sive response of Algire(12) or to the Shwartzman reaction as previously ascribed to other bacterial-derived antineoplastic agents(13-14). Likewise, a refractive state may also be induced toward both the Shwartzman reaction(15-17) and the hypotensive state(12).

Summary. Of the 3 tumor sites tested, only the subcutaneous transplants of the S-37 tumors responded to treatment with the 2 pyrogenic fractions PC-3 and PC-6. Regardless of the treatment regimen, the intracerebral S-37 transplants failed to respond to treatment with pyrogen. Animals bearing either the intraocular transplants of adenoma EO771 or the subcutaneous transplants of leukemia 1498 exhibited no increase in survival time as a result of pyrogen therapy.

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Insulin Antagonism in Serum of Untreated Diabetics and in Previously Treated Diabetics with Ketoacidosis.* (26120)

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It has already been shown that sera of uncontrolled diabetics, who have previously been treated with insulin, neutralize the activity of insulin when tested *in vitro* by the rat diaphragm assay(1). This antagonism disappears when the diabetes is brought under control. As far as we know, no one has tested the effect of added insulin to sera from new and untreated diabetics, where no insulin antibodies are present. The present communication describes studies in which the biological effect of insulin on the rat diaphragm incubated in sera of new and untreated diabetics is compared to its effect when incubated in sera of patients with ketoacidosis, who had been previously treated with insulin.

Materials and methods. Blood was obtained from: 1) recently discovered diabetics with an elevated blood sugar and glycosuria; these patients never received insulin, and 2) patients admitted to the hospital with untreated diabetic acidosis; although they had had no insulin treatment for many hours prior to admission, they all had received insulin for over 2 months in the past. The serum was removed after proper blood clotting and kept at −10°C until time of testing. Sera with a glucose content grossly over 300 mg% were dialysed against a bicarbonate buffer(2) to decrease the glucose content to this approximate range. The same buffer, with glucose added (300 mg%), was used for the bioassay. The specimens were assayed for insulin or

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TABLE I. Untreated New Diabetics.

Case No.	Age	Buffer: Insulin added (μU/cc)	Buffer: Glucose uptake above basal level ± S.E. (μg/10 mg dry diaphragm)	Serum: Insulin added (μU/cc)	Serum: Glucose uptake above basal level ± S.E. (μg/10 mg dry diaphragm)	% inhibition of added insulin	Blood sugar
10	41	1000	246 ± 28	0	0 ± 13		200
"	"	"	"	1000	168 ± 2	70	"
11	40	1000	210 ± 23	0	4 ± 8		250
"	"	"	"	1000	116 ± 13	84	"
12	58	1000	328 ± 8	0	166 ± 15		380
"	"	"	"	1000	312 ± 30	14	"
13	50	1000	280 ± 18	1000	144 ± 8	85	374
14	58	500	300 ± 13	0	196 ± 10		288
"	"	"	"	500	258 ± 15	37	"
15	56	500	162 ± 10	0	294 ± 15		392
"	"	"	76 ± 2	500	352 ± 32	0	"
16	73	500	266 ± 25	500	46 ± 40	94	382
17	42	500	214 ± 15	500	70 ± 15	68	300
18	79	500	214 ± 15	500	94 ± 22	90	360
19	39	500	314 ± 12	500	162 ± 28	87	380
20	30	500	336 ± 23	500	292 ± 13	27	480
21	65	500	270 ± 15	500	30 ± 10	95	392
22	31	500	228 ± 17	500	148 ± 32	75	585
23	33	500	230 ± 17	500	96 ± 2	88	200
24	30	500	138 ± 22	500	90 ± 10	72	278
25	51	500	274 ± 42	500	212 ± 23	47	281

anti-insulin activity by the rat diaphragm method of Vallance-Owen *et al.*(3), using the intact serum. In each experiment the glucose uptake by serum with added insulin‡ was compared with the uptake in buffer alone (basal uptake) and buffer with added insulin. When enough material was available, inherent serum insulin activity was also tested. Each category was determined in triplicate by use of hemidiaphragms from 3 different rats.

One acidotic serum and sera of 2 normal subjects were subjected to hanging curtain electrophoresis(4), using a barbital buffer, pH 8.6, ionic strength 0.05. Position of the conventional protein fractions was determined by staining the curtains with ninhydrin. The collected fractions were dialysed against 20% Plasdone C§ overnight to remove the buffer and most of the water, then reconstituted to original serum volume with bicarbonate buffer for insulin bioassay. All fractions were frozen until time of assay, and glucose was then added to make the concentration 300 mg%. Inadequate amounts of serum prevented us from testing these specimens prior to electrophoresis.

Results and discussion. Our series consists of sera from 25 diabetics: 16 with ketoacidosis and no treatment and 9 with ketoacidosis and previous insulin treatment. Table I shows that the sera of most of the new diabetics markedly blocks the action of added insulin, although in a few cases (Nos. 12, 14, 15, and 20) the loss of activity is not significant. One patient, (Case #15), showed levels above 1000 μU/cc both with and without added insulin. Since the difference in glucose uptake between 500 and 1000 microunits is relatively small, the difference from day to day in the bioassay results would not become apparent unless both concentrations were tested simultaneously. Table II gives the data obtained in diabetics with ketoacidosis. Degree of inhibition does not seem to vary much from that of the new diabetics. There seemed to be little correlation with blood sugar, CO_2, or lipemia. Case No. 2, an elderly woman who died in shock 3 hours after admission, showed very little inhibition of

‡ Purified insulin supplied by Dr. O. K. Behrens, Eli Lilly Co., Indianapolis, Ind.

§ Grade of polyvinylpyrrolidone, Antara Chemicals, New York City.

TABLE II. Diabetic Ketoacidosis.

		Buffer		Serum					
Case No.	Age	Insulin added (μU/cc)	Glucose uptake above basal level ± S.E. (μg/10 mg dry diaphragm)	Insulin added (μU/cc)	Glucose uptake above basal level ± S.E. (μg/10 mg dry diaphragm)	% inhibition of added insulin	Blood sugar	CO_2	Lipemia
1	35	1000	200 ± 23	0	74 ± 22		298	—	No
	"	"	"	1000	72 ± 10	90	"	—	"
2	74	1000	254 ± 27	1000	218 ± 10	29	1400	5.0	No
3	20	500	258 ± 15	500	212 ± 7	40	456	8.0	Yes
4	23	500	258 ± 15	500	160 ± 15	74	550	—	"
5	21	500	214 ± 15	500	72 ± 7	95	672	—	"
6	22	500	266 ± 25	500	100 ± 7	94	348	10.0	No
7	24	500	162 ± 10	500	12 ± 22	97	456	4.8	Yes
8	30	500	228 ± 17	500	120 ± 22	86	832	5.7	"
9	25	500	230 ± 17	500	154 ± 7	68	812	7.0	Lip. ret.*

* Lipemia retinalis.

the added insulin. All the others were typical juvenile-type diabetics with acetonemia and clinical symptoms of ketoacidosis at time of admission. Case No. 9 had a relatively low blood sugar, mild acetonemia, and relatively mild symptoms. All of these cases needed less than 1000 units/24 hours for ultimate control of the condition. Case No. 2 had received 300 units at time of death. Table III shows data obtained on 5 normal sera. No gross antagonism can be seen.

TABLE III. Normal Sera without and with Addition of Insulin (500 μU/cc) Compared with Buffer + Insulin (500 μU/cc).

Case No.	Age	Glucose uptake above basal level ± S.E. (μg/10 mg dry diaphragm) Buffer + insulin	Serum alone	Serum + insulin
1	26	266 ± 34	134 ± 22	322 ± 14
2	25	162 ± 26	156 ± 16	196 ± 15
3	31	280 ± 43	164 ± 17	262 ± 14
4	22	252 ± 45	152 ± 25	288 ± 13
5	25	264 ± 11	68 ± 8	260 ± 44

Table IV shows bioassay data obtained on one acidotic serum after hanging curtain electrophoresis. Since several fractions showed no antagonism to insulin added, especially in the γ and α region, they were assayed without addition of insulin. High insulin activity was found in fractions 4 (166 μU/cc), 5 (570 μU/cc), 7, and 8 (710 μU/cc) when plotted on the curve of the concurrent insulin standard. Table V shows data obtained on 2 normal sera subjected to hanging curtain electrophoresis. The total amount of insulin activity is far less than that of the patient in ketoacidosis. These findings agree closely with those of Randle and Taylor(5,6,7). The reported insulin antagonist of Field and Stetten(8) was not found, using our technic.

We feel that these data suggest the presence of substances in the blood of new diabetics, as well as diabetics in ketoacidosis which can combine with insulin to neutralize its effect on glucose uptake by the rat diaphragm. In the new diabetics this is presumably not an antibody. Recent work(9) indicates that insulin-basic protein complexes present in normal sera do not produce an insulin-like effect on glucose uptake of the rat diaphragm, while they do stimulate glucose uptake by the rat adipose tissue. Precipitation of the basic protein(s) at pH 10, or preincubation with rat adipose tissue extract (10), restores the insulin-like effect on the rat diaphragm. From the electrophoresis experiment it appears that a large quantity of insulin is similarly liberated by this technic. It is possible that new diabetics, (and diabetics in acidosis) possess excessive amounts

TABLE IV. Diabetic Ketoacidosis: Serum Insulin Activity after Hanging Curtain Electrophoresis.

Fraction No.	Protein band location*	Glucose uptake above basal level ± S.E. (μg/10 mg dry diaphragm) Buffer + insulin (1000 μU/cc)	Fraction + insulin (1000 μU/cc)	Buffer + insulin (1000 μU/cc)	Fraction alone	Insulin activity (μU/cc original serum)
2				202 ± 7	−30 ± 23	0
3				238 ± 7	−58 ± 7	0
4		204 ± 13	232 ± 33		152 ± 15	160
5	γ			238 ± 7	198 ± 18	570
6				202 ± 7	76 ± 22	40
7	β			332 ± 30	122 ± 8	50
8		204 ± 13	236 ± 27	178 ± 11	162 ± 2	710
9	α	222 ± 13	204 ± 17	200 ± 33	44 ± 22	<10
10		186 ± 10	228 ± 8	178 ± 11	84 ± 23	90
11		204 ± 13	226 ± 8			
12	alb.	186 ± 10	206 ± 32			
13		222 ± 13	220 ± 8			
14						
15	pre-alb.			268 ± 35	−26 ± 43	0

* Identification by acid ninhydrin stain of curtain.

TABLE V. Normal Sera Insulin Activity after Hanging Curtain Electrophoresis.

Fraction No.	Protein band location	Glucose uptake above basal level ± S.E. (μg/10 mg dry diaphragm) Subject R.M. Buffer and insulin*	Serum fraction	Insulin activity†	Subject D.S. Buffer and insulin*	Serum fraction	Insulin activity†
4		294 ± 58	26 ± 7	<10			
5	γ	308 ± 9	62 ± 24	<10	234 ± 27	144 ± 22	216
6		210 ± 4	10 ± 33	<10	234 ± 27	54 ± 17	15
7	β	240 ± 19	1 ± 11	<10	242 ± 24	38 ± 14	<10
8		240 ± 19	6 ± 11	<10	242 ± 24	62 ± 7	14
9	α	210 ± 4	32 ± 10	<10	326 ± 17	40 ± 23	<10
10		240 ± 19	4 ± 6	<10	326 ± 17	92 ± 13	22
11		210 ± 4	2 ± 7	<10	320 ± 7	50 ± 6	<10
12	alb.	254 ± 28	122 ± 13	100	320 ± 7	0 ± 9	0
13		210 ± 4	−28 ± 18	0			

* 1000 μU/cc. † μU/cc original serum.

of basic proteins in their sera which bind most of the insulin released from the pancreas, thus reducing the amount of free insulin available to certain tissues. Thus, the onset of diabetes may not result from underproduction of insulin, but rather from inactivation by excess binding.

Summary. Undiluted sera of new, untreated diabetics and diabetics in ketoacidosis have been shown to exhibit antagonism to added insulin when assayed by the rat diaphragm technic. One acidotic serum showed unusually high insulin activity after hanging curtain electrophoresis. The possible significance of these data is discussed.

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