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Studies on the Effect of Tolbutamide on Insulin Secretion. (27820)

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The very extensive use of the sulfonylurea drugs, particularly tolbutamide and chlorpropamide in treatment of diabetes has prompted a great deal of research on the mode of action of these drugs(1-7), yet the physiologic mechanisms responsible for the hypoglycemic action are not understood. Levine(8), examined in detail the various hypotheses on the mode of action of these drugs and finally stated, "The many *in vivo* observations compel one to accept the conclusion that the hypoglycemic sulfonamides somehow promote the release of additional insulin from the beta cells of the islets of Langerhans, a phenomenon spoken of as *beta cytotrophism*." The data presented here do not support this hypothesis. We have measured the insulin secretion rate in dogs given tolbutamide by intravenous infusion and have found that it is not significantly different from the secretion rate in control dogs given a saline infusion. In contrast to this, glucose infusion produces a secretion rate 5 to 10 times that of the saline treated controls.

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Methods. Adult female dogs were used for this study. Average body weight for the group was 30 lb, but body weights varied from 20 to 50 lb. The general state of nutrition also varied to the same extent. Fifteen dogs were treated with sodium tolbutamide, 1.0 g in 500 ml saline administered intravenously. Fourteen dogs, serving as controls, received 500 ml saline intravenously. A group of 8 dogs were infused with 500 ml of 10% glucose in saline.

Operative procedure. The animals were fasted for 24 hours before the experiment. They were anaesthetized with Nembutal and a mixture of O_2 and 5% CO_2 was administered by intra-tracheal tube throughout the entire procedure. The intravenous infusion of tolbutamide (or glucose or saline) was started at the beginning of the abdominal surgery so that the drug was present in the pancreas for about 30 minutes before collection of pancreatic venous blood was started. Rate of infusion of the 500 ml volume was so regulated that the infusion was continued throughout the entire period of blood collection.

During the abdominal operation, special care was taken to cauterize or ligate all blood vessels to minimize bleeding later in the experiment after heparin was administered. A cannula (polyethylene tubing 3 mm inner diameter) containing heparin solution (1000 U/ml) was inserted into the splenic vein, with the tip directed into the portal vein. All the tributary veins to the portal which carried blood from the stomach and intestines were ligated as far as possible so that the blood flowing from the cannula was only that which had passed through the pancreas. Immediately before starting blood collection, heparin solution (3-4 ml) was injected through the cannula into the portal vein. After a minute or two, when the heparin was in the general circulation, the portal vein was tied off at its entrance into the liver. The hepatic artery was ligated also at a point close to the liver, to exclude the liver from the general circulation and prevent any rise in the level of blood glucose. Specimens of blood for glucose determinations were collected from the cannula every 15 minutes during the experiment. The operative procedure required about 30 minutes.

Blood was collected in 250-ml centrifuge bottles which were packed in chipped ice. Collection time was approximately 50 minutes and about 500 ml blood was collected in each experiment. In the experiments in which glucose was given, more than 500 ml of blood was collected in the 50-minute period.

Extraction of insulin. Immediately after the pancreatic venous blood was collected, it was centrifuged in the cold room and the plasma removed as quickly as possible. The plasma was shell-frozen at once and then either lyophilized or stored in a freezer.

The method for extraction of insulin was one devised by Bates and has been described (9). This consists of a series of extractions with ethanol-NaCl solutions. During the first period of this study, from Nov. 1958, to Oct. 1959, the procedure consisted of lyophilization of the plasma, grinding of the dried plasma in a Wiley mill, mixing it with a filter aid (Hyflo) and removing the lipid from the mixture with 95% ethanol. The lyophilized, defatted plasma was then extracted with ethanol-NaCl solutions and the separation of residue and extract accomplished by percolation or centrifugation. A modification of this procedure was used during the second period of this study, from June 1960 to June 1961, in which the whole plasma rather than the lyophilized plasma was extracted, using the same concentrations of ethanol-NaCl. Separation of residue and supernatant was made by centrifugation. The supernatant fraction was lyophilized and extracted with 95% ethanol to remove lipid material. The insulin was extracted from the fat-free residue with water. The data from the 2 sets of experiments have been presented separately.

Method of assay. The extracts of plasma were assayed for insulin by an *in vivo* method described previously (9), but with one modification, namely the use of hypophysectomized rats instead of alloxan diabetic hypophysectomized rats as previously described. A log-dose response curve for insulin using this modification is shown in Fig. 1. This is a composite of the insulin standard curves from 28 routine assays carried out during the study. With this modification, the index of precision (λ) is 0.22, which is better than the λ of 0.36 of the previous method.

The plasma extracts were made up in saline or albumin-saline solutions so that each dose of 2 ml volume contained 100 mg of protein material. The insulin standards were

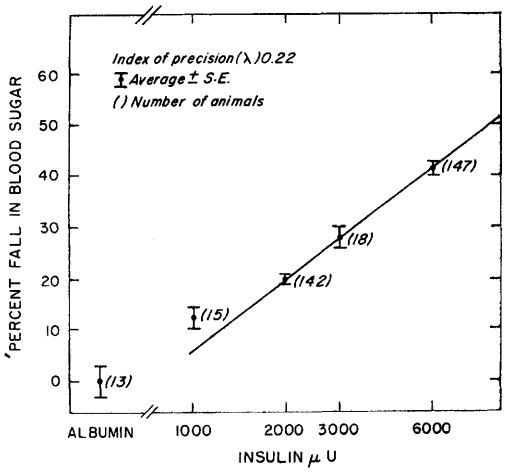

FIG. 1. Change in blood sugar for different doses of insulin in the fasted hypophysectomized rat.

TABLE I. Effect of Tolbutamide and Glucose on Insulin Secretion Rate in the Dog.

Exp	No. dogs	Blood collection			Extract of plasma		Insulin assay		Rate of secretion
		Whole, ml	Plasma, ml	Minutes	Total, mg	mg/ml plasma	μU/mg extract	μU/ml plasma	Insulin, μU/ml/min
Data from November 1958 to October 1959									
Saline (control)	10	300 ± 38*	143 ± 25	42 ± 4.5	356 ± 101	1.96 ± .29	39.0 ± 6.6	59 ± 11.1	1.52 ± .27
Tolbutamide	6	411 ± 24	210 ± 30	62 ± 10.7	392 ± 50	1.70 ± .10	56.0 ± 9.7	89 ± 9.1	1.58 ± .12
Glucose	5	506 ± 82	299 ± 55	74 ± 10.0	613 ± 122	1.99 ± .40	157.0 ± 44	288 ± 59	4.14 ± .92
Data from June 1960 to June 1961									
Saline (control)	4	637 ± 57	304 ± 7	46 ± 6.3	433 ± 108	1.42 ± .35	37.8 ± 11	48 ± 22	1.04 ± .44
Tolbutamide	9	510 ± 57	234 ± 29	51 ± 4.0	432 ± 66	1.93 ± .33	46 ± 15	75 ± 16	1.55 ± .28
Glucose	3	810 ± 83	513 ± 19	46 ± 8.3	532 ± 60	1.04 ± .13	507 ± 72	515 ± 28	12.3 ± 3.3

* Mean ± stand. error.

made in 5% albumin in saline.

Results. A summary of all experiments carried out over a 2-year period is given in Table I. Data on blood collections and yield of plasma extract are included, as well as insulin assays, to call attention to the many variables inherent in this type of physiological study. It was necessary to use dogs varying in size and age, of mixed breeds and differing in general state of nutrition. It is also important to remember that the technics of extraction of insulin from plasma and that of bioassay are certainly not as refined as one would like.

The blood sugar level in the dogs receiving tolbutamide solution or saline only did not rise over 90 mg % at any time during the experiment, and often fell as low as 30 mg %. In the dogs receiving 10% glucose solution, blood sugar levels were considerably over 350 mg % throughout experiment.

Since the method of extraction of insulin from plasma was changed after the first year of study, the data are given for the 2 periods separately (Table I).

In the first series of experiments in which the plasma was lyophilized before extraction, there were 10 control dogs which received saline only. The mean insulin level in the pancreatic vein blood of these controls was 59 ± 11.1 μU/ml plasma. The insulin level in peripheral venous blood of 5 normal fasting dogs was 26 ± 5 μU/ml plasma. Six dogs received tolbutamide, and the mean insulin level in pancreatic vein blood of this group was 89 ± 9.1 μU/ml plasma. The rate of insulin secretion for the saline treated group was 1.52 ± 0.27 μU/ml/minute and for the tolbutamide treated group 1.58 ± 0.12 μU/ml/minute.

In the second series of experiments where the insulin extractions were made on the whole plasma, there were only 4 saline treated dogs, and the yield of extract per ml plasma was low, but insulin content per mg extract was essentially the same as for the saline treated group of the first series. Insulin concentration in the pancreatic venous blood for the group averaged 48 ± 22 μU/ml plasma. There were 9 dogs treated with tolbutamide and average insulin concentration in this

group was 75 ± 16 μU/ml plasma. The mean rate of insulin secretion for the saline controls was 1.04 ± 0.44 μU/ml/minute and for the tolbutamide treated 1.55 ± 0.28 μU/ml/minute. Combining the 2 sets of data in Table I, the insulin secretion rate for the saline treated animals is 1.37 μU/ml/minute and for the tolbutamide treated animals 1.56 μU/minute. The average effect is an increase of 0.18 μU/ml/minute in the tolbutamide series with 95% confidence limits of –0.38 μU/ml/minute to + 0.74 μU/ml/minute. The results thus fail to demonstrate a stimulating effect of the tolbutamide on insulin secretion, but cannot exclude effects as large as 0.74 μU/ml/minute or about 50%.

The infusion of glucose raised the insulin secretion rate considerably above the rate obtained with saline infusion, but degree of response was quite varied. In the first series insulin concentration in the pancreatic venous blood was 288 ± 59 μU/ml plasma; in the second series it was 515 ± 28 μU/ml. In some cases, the level of insulin was more than 10 times that found in fasting saline treated dogs. This difference in response from one dog to another might well be due to differences in nutritional state of the dogs which varied from good to poor and to the range in age of the dogs, which was considerable. The average secretion rate of the 5 dogs in the first series was 4.14 ± 0.92 μU/ml/minute, and in the second series of 3 dogs 12.3 ± 3.3 μU/ml/minute. Combining the 2 series, average secretion rate of the dogs treated with glucose was 7.21 ± 1.9 μU/ml/minute.

Discussion. It is important to emphasize here that the secretion rates for insulin given in Table I are valid only for comparison within the 3 groups of dogs and do not give the physiological secretion rates, since the animals were bled in most cases to a state of circulatory failure in order to obtain sufficient amounts of blood.

Our findings do not support the *beta cytotrophic* hypothesis, but they do confirm the findings of several investigators who have measured the activity in the blood of humans following tolbutamide administration. Using an *in vitro* rat diaphragm bioassay, Renold *et al.*(10) measured the insulin-like activity in peripheral plasma of 4 normal individuals before and after a rapid intravenous infusion of tolbutamide (37.5 mg/kg). Although blood glucose decreased about 50%, in no instance did the insulin-like activity of the plasma increase. Following the same technic for assay of insulin, neither Weaver *et al.*(11), nor Seltzer and Smith(12) was able to detect changes in insulin-like activity in the sera of diabetic patients 140 minutes after tolbutamide given orally (3 g total dose). On the other hand, Vallance-Owen, Joplin, and Fraser(13), also using the rat diaphragm assay technic, and with comparable doses of tolbutamide orally, found significant increases in plasma insulin activity both in normal individuals and in those diabetics who were clinically responsive to tolbutamide. The non-responsive diabetics showed no increases in plasma insulin activity. In every case the fall in blood sugar correlated well with the rise in insulin activity. Yalow *et al.*(14) have confirmed these findings using their technic of immuno-bioassay for insulin. With another *in vitro* technic for the assay of insulin, the rat adipose tissue method, Pfeiffer *et al.*(15) reported very striking increases following tolbutamide administration, levels of insulin-like activity rising to 4000 mU per ml of serum in normal subjects, and in tolbutamide-responsive diabetics; they found no increase in juvenile diabetics after tolbutamide. It is very difficult to reconcile these findings of marked increase in insulin secretion in human beings with our failure to detect a significant change in insulin secretion rate after tolbutamide in dogs.

Summary. Rate of insulin secretion has been studied in dogs fasted for 24 hours and given an intravenous injection of 1 g sodium tolbutamide in 500 ml saline. Insulin was extracted from pancreatic venous blood and assayed by an *in vivo* bioassay method. The mean rate of insulin secretion for the 14 control dogs receiving only saline was 1.37 μU/ml/minute, and for the 15 dogs receiving tolbutamide was 1.56 μU/ml/minute. The results thus fail to demonstrate a stimulating effect of tolbutamide on insulin secretion. Administration of glucose, on the other hand, increased the insulin secretion rate in 8 dogs

as much as 5 to 10 times that of the saline treated dogs.

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Evaluation of Glycolytic and Citric Acid Cycles in Homogenates of Dystrophic Mouse Muscle.* (27821)

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The purpose of this study was to compare the two major metabolic pathways operating in the skeletal muscles of littermate normal and dystrophic mice in the hope of elucidating the underlying chemical defect in this hereditary myopathy. The affected extremity muscles in mice show histologic changes similar to those in human muscular dystrophy(1).

In both human and mouse muscular dystrophy there is abnormal distribution of such glycolytic enzymes as serum aldolase(2,3,4, 5), phosphohexoisomerase(3,4), lactic dehydrogenase(3,5), muscle aldolase(2,3,6), and phosphorylase(2). This information suggested a primary defect in glycolysis and prompted evaluation of the complete cycle. Canal(7) injected fructose into the blood stream of patients with muscular dystrophy and found abnormal retention of lactic and pyruvic acids. This observation fortified the possibility of a deficiency either in glycogenolysis in muscle or glycogenesis in the liver. It could also reflect a disturbance at or below the acetyl Co A level, resulting in a failure to oxidize pyruvic acid to carbon dioxide and water in the citric acid cycle. A failure in the cycle below the acetyl Co A level could conceivably drive the reaction in the direction of excess fat synthesis. Excessive fat is deposited in the muscles of both human beings and animals afflicted with muscular dystrophy.

Our experiments indicate that both the glycolytic cycle and the citric acid cycle, as com-

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