A Diabetogenic Polypeptide from Bovine Adenohypophysis Similar to that Excreted in Lipoatrophic Diabetes

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A polypeptide has been isolated from bovine adenohypophysis which antagonizes the hypoglycemic effect of exogenous insulin and which, per se, induces loss of carbohydrate tolerance in men and dogs. Mild acid hydrolysis of the active polypeptide yields a compound which retains the same biological properties. Characteristics of the active principle and its hydrolytic product is the long duration of their activities, the greatest intensity of the effects being observed between 34 and 60 hours after a single intramuscular injection. Both substances are devoid of ACTH activity. The active polypeptide resembles closely the insulin antagonist isolated from the urine of patients with lipoatrophic diabetes previously reported from this laboratory. Details of the isolation and physiologic effects of the active substance and its hydrolytic product are described. (Metabolism 15: No. 4, April, 308–324, 1966)

IN RECENT COMMUNICATIONS from this laboratory,[1,2] the isolation of an insulin antagonist from the urine of patients with lipoatrophic diabetes has been reported. It also was demonstrated that a similar substance was present in the urine of a maturity-onset insulin-resistant diabetic without lipoatrophy. The substance was found to be a polypeptide which when administered to either dogs or man exhibited diabetogenic and anti-insulin effects. The origin of the active principle had not been determined.

The present report describes a procedure for the isolation of a similar substance from the anterior lobes of bovine pituitary glands. Like the insulin antagonist from the urine, the material is also highly active both in dogs and in man.

MATERIALS AND METHODS

Frozen bovine anterior pituitary glands, obtained from the Armour Pharmaceutical Company, Kankakee, Illinois, were treated according to the procedure shown in Figure 1. The

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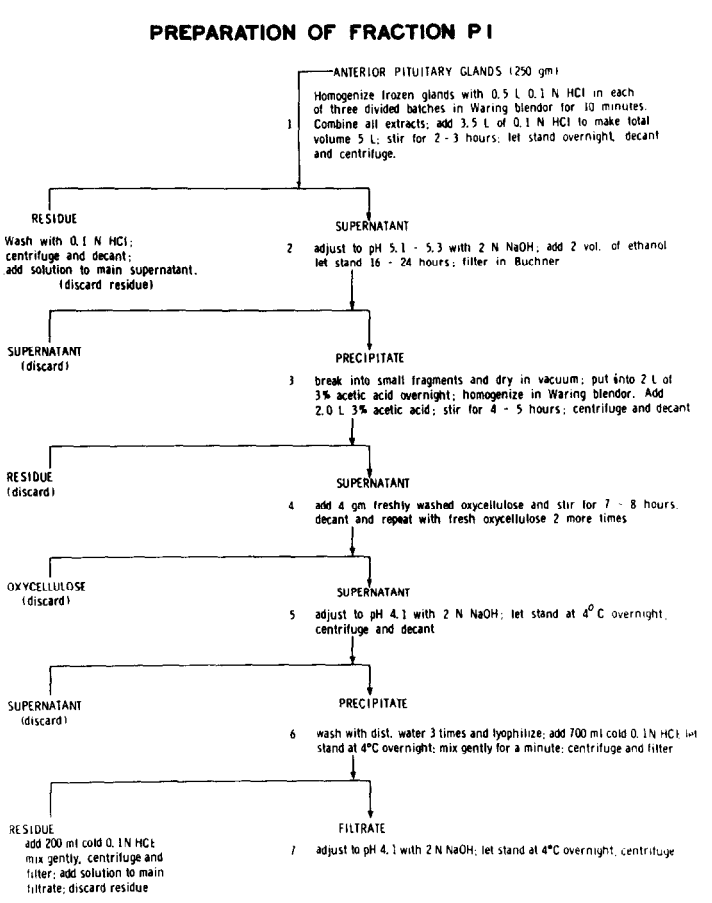

Fig. 1A

oxycellulose powder (17–21 per cent COOH, Eastman Chemical Products, Inc., Kingsport, Tennessee) employed in step 4 was kept refrigerated and before use was washed successively with distilled water, 0.1 N HCL, and distilled water. Electrophoresis of fraction PI on cellulose acetate reveals 3 bands, a prominent one with minor bands before and after. Elemental analysis of the compound gives the following; C 48.32 per cent, H 7.18 per cent, N 15.36 per cent, and S 1.41 per cent. Its isoelectric point is approximately pH 4.1, and the average yield is 0.05–0.06 per cent.

Dogs were maintained on a constant high carbohydrate diet (454 Gm. of Pard and 75 Gm. of glucose) and the human subjects on a constant 3.200 calorie diet including 300 Gm. of carbohydrate before and during all testing procedures. Glucose and insulin tolerance tests were carried out before and at various intervals after administration of the material. Twenty-four hour urine specimens were collected daily on the human subjects for the estimation of 17-ketosteroids,[3] 17-hydroxysteroids,[4] uric acid,[5] glucose,[6] and creatinine.[7] The active principle from the pituitary tissue was prepared for injection in exactly the same way as that previously described[2] for the insulin antagonist extracted from urine.

Results

Fraction PI was tested for biological activity on 3 normal dogs. Figures 2 and 3 depict examples of the effects obtained on glucose tolerance and on insulin tolerance in dogs.

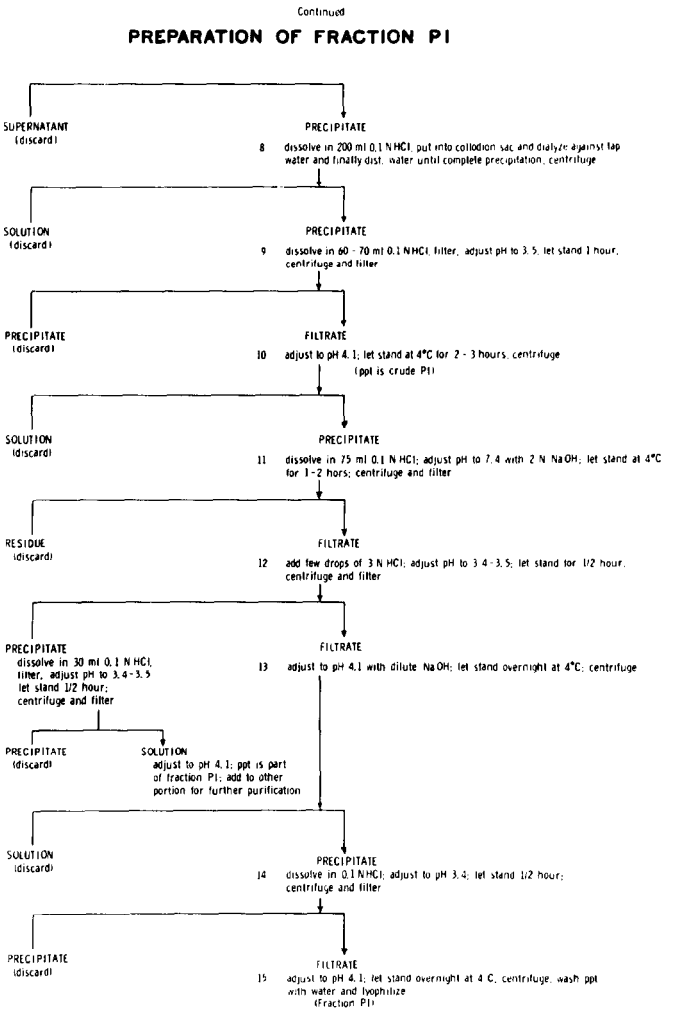

Fig. 1B

Fraction PI also induces glucose intolerance and insulin resistance in man. Tables 1, 2 and 3 show the results obtained when 20 mg. was administered intramuscularly in a single dose to each of 3 normal volunteers. In each case the largest degree of carbohydrate intolerance was observed 8 hours following administration of PI. Table 4 shows that urinary excretion of 17-hydroxysteroids, 17-ketosteroids and uric acid did not change significantly.

Insulin tolerance tests were performed on 2 more healthy subjects. This time doses of 20 and 30 mg. of fraction PI were administered intramuscularly on 2 successive days and the insulin tolerance tests were done 9 and 33 hours after the second injection. Figures 4 and 5 indicate the marked insulin resistance which was induced. No untoward reacting to this material was observed in any subject.

Further Fractionation of Fraction PI. Biological Effects of Fraction PIIIC2

Figure 6 describes the procedure by which fraction PIIIC2 is obtained from

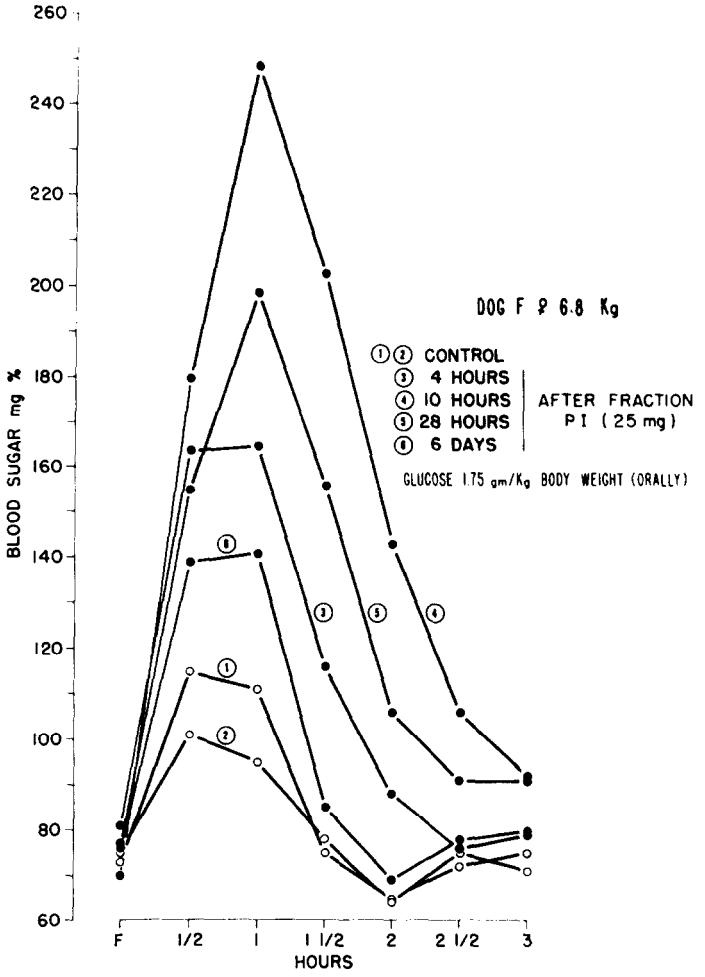

Fig. 2

fraction PI. Elemental analysis of this compound shows C 51.22 per cent, H 7.48 per cent, N 14.66 per cent, and S 1.91 per cent. Its isoelectric point is the same (approximately pH 4.7) as the urinary fraction IIIC2.

Although fraction PIIIC2 is a hydrolytic product of fraction PI, it possesses diabetogenic and anti-insulin properties. However, in one set of experiments on the same dog (Fig. 7, 8 and 9), fraction PI was found to be approximately twice as potent as fraction PIIIC2.

Biological activity of fraction PIIIC2 was tested on 7 normal young men. Three of them received a single dose of 11–12 mg. intramuscularly and all developed glucose intolerance. Figures 10 and 11 show the results of subjects W. M. B. and J. S. S. Table 5 indicates that urinary excretion of 17-hydroxysteroids, 17-ketosteroids, and uric acid did not change significantly.

Each of the other 4 subjects was given a larger single dose (40 mg.) of the

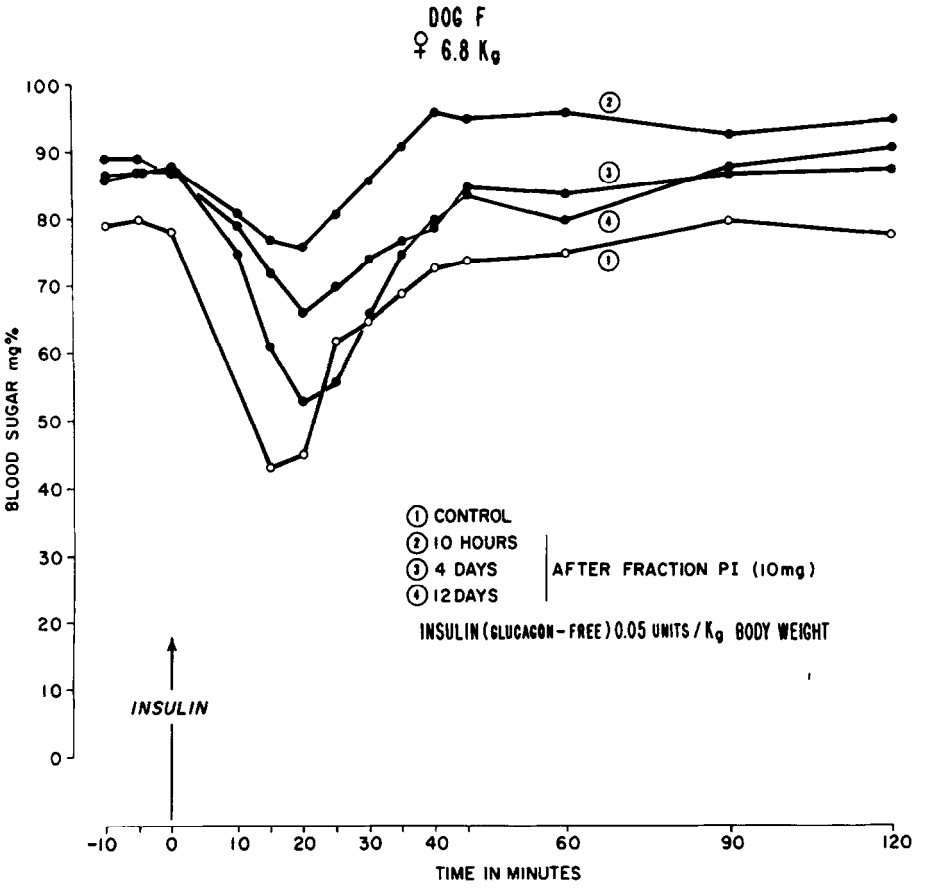

Fig. 3

same fraction. Glucose tolerance tests were performed daily for from 7 to 9 days. Tables 6, 7, 8 and 9 disclose the following: (1) the material induces carbohydrate intolerance which persists for 2 to 3 days; (2) renal glycosuria becomes evident; and (3) a resurgence of both (1) and (2) at about the sixth postinjection day.

A series of insulin tolerance tests were performed on W. M. B. following a 12 mg. dose of fraction PIIIC2, the results of which are shown on Figure 12.

Discussion

A diabetogenic polypeptide has been isolated from bovine adenohypophsis by the same procedure with which a similar diabetogenic polypeptide was previously isolated from the urine of patients with lipoatrophic diabetes.[2] The physical properties of both compounds are very similar and have isoelectric points at approximately pH 4.1. Mild acid hydrolysis of both polypeptides yields a similar active substance which in both cases has an isoelectric point at approximately pH 4.7.

Table 1.—*Effect of Fraction PI upon Glucose Tolerance on a Normal Subject J. C. M. (22 years, male, 75 Kg.)*

HOURS AFTER INJECTION 20 mg	F	1/2	1	1 1/2	2	2 1/2	3
Control	81	129	98	85	82	98	87
8	84	174	160 (trace)	127	119 (trace)	116	66
33	96	134	151	118	104	87	103
56	79	145	170	114	119	112	104
81	75	126	133	105	105	105	76
105	79	118	128	130	122	116	113
131	79	128	135 (trace)	120 (trace)	104 (trace)	85 (trace)	94 (trace)
155	73	130	130 (trace)	120 (trace)	113	75	81
179	74	155	112 (+)	119 (trace)	96 (trace)	85	95
2 (months)	84	117	147	123	103	107	78

Table 2.—*Effect of Fraction PI upon Glucose Tolerance on a Normal Subject J. C. M. (22 years, male, 75 Kg.)*

HOURS AFTER INJECTION 20 mg	F	1/2	1	1 1/2	2	2 1/2	3
Control	74	124	118	101	100	97	82
8	85	147	156 (trace)	156 (trace)	108 (trace)	106 (trace)	89
32	84	147	159 (trace)	114 (trace)	115	110	104
55	79	123	144 (trace)	104 (trace)	109 (trace)	96	86 (trace)

Table 3.—*Effect of Fraction PI upon Glucose Tolerance on a Normal Subject L. J. B. (22 years, male, 68.3 Kg.)*

Hours after injection 20 mg	F	1/2	1	1 1/2	2	2 1/2	3
Control	81	167	145	130	78	93	90
8	82	117	158	113	130	103	92
34	85	154	150	132	123	120	115
58	83	138	143	149	101	118	83
104	77	139	135	115	115	89	71

Table 4.—*Effect of Fraction PI upon Urinary 17-OHCS, 17-KS, Creatinine, Uric Acid and Glucose*

SUBJECT	DAY	INJECTION	17-OHCS mg/day	17-KS mg/day	CREATININE g/day	URIC ACID g/day	GLUCOSE g/day
B.A.G.	1	0	7.2	22.8	2.59	0.940	1.324
	2	0	6.2	22.8	2.37	0.919	1.254
	3	0	7.6	22.8	2.50	0.932	1.324
	4	20 mg (IM)	6.0	21.6	2.24	0.735	1.104
	5	0	6.6	18.6	2.50	0.795	1.216
	6	0	7.4	22.8	2.56	0.786	1.226
	7	0	7.0	19.6	2.49	0.778	1.188
	8	0	8.2	19.2	2.47	0.701	1.150
	9	0	6.6	18.0	2.52	0.769	1.234
	10	0	8.6	21.4	2.56	0.791	1.274
J.C.M.	1	0	10.8	19.0	1.91	0.772	0.838
	2	0	9.4	18.6	1.97	0.805	0.706
	3	20 mg (IM)	11.6	22.0	2.00	0.805	0.926
	4	0	9.8	19.2	2.01	0.793	0.890
	5	0	10.2	22.4	2.01	0.739	0.926
	6	0	9.4	19.2	2.12	0.759	0.890

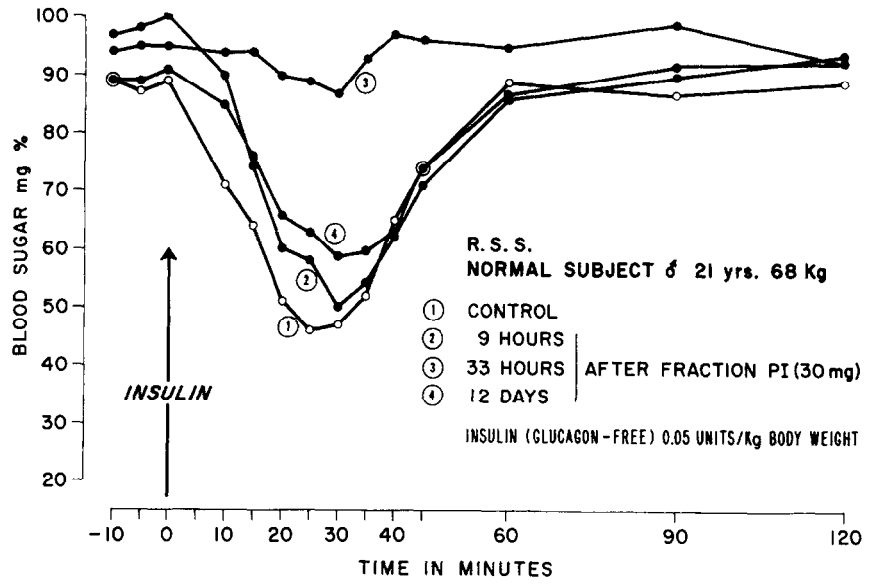
BLOOD SUGAR mg %
100
90
80
70
60
50
40
30
20
INSULIN
R. S. S.
NORMAL SUBJECT ♂ 21 yrs. 68 Kg
① CONTROL
② 9 HOURS
③ 33 HOURS
④ 12 DAYS
AFTER FRACTION PI (30 mg)
INSULIN (GLUCAGON-FREE) 0.05 UNITS/Kg BODY WEIGHT
-10 0 10 20 30 40 60 90 120
TIME IN MINUTES

Fig. 4

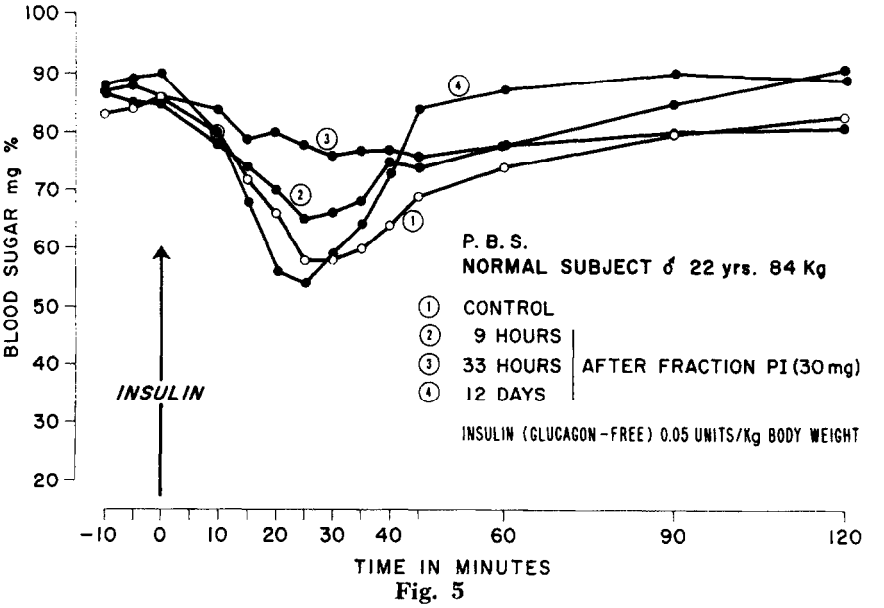
BLOOD SUGAR mg %
100
90
80
70
60
50
40
30
20
INSULIN
P. B. S.
NORMAL SUBJECT ♂ 22 yrs. 84 Kg
① CONTROL
② 9 HOURS
③ 33 HOURS
④ 12 DAYS
AFTER FRACTION PI (30 mg)
INSULIN (GLUCAGON-FREE) 0.05 UNITS/Kg BODY WEIGHT
-10 0 10 20 30 40 60 90 120
TIME IN MINUTES

Fig. 5

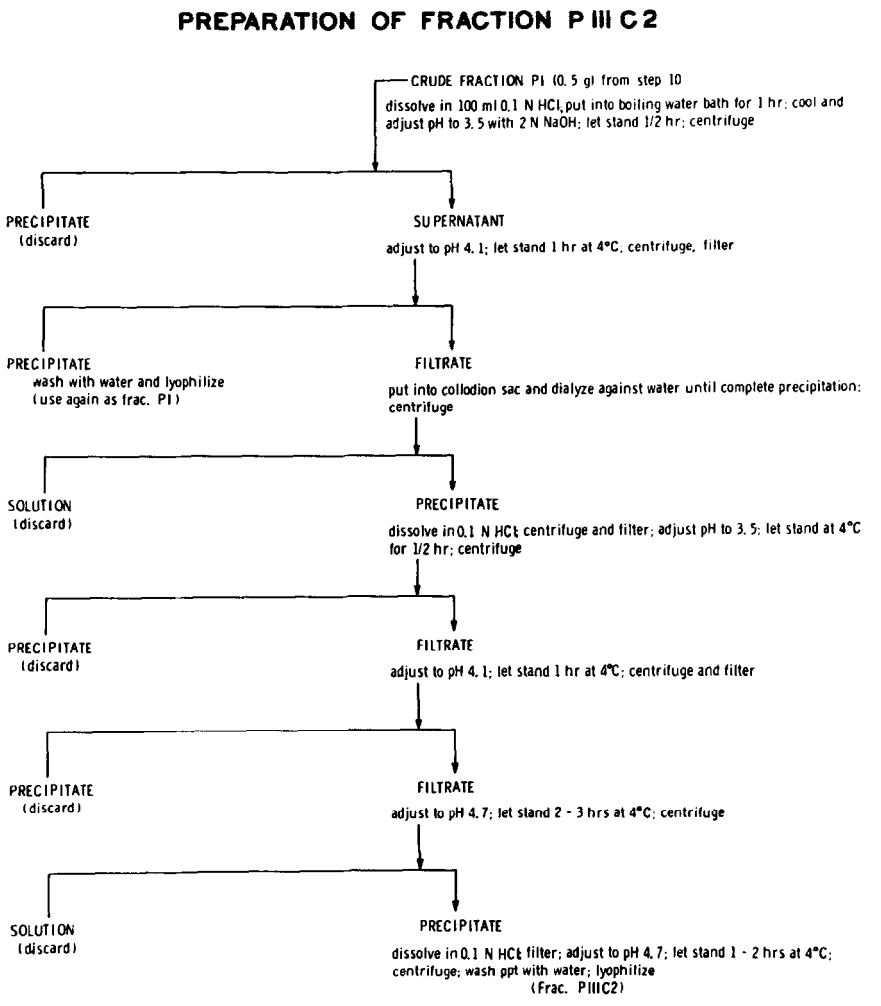

Fig. 6

The material is not bovine corticotropin since no evidence of adrenal cortical stimulation was observed. Pure bovine growth hormone does not diminish carbohydrate tolerance of man.[8] It has been shown that bovine growth hormone can be altered by chemical manipulation after which it may induce carbohydrate intolerance in man.[9] While this possibility exists, it seems an unlikely one since the isolation procedure employed in our work for the isolation of fraction PI is sufficiently mild that one would not anticipate disruption of the growth hormone molecule.* Furthermore, the prolonged reduction in

*Dr. Don S. Shalch, School of Medicine and Dentistry of the University of Rochester showed that our fraction PI has brought about one thousandth the affinity for antigrowth hormone antibody as BGH.

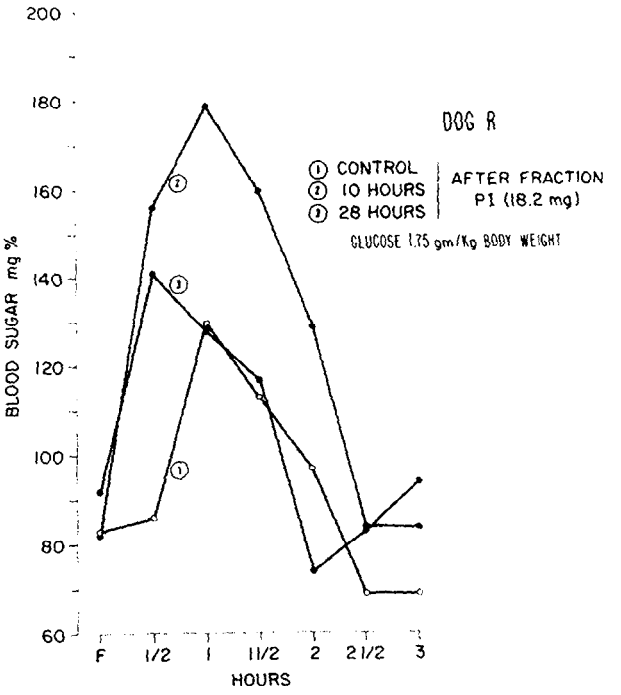
DOG R
① CONTROL
② 10 HOURS
③ 28 HOURS
AFTER FRACTION
P1 (18.2 mg)
GLUCOSE 1.75 gm/Kg BODY WEIGHT
BLOOD SUGAR mg %
200
180
160
140
120
100
80
60
F
1/2
1
1 1/2
2
2 1/2
3
HOURS

Fig. 7

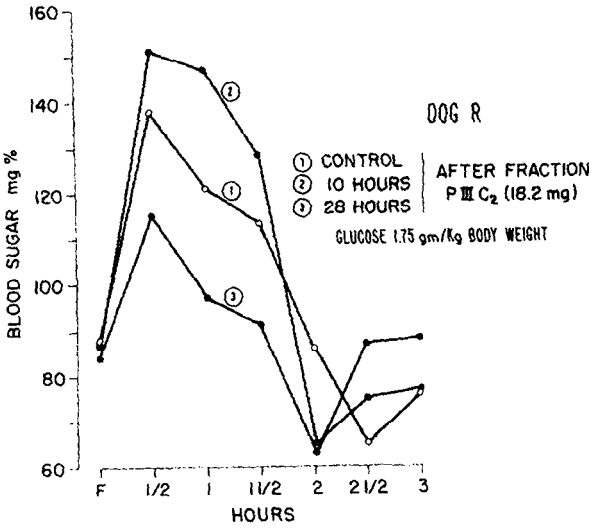
DOG R
① CONTROL
② 10 HOURS
③ 28 HOURS
AFTER FRACTION
P III C2 (18.2 mg)
GLUCOSE 1.75 gm/Kg BODY WEIGHT
BLOOD SUGAR mg %
160
140
120
100
80
60
F
1/2
1
1 1/2
2
2 1/2
3
HOURS

Fig. 8

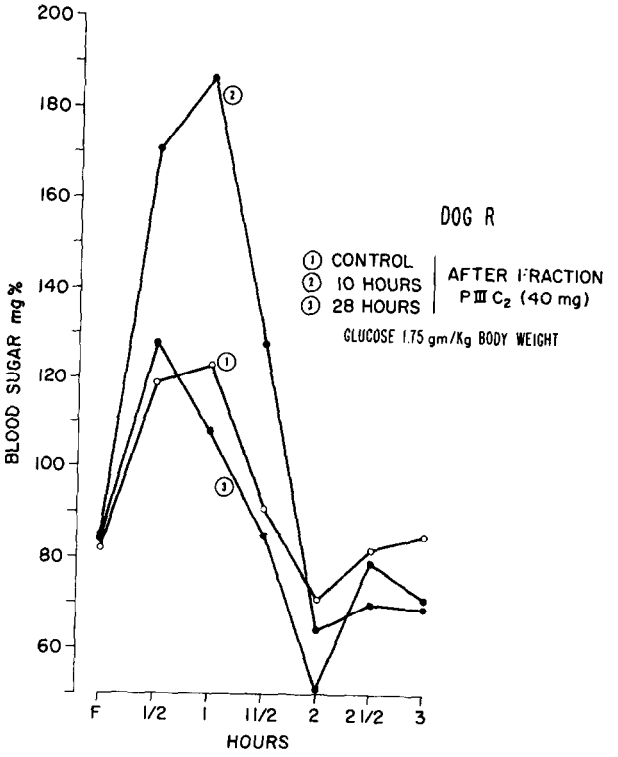
DOG R
① CONTROL
② 10 HOURS
③ 28 HOURS
AFTER FRACTION
P III C2 (40 mg)
GLUCOSE 1.75 gm/Kg BODY WEIGHT
BLOOD SUGAR mg %
HOURS
200
180
160
140
120
100
80
60
F
1/2
1
1 1/2
2
2 1/2
3

Fig. 9

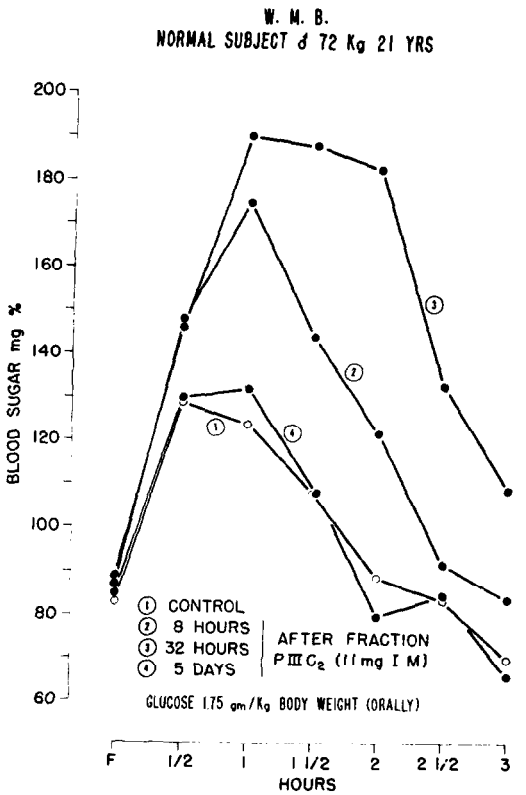
W. M. B.
NORMAL SUBJECT ♂ 72 Kg 21 YRS
① CONTROL
② 8 HOURS
③ 32 HOURS
④ 5 DAYS
AFTER FRACTION
P III C2 (11 mg I M)
GLUCOSE 1.75 gm/Kg BODY WEIGHT (ORALLY)
BLOOD SUGAR mg %
HOURS
200
180
160
140
120
100
80
60
F
1/2
1
1 1/2
2
2 1/2
3

Fig. 10

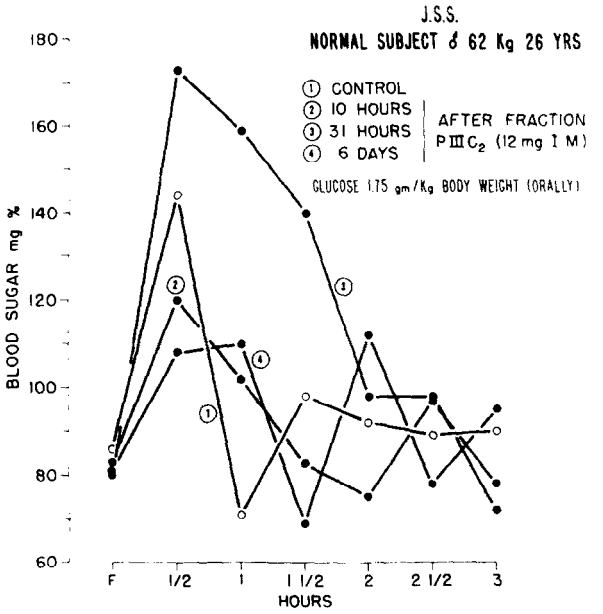

Fig. 11

Table 5.—*Effect of Fraction PIIIC2 upon Urinary 17-OHCS, 17-KS, Creatinine, Uric Acid and Glucose*

SUBJECT	DAY	INJECTION	17-OH CS mg/day	17-KS mg/day	CREATININE g/day	URIC ACID g/day	GLUCOSE g/day
W.M.B.	1	0	5.8	6.2	1.68	0.834	1.16
	2	0	6.0	11.0	1.87	0.795	1.32
	3	11 mg (IM)	6.0	8.2	1.83	0.773	1.24
	4	0	5.4	7.3	1.81	0.729	1.28
	5	0	5.2	5.7	1.73	0.585	1.40
	6	0	6.2	8.9			1.36
	7	0	6.4	9.3			1.28
J.S.S.	1	0	6.0	12.8	1.59	0.742	0.760
	2	0	7.1	10.9	1.85	0.838	0.905
	3	12 mg (IM)	6.2	13.4	1.87	0.671	0.900
	4	0	8.2	16.2	2.27	1.047	1.280
	5	0	6.3	14.4	2.10	0.903	0.987
J.G.E.	1	0	8.4	19.2	2.40	1.009	1.348
	2	0	8.4	15.8	1.85	0.761	1.240
	3	0	7.0	18.2	1.94	0.803	0.960
	4	12 mg (IM)	8.2	16.4	2.36	1.042	1.360
	5	0	8.2	15.8	2.48	0.944	1.400
	6	0	9.2	17.8	2.23	0.868	1.400

Table 6.—*Effect of Fraction PIIIC2 upon Glucose Tolerance on a Normal Subject J. C. M. (22 years, male, 75 Kg.)*

HOURS AFTER INJECTION 40 mg	F	1/2	1	1 1/2	2	2 1/2	3
Control	83	147	134	102	101	110	88
8	79	151	152 (trace)	126 (trace)	115	106	94
32	83	137	165	134	111	114	106
55	74	150	158 (trace)	130	125	123	100
64	80	148	188 (trace)	189 (+)	164 (trace)	132	116
78	74	151	158	137	120	110	105
102	75	141	185 (+)	130 (+)	108 (trace)	113	89
126	76	120	139	106	91	87	100

Table 7.—*Effect of Fraction PIIIC2 upon Glucose Tolerance on a Normal Subject R. F. J. (22 years, male, 59 Kg.)*

HOURS AFTER INJECTION 40 mg	F	1/2	1	1 1/2	2	2 1/2	3
Control	76	121	101	72	92	105	87
14	78	98	117	79	78 (+)	89	83
38	80	128	133 (+)	83	98	90	75 (+)
62	75	122	110 (trace)	71	67	74 (trace)	71
86	83	126	134 (trace)	106	78 (+)	76	77
110	75	102	94	/4	68	94	79
134	76	129	143 (+)	101	77 (+)	83	89
142	91	147	195 (++)	222	210 (++++)	158	109
158	69	108	144	77	101 (+)	92	75
3 (months)	81	109	95	87	82	88	93

Table 8.—*Effect of Fraction PIIIC2 upon Glucose Tolerance on a Normal Subject J. S. B. (22 years, male, 79 Kg.)*

HOURS AFTER INJECTION 40 mg	F	1/2	1	1 1/2	2	2 1/2	3
Control	84	167	131	112	118	68	84
12	87	183	162	144	115	84	91
36	82	172	138	102	107	65	93
60	76	162	131	72	118	53	57
84	78	168	119	87	65	61	65
108	81	168	129	99	74	78	52
116	94	145	179	118	110	93	57
135	78	190	192 (+)	157 (+)	134 (trace)	77	110 (trace)
160	89	219	203	118 (+++)	116 (++)	71	83
184	82	211	126	150	85	86	73

Table 9.—*Effect of Fraction PIIIC2 upon Glucose Tolerance on a Normal Subject L. J. B. (22 years, male, 68 Kg.)*

HOURS AFTER INJECTION 40 mg	F	1/2	1	1 1/2	2	2 1/2	3
Control	84	156	129	132	106	108	101
14	83	148	142 (+)	114 (+)	92 (trace)	95	104
36	84	153	168	172 (+++)	140 (++)	125 (trace)	86
60	86	178	190 (++)	162 (+++)	140 (++)	94 (+)	77 (trace)
84	79	149	144 (trace)	132 (trace)	90 (trace)	121	121
106	83	148	155 (trace)	142 (trace)	134 (trace)	99 (trace)	107
130	79	147	131 (+)	115 (trace)	105	101	101
156	79	132	122	108	80	99	80
228	86	152	112	133	88	93	98

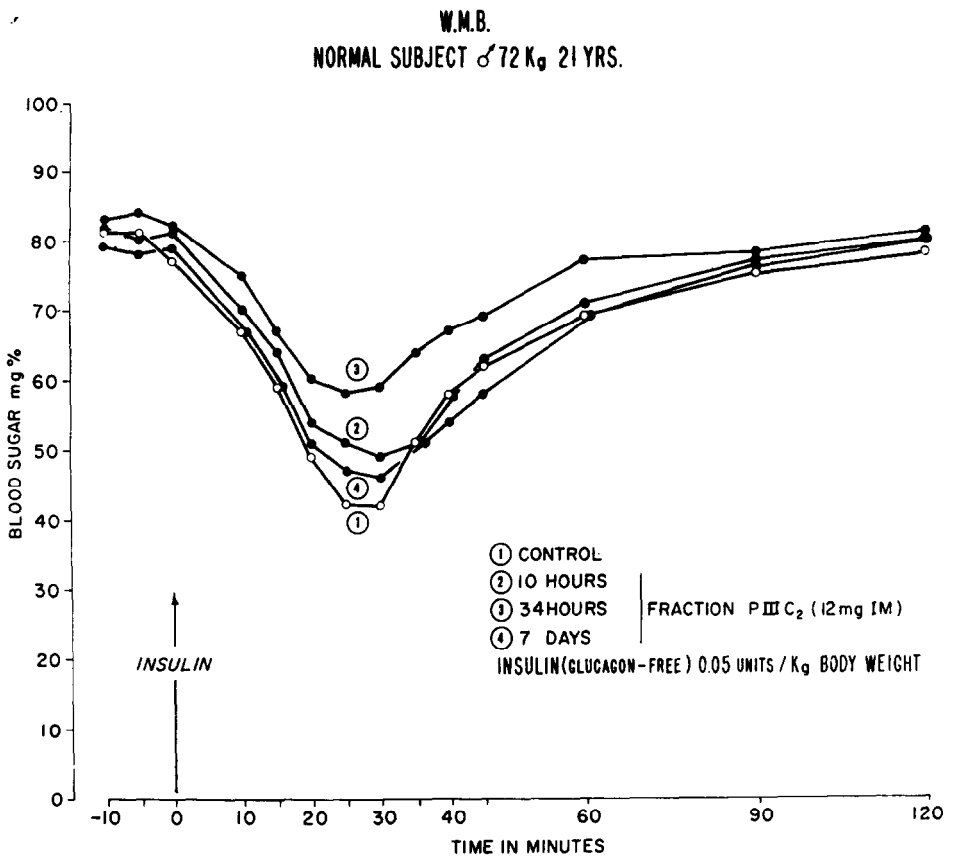

Fig. 12

carbohydrate tolerance induced by fraction PIIIC2 should have been accompanied by a rise of plasma free fatty acids and a fall of plasma amino nitrogen had the former been the result of a growth hormone effect.

It thus seems likely that the diabetogenic polypeptide isolated from the urine of patients with lipoatrophic diabetes is of adenohypophyseal origin and that it is different from the other anterior pituitary polypeptides known to diminish carbohydrate tolerance in man.

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