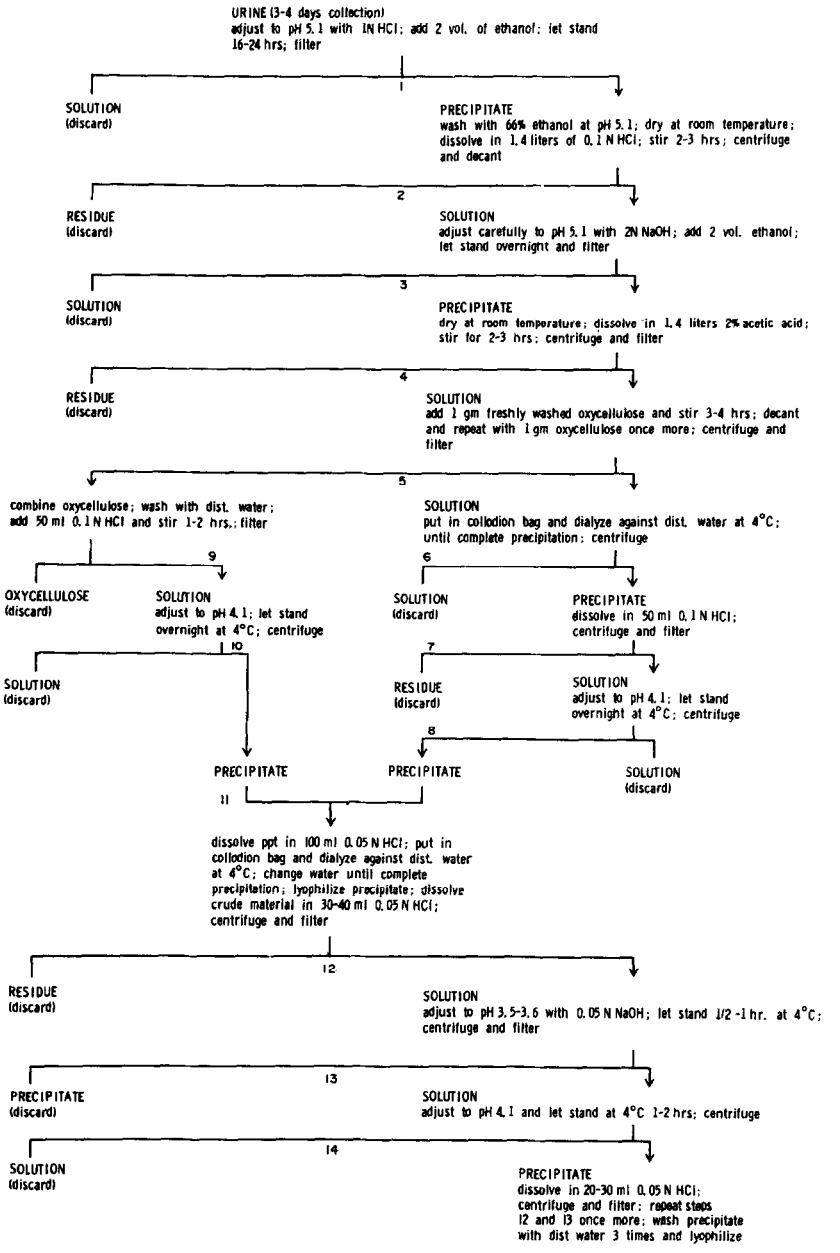

Fig. 1

Subject	C %	H %	N %	S %
M. D.	48.41	6.96	13.58	1.72
R. T.	48.53	6.95	13.26	1.59

Table 1.—Elemental Analysis of Fraction One Isolated from Urine of Patients with Lipoatrophic Diabetes

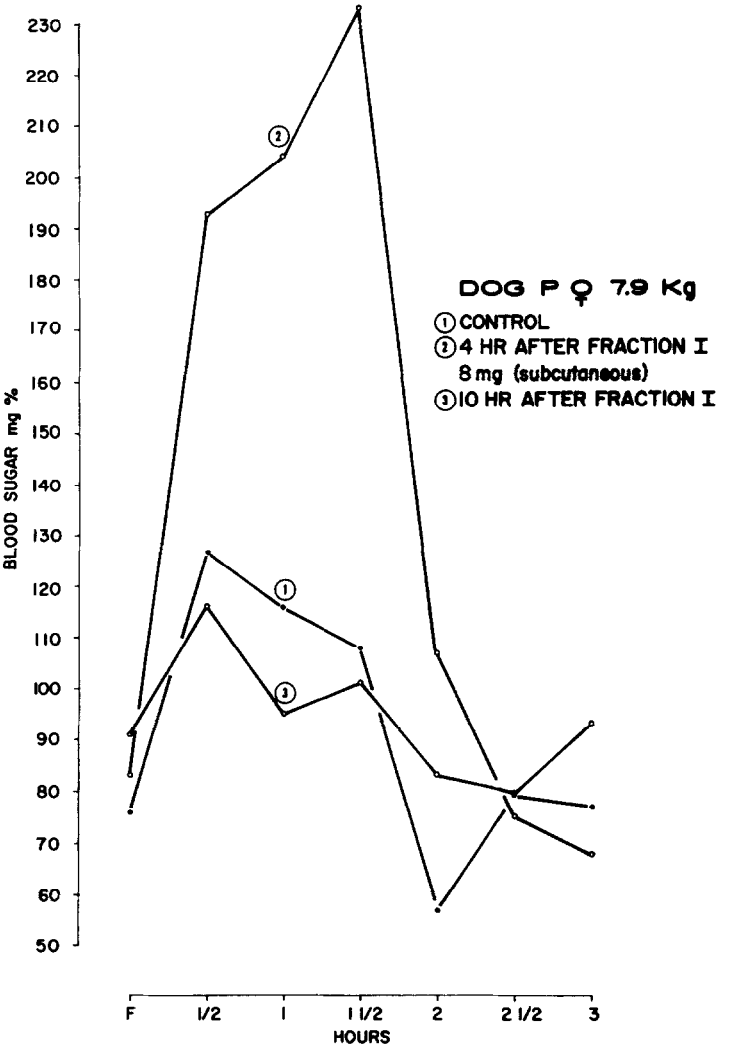

Fig. 2

to exogenous insulin and Dog O had a significantly elevated fasting blood sugar. No significant change in rectal temperature was observed.

The biological effects of the polypeptide were also studied in two human volunteers (R.C.M. and G.W.). After control glucose and insulin tolerance tests had been obtained, a single dose of 25 mg. was administered intramuscularly to each of the subjects. Glucose tolerance tests were then carried out daily for eight days except for the second day (45 hours) during which an insulin tolerance test was done. The results are presented in Tables 2 and 3. Table 4 shows the effect of the peptide upon insulin tolerance 45 hours after injection. No significant changes in urinary 17-hydroxysteroids and 17-ketosteroids were observed in either subject.

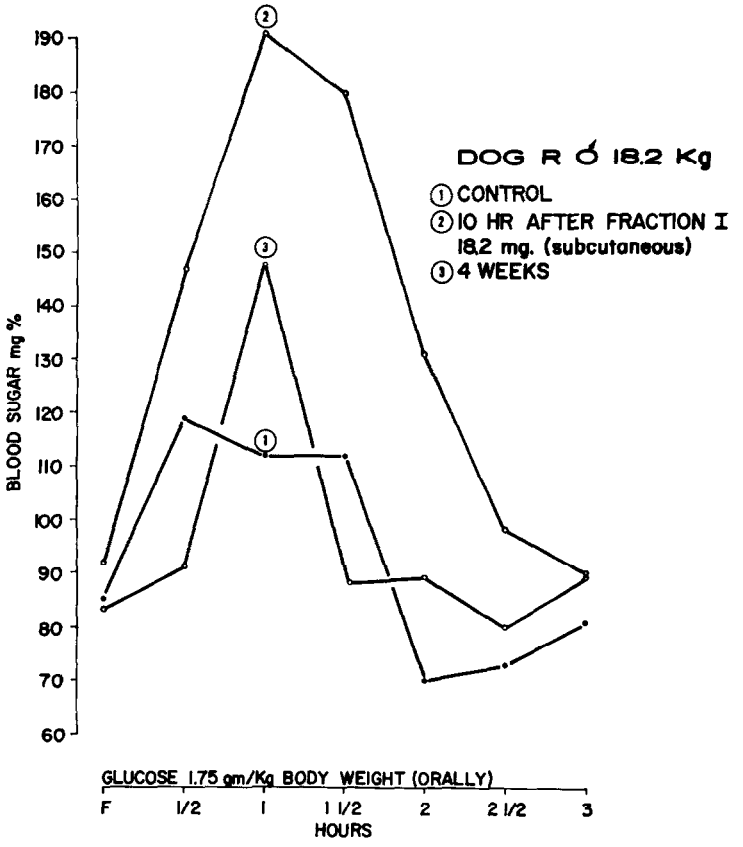

Fig. 3

Table 2.—Effect of Fraction One on Glucose Tolerance of Normal Subject (R.C.M., 23 years, M, 84.8 Kg.)

Hours after injection 25 mg (IM)	F	1/2	1	1 1/2	2	2 1/2	3
Control	95	165	121 trace	98	83	83	72
22	107	184	160 +++	118	115	105 trace	107
69	92	176	98 trace	98	90	90	95
93	92	154	99	93	82	93	94
117	87	159	129	92	105	114	108
141	85	151	123	78	88	88	90
166	91	159	104	90	73	95	104
190	91	146	141	107	88 trace	93	80

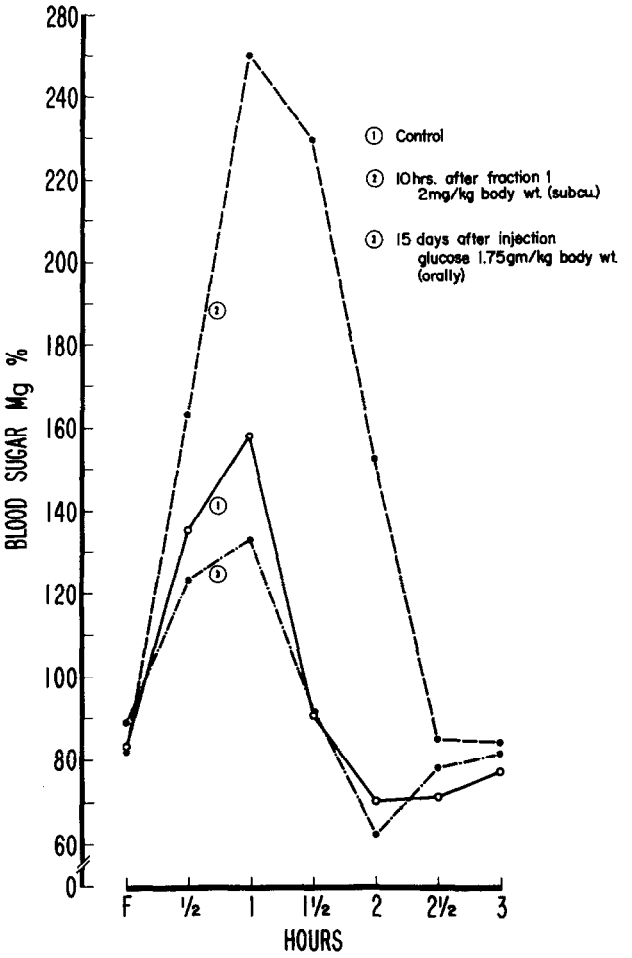

Fig. 4.—Dog P (F), 7.4 Kg. body weight.

Table 3.—Effect of Fraction One on Glucose Tolerance of Normal Subject (R.C.M., 33 years, M, 82.2 Kg.)

Hours after injection 25 mg (IM)	F	1/2	1	1 1/2	2	2 1/2	3
Control	85	121 +	96 ++	94 +	95 trace	84	50
22	98	174 trace	139 +++	97 +	104 trace	100 trace	80
69	90	129	167 ++	113 +	106 +	112 trace	100
93	84	136	180 +	131 trace	111 trace	114	114
117	85	163	163 ++	118 ++	78 +	75 +	79 trace
141	88	136	141 trace	87	112	111	107
166	86	132	138	97 trace	92	81	83
190	84	141	122 trace	87	92	84	106

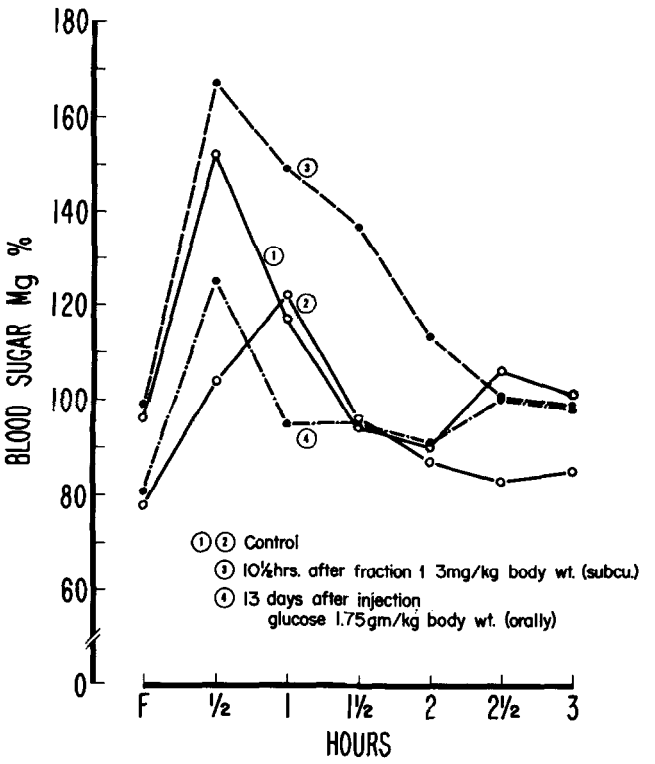

Fig. 5.—Dog L (M), 10.6 Kg. body weight.

Table 4.—Effect of Fraction One on Insulin Tolerance of Normal Subjects (Glucagon-free Insulin, 0.05 units/Kg.)

		Time in minutes								
Subject	Hours after injection 25 mg (IM)	-10	-5	0	20	30	45	60	90	120
R. C. M. 84.8 Kg	Control	85	85	86	30	24	46	69	81	90
	45	91	90	91	64	53	61	71	85	84
G. W. 82.2 Kg	Control	86	89	89	32	20	50	59	74	83
	45	93	90	94	55	49	60	67	88	85

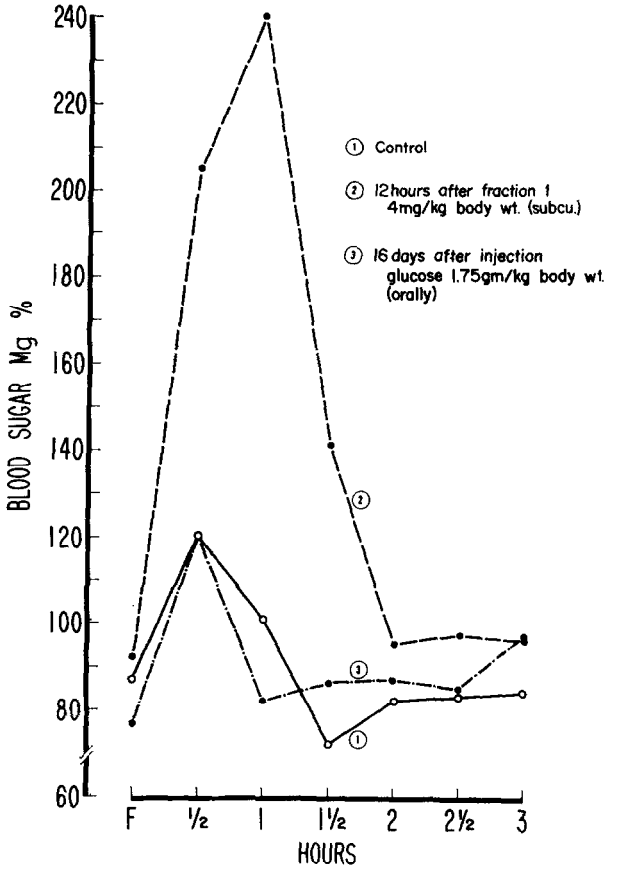

Fig. 6.—Dog L (M), 10.6 Kg. body weight.

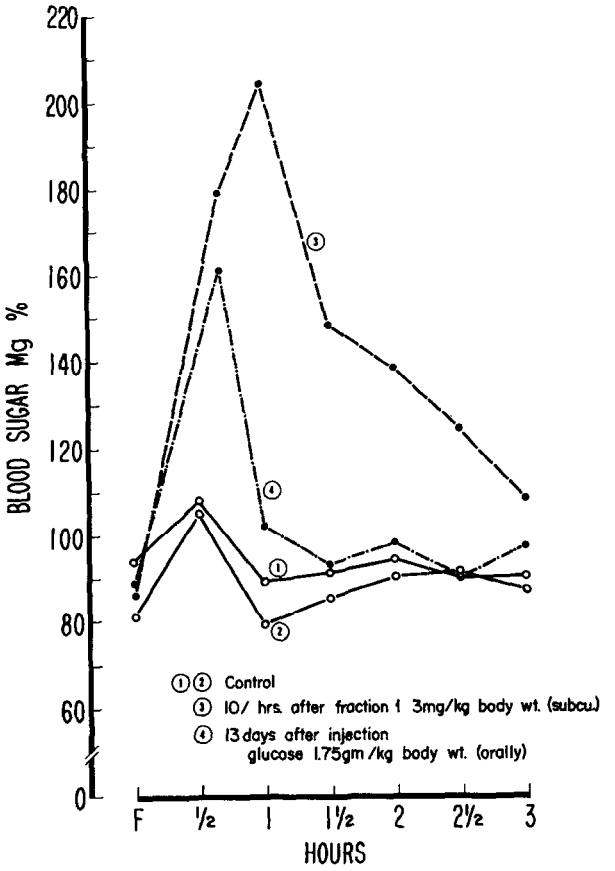

Fig. 7.—Dog X (M), 10.1 Kg. body weight.

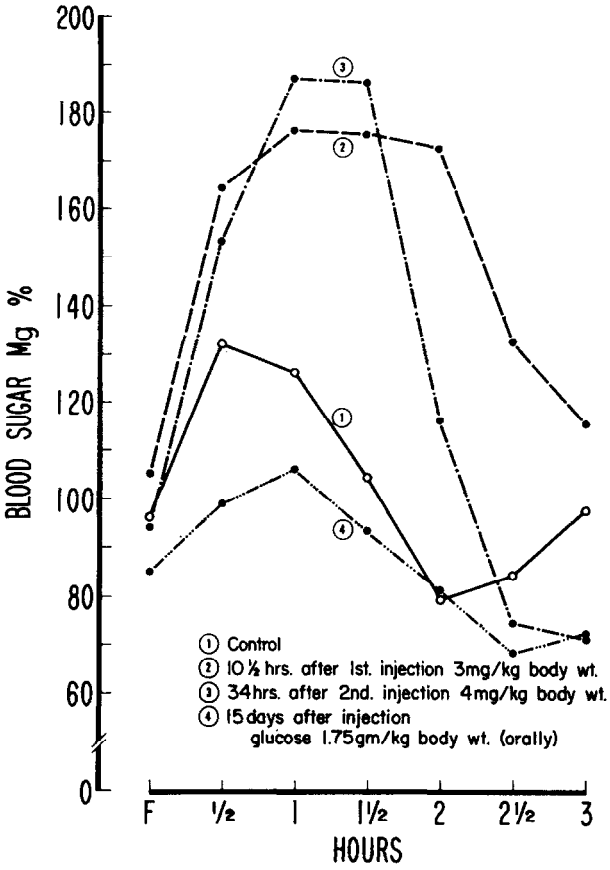

Fig. 8.—Dog O (F), 10.3 Kg. body weight.

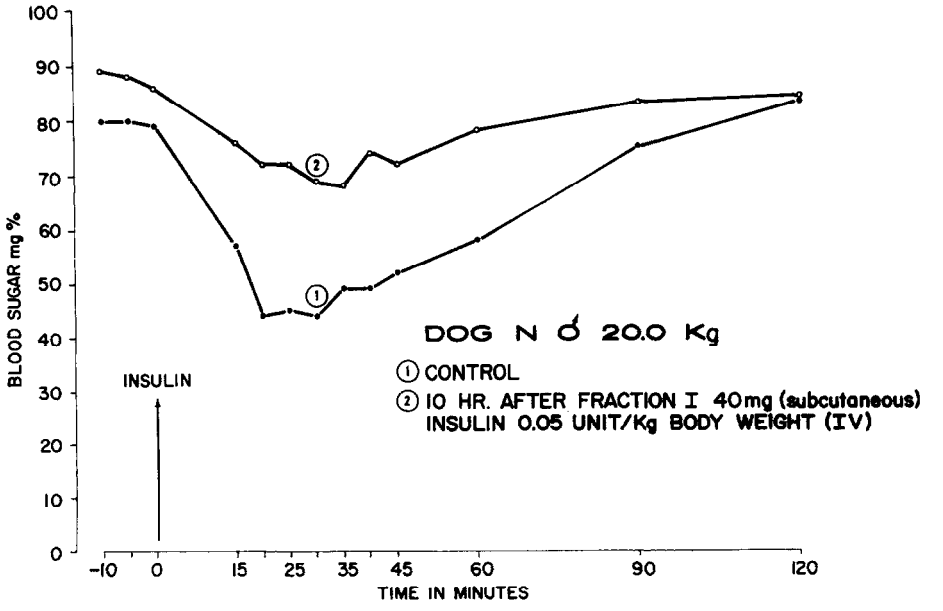

Fig. 9

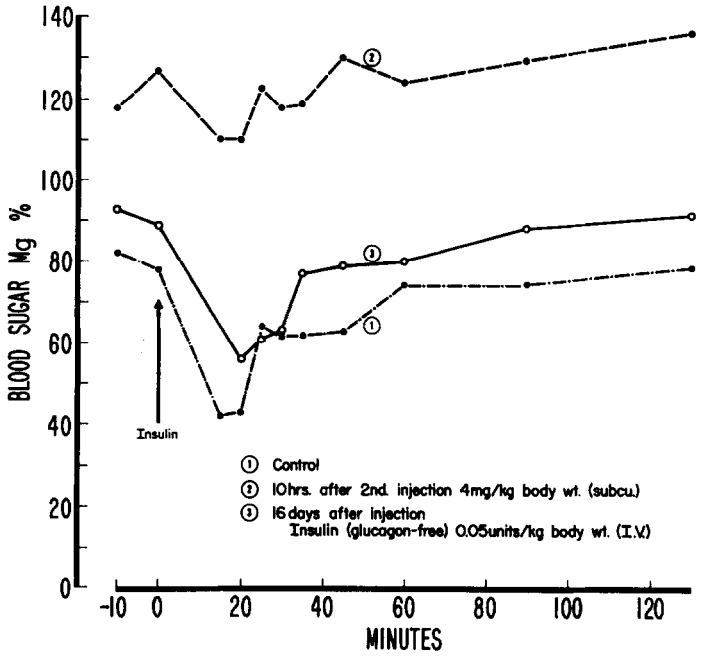

Fig. 10.—Dog O (F), 10.3 Kg. body weight.

COMMENTS

Isolation from urine of the active polypeptide by the present procedure appears to be more complete. In the previous method[1] a substantial quantity of the active compound was still present in the acetic acid solution even after three successive treatments with oxycellulose. Because we were not aware of this, these solutions were discarded. This was very unfortunate because much of the peptide (isoelectric point, approximately pH 4.1) was lost. The toxic material was removed at pH 3.5–3.6 (step 12 of the new method).

Disc electrophoresis of the active material reveals one prominent band together with two minor ones. Further purification of this substance by Sephadex column chromatography is now in progress.

ACKNOWLEDGMENTS

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