EVIDENCE FOR THE PRESENCE OF INSULIN IN BLOOD SERUM. A METHOD FOR AN APPROXIMATE DETERMINATION OF THE INSULIN CONTENT OF BLOOD [1]

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In recent years the carbohydrate metabolism of the isolated rat diaphragm has been widely studied (1–8). Using this preparation, Gemmill (2, 3) succeeded in demonstrating *in vitro* the stimulating effect of insulin on the glucose utilization and glycogen synthesis of an isolated muscle. It has since been shown that a quantitative relationship exists between the amount of insulin present in the incubation medium and its effect on the glucose metabolism of the diaphragm (5, 6). Furthermore, it was found that the diaphragms of young rats are sensitive to extremely small concentrations of insulin (9). This investigation was intended to determine if the glucose metabolism of the isolated rat diaphragm could be used to demonstrate the presence of insulin in blood serum and to determine the concentration of this hormone in blood.

METHODS

The standard technique used in these experiments is as follows: Four young rats of 80–100 gr. body weight, fasted for 24 hours, are killed by decapitation. The diaphragms are taken out quickly and carefully, to avoid trauma as much as possible, and divided in half. The hemi-diaphragms are washed in ice cold buffer solution (without glucose), and dried between filter paper. Four of the hemi-diaphragms, each from a different rat, are then placed in a small flask; the remaining four are placed in a similar flask. Into each flask, beforehand, have been pipetted 2 ml. of an ice cold buffer solution with a mineral composition closely resembling blood plasma (10), and after equilibration with a 7% CO_2 – 93% O_2 mixture, a pH of 7.4. In addition the buffer contained 200 mg.% glucose. The glucose buffer solution in one of the flasks contained insulin in the concentration to be tested; the other served as control. Following equilibration with the gas mixture of 93% oxygen plus 7% carbon dioxide, both flasks are incubated at 37° with shaking at a rate of 120/minute. After 90 minutes the flasks are removed and cooled. The muscle tissue, having been dried between filter paper, is weighed and the amount of glucose is determined in the medium (11). The difference in glucose utilization per 100 mg. of wet diaphragm between the flask containing insulin and the control flask indicates the effect of the added insulin.

In other experiments, four flasks containing the quarter-diaphragms of eight rats are used. Each diaphragm is cut in four pieces and the four quarters are distributed among the four flasks. Here, each flask contains eight quarter-diaphragms of eight different rats. This procedure is chosen so that the variation in glucose consumption and in sensitivity to insulin between individual diaphragms will be neutralized. The four flask technique enables us to compare the glucose utilization in glucose buffer solutions of three different insulin concentrations with the glucose utilization in a control vessel containing only glucose buffer. Generally speaking, the glucose utilization of hemi-diaphragms in buffer solutions without insulin is lower than that of quarter-diaphragms (on an equal weight basis), but the effect of added insulin is usually more evident when hemi-diaphragms are used. The glucose utilization of the diaphragm increases even further when it is divided into more fragments, but the effect of insulin becomes progressively less. For this reason, pieces of diaphragm smaller than a quarter-size have not been used in this work.

Figure 1, a concentration action curve, illustrates the effect of different concentrations of a sample of purified insulin (expressed as units/ml. of the medium) on the glucose utilization (in mg./100 mg. wet tissue) of the rat diaphragm. The graph illustrates that, as the insulin concentration is increased, the insulin effect increases until, at about 10^{-1} units/ml., it tends to become more or less constant. In the region of lower concentrations, it was found that amounts as low as 5×10^{-6} still produced a significant increase in glucose utilization. The sample of pure insulin used in these experiments contained 28 units/mg.[2] Assuming a molecular weight of insulin of 48,000, a concentration of 5×10^{-6} units/ml. is equal to 4.5×10^{9} molecules per flask. This number of insulin molecules enabled the diaphragms to utilize about 10^{18} more molecules of glucose than diaphragms in the control flask without insulin.

[1] These researches were supported by grants from the Organon Laboratories, Inc., and the Netherlands Diabetics Association.

[2] We are indebted to Dr. J. Lens of Organon Laboratories, Inc. for a gift of pure insulin.

In the course of further studies, it was found that whereas the sensitivity of the insulin-glucose diaphragm system is usually of the same order as given in Figure 1, variations in the sensitivity are occasionally encountered. In addition to the curve of Figure 1, Figure 2 shows the least sensitive concentration action curve. This was obtained in the summer of 1950, when the sensitivity of the diaphragms used was obviously much less, especially in the region of the lower insulin concentrations. The cause of the phenomenon is unknown to us. Other investigators have probably refrained from using the isolated rat diaphragm as a test object for the standardization of insulin because of this variation in sensitivity. It seems to us, however, that occasional evidence of a smaller sensitivity need not prohibit the use of the rat diaphragm for this purpose, providing proper control standardizations are carried out with every experiment.

Demonstration of the presence of insulin activity in normal serum

The procedure for this experiment was the same as that used for the standardization curve with the difference that here, one vessel contained the diaphragm suspended in 2 ml. of a glucose buffer, whereas the other vessel contained 1.68 ml. of buffer to which 0.32 ml. of serum had been added. The initial concentration of glucose in both vessels was adjusted to 200 mg.%. After incubation and shaking for 90 minutes, the amount of glucose utilized in both vessels was determined and expressed as mg./100 mg. of wet tissue. The difference between the glucose utilization in the medium with serum and in the buffer without serum was taken as the "insulin effect" of the serum.

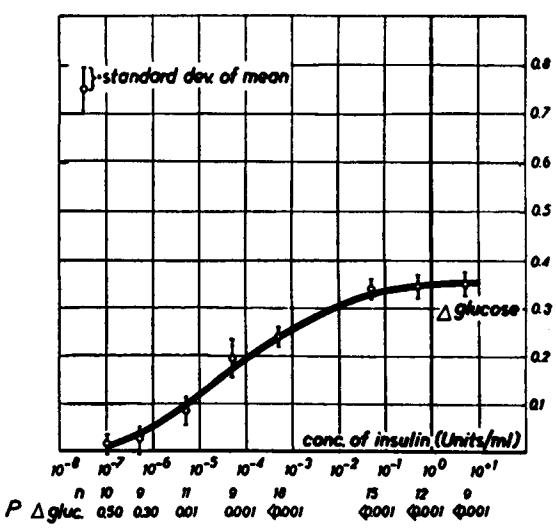

FIG. 1. CONCENTRATION ACTION CURVE, SHOWING THE INCREASE IN GLUCOSE UTILIZATION OF THE ISOLATED RAT DIAPHRAGM BY DIFFERENT CONCENTRATIONS OF INSULIN IN THE MEDIUM

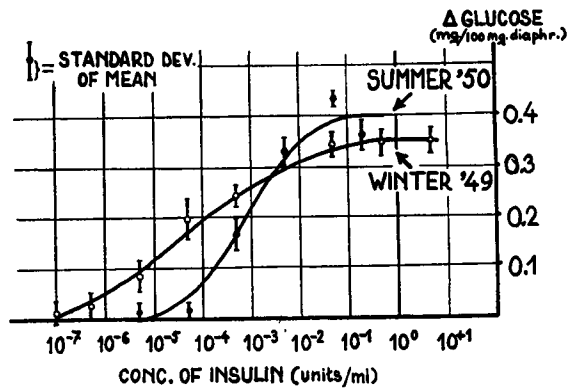

FIG. 2. VARIABILITY OF THE CONCENTRATION ACTION CURVE OF THE QUANTITATIVE EFFECT OF INSULIN UPON THE GLUCOSE UTILIZATION OF THE ISOLATED RAT DIAPHRAGM

RESULTS

Table I gives the results of eight experiments on normal human serum and five on normal dog serum. From these figures it appears that normal serum produces an "insulin effect" in the sense that it produces an extra utilization of glucose of 0.1–0.2 mg./100 mg. of diaphragm after one and one-half hours.

Table II gives the results of similar experiments carried out with the serum of four cases of diabetic coma and the serum of two dogs after pancreatectomy. The first dog (R 75) died in severe coma six days after the operation. The second dog (F 78) was maintained on insulin after the operation. It died in coma 20 days after insulin was withdrawn, and at postmortem examination, only minute amounts of pancreatic tissue were found. It will be seen that all these sera did not significantly increase the glucose utilization of the diaphragm.

Figure 3 shows the results of repeated determinations on the serum of dog F 78 after insulin was withdrawn. A progressive decrease of the insulin effect occurred as the diabetes developed until, on the 20th day, when the dog died in coma, there was almost no insulin content.

Table III gives the results of five experiments carried out with the serum of diabetic patients who were under treatment with insulin. It will be seen that the values obtained were all within the normal range. It would seem that the value from patient S was the lowest of this series because it was obtained with a serum sample taken

TABLE I

Difference in glucose utilization (mg.) by 100 mg. diaphragm in: a) buffer + serum of normal humans or dogs, b) buffer without serum

Serum from	Av. extra glucose utilization (a−b)	Standard dev.	No. of observations
	(mg.)		
K.	+0.171	0.075	3
H.	+0.181	0.092	6
W.	+0.186	0.038	6
G.	+0.131	0.046	6
K.	+0.103	0.050	6
W.	+0.175	0.049	6
K.	+0.159	0.065	5
K.	+0.207	0.060	6
Dog X.	+0.168	0.047	6
Dog R.75	+0.226	0.039	6
Dog F.78	+0.202	0.050	6
Dog S.	+0.182	0.072	6
Dog 105	+0.098	0.049	6

TABLE II

Difference in glucose utilization (mg.) by 100 mg. diaphragm in: a) buffer + serum during diabetic coma, b) control buffer without serum

Serum from	Av. extra glucose utilization (a−b)	Standard dev.	No. of observations
	(mg.)		
Pat. J.	−0.043	0.050	3
Pat. D.	−0.014	0.042	5
Pat. B.	+0.028	0.037	6
Pat. P.	+0.027	0.024	5
Dog R.75*			
2 days without ins.	−0.048	0.067	6
4 days without ins.	0	0.031	6
Dog F.78*			
20 days without ins.	+0.033	0.056	6

* After pancreatectomy.

after insulin had been withheld for 24 hours. However, in other experiments, not recorded here, there was only a small and not always statistically significant difference between the insulin effect of diabetic serum taken before and after insulin was withheld.

Table IVA gives the results of three sets of experiments, all carried out on the serum of patient K, who was admitted with diabetic coma. The technique of these experiments was so arranged that the diaphragms were incubated (a) in buffer with serum obtained after the patient had recovered from coma, and (b) in serum obtained during the coma, before the beginning of treatment.

This procedure was chosen so as to make all other conditions in the two flasks as equal as possible. It will be seen that there was a definitely higher glucose utilization in the medium containing the serum taken after recovery, as compared to that in the diluted coma serum. Table IVB also gives the results of two similar experiments carried out with the sera of dogs, before and after pancreatectomy, showing the same effect.

Figure 4 represents a "time curve" in which the rat diaphragms were incubated (a) in serum

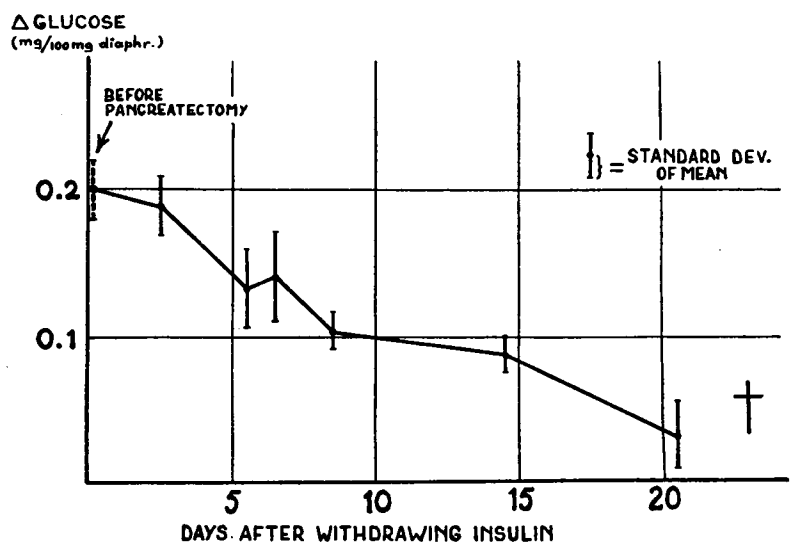

FIG. 3. DIMINUTION OF THE "INSULIN EFFECT" OF THE SERUM OF THE DEPANCREATIZED DOG, TESTED ON THE ISOLATED RAT DIAPHRAGM

TABLE III

Difference in glucose utilization (mg.) by 100 mg. diaphragm in: a) buffer + serum of diabetic patient while under insulin treatment, b) buffer without serum

Serum from	Av. extra glucose utilization (a−b)	Standard dev.	No. of observations
	(*mg.*)		
J.	+0.207	0.035	3
S.	+0.095	0.053	6
D.	+0.222	0.077	6
H.	+0.124	0.033	6
B.	+0.216	0.059	6

obtained during coma, and (b) in serum obtained after recovery by treatment with insulin. During the incubation, samples were taken from the flasks at regular intervals, and the amounts of glucose compared. It will be seen that the glucose utilization was consistently higher in the "recovery serum" than in the coma serum.

TABLE IV A

Difference in glucose utilization (mg.) by 100 mg. diaphragm in serum from Patient K during coma and after recovery

a = buffer plus serum obtained after recovery
b = buffer plus serum obtained during coma

No. of observations	(a−b)	Standard deviation
	(*mg.*)	
2	+0.100	0.028
6	+0.067	0.044
6	+0.102	0.041

The above results, summarized in Figure 5, seem to indicate that normal human or dog serum contains a substance which is able to stimulate the utilization of glucose, and which is absent from the serum of patients with diabetic coma or dogs after complete pancreatectomy. The possibility was considered that this difference between normal serum and serum of diabetic coma was due to an inhibition of the glucose utilization by the presence of ketone bodies. *A priori* this did not seem probable as the values obtained with the coma serum were not significantly lower than those obtained when the diaphragms were incubated in buffer without serum. However, in order to investigate the possibility of a toxic effect of the ketone bodies in coma serum on the glucose utilization, the experiments given in Table V were carried out. Each experiment consisted of four

TABLE IV B

Difference in glucose utilization (mg.) by 100 mg. diaphragm in serum from two dogs before and after pancreatectomy

a = buffer plus normal serum
b = buffer plus serum after pancreatectomy

Serum from	No. of observations	(a−b)	Standard deviation
		(*mg.*)	
Dog R.75	6	+0.159	0.053
Dog F.78	6	+0.146	0.025

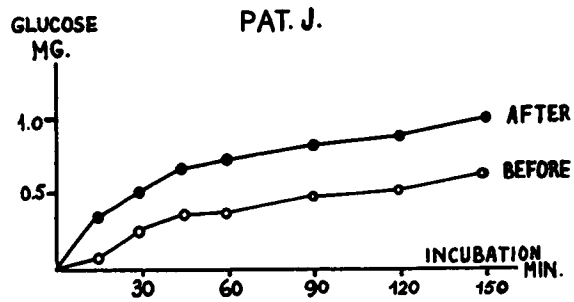

FIG. 4. COMPARISON OF THE GLUCOSE UTILIZATION OF THE ISOLATED RAT DIAPHRAGM INCUBATED IN SERA OBTAINED FROM ONE PATIENT BEFORE AND AFTER INSULIN TREATMENT FOR DIABETIC COMA

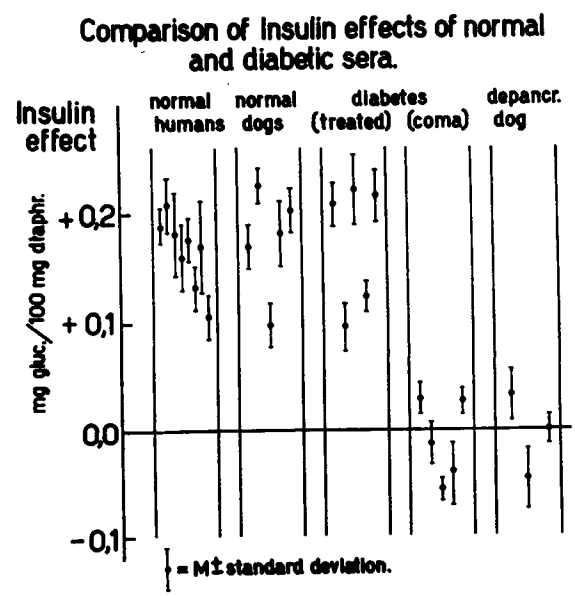

FIG. 5. SUMMARY OF THE DATA ILLUSTRATING THE DIFFERENCE IN "INSULIN EFFECT" BETWEEN NORMAL SERA AND SERA FROM PATIENTS OR DOGS IN UNTREATED DIABETIC COMA

TABLE V

Influence of acetone and β-hydroxybutyric acid on the glucose utilization (mg.) by 100 mg. diaphragm

	Medium	Acetone	β-hydroxy butyric ac.	Av. glucose util. (*mg./100 mg. tissue*)	"Insulin" effect of serum	No. of observations
I	a) 2 ml. buffer	−	−	0.534		6
	b) 0.32 ml. serum + 1.68 ml. buffer	−	−	0.590	0.056±0.062	6
	c) 2 ml. buffer	+	+	0.532		6
	d) 0.32 ml. serum + 1.68 ml. buffer	+	+	0.612	0.080±0.049	6
II	a) 2 ml. buffer	−	−	0.571		6
	b) 0.32 ml. serum + 1.68 ml. buffer	−	−	0.669	0.098±0.049	6
	c) 0.32 ml. serum + 1.68 ml. buffer	+	+	0.683	0.112±0.089	6
III	a) 2 ml. buffer	−	−	0.694		6
	b) 0.32 ml. serum + 1.68 ml. buffer	−	−	0.786	0.092±0.065	6
	c) 2 ml. buffer	+	+	0.691		6
	d) 0.32 ml. serum + 1.68 ml. buffer	+	+	0.748	0.057±0.024	6

sets of observations carried out on diaphragms suspended (a) in buffer solution, (b) in buffer solution to which serum had been added, (c) in buffer with added acetone and β hydroxybutyric acid, both in a concentration of 30 mg.%, and (d) in buffer with serum and the same concentration of ketone bodies. If the difference observed between the "insulin activity" of normal and coma serum was due to the presence of ketone bodies, one would expect that the insulin activity of normal serum would disappear when acetone and β hydroxybutyric acid were added. It was found that this addition had no influence on the glucose utilization of the diaphragm, and did not diminish the "insulin effect" of normal serum.

A concentration of both ketone bodies of 30 mg. % in the medium in these experiments corresponds to a concentration in the undiluted serum of 200 mg.% which is about the concentration one may find in the serum during diabetic coma.

Table VI gives the results of two experiments in which insulin in concentrations of 10^{-2} and 10^{-4} units/ml. were added to coma serum. This was done to test the possibility that the coma serum might contain substances which inhibited the normal insulin effect. The effect of added insulin was apparently not influenced by the presence of the serum. Thus it appears that the absence of an insulin effect in the coma serum is not so much due to the presence of substances that counteract or inhibit insulin activity, but to the absence of insulin itself.

TABLE VI

Effect of added insulin on glucose utilization (mg.) by 100 mg. diaphragm in buffer + serum

	Medium	Added insulin (*U./ml.*)	Glucose utilization (*mg./100 mg. tissue*)	"Insulin" effect	No. of observations	Remarks
I	a) 2 ml. buffer	−	0.627	−	5	
	b) 0.24 ml. serum + 1.76 ml. buffer	−	0.613	−0.014±0.042	5	coma-serum
	c) 2 ml. buffer	10^{-2}	0.770	+0.143±0.053	5	
	d) 0.24 ml. serum + 1.76 ml. buffer	10^{-2}	0.769	+0.142±0.047	5	
II	a) 0.265 ml. serum + 1.735 ml. buffer	−	0.586	−	6	coma-serum
	b) 0.265 ml. serum + 1.735 ml. buffer	10^{-4}	0.689	0.103±0.032	6	
III	a) 2 ml. buffer	−	0.813	−	6	
	b) 0.32 ml. serum + 1.68 ml. buffer	−	0.863	+0.050±0.042	6	coma-serum
	c) 2 ml. buffer	10^{-2}	1.031	+0.218±0.031	6	
	d) 0.32 ml. serum + 1.68 ml. buffer	10^{-2}	1.011	+0.198±0.030	6	

Influence of serum on glycogen synthesis

Gemmill (2, 3), Stadie and Zapp (5), and Tuerkischer and Wertheimer (7) demonstrated that insulin not only increases glucose utilization of the isolated rat diaphragm, but also promotes glycogen synthesis. If the substance which is present in normal serum and stimulates the utilization of glucose is really insulin, it should also have a positive influence on glycogen synthesis. This actually proved to be the case. Table VII gives the results of five experiments, three on normal serum and two on serum of diabetic patients under insulin treatment. In all cases there was a significant increase in glycogen synthesis in comparison to the flasks in which the diaphragms were incubated in buffer without serum.

TABLE VII

"Insulin effect" of serum on glucose utilization and on glycogen synthesis

Experiment	Extra glucose utilized (*mg./100 mg. diaphragm tissue*)		Extra glycogen synthesized (*mg./100 mg. diaphragm tissue*)		No. of observations
1	0.092	±0.026	0.086	±0.013	6
2	0.207	±0.024	0.171	±0.020	5
3	0.183	±0.014	0.072	±0.028	6
4*	0.157	±0.014	0.097	±0.015	6
5*	0.124	±0.013	0.121	±0.008	6

* Diabetic sera.

Inhibition of insulin effect of serum by addition of cysteine or glutathione

Insulin loses its activity in the presence of SH compounds such as cysteine or glutathione. Table VIII gives the results of experiments which show this inactivation by cysteine or glutathione on the isolated rat diaphragm. It will be seen that the effect of both pure insulin and serum either disappeared or diminished considerably when the insulin or serum had been treated with one of these SH compounds. The results of these experiments are a further proof that the stimulating effect of the serum on the glucose utilization of the isolated rat diaphragm is indeed due to the presence of insulin contained therein.

Approximate estimation of the concentration of insulin in normal human serum

The figures given in Table I for the insulin effect of normal serum cannot be used for a computation of the actual insulin content. Some of these figures were obtained before we were aware of the variability in the sensitivity of the diaphragm in the lower range of the concentration action curve. For this reason, in further ex-

TABLE VIII

Inhibition of the effect of insulin or serum on glucose utilization of the isolated rat diaphragm by cysteine or glutathione

Exp. no.	Medium (2 ml. per flask)						Glucose utilization (*mg./100 mg. tissue*)	
	Buffer	Insulin	Serum	Cysteine	Glutathione	Init. gluc. conc.	Average utiliz.	Effect of insulin or serum
	(*ml.*)	(*U./ml.*)	(*ml.*)					
I a	2	–	–	–	–	200	0.628	
b	2	10^{-2}	–	–	–	200	0.826	b−a=0.198±0.026
c	2	–	–	+	–	200	0.644	
d	2	10^{-2}	–	+	–	200	0.658	d−c=0.014±0.013
II a	2	–	–	–	–	175	0.555	
b	1.73	–	0.27	–	–	175	0.639	b−a=0.084±0.022
c	2	–	–	+	–	175	0.563	
d	1.73	–	0.27	+	–	175	0.610	d−c=0.047±0.018
III a	2	–	–	–	–	200	0.578	
b	2	10^{-2}	–	–	–	200	0.738	b−a=0.160±0.012
c	2	–	–	–	+	200	0.580	
d	2	10^{-2}	–	–	+	200	0.686	d−c=0.106±0.012
IV a	2	–	–	–	–	185	0.662	
b	1.68	–	0.32	–	–	185	0.767	b−a=0.105±0.011
c	2	–	–	–	+	185	0.647	
d	1.68	–	0.32	–	+	185	0.688	d−c=0.041±0.037

TABLE IX

Approximate estimation of insulin concentration in serum

	Medium	Serum dil.	No. of observations	"Insulin" effect	Estimated insulin concentration	Source
					(*U./ml. serum*)	
I	a) buffer + serum	1:5	6	$+0.182 \pm 0.072$	$\pm 5 \times 10^{-4}$	normal dog
	b) buffer + serum + 10^{-4} U./ml.		6	$+0.185 \pm 0.061$		
II	a) buffer + serum	4:25	6	$+0.175 \pm 0.049$	$< 6.25 \times 10^{-2}$	normal human
	b) buffer + 5×10^{-3} U./ml.		6	$+0.327 \pm 0.059$	($\pm 10^{-4}$?)	
III	a) buffer + serum	4:25	6	$+0.187 \pm 0.035$	$\pm 6.25 \times 10^{-4}$	normal human
	b) buffer + 10^{-4} U./ml.		6	$+0.181 \pm 0.028$		
IV	a) buffer + serum	4:25	6	$+0.095 \pm 0.053$	$\pm 6.25 \times 10^{-5}$	human diabetic under insulin treatment
	b) buffer + 10^{-5} U./ml.		6	$+0.097 \pm 0.060$		
V	a) buffer + serum	4:25	6	$+0.222 \pm 0.077$	$> 6.25 \times 10^{-5}$	human diabetic after insulin injection
	b) buffer + 10^{-5} U./ml.		6	$+0.096 \pm 0.034$	($\pm 10^{-3}$?)	

periments, we have taken care to include one or two control observations with pure insulin of known concentration with every serum determination. From the figures thus obtained, Table IX has been constructed. It summarizes the results of five experiments in which the insulin effects of different sera were compared with the effects of a known quantity of insulin which had been added to the glucose buffer solution in the control vessel. From these observations it will be seen that a normal dog serum contained about 5×10^{-4} units/ml. One normal human serum was found to contain about 10^{-4} units/ml., in any event less, in this particular experiment than 6.25×10^{-2} units/ml. The value obtained for the serum of a human diabetic under insulin treatment corresponded very well with the figures of the control experiment, which were 6.25×10^{-5} units of added insulin /ml. In the last experiment, in which the control vessel contained 10^{-5} units/ml., the serum produced a much higher glucose utilization than the control which, according to our standardization curve, would yield about 5×10^{-4} units/ml. This high value was obtained in a serum of a diabetic patient taken 15 minutes after an insulin injection of 40 units.

From these figures and several others, we tentatively suggest that the insulin concentration in normal serum is between 6.25×10^{-5} and 6.25×10^{-4} units/ml. In diabetic coma, the concentration is lower, being well below 10^{-5} units/ml. In cases of diabetes, while under insulin treatment, we have found a normal or slightly higher insulin content of the serum. The data from some of our experiments suggest that shortly after an injection of insulin, the insulin content of the serum may be higher than normal, but a sufficient number of observations have not yet been made to establish this as fact. On the other hand, we have repeatedly determined the insulin content in the serum of diabetic patients who, for some reason or other, had stopped taking insulin. One of these was a 78 year old woman who had discontinued her insulin injections after a stroke, and was admitted four days later with moderate diabetic acidosis, but not in coma. The insulin content of her blood was found to be 10^{-5} units/ml. It must be pointed out that the determination of the insulin content of the serum by the rat diaphragm method may not be sensitive enough to detect small differences in insulin content, but as it is, we have found a significant lowering of the insulin concentrations of the serum only in diabetic coma, not in patients with simple diabetes.

Hyperinsulinism and functional hypoglycaemia

The estimation of the insulin in serum offers possibilities for a direct demonstration of true hyperinsulinaemia and a differential diagnosis of this condition from "functional hypoglycaemia." We have tested two sera of cases of functional hypoglycaemia.[3] In both cases the insulin con-

[3] We are indebted to Dr. Lubsen and Dr. Meyer for these serum samples.

TABLE X

Estimation of insulin content of serum of patient with "functional" hypoglycaemia

Addition	Glucose effect (*mg./100 mg. tissue*)	Glycogen effect (*mg./100 mg. tissue*)
Insulin 10^{-5} U./ml.	-0.012 ± 0.090 $>\$$	$+0.066 \pm 0.024$ $>S$
Pat. serum 1 : 6	-0.001 ± 0.051 $>S$	$+0.122 \pm 0.018$ $>S$
Insulin 10^{-3} U./ml.	$+0.217 \pm 0.067$	$+0.187 \pm 0.061$
Conclusion:	Ins. serum $< 6 \times 10^{-3}$	$6 \times 10^{-5} <$ Ins. serum $< 6 \times 10^{-3}$

S = statistically significant difference.
$ = no statistically significant difference.

tent of the serum was found to be within normal limits. Table X summarizes the estimation of the insulin content in one of these cases. Quarter-diaphragms were used. One flask contained glucose-buffer solution, the second diluted serum. To the glucose-buffer solution in the third and fourth flasks, insulin was added in quantities of 10^{-5} and 10^{-3} units/ml., respectively. The diaphragms in this experiment were relatively insensitive, with the result that neither the addition of 10^{-5} units/ml. nor the addition of the patient's serum had a significant effect on glucose utilization. Judging from the glucose utilization, the insulin content of this patient's serum was significantly below 6.25×10^{-3} units of insulin, but was not significantly higher than 6.25×10^{-5} units/ml. A significant increase in glycogen synthesis was produced by the addition of 10^{-5} units of insulin /ml. The serum effect was significantly higher, but the effect of 10^{-3} units was

TABLE XI

Estimations of insulin content in serum of patient with hyperinsulinism

Exp.	Medium	Glucose effect (*mg. glucose/100 mg. wet tissue*)	Glycogen effect (*mg. glucose/100 mg. wet tissue*)
I	a) Patient's serum 4 : 25	0.219 ± 0.046 $>S^*$	0.136 ± 0.061 $>S$
	b) Normal serum 4 : 25	0.187 ± 0.035 $>\$$	0.086 ± 0.047 $>\$$
	c) Insulin 10^{-4} U./ml.	0.181 ± 0.028	0.072 ± 0.028
II	a) Patient's serum 4 : 25	0.218 ± 0.061 $>\$$	0.120 ± 0.070 $>\$$
	b) Redissolved lyophylized patient's serum 4 : 25	0.217 ± 0.028 $>\$$	0.123 ± 0.060 $>\$$
	c) Insulin 10^{-4} U./ml.	0.199 ± 0.051	0.098 ± 0.040

Conclusion: From experiment I: Insulin concentration in first serum sample $> 6.25 \times 10^{-4}$ U./ml. ($\pm 10^{-3}$ U./ml. ?).
From experiment II: Insulin concentration in second serum sample $\pm 6.25 \times 10^{-4}$ U./ml. Insulin content did not decrease by lyophylisation.
* S = Values differ significantly.
$ = No significant difference.

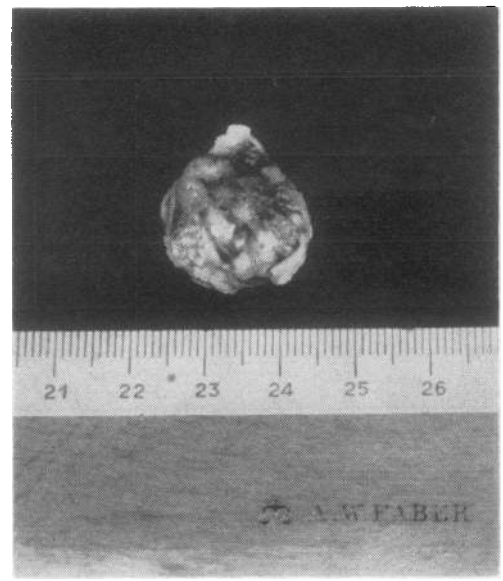

FIG. 6. PANCREATIC ISLET CELL ADENOMA, DIAGNOSED BY DIRECT ESTIMATION OF THE INSULIN CONTENT IN THE PATIENT'S SERUM

higher than that of the serum. From these results it appeared that the insulin content of the serum was lower than 6.25×10^{-3}, but higher than 6.25×10^{-5} units/ml. The conclusion that the insulin content in this case, as far as could be ascertained by our method, was about normal was supported later by the absence of an islet cell tumor at the operation and the success of a high protein, high fat diet.

We have examined only one case of hyperinsulinism thus far. The results of two determinations are given in Table XI. In both experiments the insulin content was found to be higher than that of normal serum, which was tested on the same diaphragms, and also significantly higher than the effect of 10^{-4} units of insulin. As the serum had been diluted 4 : 25, the insulin content of the serum, read from the concentration curves, was estimated at about 3×10^{-3} units/ml., which is almost five times as much as the highest level of normal. At operation an islet cell adenoma was discovered and extirpated [4] (Figure 6).

DISCUSSION

The quantitative aspects of the action of insulin on the isolated rat diaphragm have been

[4] We are indebted to Dr. Lips for sending us the serum of this case.

studied by Stadie and Zapp (5), Krahl and Park (6), and Willebrands and associates (9). The first named investigators found the lowest limits at which an effect could be detected to be 10^{-3} units/ml.; Krahl and Park found significant effects at 10^{-4} units/ml., and Willebrands and coworkers, at 5×10^{-6} units/ml. The difference between the results of these authors are best explained by the variations in the sensitivity of the diaphragms as illustrated in Figure 2. Tuerkischer and Wertheimer (7) and Perlmutter and Greep (12) have been, as far as we are aware, the only authors who have demonstrated a stimulating effect of human blood serum upon the glycogen synthesis of the isolated rat diaphragm. However, they have not followed up their observations and we suppose that the great variability in sensitivity of the diaphragms has discouraged them from using this relatively simple *in vitro* technique for the determination of the insulin concentration of serum.

Several attempts have been made, however, to determine the insulin concentration of serum by testing the drop in blood sugar after the injection of serum into animals which had been made especially sensitive to small amounts of insulin (13). For this purpose Gellhorn, Feldman, and Allen (14, 15) and Eston and Eston (16) have used hypophysectomized adreno-demedulated rats. Anderson and associates (17–19) and Bornstein (20, 21) introduced a further improvement when they found that the alloxan-diabetic hypophysectomized adreno-demedulated or adrenalectomized rat was an even better test object for the detection of minute amounts of insulin. The last named author found the insulin content of human plasma to be about 10^{-4} units/ml. in the fasting state, rising to about 3×10^{-4} units/ml. after oral administration of glucose. These figures agree very well with our finding that the insulin content of normal serum must be somewhere between 6.25×10^{-4} and 6.25×10^{-5} unit/ml., although our technique does not enable us to distinguish significantly between concentrations of 10^{-4} and 3×10^{-4} units/ml. For this reason, we are also unable to pass a judgment on the recent claim of Bornstein and Lawrence (22) that there exist two types of human diabetes, one with and the other without insulin in the serum.

SUMMARY

The results of a quantitative study of the effect of insulin on the glucose utilization of the isolated rat diaphragm are reported. Although the sensitivity of the isolated rat diaphragm to small amounts of insulin is variable, it can be demonstrated by this method that normal human and dog sera contain a substance which has an insulin-like action on glucose utilization and glycogen synthesis of the isolated rat diaphragm. In sera of patients with diabetic coma, and of dogs after complete pancreatectomy, the insulin-like activity was either absent or considerably diminished.

The increase in the glucose utilization and glycogen synthesis upon the addition of serum may be used as a means of estimating the insulin content of blood. Normal blood serum contains between 6.25×10^{-5} and 6.25×10^{-4} units of insulin per ml. Using this method, accurate diagnoses of "functional" hypoglycaemia (two cases) and of islet cell adenoma (one case) were made. The possibilities and limitations of the method are discussed.

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