A Urinary Diabetogenic Peptide in Proteinuric Diabetic Patients

By LAWRENCE H. LOUIS AND JEROME W. CONN

A polypeptide exhibiting diabetogenic and anti-insulin properties has been isolated from the urine of 33 of the 35 proteinuric diabetic patients. This material could not be found in the urine of 34 normal subjects and 32 diabetic patients without proteinuria. It was, however, detected chemically in one of seven nondiabetic patients with proteinuria, but the amount present was insufficient for biological testing. This polypeptide closely resembles, in physiochemical properties, that obtained from patients with lipoatrophic diabetes, as well as that isolated from the adenohypophyses of beef, sheep, and swine. The isoelectric point of the active polypeptide from all the above sources is approximately pH 4.1. In one diabetic subject who underwent hypophysectomy, this material disappeared from the urine following the operation. It is suggested that the source of the active principle isolated from the urine of diabetic patients with proteinuria is probably the pituitary gland. (Metabolism 18: No. 7, July, 556–563, 1969)

IN 1963 WE REPORTED that the urine of patients with lipoatrophic diabetes contains a diabetogenic polypeptide.[1] This has recently been confirmed by others.[2] We demonstrated that the peptide was capable of impairing glucose tolerance and of inducing insulin resistance when administered either to dogs or humans. The present study was designed to explore the possibility that a similar substance might be found in the urine of diabetic patients without lipodystrophy since a chance observation had disclosed its presence in an obese, maturity-onset diabetic.[1] Freshly collected urine samples from 67 diabetic patients and 34 healthy subjects have been studied. The diabetogenic peptide was found in the urine of 33 of 35 diabetics with proteinuria but it was absent in all of 32 diabetics without proteinuria, as well as in the normal subjects. Seven additional subjects with proteinuria but without diabetes were studied. One of them exhibited a similar urinary material.

MATERIALS AND METHODS

Collection and preservation of urine samples were the same as previously described.[1] Clinical information on the proteinuric diabetic subjects studied are shown in Table 1. Our

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Table 1.—Clinical Data

Patient	Age	Sex	Duration of diabetes years	FBS mg%	Insulin or anti-diabetic drugs	Urinary Protein	Diabetogenic peptide mg/day	*Retinopathy	*Neuropathy	*Nephropathy
D. E. S.	24	M	18	171–366	30 lente 20 reg.	++	8.6	+++	+++	+++
W. W.	51	M	10	120–360	30 lente	+++	4.8		++	+++
L. B.	63	F	4	106–288	40 lente	+++	7.1			+++
C. A. M.	57	M	15	106–248	31 lente	+++	6.4	+	++	++
E. Z.	42	M	11	50–267	O. B. I. 50 mg bid Dymelor 0.5 g	++++	0.6	+++	++	+++
R. L.	46	M	14	46–350	22 lente	+++	0.8	++	++	+++
J. P.	45	M	14	90–233	25 reg. 30 NPH	++++	0.2	++	++	+++
D. A. B.	21	M	19 1/2	102–250	66 lente	++	7.7	+++	++	++
G. S.	54	F	13	76–205	diabinese 375 mg	+	3.4	+		++
H. E. R.	54	F	24	90–168	Orinase 0.5 g TID	++++	3.0	++	++	++
J. K. L.	24	F	1 1/2	68–84	22 lente	++	1.8		+	++
M. M. S.	61	F	18	73–306	30 NPH	++	1.4	+++	+++	+++
C. G. S.	58	M	16	106–226	Orinase ig TID	+	10.6		+	++
A. H. C.	66	M	25	115–202	40 NPH	+	4.3		+	++
P. C. N.	23	M	20 1/2	120–370	25 lente	+++	4.3	++	+++	++
R. R.	44	F	6 mos.	90–140	Orinase ig bid	++	3.2			++
S. G. T.	24	F	2	76–325	45 lente	+	7.2			
B. E. C.	54	M	15	167–385	48 P21	++	7.8	++	++	++
J. C.	64	M	5 mos.	90–105	Orinase 0.5 g qd.	++	3.5		+	+
F. A.	62	M	20	98–277	diabinese 0.5 qd.	+++	6.3	++	++	++
R. A. R.	59	F	15	124–335	50 lente	++	1.2		++	++
T. H.	54	M	33	28–450	20–50 lente	+++	0.4	+	++	++
B. B.	55	M	4	90–305	40 lente	+++	0.6			++
F. F. A.	58	F	9	79–280	20 lente	+++	2.3	+	+	+++
K. R. M.	22	M	16	76–450	32 NPH	++	8.8	+++	++	++
G. B. B.	21	M	14	94–360	30 NPH	+++	2.2	++	++	++
K. W.	79	F	26	98–194	46 NPH	+	3.3		++	+
W. O. G.	79	M	21	93–170	22 lente	+	3.2		+	+
G. A. Z.	54	F	1	80–105	none	+	1.1			+
K. H. J.	38	M	20	238–560	38 lente	+++	1.8	++	+	+++
L. A. S.	28	F	20	44–280	30 lente	+++	0.6	+		+++
R. L. A.	19	M	8	120–380	88 NPH	++++	3.5	++	+	+++
H. M. Z.	36	F	1 mo	102–118	none	+	4.1			+

* + = Mild ++ = Moderate +++ = Severe

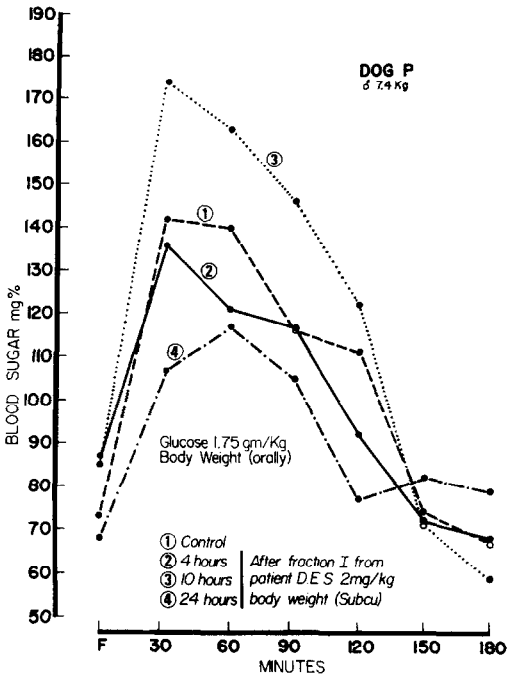


Fig. 1

more recent procedure for the isolation of the diabetogenic polypeptide (fraction 1) from urine was employed.[3]

Maintenance and preparation of dogs for glucose and insulin tolerance tests have been previously described.[6,7] In the present studies, two well-trained dogs were employed. However, because of the scarcity of the purified material, most of the tests were done on a small animal (Dog P, 7.4 Kg.). The assay procedure consisted of (a) a control glucose tolerance test done on day 1, and (b) an injection of the substance 10 hours before the second glucose tolerance test which was done on day 2. An interval of at least 13 days elapsed before the procedure was repeated in the same animal with the isolated peptide from another patient.

Blood glucose was measured by the Somogyi-Nelson procedure.[4] Disc electrophoresis was performed in a Model 6 apparatus* with a single bath assembly. The power source was Model 200.* Analyses were carried out according to the procedure of Ornstein and Davis.[5]

Electrophoresis was carried out at room temperature at a current of 4 ma per tube for 30 minutes. Polypeptide samples used were 60–100 μg. per tube. The buffer was of pH 8.3. Destaining was performed at a current of 12.5 ma per tube for 60 minutes.

Results

An effort to determine the time of maximal biological effect was made by carrying out glucose tolerance tests 4 hours, 10 hours and 24 hours after an injection of the peptide (2 mg. per kilogram of dog body weight) from patient D.E.S. The results are presented in Fig. 1. In Dog P, it appears that the greatest effect occurred 10 hours after injection. Subsequently, all tests were done 10 hours after administration of the peptide. Table 2 shows the effect

*Canal Industrial Corp., Rockville, Md.

Table 2.—Effect of Urinary Polypeptide (Fraction 1) on Glucose Tolerance*

Patient	Polypeptide mg./Kg. body wt.	F	½	1	1½	2	2½	3
	0	73	142	140	116	111	74	67
D.E.S.	2	85	174	163	146	122	71	59
	0	93	154	143	111	75	93	88
W.W.	2	95	143	243	237	188	119	87
	0	88	124	148	138	81	74	88
L.B.	2	93	168	238	175	118	106	86
	0	77	138	166	156	80	59	72
C.A.M.	2	95	171	197	152	122	83	80
E.Z.	0	84	119	161	122	74	72	73
R.L.	1.65	104	196	250	166	124	96	99
J.P.	0	84	139	128	118	67	71	89
D.A.B.	2	94	144	238	285	234	199	131
	0	67	102	132	126	74	62	60
	0	89	123	133	91	62	78	81
G.S.	2	99	178	229	193	143	93	66
	0	78	125	151	117	66	74	81
H.E.R.	2	98	177	235	217	118	86	76
	0	83	130	156	122	104	60	63
J.K.L.	2	78	157	182	165	118	76	61
	0	75	122	117	109	62	76	78
M.M.S.	2	86	115	148	176	160	173	142
	0	77	96	101	80	88	62	60
C.G.S.	2	88	146	193	192	211	165	118
	0	74	118	115	109	98	51	71
	pH 3.5–3.6 material							
A.H.C.	2	73	115	137	86	83	65	76
A.H.C.	2	76	131	187	226	204	177	96
	0	72	103	100	87	53	61	66
P.C.N.	2	77	99	140	126	108	53	58
	0	56	121	161	105	66	51	60
R.R.	2	71	93	160	201	192	172	105
	0	70	105	92	82	87	60	68
S.G.T.	2	71	124	168	198	159	96	44
	0	89	104	141	129	110	51	81
	0	64	113	138	120	51	62	67
B.E.C.	2	86	152	193	205	172	95	76
	0	68	105	185	60	59	—	63
J.C.	2	63	151	273	180	95	64	65
	0	77	133	167	113	60	62	65
F.A.	2	75	175	233	206	111	63	69
R.A.R.	0	66	132	153	122	66	60	75
T.H.	2	72	128	179	164	132	67	66
B.B.	0	86	115	141	111	83	50	66
F.A.A.	2	81	116	144	174	135	94	75
	0	62	104	168	104	79	65	58
K.R.M.	2	61	113	151	183	174	133	105
	0	60	114	154	103	109	68	57

* Dog P, 7.4 Kg.; glucose 1.75 Gm./Kg. body weight (orally).

Table 2.—continued

Patient	Polypeptide mg./Kg. body wt.	F	½	1	1½	2	2½	3
G.B.B.	2	—	107	167	145	101	85	63
	0	68	112	162	85	67	63	69
	0	86	115	141	111	83	50	66
K.W.	2	80	125	177	183	173	152	120
	0	73	115	146	111	73	63	64
W.O.G.	2	75	126	167	176	142	91	55
G.A.Z.	0	75	142	175	136	86	59	68
K.H.J.	0	73	108	107	102	81	73	66
L.A.S.	1.47	84	137	184	159	154	122	88
	0	82	123	162	158	102	86	98
R.L.A.	2	79	105	165	176	149	85	74
	0	72	106	149	145	75	58	60
H.M.Z.	2	77	144	172	163	109	70	66
	0	77	105	108	102	85	85	72

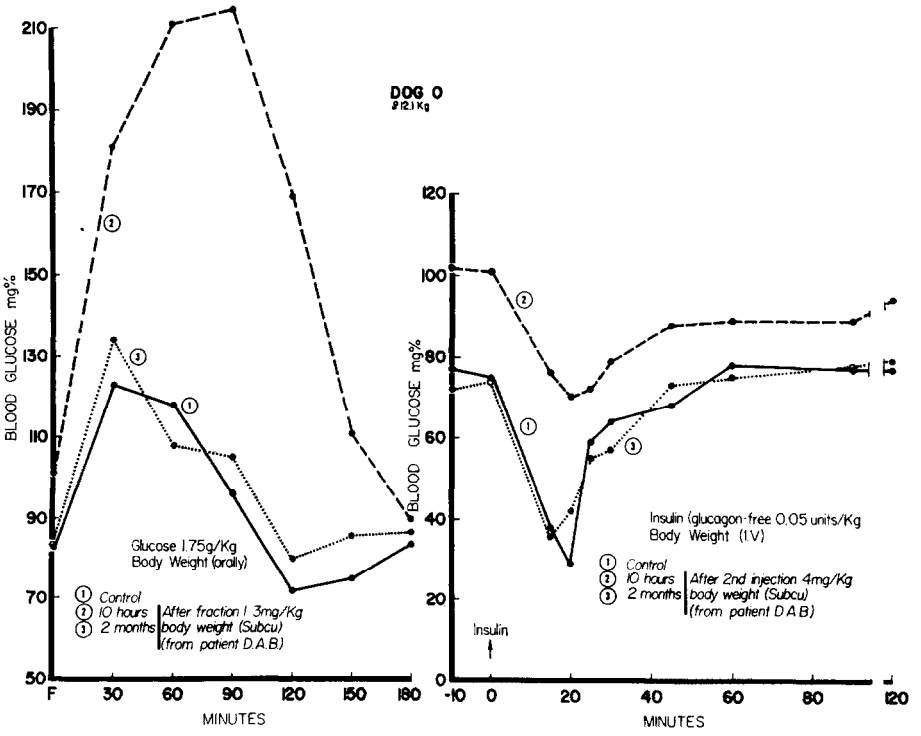


Fig. 2

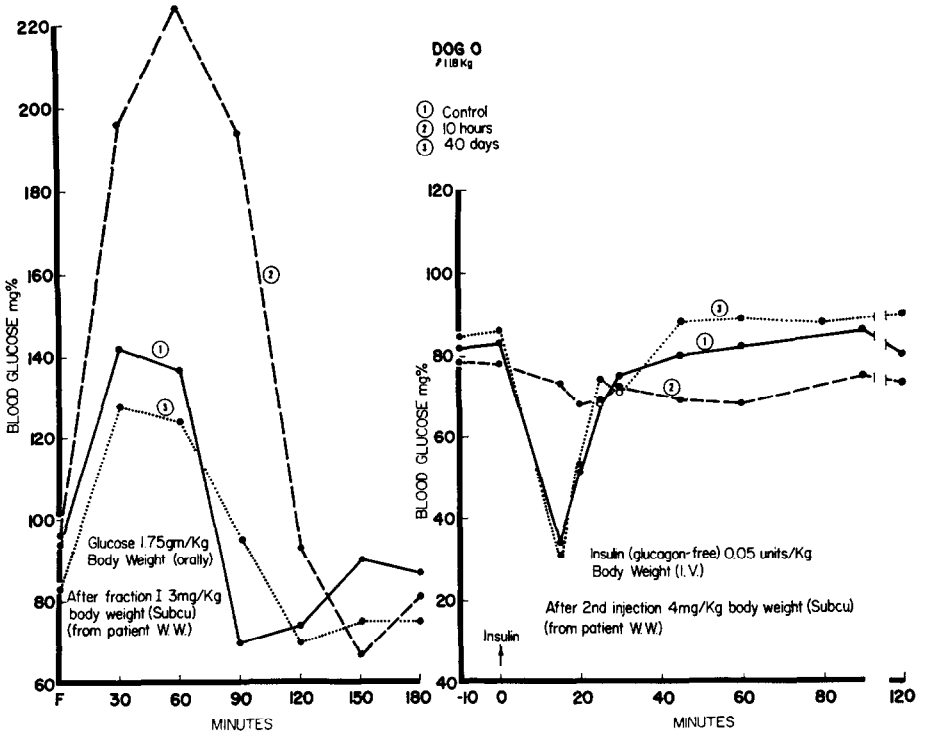


Fig. 3

upon glucose tolerance of the polypeptide (fraction 1) extracted from the urine of each of the 33 diabetic proteinuric patients in which it was found.

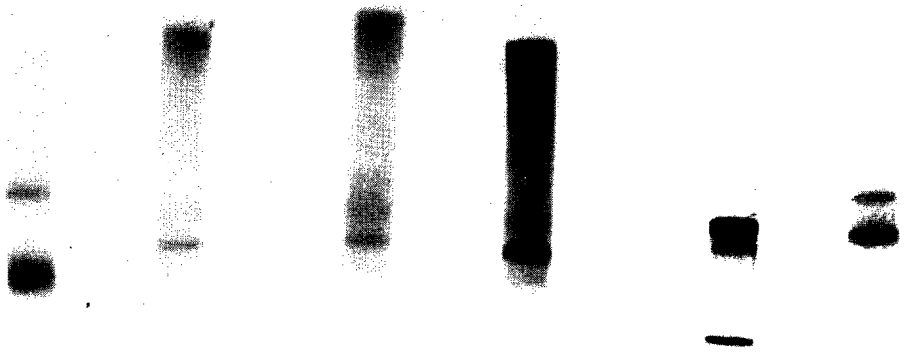
Reduced glucose tolerance and insulin resistance induced by the polypeptide from patients W.W. and D.A.B. were demonstrated in another animal as well (Dog O). The procedure for these tests was the same as previously described.[7] Figures 2 and 3 show the results. Of interest is the fact that the peptide found in patient D.A.B. was no longer present after he had undergone surgical hypophysectomy.

Elemental analysis of the diabetogenic polypeptide from patient W.W. shows the following: C 48.14 per cent, H 6.89 per cent, N 14.68 per cent, and S 1.49 per cent. These values are almost the same as those obtained upon the peptide isolated from the urine of patients with lipoatrophic diabetes.[3]

Figure 4 shows the polyacrylamide gel electrophoretic patterns of fraction 1 from a lipoatrophic diabetic (pattern 3) and from 3 diabetics with proteinuria (patterns 4, 5 and 6). For comparison (Fig. 5) disc electrophoresis was also performed on fraction 1 isolated from bovine (pattern 7) and human (pattern 8) pituitary glands. Further purification of these fractions by Sephadex column chromatography is now in progress.

Discussion

Since our report[1] on the isolation of a diabetogenic polypeptide from the urine of patients with lipoatrophic diabetes and the chance observation that



3 4 5 6

Fig. 4

7 8

Fig. 5

it was present in a proteinuric maturity-onset diabetic, we have been seeking to find the material in other diabetic patients. The significance of the associated proteinuria was not appreciated initially. It is now apparent that the peptide was present in the urine of 33 of the 35 diabetics with proteinuria but was present neither in 32 nonproteinuric diabetics nor in 34 healthy subjects. In 1 of 7 nondiabetics with proteinuria, a small amount of material which precipitated at pH 4.1 was found but it was insufficient to test for biological activity. When the material is present in proteinuric urine, it is not related quantitatively to the amount of protein excreted. Nineteen (58%) of the 33 patients excreting this peptide had detectable diabetic retinopathy of various grades and it is evident that all had some form of nephropathy. The observation that the diabetogenic peptide disappeared from the urine following hypophysectomy in the one such case in which it was studied, together with our ability to isolate a similar material from the adenohypophysis of beef, sheep, and swine, suggests that it originates in the pituitary gland. It is tempting to suggest that this material might somehow be involved in the microangiopathy of diabetes mellitus. In recent studies with frozen human pituitary glands, we have succeeded in isolating a polypeptide (isoelectric point approximately pH 4.1) which closely resembles the substance isolated from the urine of patients with proteinuria and diabetes mellitus. These studies will be detailed in another communication.

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REFERENCES

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

2. Hipolito-Reis, C., Sobrinho-Simoes, M., Ferraz, A., Jr., Hargreaves, M. P., and Cerqueira-Magro, F.: Peptide Urinaire de Type Anti-Insulinique dans le Diabéte Dit Lipo-atrophique de Lawrence. Rev. Franc. Endocr. Clin. 9:373–380, 1968.

3. Louis, L. H.: Lipoatrophic diabetes: An improved procedure for the isolation and purification of a diabetogenic polypeptide from urine. Metabolism 18:545–555, 1969.

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

5. Ornstein, L., and Davis, B. J.: Disc Electrophoresis. Rochester, N. Y., Distillation Products Industries, 1958.

6. Louis, L. H., Conn, J. W., and Minick, M. C.: A diabetogenic polypeptide from bovine adenohypophysis similar to that excreted in lipoatrophic diabetes. Metabolism 15:308, 1966.

7. —, and Conn, J. W.: A diabetogenic polypeptide from hog and sheep adenohypophysis similar to that found in lipoatrophic diabetes. Metabolism 17:475, 1968.