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Received November 27, 1961. P.S.E.B.M., 1962, v109.

Insulin Content of Pancreas after Sodium Fluoroacetate-Induced Hyperglycemia.* (27233)

JOHN H. KARAM AND GEROLD M. GRODSKY
Metabolic Research Unit and Departments of Medicine and Biochemistry, University of California Medical Center, San Francisco

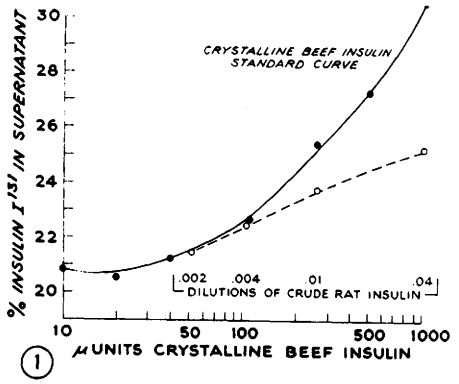
Studies of the basic metabolic disturbance induced by sodium fluoroacetate (SFA) suggest that it undergoes a "lethal synthesis" forming fluorocitrate which by interfering with the action of aconitase blocks the Krebs cycle(1,2). Citrate then accumulates in all tissues. Elliott and Phillips(3) and Engel *et al.*(4) noted that rats given SFA *in vivo* also developed hyperglycemia and ketosis, "SFA diabetes." It was not known whether the diabetes was due to a general block of the Krebs cycle resulting in secondary hyperglycemia, or a specific inhibition in the mitochondria of beta cells of the pancreatic islets, causing a deficiency of insulin production. While much of the data correlated better with a general peripheral block of the Krebs cycle, persistent diabetes in one rat after an injection of SFA(5) left open the possibility of specific damage to insulin-producing cells.

To investigate the action of SFA in rats, we applied a recently developed method for the immunochemical assay of insulin in pancreatic tissue(6).

Methods. Twenty female Long-Evans rats weighing 100-170 g were divided into 3 groups as follows: 1) 5 normal controls were fasted for 12 hr, then decapitated. 2) Five rats received rapid infusions of alloxan (Eastman Organic Chemicals, Rochester, N. Y.), 50 mg/kg, in the tail vein after a 72-hr fast. Diabetes was established by urinary glucose level of 2-9 g per day while rats were fed *ad libitum*. On the third day after alloxan administration the animals were killed as above after a 12-hr fast. 3) Ten rats were given an intraperitoneal injection of 0.6 mg per 100 g body weight freshly dissolved SFA, 0.06% in saline. Three animals died within 6 hr of the injection. The 7 surviving rats were fed *ad libitum* for 12 hr after SFA injection, then fasted for 12-18 hr and decapitated. Within 6 hr after receiving SFA, all animals manifested glycosuria and ketonuria as measured with Clinistix and Acetest tablets (Ames Co., Elkhart, Ind.). Jugular vein blood was collected at decapitation and fasting blood glucose levels were measured by the Somogyi method(7).

Insulin assay. The tail and body of the pancreas were immediately removed, blotted, weighed and placed in 10% trichloroacetic acid. After homogenization with mortar and pestle and centrifugation, the insulin was extracted from the precipitated protein with acid alcohol (15 ml of 12 N HCl diluted to 1 L with 75% ethanol) and further purified by the method of Grodsky and Tarver(8).

* This work was supported by a grant from Nat. Inst. of Arthritis and Metab. Dis., U.S.P.H.S.



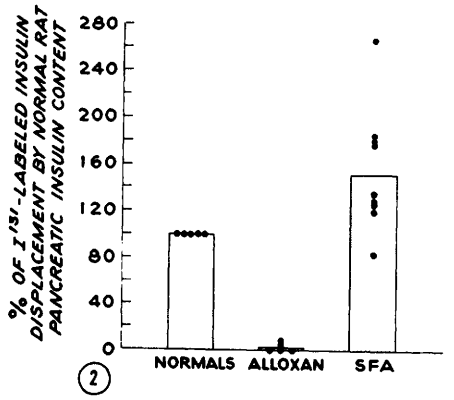


FIG. 1. Comparison of displacement of beef insulin-I^{131} from antibeef guinea pig serum between varying dilutions of crude rat insulin and known concentrations of crystalline beef insulin.

FIG. 2. Pancreatic insulin content expressed as change in % displacement of beef insulin-I^{131} from incubation systems containing antibeef guinea pig serum and identical dilutions of pancreatic extracts from normal, alloxan-diabetic and SFA-diabetic rats.

The final precipitate of crude insulin was dissolved in 0.5 ml 0.01 N HCl.

Pancreatic insulin was assayed by the method of Grodsky and Forsham(9) based on per cent of change in binding of insulin-I^{131} to insulin antibodies induced by varying quantities of unlabeled insulin. The degree of binding is measured quantitatively after preferential precipitation of bound insulin with sodium sulfate. The assay used a guinea pig antibody to beef insulin, beef insulin-I^{131}, 5-10 mc per mg (Abbott Laboratories) and standard solutions of crystalline beef insulin (Eli Lilly Lot No. T-2842).

As purified rat insulin was not available as a standard for the insulin assay system, it was necessary to compare the immunologic proportionality of rat insulin to the standard crystalline beef insulin preparation. Displacement of beef insulin-I^{131} by serial dilutions of acid alcohol extracts of rat pancreas was compared to the displacement by known concentrations of crystalline beef insulin. Representative curves are shown in Fig. 1. The divergence of these curves demonstrates that rat insulin does not quantitatively cross-react with beef insulin.

Because of the poor cross-reaction between crude rat insulin and beef insulin, determination of the absolute values of pancreatic insulin content in the rat was not feasible. However, since this experiment compared relative insulin content of the pancreas of normal, alloxan-diabetic and SFA-diabetic rats, the absolute content was not vital for evaluation of results. Accordingly, identical dilutions (1:250) of pancreatic extracts were used for all determinations in all groups of animals.

To insure proper control of assay conditions, at least one alloxan and one normal pancreatic extract were assayed with pancreatic extracts of SFA-treated animals on a given day. The change in per cent of displacement was expressed by measuring the insulin-I^{131} displaced into the supernatant by the pancreatic extract tested and comparing it to the displacement by an identical dilution of normal rat pancreas, taken as 100.†

Results. Both alloxan and SFA produced hyperglycemia in rats (Table I). After alloxan, however, the blood sugar levels were about 3 times that after SFA treatment. Both groups had glycosuria but the SFA-treated animals excreted qualitatively detectable ketone bodies in urine more consistently.

Insulin content of the pancreas of alloxan-

† Change in % displacement $= \frac{\text{Unk} - \text{Bl}}{\text{N} - \text{Bl}}$, where

Unk = % free insulin-I^{131} (supernatant fraction) after incubation with pancreatic extract from experimental rats.

N = % displacement of insulin-I^{131} (supernatant fraction) after incubation with pancreatic extract from normal rats.

Bl = % free insulin-I^{131} after incubation without added unlabeled insulin.

TABLE I. Effect of Intravenous Alloxan and Intraperitoneal Sodium Fluoroacetate on Fasting Blood Sugar, Urinary Glucose and Ketone Levels in Rats.

Animals	Body wt, g	Fasting blood sugar, mg/100 ml	Qualitative urine sugar	Urine acetone
Normal				
1	155	86	0	0
2	160	57	0	0
3	135	97	0	0
4	120	87	0	0
5	150	—	—	—
Alloxan (5 mg/100 g)				
1	124	317	+	0
2	138	248	+	+
3	100	800	+	+
4	113	755	+	0
5	105	790	+	0
Sodium fluoroacetate (0.6 mg/100 g)				
1	155	198	+	+
2	155	224	+	+
3	137	174	+	+
4	170	169	+	+
5	125	143	+	+
6	153	164	+	+
7	108	241	+	+

diabetic and SFA-treated rats is shown in Fig. 2, expressed as change in per cent displacement of insulin-I^{131}. Pancreatic extracts from SFA-treated rats, assayed on the same day and with dilutions identical to the pancreatic extracts from normal rats, had a mean insulin-I^{131} displacement equivalent to 152% of normal rat pancreas. This difference was statistically significant ($p = 0.015$).

Pancreatic extracts of the alloxan-treated, diabetic rats averaged only 2% of the insulin-I^{131} displacement of normal rats. Extracts from 3 of the alloxan-treated rats contained too little insulin to cause significant displacement of any insulin-I^{131} from antibody.

Discussion. The poor immunochemical cross-reaction between rat and beef insulin contrasts with the close cross-reaction of beef, pork, and human insulins observed with the same antibeef guinea pig serum(10), suggesting that insulin from rats differs grossly in chemical structure from insulin from these other species.

The 95-100% reduction of insulin in the pancreas of alloxan-treated rats was expected and validated the immunochemical insulin assay used.

Results of this study using a direct assay of pancreatic insulin content indicate that the diabetogenic action of SFA is different from alloxan since insulin content is not reduced. Pancreatic insulin seems to increase compared to normal controls. While this experiment does not rule out impaired release of insulin by beta cells of the pancreas as a primary action of SFA, increased insulin content of the pancreas is a likely consequence of the stimulatory hyperglycemia resulting from the peripheral blockade of the Krebs cycle(1,2), which agrees with the data of Engel *et al.*(11). They demonstrated that the diabetes produced by SFA was characterized by an associated slight insulin insensitivity and that the diabetic action of SFA was present in alloxan-treated rats, resulting in a more severe diabetic state in these animals.

Summary. A specific immunochemical assay was used to measure extractable insulin from the pancreas of normal rats, alloxan-diabetic rats, and rats made diabetic with sodium fluoroacetate (SFA). Rat insulin proved immunologically different from crystalline beef insulin, suggesting a significant chemical difference between these insulins. Pancreatic insulin content was decreased or absent in alloxan-treated diabetic rats, but appeared to be increased in rats with SFA diabetes, compared with normal controls. These findings demonstrate that the diabetes produced by SFA is not due to an alloxan-like action on beta cells resulting in insulin deficiency, but is secondary to a peripheral block in glucose utilization.

The authors gratefully acknowledge the invaluable advice and criticism of Dr. P. H. Forsham.

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Received November 27, 1961. P.S.E.B.M., 1962, v109.

Connective Tissue VI: Dermal Lipid Response to Diphenylhydantoin.* (27234)

A. C. CHUNG† AND J. C. HOUCK

Division of Research, Lankenau Hospital, Philadelphia, Pa., and Biochemical Research Laboratory, Children's Hospital, Washington, D.C.

Diphenylhydantoin (Dilantin) is known to produce changes in the chemistry of the connective tissue, particularly with regard to dermal concentration of collagen, scleroprotein and hexosamine(1,2). One of us (JCH) has suggested that from the increases in dermal collagen produced by diphenylhydantoin administration there might be a decrease in dermal lipid as well as dermal water concentration(1). This paper describes the results of an analysis of lipid concentration of rat skin from animals which had received diphenylhydantoin, and compares these results with 3 different groups of control animals.

Materials and methods. Twenty-four male Sprague-Dawley rats (220-250 g) were grouped into 4 groups of 6 animals each. One group was subjected to daily subcutaneous injections of 0.65 mg of cortisol (Cortil, C. Pfizer Co.), another to daily intraperitoneal injections of one ml of 0.15 M NaCl in which was suspended 25 mg of Dilantin sodium (Parke, Davis & Co.). The third group was similarly injected with 25 mg of α,α-diphenyl α-aminoacetic acid. (Parke, Davis & Co.), an inactive analogue of Dilantin, and the last group served as a normal control. After 10 daily doses of Dilantin, cortisol or diphenyl aminoacetic acid, all animals were sacrificed and about 1 g of abdominal skin from each animal was removed, shaven and dissected free of adhering fat, fascia and muscle. The cleaned skin from each rat was minced, pooled with the tissue from the other members of each experimental group and lyophilized. One g of pooled, lyophilized tissue from each group was further minced, allowed to stand in 10 ml of 0.15 M NaCl for 2 hours and then extracted in a Virtis homogenizer for one hour. The aqueous suspension of macerated tissue from each group was then extracted with 250 ml of a mixture of chloroform and methanol (2:1, v/v) in a Waring blendor according to Folch (3). The chloroform layer of the extract was analyzed with respect to total and esterified cholesterol(4), lipid phosphorus(5) and neutral fats (glycerides) according to Van Handel and Zilversmitt(6). Total lipids were determined gravimetrically by drying an aliquot *in vacuo,* redissolving the residue in petroleum ether (B.P. 40-60°C), filtering, redrying and determining the weight of the residues in a semi-micro balance. The isolation of neutral glycerides(7) and their separation into tri-, di-, and mono-glycerides was accomplished by silicic acid column chromatography(8).

Results. The results of dermal lipid analysis are shown in Table I. None of the 3 control groups demonstrated any meaningful differences in dermal lipids. These results are in very close agreement with those reported by Kao *et al.*(9) for normal male rats of this weight range when expressed in terms of wet weight (50-55% water).

Mean concentrations of phospholipid, neutral and total fat in the pooled tissues obtained from 3 groups of control animals were 13.08, 205 and 240 mg/g respectively. The standard deviations of each of these means, representing 3 pools of 6 rats each, were ±

* Supported in part by a grant-in-aid from the N.I.H., U.S.P.H.S.

† Present address: Surgical Research Lab., Georgetown University Hospital, Washington, D.C.