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A Diabetogenic Polypeptide from Hog and Sheep Adenohypophysis Similar to that Found in Lipoatrophic Diabetes

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A polypeptide possessing physical and biological properties similar to that excreted in the urine by patients with lipoatrophic diabetes was previously isolated from bovine adenohypophysis. A similar principle has now been obtained from the anterior lobes of hog and sheep pituitaries by the same procedures of isolation. The material from these animals also induces hyperglycemia and insulin resistance both in humans and dogs. The substance exhibits no similarity in physicochemical properties to either growth hormone or prolactin. Its presence in the pituitary gland of all three species of animals so far studied suggests that the peptide is a naturally occurring substance but its physiological significance is unknown. Its physicochemical and biological similarity to the urinary polypeptide found in lipoatrophic diabetes suggests a relationship. (Metabolism 17: No. 6, June, 475-484, 1968)

WE HAVE REPORTED upon the isolation from the urine of patients with lipoatrophic diabetes of a polypeptide which antagonizes the hypoglycemic effects of exogenous insulin and which induces loss of carbohydrate tolerance in dogs and humans.[1,2] More recently, we have described the isolation of a similar substance from the anterior lobe of bovine pituitary glands.[6,7] This material also induces hyperglycemia and insulin resistance

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Table 1.—*Elemental Analysis of Fraction PI Isolated From Bovine, Porcine and Ovine Adenohypophysis*

Source	C %	H %	N %	S %
Bovine	48.32	7.18	15.36	1.41
Porcine	50.14	6.56	15.95	1.33
Ovine	50.57	6.99	16.23	1.59

when administered to dogs and humans. Because the substance from both sources was found to be similar in physicochemical and biological properties, it was concluded that the origin of the diabetogenic polypeptide isolated from the urine of patients with lipoatrophic diabetes is probably the pituitary gland.

The present paper describes the isolation of a similar substance from porcine and ovine adenohypophyses. This material is also diabetogenic and antagonizes exogenous insulin when administered either to dogs or to humans.

Materials and Methods

Frozen porcine and ovine anterior pituitary glands, as well as pancreas, posterior pituitary and thyroid gland (Pharmaceutical Division of Wilson and Company, Inc., Chicago, Illinois), were subjected to the isolation procedure previously described[7] for obtaining the diabetogenic material from bovine adenohypophysis.

The procedures employed for testing diabetogenicity and insulin resistance have been described previously.[2,7] In the present studies four trained dogs, two males and two females, were used. They were maintained between tests in a diet of "Friskies Mix" dog food but for three days prior to and throughout the testing periods were given a daily diet consisting of 454 Gm. of "Pard," 100 Gm. "Friskies," and 60 Gm. of sucrose.

Four young healthy male volunteers who had no family history of diabetes mellitus served as subjects. They were maintained on a constant 3000 calorie diet including 300 Gm. of carbohydrate throughout their studies. Preparation and sterilization of the peptide for intramuscular injection was the same as previously described.[2]

Blood glucose was measured by the Somogyi-Nelson procedure.[3] 17-hydroxysteroids and 17-ketosteroids were determined by the Silber-Porter[4] and Chaney methods,[5] respectively.

Results

The elemental composition of the polypeptide isolated from bovine, porcine and ovine pituitaries is presented in Table 1. The average yield of this material from these glands is 0.05–0.07 per cent. Isoelectric point of the substance from all three species of animals is approximately pH 4.1. No similar polypeptide was found in the posterior lobes of bovine pituitaries, thyroid gland or pancreas.

Fraction PI obtained from porcine adenohypophysis was tested for biological activity on four normal dogs and two men. Figures 1 through 4 show typical effects upon glucose and insulin tolerance in dogs. Porcine fraction PI, similar to the bovine fraction PI, induces glucose intolerance in man. Tables 2 and 3 demonstrate the results obtained when 40 mg. of the substance was administered intramuscularly in a single dose to each of the two normal men. The greatest initial effect after administration of the peptide was 10 hours in subject R.H.J. and 33 hours in subject J.P. Of great interest is the recurrence

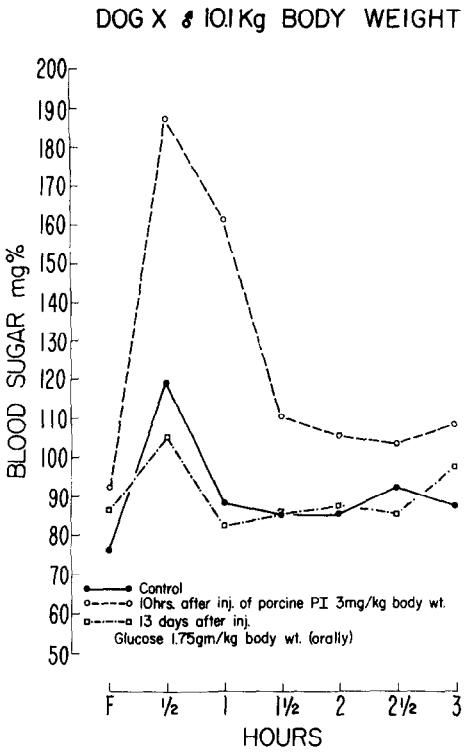
DOG X ♂ 10.1 Kg BODY WEIGHT
BLOOD SUGAR mg%
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
Control
10hrs. after inj. of porcine PI 3mg/kg body wt.
13 days after inj.
Glucose 1.75gm/kg body wt. (orally)
F
½
1
1½
2
2½
3
HOURS

Fig. 1.

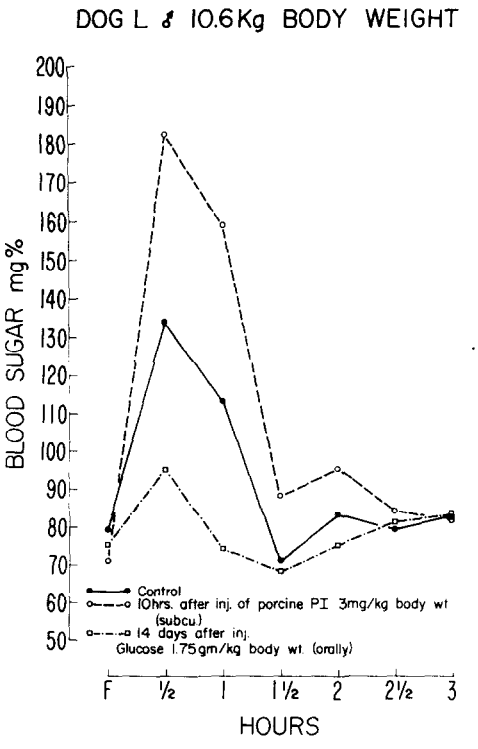
DOG L ♂ 10.6 Kg BODY WEIGHT
BLOOD SUGAR mg%
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
Control
10hrs. after inj. of porcine PI 3mg/kg body wt (subcu.)
14 days after inj.
Glucose 1.75gm/kg body wt. (orally)
F
½
1
1½
2
2½
3
HOURS

Fig. 2.

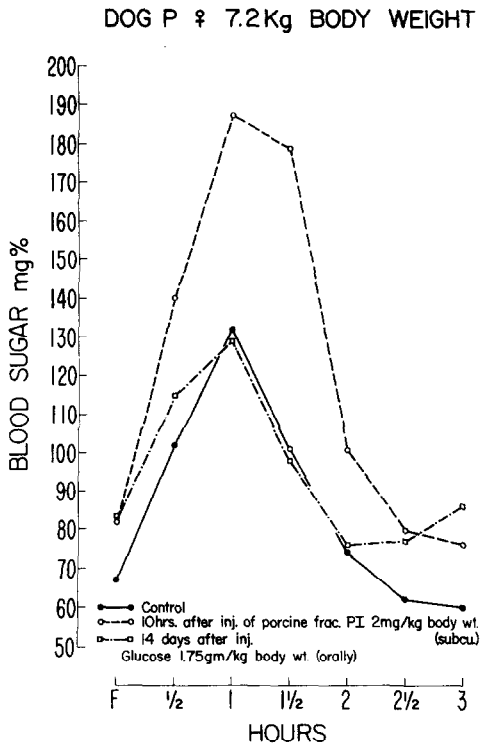

Fig. 3.

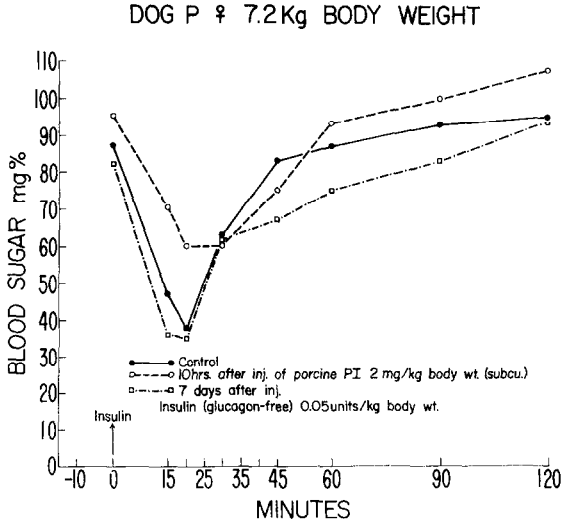

Fig. 4.

of carbohydrate intolerance at about the sixth post-injection day in both of the subjects. This phenomenon has been observed previously.[7]

Ovine pituitary fraction PI was tested for biological activity on the same animals used for testing the porcine fraction. Figures 5 and 6 show the effect of a single injection of 3 mg. per Kg. in two dogs.

Ovine fraction PI, 40 mg. intramuscularly in a single dose, was administered

Table 2.—*Effect of Porcine Fraction PI Upon Glucose Tolerance of a Normal Subject R.H.J. (24 years ♂ 64.7 Kg.)*

Hours After Injection 40 mg. (I.M.)	F	½	1	1½	2	2½	3	4
Control	90	120	91	90	88	72	75	
10	86	142	164 (+)	129 (trace)	111	113	91	
34	89	155	147 (trace)	113	104	78	78	
58	85	116	123	99	88	87	68	
82	84	135	105	111	93	108	79	47
106	76	119	102 (trace)	67 (trace)	96	90	81	84
114	87	134	213 (trace)	218 (++++)	201 (+++)	162 (+++)	110	—
130	85	147	166 (++)	149 (++)	154 (+)	119 (trace)	97	68
154	83	141	88 (trace)	103	89	81	81	
176	76	142	96	82	96	92	92	

Table 3.—*Effect of Porcine Fraction PI Upon Glucose Tolerance of a Normal Subject J.P. (22 years ♂ 65.0 Kg.)*

Hours After Injection 40 mg. (I.M.)	F	½	1	1½	2	2½	3
Control	80	165	100 (trace)	80	82	74	60
8	84	161	114	117	79	100	93
33	82	168	165 (+++)	136 (+)	113 (trace)	86	102
55	88	154	144	99	135	100	104
79	79	149	161	120	103	98	69
103	83	138	138	132	132	114	90
127	82	147	134	151	133	140	107
151	83	145	179	132	134	112	107
175	82	131	128	93	110	86	88

to subjects S.N. and V.L. Glucose tolerance tests were performed daily for eight consecutive days. Tables 4 and 5 show the results. Greatest initial impairment of glucose tolerance occurred at 31 hours. The later effect upon carbohydrate tolerance occurred again on the sixth post-injection day.

Urinary excretion of 17-hydroxysteroids and 17-ketosteroids, carried out only on the human subjects, did not change significantly during or after the administration of either porcine or ovine fraction PI. No untoward local or systemic effects were observed.

An attempt was made to compare the diabetogenic potencies of the polypeptides isolated from bovine, porcine and ovine anterior pituitaries by testing each in the same animal according to a fixed protocol. The procedure consisted of (a) control glucose tolerance test done on day 1; (b) control insulin tolerance test done on day 2; (c) first subcutaneous injection of the substance

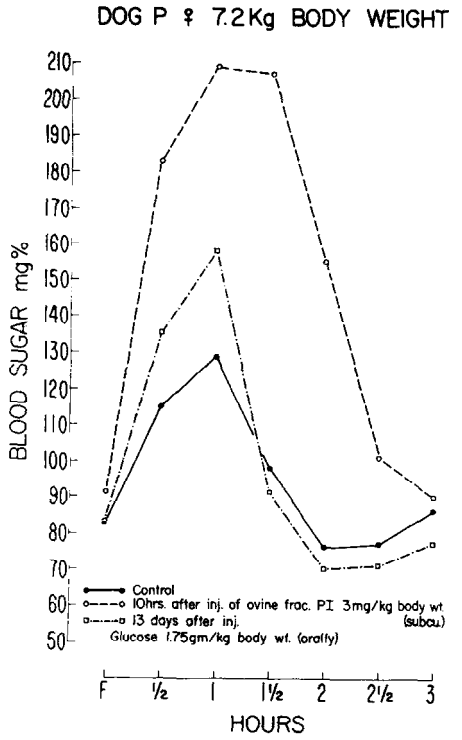
DOG P ♀ 7.2Kg BODY WEIGHT
BLOOD SUGAR mg%
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
Control
10hrs. after inj. of ovine frac. PI 3mg/kg body wt. (subcu.)
13 days after inj.
Glucose 1.75gm/kg body wt. (orally)
F
½
1
1½
2
2½
3
HOURS

Fig. 5.

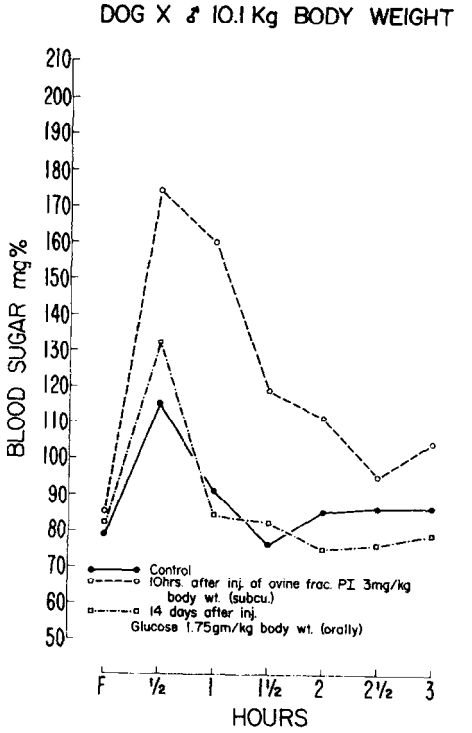
DOG X ♂ 10.1 Kg BODY WEIGHT
BLOOD SUGAR mg%
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
Control
10hrs. after inj. of ovine frac. PI 3mg/kg body wt. (subcu.)
14 days after inj.
Glucose 1.75gm/kg body wt. (orally)
F
½
1
1½
2
2½
3
HOURS

Fig. 6.

Table 4.—*Effect of Ovine Fraction PI Upon Glucose Tolerance of a Normal Subject S.N. (33 years ♂ 60.3 Kg.)*

Hours After Injection 40 mg. (I.M.)	F	½	1	1½	2	2½	3
Control	93	121	94	85	86	86	90
10	93	128	113	113	109	95	70
31	90	169	144 (++)	147 (+)	116 (+)	116 (trace)	82
55	94	109	110	96	124	108	84
79	91	139	127	119	101	90	86
103	92	138	155	125	92 (trace)	89	90
127	83	136	158	142 (trace)	116 (trace)	102	79
151	89	146	134 (trace)	117 (trace)	113 (trace)	95 (trace)	99
175	83	99	125	94	84	80	80

Table 5.—*Effect of Ovine Fraction PI Upon Glucose Tolerance of a Normal Subject V.L. (21 years ♂ 70.4 Kg.)*

Hours After Injection 40 mg. (I.M.)	F	½	1	1½	2	2½	3
Control	91	174	109 (trace)	98	79	75	64
7	99	166	126 (trace)	124 (trace)	121 (trace)	110	48
31	96	167	145 (trace)	126 (trace)	98	62	67
55	94	195	128 (trace)	92	—	—	—
79	99	161	147 (trace)	97	87	76	61
103	96	144	123 (trace)	86 (trace)	84 (trace)	66 (trace)	59
111	85	155	217 (+)	162 (++)	117 (trace)	101 (trace)	84 (trace)
127	100	168	186 (+)	105 (+)	101 (trace)	69	69
151	97	129	123	97	101	98	56
175	95	137	150	94	93	89	54

(3 mg. per Kg. body weight) 10 hours before the second glucose tolerance test done on day 3; and (d) a second injection of the same substance (4 mg. per Kg. body weight) 10 hours before the second insulin tolerance test done on day 4. Then at least 13 days elapsed before the protocol was repeated with fraction PI from another species. The results are depicted in Figures 7, 8, and 9. It is difficult to discern any differences. It should be observed that the level of the fasting blood sugar rose mildly after the second dose of the compound from each species.

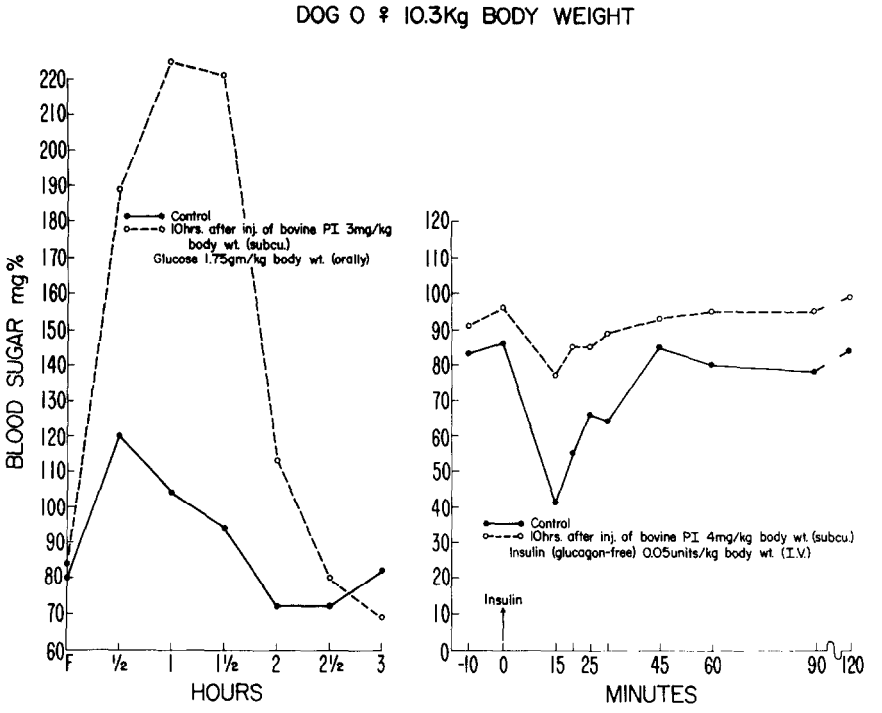
DOG O ♀ 10.3Kg BODY WEIGHT
BLOOD SUGAR mg%
Control
10hrs. after inj. of bovine PI 3mg/kg body wt. (subcu.)
Glucose 1.75gm/kg body wt. (orally)
HOURS
F ½ 1 1½ 2 2½ 3
Control
10hrs. after inj. of bovine PI 4mg/kg body wt. (subcu.)
Insulin (glucagon-free) 0.05units/kg body wt. (I.V.)
Insulin
MINUTES
-10 0 15 25 45 60 90 120

Fig. 7.

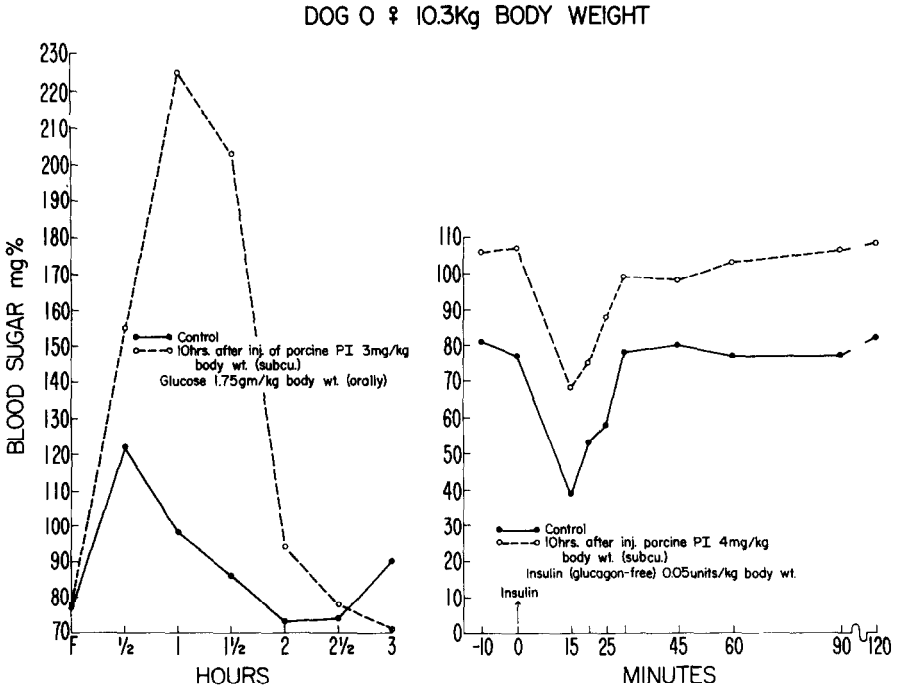
DOG O ♀ 10.3Kg BODY WEIGHT
BLOOD SUGAR mg%
Control
10hrs. after inj. of porcine PI 3mg/kg body wt. (subcu.)
Glucose 1.75gm/kg body wt. (orally)
HOURS
F ½ 1 1½ 2 2½ 3
Control
10hrs. after inj. porcine PI 4mg/kg body wt. (subcu.)
Insulin (glucagon-free) 0.05units/kg body wt.
Insulin
MINUTES
-10 0 15 25 45 60 90 120

Fig. 8.

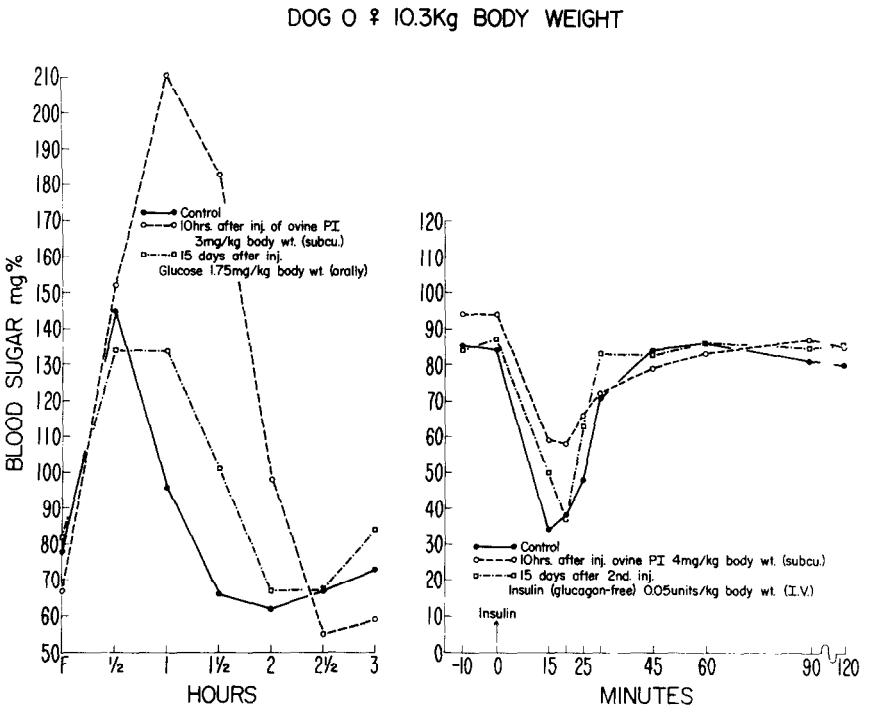

Fig. 9.

Comment

A diabetogenic polypeptide has been isolated from porcine and ovine adenohypophyses by the same procedures with which bovine pituitaries have yielded a similar substance.[7] All three pituitary peptides are very similar to one which we have reported[2] to be in the urine of patients with lipoatrophic diabetes. The isoelectric point of all four biologically active peptides is approximately pH 4.1.

These diabetogenic peptides are not ACTH-like since they do not increase urinary excretion of 17-hydroxysteroids and 17-ketosteroids. One would not expect them to be somatotrophin-like since nonprimate growth hormone exhibits no significant metabolic effects in man[8] and the mild procedures employed in our isolation would not have altered somatotropin.

Comparison of the isoelectric points of ovine and bovine prolactin* with our ovine and bovine fraction PI shows them to be greatly different. However, in one test of our ovine fraction PI, prolactin activity (Reece-Turner indermal pigeon crop technique) of approximately 4 IU per mg. was found.†

The tentative conclusion seems justified that the polypeptide isolated from bovine, porcine, and ovine adenohypophysis is different from other known diabetogenic pituitary hormones. Whether it has physiological significance

*Ovine prolactin (NIH-P-S-7); bovine prolactin (NIH-P-B1).

†We are grateful to Dr. Raymond Kahn, Department of Anatomy, University of Michigan, Ann Arbor, Michigan, for the performance of the prolactin assay on our ovine fraction PI.

remains to be determined. It is, however, very similar to the peptide excreted in the urine by patients with lipoatrophic diabetes.

REFERENCES

1. Louis, L. H., and Minick, M. C.: Isolation of an insulin antagonist from urine of patients with lipoatrophic diabetes. J. Lab. Clin. Med. 60:995, 1962.

2. —, Conn, J. W., and Minick, M. C.: Lipoatrophic diabetes: Isolation and characterization of an insulin antagonist from urine. Metabolism 12:867, 1963.

3. Somogyi, M.: Notes on sugar determination. J. Biol. Chem. 195:19, 1952.

4. Silber, R. H., and Porter, C. C.: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma. J. Biol. Chem. 210:923, 1954.

5. Chaney, A. L.: 17-ketosteriods in urine. Standard methods. Clin. Chem. 2:79, 1958.

6. Louis, L. H., Conn, J. W., and Minick, M. C.: Isolation of a peptide from bovine adenohypophysis which induces hyperglycemia and insulin resistance in men and dogs. Diabetes 14:445, 1965.

7. —, —, and —: A diabetogenic polypeptide from bovine adenohypophysis similar to that excreted in lipoatrophic diabetes. Metabolism 15:308, 1966.

8. Bergenstal, D. M., and Lipsett, M. B.: Metabolic effects of human growth hormone and growth hormone of other species in man. J. Clin. Endocr. 20:1427, 1960.