SERUM INSULIN IN EARLY CASES OF SEVERE DIABETES

BY

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Since the initial studies on this subject by Bornstein and Lawrence (1951) much interest has been shown in serum insulin levels in early diabetes. While most of the many methods devised for the assay of insulin in serum suggest that it is present in at least normal quantities in the blood of obese diabetics without ketosis, there is no unanimity about its levels in the serum of patients with loss of weight who have severe ketoacidosis. Differing modes of classification of patients, as well as differing modes of assay of insulin, have contributed to confusion in this regard. The studies described below suggest that insulin is sometimes present in considerable quantities in the serum of untreated ketosed patients with blood sugars below 400 mg./100 ml., though it may be absent in some untreated patients who have very much higher blood-sugar levels, and in diabetic coma. Further evidence of insulin antagonism in diabetic acidosis is given.

Methods

Insulin activity was assayed by effects of serum or serum-protein fractions on glucose uptake by isolated rat diaphragm (Randle, 1956), though in some instances glucose was estimated by the method of Hugget and Nixon (1957). Where whole serum was used this was diluted to 25% with bicarbonate buffer. Glucose concentrations were adjusted so as to give a final glucose concentration of 250 mg./100 ml.

Serum-protein fractions were prepared by electrophoresis of serum on treated cellulose columns, by methods already published in detail (Randle and Taylor, 1958; Naughton and Taylor, 1960). After electrophoresis the fractions were dialysed against distilled water, using Visking 32/24 casing. Loss of insulin had previously been checked through the batch of Visking tubing employed by measuring loss of ^{131}I -labelled insulin, under conditions of dialysis identical with those employed with serum-protein fractions. It appeared to be less than 25% under the conditions used.

Acid-alcohol extracts were prepared by a modification of the method of Baird and Bornstein (1957). In this modification the acetone-precipitated proteins, after crude acid-ethanol extraction, were washed with ether and taken up in a small volume of N/100 HCl. The acidic extract was then mixed with the bicarbonate buffer in a volume four times that of the original serum taken. After vigorous stirring, any undissolved protein was centrifuged off and the clear supernatant, containing the insulin, used for assay.

Patients had either attended the Diabetic Clinic, King's College Hospital, London, or the Joslin Clinic, Boston, Mass., U.S.A., and blood samples were removed at a first visit. None had had either oral hypoglycaemic or previous insulin therapy. All showed ketosis on presentation (at least +++ by Rothera's test) and some loss of weight. Obese patients were carefully excluded from the series.

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Results

Table I shows the effects of 25% serum from diabetics in ketosis on glucose uptake of isolated rat diaphragm. Effects of serum taken from normal people are shown for comparison. The effects due to the diabetic serum are small, and in most cases are not significantly different from those due to buffer incubated alone without serum. One serum sample (sample 10) caused inhibition of glucose uptake by diaphragm to a marked extent.

TABLE I.—Effect of 25% Serum from Untreated Diabetics Presenting with Severe Ketosis on Glucose Uptake of Isolated Rat Diaphragm, *in vitro*

Patient	Effect	Blood Sugar (mg./100 ml.)
1	+1.0* ± 0.27	410
2	+0.5 ± 0.30	300
3	+0.8* ± 0.21	340
4	+0.3 ± 0.30	—
5	+0.6 ± 0.50	259
6	+0.6 ± 0.33	384
7	+0.5 ± 0.32	400
8	+0.6 ± 0.31	—
9	+2.2* ± 0.50	—
10	-0.8* ± 0.21	650
Normals (8)	+1.1* ± 0.15	—

Results are expressed as differences ± S.E. of differences in glucose uptake for diaphragms suspended in medium alone, in comparison with diaphragms suspended in medium + serum. Glucose uptake was calculated as mg. glucose taken up/g. wet diaphragm/hr. incubation. At least six diaphragms were used in each group. Differences marked with asterisks are statistically significant ($P < 0.05$).

In Table II are shown the effects of serum-protein fractions made by zone electrophoresis on glucose uptake. For this purpose serum-protein fractions were pooled and effects tested in samples corresponding to four main fractions. These were: whole albumin and α_1 -globulins, α_2 -globulins, beta-globulins, and

TABLE II.—Effect of Serum-protein Fractions Made by Column Electrophoresis of Serum of Untreated Diabetics in Ketosis on Glucose Uptake of Isolated Rat Diaphragm, *in vitro*

Patient	Albumin	Globulins		
		α_2	Beta	Gamma
3	1.3 ± 0.48*	0	—	0.4 ± 0.35
5	1.1 ± 0.32*	2.1 ± 0.47*	1.2 ± 0.30*	1.8 ± 0.36*
8	-0.4 ± 0.34	0.1 ± 0.40	0	0.1 ± 0.41
10	2.3 ± 0.64*	-1.8 ± 0.57*	0.4 ± 0.66	—
11	1.9 ± 0.30*	1.0 ± 0.41	1.6 ± 0.30	0.4 ± 0.32
12	0.7 ± 0.39	0.9 ± 0.44	0.7 ± 0.37	0.9 ± 0.32*

Results are expressed as in Table I. For details of electrophoresis, see text. * Differences are derived from experiments in which at least six observations were made in both control and experimental groups. Statistically significant differences are marked with an asterisk ($P < 0.05$).

Effects of acid-ethanol fractions of serum taken from untreated patients in ketoacidosis on glucose uptake of isolated rat diaphragm. Effects expressed as in Table IV. Abscissa: Blood sugar mg./100 ml.; uptakes which are statistically significant are shown with an asterisk. Ordinate: Glucose uptake of diaphragm, as an index of insulin activity.

gamma-globulins. Table II shows that the albumin and alpha₁-globulin fractions, as well as the beta- and gamma-globulins, stimulated glucose uptake in most instances. Effects in the alpha₂-globulins were, however, variable, and while stimulation was observed with one sample there was none in others, and in one instance there was a significant inhibition of uptake in this fraction. No insulin activity was detected in fractions derived from one serum specimen (sample 8) from a ketosed diabetic.

In the Chart are shown effects of acid-ethanol extracts of serum from a further 10 ketosed diabetic patients (age range 16–63 years) on glucose uptake of isolated rat diaphragm. In four instances large effects were noted which were statistically significant. These were found in patients whose blood sugars were less than 400 mg./100 ml. Small effects on glucose uptake or no effects were observed with the other patients in this group. No insulin was detectable in a case of diabetic coma with a blood sugar of 1,250 mg./100 ml.

Discussion

The results shown in Table I are in broad agreement with those of other workers who have investigated effects of serum from ketosed diabetic patients on glucose uptake on isolated rat diaphragm (Vallance-Owen *et al.*, 1955; Baird and Bornstein, 1957; Seltzer and Smith, 1959; Gundersen and Williams, 1960; Willebrands and Sandwijk, 1960). With a variety of differing techniques, insulin effects of such serum are in general much less than those of normal human serum or may be absent altogether. It has been emphasized by many workers that insulin may be present in such serum, though its effects may be masked, so far as muscle tissue is concerned, by circulating insulin antagonists. This distinction does not, however, apply to adipose tissue, where the muscle antagonists to insulin appear to be ineffective (cf. Lowy *et al.*, 1961). Using adipose tissue, insulin effects with serum from ketosed patients may be very large (Steinke *et al.*, 1961a, 1961b; Beigelman and Tranquada, 1962).

In a further examination of insulin effects with this serum an attempt was made to separate insulin from circulating antagonists by column electrophoresis. When serum from ketosed diabetic patients was fractionated in this way, insulin effects were found in the same fractions as they appeared in normal human serum—that is, in both the albumin-alpha₁-globulin fractions and the beta- and gamma-globulins. The magnitude of these effects was, however, variable. In one specimen of serum (sample 8) no insulin was detectable in any of the fractions. In most samples effects were considerable in all fractions except the alpha₂-globulins. These large effects in serum-protein fractions are in contrast with the very small effects frequently obtained with whole serum.

In the fractionation experiments it appears that insulin has been separated from its circulating antagonists, and consequently was rendered detectable. Both Gundersen and Williams (1960), who used curtain electrophoresis, and Willebrands and Sandwijk (1960), who used starch column electrophoresis, have reported considerable insulin effects in the beta- and gamma-globulin fractions derived from ketosed diabetics, as assessed by effects of such fractions on glucose uptake of isolated rat diaphragm. Reasons for regarding effects of this kind, as well as effects in albumin fractions, as due to insulin have been published elsewhere (Taylor

and Randle, 1959; Vargas *et al.*, 1960). In addition, the effects of beta- and gamma-globulin fractions on glucose uptake could be reduced by dialysis with membranes known to facilitate the passage of insulin through them (Taylor, 1962). It is concluded that insulin is found in the beta- and gamma-globulins of serum from many untreated diabetics who present with ketosis, as well as in the albumin fractions. The qualitative distribution of biological activity did not differ from that found in similar fractions derived from the sera of normal people.

In one specimen of serum from a ketosed patient material was found which inhibited glucose uptake by muscle. This was noted both in the whole serum, when tested, and in the alpha₂-globulins derived by fractionation of whole serum. Effects of this kind resemble those found in the alpha₂- and beta-globulins of normal human serum (Taylor *et al.*, 1960). Several other workers have now noted the presence of material with this property in the serum of severely ketosed diabetics (Baird and Bornstein, 1957; Seltzer and Smith, 1959; Willebrands and Sandwijk, 1960). Since acetoacetate, as well as some unesterified fatty acids, is capable of depressing glucose uptake by muscle (Ottaway, 1961; Williamson and Krebs, 1961; Newsholme *et al.*, 1962), the excess of these substances in diabetic serum might explain the inhibitory effects noted above.

Further strong evidence that insulin is present, often in considerable quantities, in the sera of ketosed diabetics is seen in the Chart, which shows the effects of acid-ethanol extracts of serum on glucose uptake by diaphragm, using serum from a further series of patients. Clinical details of this group are given in Table III. Extensive proof that effects of such extracts are due to insulin has already been provided for normal human serum by Baird and Bornstein (1957, 1959). That this is also the case for extracts of serum from ketosed diabetic patients is shown in Table IV; it is seen that these effects are substantially neutralizable by antisera made against pig insulin.

TABLE III.—Clinical Details of Patients in Chart

Patient No.	Age	Sex	Weight Loss
1	26	M	+
2	16	F	+
3	22	M	++
4	22	F	++
5	50	F	++
6	42	M	+
7	63	M	+++
8	28	M	+++
9	49	F	++
10	21	M	Not known

TABLE IV.—Neutralization of Effects of Acid-Alcohol Extracts of Serum (Taken from Diabetics with Ketosis) on Glucose Uptake of Isolated Rat Diaphragm, Using Antisera Made Against Pig Insulin

Addition to Buffer	Glucose Uptake (Mean ± S.E.M. mg. Glucose/g. Wet Diaphragm/90 min.)	Significance of Differences between Means, P
None	(a) 3.8 ± 0.22 (22)	(b-a) < 0.01 > 0.001
Extract	(b) 5.0 ± 0.22 (20)	(b-c) < 0.05 > 0.02
Extract + antiserum (1/20)	(c) 4.2 ± 0.25 (20)	

Figures in parentheses refer to numbers of observations from which means were derived.

Nevertheless, insulin was not always detected in the serum of patients with blood sugars greater than 400 mg./100 ml., nor in diabetic coma. It seems likely, therefore, that some cases of severe diabetes presenting with ketosis suffer at the onset of their disease from

severe insulin deficiency of an absolute type, as Bornstein and Lawrence (1951) have suggested. While others possess levels of insulin which are not very different from those of normal subjects in the post-absorptive state, this does not imply that the pancreatic islet tissue is normal in patients of this type. The islet tissue in this type of patient is in any case secreting insulin under the stimulus of a raised blood sugar, probably to a maximal extent. Indeed, such patients appear to be unable to respond to an additional glucose load by secreting additional insulin (Seltzer and Smith, 1959). Failure of blood pyruvate to rise after glucose ingestion in this class of diabetic patients (Smith and Taylor, 1956) may also be related to their inability to secrete insulin. It may be, therefore, that most patients in this group are suffering from some degree of impairment of islet-cell function.

This group ultimately seems to suffer a complete exhaustion of the islet cells, since insulin is not detectable in their pancreases at necropsy (Wrenshall *et al.*, 1952), nor does endogenous insulin (assayed by immunological methods) appear to be present in their serum some time after the beginning of insulin treatment (Yalow and Berson, 1961). Insulin is also present at very low levels in the protein fractions of serum from these patients when the blood is taken many hours after insulin injections (Taylor, 1963, unpublished).

This type of diabetes, associated at the time of onset with relatively high blood-insulin levels, shows some analogies with that induced in animals by growth-hormone administration (Randle and Young, 1956). Nevertheless, in this form of human diabetes (as opposed to that of the obese) growth-hormone levels do not seem to be raised (Erlich and Randle, 1961). It is uncertain, therefore, if growth hormone has any direct role in the genesis of this type of diabetes, though its presence might be necessary for the diabetes to develop.

In conclusion, it is suggested that severe diabetes presenting with loss of weight and ketosis is predominantly a disease in which insulin secretion is either deficient or becoming deficient. The high blood-insulin levels sometimes encountered in the early stages of this condition may reflect a stage of overstimulation of beta cells preceding final exhaustion. It is possible that in at least some of these patients the insulin antagonism is a consequence and not the cause of the insulin deficiency.

Summary

Serum from 22 cases of untreated diabetes presenting with ketosis has been examined for insulin activity.

Insulin was found in serum or protein fractions of many of these cases, though it was not detectable in a minority of cases with high blood sugars or in diabetic coma. Effects of this insulin in whole serum often appeared to be masked by insulin antagonists. One such antagonist, with its possible relation to diabetic ketosis, is discussed.

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PROGNOSIS IN THE ANARTHROTIC RHEUMATOID SYNDROME

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The anarthrotic rheumatoid syndrome (Bagratuni, 1953, 1956, 1957), or polymyalgia rheumatica as Stuart Barber (1957) named the condition, has now been accepted as a clinical entity under various names (*British Medical Journal*, 1957; *Lancet*, 1961). Todd (1961) has given the most recent survey of the disorder and presented 20 new cases.

The present article deals with the fate of 50 patients with this syndrome who have been followed up in the period 1945–61. The symptomatology and principal findings in 28 patients have already been described (Bagratuni, 1953, 1956) and the blood-protein changes analysed (Bagratuni, 1957).

By 1958 22 more cases had been collected and followed up. The principal findings in these 50 patients are shown in Tables I and II.

Follow-up

A comprehensive review of the 50 patients was undertaken in 1961 with recall for clinical reassessment of all patients who had not been regularly seen. Muscle tenderness, joint swelling or tenderness, limitation of joint movement, and rheumatic nodules were especially looked for.