TOLBUTAMIDE HYPOGLYCEMIA IN ACUTELY DEPANCREATIZED DOGS * †

By MARTIN KURTZ, CHARLES M. HOLTZMAN AND EDWARD MEILMAN

(From The Long Island Jewish Hospital, New Hyde Park, Long Island, N. Y.)

(Submitted for publication December 1, 1958; accepted February 12, 1959)

The hypoglycemic activity of tolbutamide and related compounds is well established; however, their mode of action is still debated. Several theories have been postulated (1–5). One of the most widely held is that tolbutamide stimulates the beta cells of the pancreas and the resulting insulin secretion produces the fall in blood sugar (1). The reported ineffectiveness of tolbutamide in depancreatized animals (1) and humans (6) is taken as strong evidence for this theory. With few exceptions these studies were done sometime after recovery from the pancreatectomies. One of us (M.K.) in the past studied the possible pancreatropic effect of another substance, *i.e.*, anterior pituitary growth hormone (7). It was observed that growth hormone had a hypoglycemic effect in acutely depancreatized dogs but not in dogs several days after pancreatectomy. Accordingly, it was thought worthwhile to study the effect of tolbutamide in acutely depancreatized dogs.

METHODS

Adult male and female mongrel dogs were used. The animals were in the postabsorptive state. All the experiments were performed under sodium pentobarbital anesthesia. To assure complete removal of the pancreas the duodenum and pancreas were removed "en bloc." In the dog, the pancreas can be divided into three parts: a middle portion and two limbs. The middle portion is intimately adherent to that portion of the duodenum equivalent to the second, third and fourth parts of the human duodenum. The two "limbs," one to the right and the other to the left of the duodenum, are completely enveloped in omentum, well defined and easily mobilized. Since it is very difficult to remove completely and with certainty that part of the pancreas adherent to the duodenum and related structures, *i.e.*, bile duct, cystic artery, and so fourth, we resected the duodenum and attached pancreas as a whole. The bowel was cut at least one to two inches beyond the junction of duodenum and pancreas (through pyloric end of stomach and through jejunum). Since these were acute experiments no attempt was made to re-establish continuity of bowel. Once having mobilized and resected the duodenum and attached pancreas, the rest of the operation was relatively simple. Since both "limbs" of pancreatic tissue are free in omentum (except at one point, noted below) we could easily make our line of resection at least one-half inch away from, and parallel to the border of, the pancreas. The exception mentioned above is that the distal one-half to one inch of the left wing is closely applied to branches of the splenic artery and vein. At that point two or three tiny arteries and veins communicate between the vessels and pancreatic parenchyma. When these are ligated, the omentum containing the pancreas is easily stripped off the vessels. When the tip of the limb is mobilized the line of resection can be continued. In essence, then, the pancreas is removed intact and certainly completely. Early in the operative series, we did postmortem examinations. However, the difference between pancreas and omental fat is obvious enough at the time of operation to make postmortem examination unnecessary.

Blood samples were collected after administration of the anesthesia, immediately after removal of the pancreas and approximately every 30 minutes thereafter for four to seven hours. The blood glucose was determined by the Nelson-Somogyi method (8). Almost all of the blood specimens were taken from peripheral veins. On several occasions cardiac punctures were done, without deleterious effects. The sodium tolbutamide (2), 200 mg. per Kg., was dissolved in 15 to 20 ml. of normal saline and rapidly injected intravenously at the times noted.

RESULTS

Two unoperated dogs were given tolbutamide and the results paralleled those reported by others (9). In both cases the blood sugars fell approximately 60 per cent and the maximum effect was noted within two to four hours after injection.

Five dogs were subjected to pancreatectomy but did not receive tolbutamide (Table I and Figure 1). Within 90 minutes each dog's blood sugar reached hyperglycemic levels. The values then either stayed on a plateau for the next one to one and one-half hours or dipped to lower levels. Similar

* This investigation was supported by a grant from the American Heart Association.

† We are greatly indebted to Dr. C. J. O'Donnovan of Upjohn Company for their generous supply of the sodium tolbutamide.

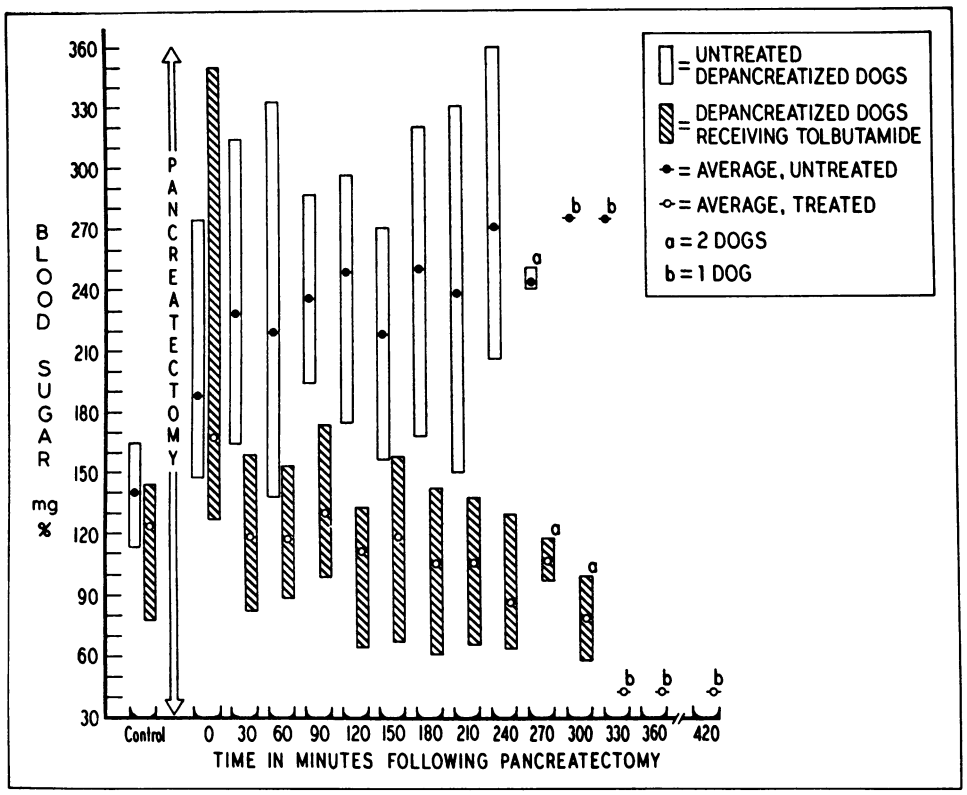

FIG. 1. COMPARISON OF THE BLOOD SUGARS OF UNTREATED DEPANCREATIZED DOGS WITH THOSE OF DEPANCREATIZED DOGS RECEIVING TOLBUTAMIDE

The tolbutamide was administered within 10 minutes of pancreatectomy. The top of each bar is equal to the greatest blood sugar value, at the time noted. The bottom of the bar is equal to the lowest blood sugar value.

TABLE I

The blood sugar curves of depancreatized dogs with and without tolbutamide

Dog	Blood sugar		Time in minutes												
	Control*	Zero time	30	60	90	120	150	180	210	240	270	300	330	360	420
			Blood sugar (*mg.* %)												
No.															
			Pancreatectomized dogs (no tolbutamide)												
1p	156	230	260	230	275	260	260	320	330	330					
2p	165	147	194	138	197	174	156	168	150	206					
3p	112	118	165	177	224	278	270	312	300	360					
4p	137	274	314	332	286	296	246	212	194	230	240	274	274		
5p	131	166	212	218	194	234	160	240	218	222	246				
			Pancreatectomized dogs (immediate tolbutamide)‡												
1p–T	78	95†	82	105	105	101	92	61	73	74					
2p–T	125	350†	134	110	173	127	158	142	129	96	118	100			
3p–T	132	147†	110	153	147	125	67	66		64					
4p–T	135	144†	159	121	150	124	150	118	100	130					
5p–T	131	127†	110	88	99	103	114	144	66	66					
6p–T	120	165†	85	125	133	133	133	133	128	72					
7p–T	144	142†	146		100	64	115		138	93	97	58	43	45	43

* Before pancreatectomy.
† After this specimen, tolbutamide given.
‡ Within one to 10 minutes after removal of the pancreas.

TABLE II

The effect of delayed tolbutamide administration on the blood sugar of depancreatized dogs

Dog	Blood sugar		Time in minutes											
	Control*	Zero time	30	60	90	120	150	180	210	240	270	300	330	360
								Blood sugar (*mg.* %)						
No.														
								Tolbutamide, 75 minutes after pancreatectomy						
8p–T	133	122		250†	182	131	128	175	186	161	163	150		
								Tolbutamide, 120 minutes after pancreatectomy						
9p–T	65	152		82		156*		209		222	152	176	225	287
10p–T	152	164		163		209*	222	180	221	209	213	193	209	197
11p–T	102	131		121		194*	173	135	137	96	59	94	80	80

* Before pancreatectomy.
† Time of administration of tolbutamide.

delayed declines of the blood sugar in depancreatized dogs have been noted previously (7). After approximately three hours, the blood sugar values started to rise again in those dogs in which a fall had occurred. At the end of the experiments, three and one-half to four hours after pancreatectomy, the blood sugars ranged from 206 to 360 mg. per cent.

The "control" blood sugars obtained in several dogs were higher than are usually found in fasting dogs. Increased adrenalin output in frightened, untrained animals is the most likely explanation. The marked hyperglycemia recorded within minutes of removal of the pancreas (zero time) in three dogs (1p, 4p and 2p-T) is also probably explained by excessive adrenalin secretion. It should be noted that the height of the blood sugar following pancreatectomy could not account for the difference in results between the untreated and treated dogs. Dog 2p-T had the highest zero time blood sugar, 350 mg. per cent, immediately after pancreatectomy and yet responded to the tolbutamide with a fall in blood sugar. Dog 11p-T which had a preinjection level of 194 mg. per cent also responded to the tolbutamide.

Seven dogs were given tolbutamide within two to 10 minutes after removal of the pancreas (Table I and Figure 1). Examination of the data reveals that during the first two hours the blood sugars did not rise as would normally be expected after pancreatectomy. Two to four hours after administration of the tolbutamide, the blood sugars started to fall. One dog (7p-T) was followed for seven hours and the hypoglycemic effect was found to persist throughout the period of observation. Figure 1 illustrates the difference between the treated and untreated depancreatized dogs. The blank bars represent the range of blood sugar values, at the times noted, for the untreated group. The cross-hatched bars depict similar values for the tolbutamide treated group. The average values for each set of readings are also noted. It is apparent that there is a striking difference in blood sugar values between the two groups. In every treated dog the last observed blood sugar, four to seven hours after pancreatectomy, was less than the preoperative (control) value. Final blood sugars ranged from 43 to 130 mg. per cent, a marked contrast to those seen in the untreated depancreatized dogs.

The administration of tolbutamide was delayed in four dogs. One, 8p-T, received the drug one hour after pancreatectomy and the others received it two hours after removal of the pancreas (Table II). Dog 8p-T showed a response in that five hours after pancreatectomy the blood sugar was only 17 mg. per cent higher than the preoperative value. The blood sugar curves of Dogs 9p-T and 10p-T do not seem to differ significantly from the depancreatized dogs not receiving tolbutamide. However, Dog 11p-T which, like Dogs 9p-T and 10p-T, received the drug two hours after pancreatectomy, showed a response similar to the dogs receiving the tolbutamide promptly after the pancreas was removed.

DISCUSSION

It is apparent that when tolbutamide is administered promptly after removal of the pancreas, a distinct hypoglycemic effect results. The blood sugars not only do not rise to the levels seen in untreated depancreatized dogs, but significant decreases in blood sugar occur. The decreases are comparable to those obtained in intact dogs receiving tolbutamide.

Fritz, Morton, Weinstein and Levine (10) have reported somewhat similar studies on three dogs and concluded that no effect occurred when the sulfonylureas (carbutamide) were administered to the acutely depancreatized dog. In our series of 11 dogs, those dogs exhibiting the most consistent effect were given the tolbutamide within 10 minutes of pancreatectomy. Fritz states that "carbutamide was administered 20 to 60 minutes after pancreatectomy." They state that no change was noted in the course of the blood sugar in the three hours that the dogs were followed. All of our dogs but one were followed for four hours or more. It is important to note that the effect became obvious in five of the seven dogs only at the fourth hour or later. One dog received tolbutamide 75 minutes after pancreatectomy and did not clearly show any effect at the end of three hours but did by the fifth hour.

Obviously, the hypoglycemic effect noted in our dogs was not mediated through the pancreas. However, these experiments do not clarify the relationship of insulin to tolbutamide activity. It is likely that the depancreatized dogs were not free of endogenous insulin and insulin may be a requirement for tolbutamide activity. The experiments in which the administration of tolbutamide was delayed were done to test this hypothesis. Turnover studies with labeled insulin (11) indicate that most if not all of the endogenous insulin would have disappeared within the two hours after pancreatectomy. The variable results obtained in the four dogs (two showing hypoglycemic effect and two no change) that received the delayed tolbutamide would then be explained by a lack of an adequate amount of recently secreted insulin. This is consistent with the concept that tolbutamide hypoglycemia requires the presence of some insulin.

However, even if tolbutamide hypoglycemia will not occur in the absence of insulin (1), it need not mean that there is a direct relationship or interactivity between them. The two could effect the utilization of glucose at completely different points in the metabolic cycle, but those steps controlled by insulin might have to be intact for tolbutamide to produce hypoglycemia.

These experiments do not exclude the possibility that, in the intact animal, tolbutamide does in addition act by stimulating the pancreas. However, the data presented here do not require that such an explanation be invoked.

SUMMARY

Tolbutamide effects a significant lowering of blood sugar when administered to acutely depancreatized dogs. In these experiments the hypoglycemic action therefore cannot be attributed to a pancreatropic effect. These studies are consistent with the thesis that insulin must be present for tolbutamide to act.

ACKNOWLEDGMENT

We would like to express our appreciation to Dr. Eli Seifter of the Department of Laboratories, the Long Island Jewish Hospital, New Hyde Park, L. I., for his help in the blood sugar determinations.

REFERENCES

1. Loubatières, A. L'utilisation de certaines substances sulfamidées dans le traitement du diabète sucré expérimental: Recherches personelles (1942–1946). Presse méd. 1955, **63**, 1701.
2. Holt, C. von, Holt, L. von, Kröner, B., and Kühnau, J. Chemische Ausschaltung der A-Zellen der Langerhansschen Inseln. Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. 1955, **224**, 66.
3. Mirsky, I. A., Perisutti, G., and Diengott, D. The inhibition of insulinase by hypoglycemic sulfonamides. Metabolism 1956, **5**, 156.
4. Moorhouse, J. A., and Kark, R. M. Physiologic actions of orinase and their relationship to the types of diabetes in man. Metabolism 1956, **5**, 847.
5. Caren, R., and Corbo, L. The potentiation of exogenous insulin by tolbutamide in depancreatized dogs. J. clin. Invest. 1957, **36**, 1546.

6. Goetz, F. C., Gilbertsen, A. S., and Josephson, V. Acute effects of orinase on peripheral glucose utilization. Metabolism 1956, **5**, 788.
7. Kurtz, M., de Bodo, R. C., Kiang, S. P., and Ancowitz, A. Hypoglycemia produced by purified anterior pituitary growth hormone and its relationship to the pancreas. Proc. Soc. exp. Biol. (N. Y.) 1951, **76**, 21.
8. Nelson, N. Photometric adaptation of Somogyi method for determination of glucose. J. biol. Chem. 1944, **153**, 375.
9. Houssay, B. A., Penhos, J. C., Urgoiti, E., Teodosio, N., Apelbaum, J., and Bowkett, J. The role of insulin in the action of the hypoglycemic sulfonyl compounds. Ann. N. Y. Acad. Sci. 1957, **71**, 25.
10. Fritz, I. B., Morton, J. V., Weinstein, M., and Levine, R. Studies on the mechanism of action of the sulfonylureas. Metabolism 1956, **5**, 744.
11. Prout, T. E., and Evans, I. E. Determination of the rate of insulin destruction *in vivo* (abstract). J. clin. Invest. 1958, **37**, 922.