Hepatic Enzyme Activities in Rats Made Diabetic with Alloxan and with Guinea Pig Anti-insulin Serum

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SUMMARY

In diabetes abnormally high rates of gluconeogenesis have been demonstrated by various methods. Here it is confirmed that in rats which have been fasted or treated with alloxan, changes occur in hepatic enzyme activity which are compatible with an adaptation to increased rates of gluconeogenesis; there are increased glutamic-pyruvic and glutamic-oxalacetic transaminase and glucose-6-phosphatase activities and reduced lactic dehydrogenase activity. Comparable effects were demonstrated in the livers of rats killed in a diabetic state twenty-four to sixty hours after injection of guinea pig anti-insulin serum, with the exception that glutamic-pyruvic transaminase activity was not increased and glutamic-oxalacetic transaminase activity was increased when expressed per liver protein or per body weight, but there was no change in the activity of the total liver. This finding provides suggestive evidence that increased gluconeogenesis is also characteristic of this experimental diabetic syndrome produced by anti-insulin serum.

Metabolic studies in experimental diabetes produced by pancreatectomy or the administration of alloxan have certain disadvantages. Pancreatectomy eliminates not only insulin production but also the exocrine secretions and the secretion of islet cells other than beta cells. Alloxan, although producing selective beta cell destruction, has nephrotoxic and hepatotoxic effects. The opportunity to produce experimental diabetes with guinea pig anti-insulin serum as described by Wright[1] was thought to provide a means to observe metabolic alterations in a state of true insulin deficiency.

Increased gluconeogenesis by the liver is one of the significant metabolic adaptations in uncontrolled diabetes, as well as in other situations inducing protein catabolism, such as fasting or cortisone administration. The interconversion of glucose and glucose-6-phosphate by the liver may be measured by determination of hepatic glucokinase and glucose-6-phosphatase activity. Evidences for decreased glucokinase and increased glucose-6-phosphatase activity in the alloxan diabetic liver have been summarized by Ashmore et al.[2] Pyruvic acid is the crucial substrate in the metabolic pathway of transamination in the protein-carbohydrate interconversion. Because pyruvate is the end product of both glycolytic cycles and the starting point of the Krebs cycle, it may enter into protein metabolism either directly by transamination by glutamic-pyruvic transaminase or indirectly after conversion to oxalacetate and transamination by glutamic-oxalacetic transaminase. Both glutamic-pyruvic and glutamic-oxalacetic transaminase activities were elevated in the alloxan-diabetic rat liver.[3-5] Glutamic-oxalacetic transaminase was elevated in human diabetic liver without insulin treatment when compared with those values found in the same patients with insulin treatment.[6] An increase in these enzymes may, therefore, indicate a higher rate of gluconeogenesis. In addition to transamination, another important pathway for pyruvic acid is its reduction to lactic acid, mediated by the enzyme, lactic dehydrogenase. This enzyme activity was decreased in the alloxan diabetic rat liver.[7,8]

Evidence for the characteristic increased gluconeogenesis in rats made diabetic with guinea pig anti-insulin serum is still lacking. The present investigations were undertaken to determine whether there were indications of such a metabolic adaptation in the anti-insulin serum diabetic rat as compared to normal fed, normal fasted, and alloxan diabetic rats.

METHODS

Male rats (180 to 220 gm.) of a Sprague-Dawley strain were used. They were allowed free access to food (Rockland diet) and water unless otherwise stated. Normal fed rats served as control. Fasting rats were

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deprived of food for twenty-four hours, unless otherwise stated. Alloxan diabetic rats were killed eleven to twelve days after a single intravenous injection of alloxan solution (4.5 per cent, 45 mg. per kilogram of body weight). Serum-diabetic animals were injected intraperitoneally every twelve hours with 4 to 6 ml. of guinea pig anti-insulin serum and killed twenty-four, thirty-six, forty-eight and sixty hours after the first injection; the preparation and assay of this serum are described elsewhere.[9] Four normal rats were included in each of these groups: two rats were fed and two rats were fasted for twenty-four-, thirty-six-, forty-eight- and sixty-hour periods. These control animals were injected with comparable volumes of either saline or normal guinea pig serum, simultaneously with the anti-insulin serum administration to the rats being made diabetic.

All animals were sacrificed by decapitation at the same time of the morning and blood was collected from the severed neck for blood glucose determination. Other blood glucose estimations had been carried out during the life of the alloxan diabetic rats. At death the blood glucose concentrations in the alloxan diabetic rats ranged between 324 and 607 (mean: 500) mg. per 100 ml., and in the antiserum-diabetic rats it lay between 266 and 585 (mean: 360) mg. per 100 ml. Other findings in these antiserum-diabetic rats have been published elsewhere,[1] and it is sufficient to repeat here that, unlike alloxan diabetic rats, they all developed ketonuria. Immediately after death, portions of liver (200 to 500 mg.) were chilled and homogenized in distilled water (20 vol.). Glucose-6-phosphatase activity was estimated immediately, and the other hepatic enzymes were assayed on the following day, using homogenates stored in the frozen state.

Glucose-6-phosphatase (3.1.3.9; D-glucose-6-phosphate phosphohydrolase[10]) activity was assayed by a method based on those of Langdon and Weakley[11] and of Ashmore, Hastings, Nesbett and Renold.[12] The inorganic phosphate liberated from glucose-6-phosphate (0.02 M) in thirty minutes at 37° C. at *p*H 6.3 (citrate buffer, 0.1 M) was estimated by the method of Gomori.[13] Glutamic-pyruvic transaminase (2.6.1.2.; L-alanine:2-oxoglutarate aminotransferase[10]) and glutamic-oxalacetic transaminase (2.6.1.1.; L-aspartate:2-oxoglutarate aminotransferase[10]) activities were estimated by methods based on those described for serum by Wroblewski and LaDue[14] and Karmen,[15] respectively. For lactic dehydrogenase (1.1.1.27.; L-lactate: NAD oxidoreductase[10]) estimation, the method used for serum by Wroblewski and LaDue[16] was adapted for use with dilute liver homogenates. In each of these spectrophotometric assays, the buffered liver homogenate (1/100 to 1/2,000 w/v) was incubated with the necessary substrate, enzymes and cofactors in a cuvette (1 cm. light path) at 25° C. and the decrease of the optical density of the mixture ($\lambda = 340$ mμ) noted at fifteen-second intervals for five minutes. For all four enzymes, activity is expressed as micromoles of substrate converted per minute per gm. liver protein (specific), per total liver, and as total hepatic activity per 100 gm. body weight.

RESULTS

Twelve untreated normal fed rats were utilized for calculating normal values, since these values were similar to those of normal fed rats receiving saline or normal serum injection in the four antiserum groups (see Methods). One exception was the glutamic-oxalacetic transaminase activity in which the saline injection caused a 20 per cent increase in the normal animals of the four groups at twenty-four, thirty-six, forty-eight and sixty hours as compared to the untreated normal fed animals. Therefore, for this particular enzyme, antiserum diabetic enzyme values were compared with this elevated normal value.

Fasted values were calculated from eighteen untreated animals fasted for twenty-four hours. Longer periods of fasting up to sixty hours and/or saline injections did not cause any significant variation.

The activity of glutamic-pyruvic transaminase and lactic dehydrogenase of the antiserum-diabetic rat liver showed similar values regardless of the duration of the diabetes, from twenty-four to sixty hours. The activities of the other two enzymes, glucose-6-phosphatase and glutamic-oxalacetic transaminase were higher in the livers of the four rats in the group which was antiserum-diabetic for sixty hours as compared with those of the twenty-four-, thirty-six-, and forty-eight-hour groups.

Table 1 contains the four enzyme activities. Alterations in enzymatic activity tend to be minimized when results are referred to total liver in the serum-diabetic rats, because of the smaller livers of these animals (see Discussion). Enzyme activity per gram wet liver for these animals was much higher than the respective normal values.

Fasting caused a slight increase in glucose-6-phosphatase, glutamic-pyruvic transaminase, and glutamic-oxalacetic transaminase, and a slight decrease in lactic dehydrogenase activities.

Glucose-6-phosphatase activity was much higher in serum-diabetes than in alloxan diabetes, based upon liver protein. Expressed per total liver or per 100 gm. body weight, serum-diabetes of twenty-four- to forty-

TABLE 1

Changes in hepatic enzyme activity in experimental diabetes

Enzyme	Group*		Enzyme activity					
			μmoles per minute (gm. protein)	Per cent	μmoles per minute (total liver)	Per cent	μmoles per minute (100 gm. body weight)	Per cent
Glucose-6-phosphatase	N		79± 2.4†	100	118± 4.5	100	62± 2.1	100
	F		111± 2.4	141	146± 2.7	124	77± 1.9	124
	AD		113± 5.5	144	173± 10.5	147	94± 4.6	152
	SD	24-48	156± 3.2	198	186± 4.2	158	103± 2.5	165
		60	202± 1.4	256	208± 10.9	175	133± 3.9	214
Glutamic-pyruvic transaminase	N		170± 3.1	100	254± 5.5	100	135± 2.8	100
	F		200± 3.9	118	270± 8.6	(n) —	140± 3.6	(n) —
	AD		210± 7.8	122	330± 23.0	(s) 130	180± 9.2	131
	SD		200± 6.2	118	232± 7.7	— —	130± 4.4	(n) —
Glutamic-oxalacetic transaminase	N		510±10	100	740± 19	100	396±10.0	100
	F		700±14	136	920± 21	124	480± 8.3	122
	AD		910±77	179	1,450±130	196	770±49.0	195
	SD‡	24-48	820±14	135	1,020± 43	(n) —	540± 8.7	113
		60	1,170±32	192	1,200± 76	(n) —	770±35.0	160
Lactic dehydrogenase	N		1,500±32	100	2,300± 55	100	1,200±33	100
	F		1,400±25	(n) —	1,900± 55	83	970±30	80
	AD		890±73	59	1,450±174	64	720±58	60
	SD		1,250±19	85	1,500± 63	66	850±18	71

*N = normal (12 rats), F = fasting (18 rats), AD = alloxan diabetic (9 rats), SD = serum diabetic (15 rats). In two enzymes SD group is divided into 24 to 48 hrs. and 60 hrs. Explanation is in the text.

†The mean values (±Standard error of the means) are significantly different from those of normal rats ($P < 0.001$ unless otherwise stated. n = not significant; s = significant with $P < 0.05$).

‡In glutamic-oxalacetic transaminase the SD groups are compared to injected normal rats. See text.

eight-hour duration caused increases similar to alloxan diabetes but activity was higher in the sixty-hour group.

Glutamic-pyruvic transaminase showed small but significant elevations equally in both types of diabetes, when expressed per unit of liver protein. The change in total activity was not significant, and when activity was referred to body weight only alloxan diabetes caused some increase.

Glutamic-oxalacetic transaminase activities were considerably increased in alloxan diabetes and in the sixty-hour-serum diabetes: the twenty-four-, thirty-six-, and forty-eight-hour groups showed identical values indicating a rapid change between forty-eight and sixty hours. Total activity of this enzyme was either unchanged or slightly elevated in the serum-diabetic animals (see smaller liver weights in serum-diabetes in Discussion).

Lactic dehydrogenase activity was decreased in both types of diabetes when calculated either per total liver or per 100 gm. body weight. On the basis of liver protein, alloxan caused a more marked reduction in activity than did antiserum.

DISCUSSION

In comparing alloxan diabetic animals with antiserum-diabetic animals, there are a few dissimilarities. Alloxan diabetic rats eat more and drink more than normal rats. Although antiserum-diabetic rats ingested less food and water than normals, they received a considerable volume of fluid in the series of intraperitoneal injections. In this experiment the alloxan diabetic rats were hyperglycemic for ten to twelve days before being killed whereas in the antiserum-diabetic rats hyperglycemia lasted only for the duration of the experiment, that is twenty-four to sixty hours. Antiserum-diabetic rats lost the same amount of body weight as fasting rats; alloxan diabetic rats lost much more, considering the weight gain which normally would occur during ten to twelve days. Nitrogen concentration in the liver was similar in both types of diabetes, but the livers of the antiserum-diabetic groups were somewhat smaller than in the alloxan diabetic group.

The results of these experiments compare favorably with those reported by Fitch and Chaikoff using homogenates of livers from fasting[17] and alloxan diabetic[3] rats. They showed changes from normal in the specific activities (per gm. liver protein) of liver enzymes in alloxan diabetic rats for glucose-6-phosphatase (+60 per cent), for glutamic-pyruvic transaminase (+38, +35 per cent), for glutamic-oxalacetic transaminase (+107, +95 per cent), for lactic dehydrogenase (−34,

−28 per cent). The authors concluded that the effects of alloxan diabetes, although similar, are more pronounced than those induced by fasting, and may be correlated with the increased rate of gluconeogenesis characteristic of these conditions. This conclusion is supported by abundant evidence that glucose is formed at a rapid rate from other sources in uncontrolled human diabetes[18] and in various forms of experimental diabetes.[19] In alloxan diabetes this probably occurs at an early stage of the syndrome as elevated glutamic-oxalacetic transaminase activity has been reported two days after the injection of alloxan into rats[3] and increased glucose-6-phosphatase activity four hours after withdrawal of insulin therapy in such animals.[20]

In rats made diabetic with guinea pig anti-insulin serum, the pattern of hepatic enzyme activity is also abnormal (table 1). Livers obtained twenty-four to sixty hours after the first injection of anti-insulin serum exhibited abnormally large amounts of glucose-6-phosphatase and glutamic-oxalacetic transaminase activity. In one experiment (six rats) glucose-6-phosphatase activity was found elevated to 148 per cent five hours after the guinea pig anti-insulin serum administration. The changed enzyme pattern together with evidence of increased glucose formation from other sources would indicate that increased gluconeogenesis occurs in antiserum diabetes as in alloxan diabetes. Wagle and Ashmore[21] found an elevated CO_2 incorporation into glucose in in vivo studies in alloxan diabetic rats, and in antiserum diabetic rats, when the antiserum was given thirty minutes before the CO_2 administration. In vitro, however, only the alloxan diabetic rat liver showed increased CO_2 fixation. In these experiments the duration of antiserum diabetes was no longer than two hours and the amount of the injected antiserum would increase the blood glucose to 180 to 220 mg. per 100 ml. within thirty minutes.

Wagle and Ashmore[21] as well as Fitch and Chaikoff[8] have implied that the mechanism of increased gluconeogenesis in diabetes is not explained by insulin deficiency alone. The role of the adrenal steroids, and the availability of reduced pyridinenucleotide coenzymes have to be considered in this metabolic disturbance, whether the experimental diabetes was produced by means of alloxan or guinea pig anti-insulin serum.

SUMMARIO IN INTERLINGUA

Activitates de Enzyma Hepatic in Rattos Rendite Diabetic con Alloxano o con Sero Anti Insulina ab Porcos de India

In casos de diabete, anormalmente alte nivellos de gluconeogenese ha essite demonstrate per medio de varie methodos. In le presente communication il es confirmate que in rattos subjicite a jejunation o tractate con alloxano, alterationes occurre in le activitate de enzyma hepatic le quales es compatibile con le notion que illos corresponde a un accelerate gluconeogenese. Le activitates de transaminase glutamic-pyruvic e de transaminase glutamic-oxaloacetic e etiam de glucosa-6-phosphatase es augmentate e illo de dishydrogenase lactic es reducite. Comparabile effectos esseva demonstrate in le hepates de rattos occidite in stato diabetic inter vinti-quatro e sexanta horas post le injection de sero anti insulina ab porcos de India, con le exception que le activitate de transaminase glutamic-pyruvic non esseva augmentate e que le activitate de transaminase glutamic-oxaloacetic se monstrava augmentate quando illo esseva exprimite in relation al proteina hepatic o al peso corporee, durante que le activitate absolute del hepate total non esseva alterate. Iste constatation provide evidentia suggestive que augmento del gluconeogenese es etiam characteristic de iste syndrome diabetic producite experimentalmente per sero anti insulina.

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Sodium Chloride and Myocardial Infarcts in Rats

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ployed, hypertension may have been masked. In any event, there was no significant difference in average initial and final blood pressures among groups.

The amount of fat accumulating in arterial walls of animals fed a high fat diet increased as a result of salt ingestion. Infarction resulting from occlusion of the arteries, either by narrowing due to fat accumulation or thrombus, was promoted by salt. The mechanism is unknown.

In a recent study reported by Thomas, Hartroft, and R. M. O'Neil (J. Nutrition *69*:325, 1959), the addition of 1 per cent NaCl to a thrombogenic diet containing propylthiouracil resulted in no infarcts, but no animal survived longer than two months. However, in this study the incidence of myocardial infarcts (23 per cent) in rats receiving the thrombogenic diet and surviving two months, compared with the incidence in rats receiving three times as much salt mixture (incidence 38 per cent), suggests that minerals other than sodium chloride may have caused the rise in incidence of infarcts. Clearly the relationship of minerals to experimental arterial disease merits further study. Furthermore, the blood and tissue lipids should be analyzed in this type of experiment and the blood pressure should be measured at closer intervals.

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