per cent; three showed crystalluria. Microscopic examination of tissues was not deemed profitable in three rats because of advanced post-mortem change; one rat died after twenty-nine days with necrosis of some cells near the center of every liver lobule, and another had unilateral pyelitis with necrosis of the apex of the renal pyramid.

Two of three dogs given daily doses of 50 mg. per kg. showed minute erosions of the gastric mucosa. All showed hypertrophy of the thyroid gland. One of the three given doses of 100 mg. per kg. had several gastric ulcers, and all showed thyroid hypertrophy. Two also showed fatty metamorphosis of the liver. The beta cells of the pancreatic islands of four of these six dogs showed partial degranulation. This change is one that I have seen in animals other than those included in Dr. Anderson's tables, but it has been quite variable in extent and percentage incidence.

Four monkeys died at various intervals after the daily administration of 500 mg. per kg. doses was begun. All showed pulmonary edema and hydrothorax. Three also showed fat vacuolization of liver cells, but the significance of this is not clear, since this is frequently true of monkey livers.

Hypoglycemic Sulfonylureas in Various Types of Experimental Diabetes

M. F. Gordon, M.D., J. F. Buse, M.D.,† F. D. W. Lukens, M.D.,‡ Philadelphia*

Recent articles on the capacity of certain sulfonylureas to lower the level of blood glucose have reviewed the early observations and summarized the first experiences with their use in man.[1-5] A special number of the *Canadian Medical Association Journal*[6] presents nineteen articles on the sulfonylureas and includes studies on two depancreatized and one Houssay dogs. One of the first questions concerning these compounds has been: Does their action depend upon the presence of insulin? In other words, will they act in the absence of insulin? They are apparently ineffective in depancreatized dogs[6, 7] and man.[6, 8] The following observations support this conclusion and extend the conditions in which the drugs have been tested.

METHODS

Alloxan diabetic rats. Rats of the Wistar strain of both sexes weighing 140 to 180 gm. were made diabetic by the intraperitoneal administration of 175 mg. per kg. of alloxan. One to two weeks later animals which excreted several grams of sugar in the urine daily, while eating an adequate constant diet, were selected for the experiments. They were kept in metabolism cages and fed a weighed amount of Purina dog chow daily. The urinary glucose was determined[9] daily and the blood sugar[10] at times. When given orally for periods of several days, the sulfonylurea was added to the weighed amount of food, which had been pulverized and moistened to make a thick paste.

Cats have been tested by observing the effect of a single intraperitoneal dose of a sulfonylurea on the blood sugar during the subsequent six to eight hours. Some of these tests were made under sodium pentobarbital anesthesia and some in unanesthetized animals. The results were not affected by anesthesia. Such tests were performed in normal, hypophysectomized, depancreatized, and Houssay (hypophysectomized and depancreatized) animals. There was one death from barbiturate anesthesia in the first Houssay cat tested so that anesthesia was not used in any hypophysectomized animals thereafter. All cats were kept in metabolism cages, were fed weighed amounts of fresh horse meat daily, and in both types of depancreatized animals urinary glucose was determined daily and the presence of ketone bodies tested by the nitroprusside method.

The complete removal of pancreas and pituitary was determined by physiological criteria and by autopsy. The principal criteria were: for pancreatectomy, the amount of glucose excreted during fasting, ketonuria, and at

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia. This work was aided by grants from the Upjohn Company and Eli Lilly and Company.

* Fellow in Medicine, Department of Medicine, University of Pennsylvania, sponsored by the American Association of University Women.

† Trainee, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland.

‡ Professor of Medicine; Director, George S. Cox Medical Research Institute, University of Pennsylvania School of Medicine, Philadelphia.

autopsy a fatty liver and no pancreatic tissue. Hypophysectomy: There was no gross pituitary tissue at autopsy and sections of the thyroid and adrenal glands showed the characteristic atrophy. Houssay animals fulfilled the criteria for complete hypophysectomy and had the usual reduction in the amount of glucose excreted after pancreatectomy.

The arylsulfonylureas used were Compound U-2043 (tolbutamide; Orinase^{R}) and BZ-55 (carbutamide), which were kindly provided respectively by the Upjohn Company and Eli Lilly and Company. U-2043 was in the form of a soluble sodium salt which was mixed directly with food or given parenterally in saline solution. BZ-55 was given in suspension in saline and a small amount of Tween 80. In control experiments the medium was given without the drug.

RESULTS

Table 1 summarizes the results of feeding sulfonylureas to alloxan diabetic rats. The amount of food eaten was constant for all control and treatment periods which are compared. Some control periods preceded and some followed the treatment periods, a procedure which emphasized the stability of the diabetes in each rat. On a constant diet, the proportion of the available glucose which was excreted differed in different animals. Thus, on the 15 gm. diet, rat 13 excreted 3 to 3.5 gm. of glucose per day whereas rat 16 excreted 5 to 6 gm. daily. As used in these experiments, the sulfonylureas had no effect regardless of these variations in the severity of the diabetes. Since the rat with milder diabetes (No. 13) showed no significant diminution in glycosuria and since this animal probably produced some insulin, it seems that the mere presence of insulin is not enough to insure the effect of the drugs as administered.

In the last line of table 1, the average glycosuria for all experiments means that for a total of 64 control days and 111 treatment days in four diabetic rats, there was no change in the amount of glucose excreted. The changes in weight appear to be the random variations of animals which maintained an essentially constant weight during the observations.

In addition to the above effort at treatment, a few observations were made on the response of the blood sugar to single intraperitoneal doses of 20 mg. of U-2043 per rat. In three normal rats so treated the blood sugar fell 12, 18 and 36 mg. per 100 ml. (19, 37 and 51 per cent of the initial levels). This agrees with a report from the Upjohn Laboratories (unpublished) that in twenty normal rats, given 20 to 160 mg. of U-2043, the blood sugar fell from 13 to 42 per cent of the initial level. Four diabetic rats were tested. In three there was no reduction of hyperglycemia. In the fourth, the initial blood sugar was very high, but from two to eight hours the blood sugar was constant in contrast to the decline at two to six hours in normal animals. This agrees with Achelis and Hardebeck[2] who state that alloxan diabetic rabbits do not respond uniformly and that BZ-55 appears to be without effect and might even aggravate severe alloxan diabetes. Our conclusion is that the alloxan diabetic rats did not respond to the single dose, or to the feeding, of sulfonylureas.

Table 2 shows the responses of normal cats to intraperitoneal saline and to varying doses of U-2043. The cat is clearly one of the species which responds to this drug. Cat 17 illustrates the occasional absence of the usual fall in blood sugar, an irregularity which has been encountered by other investigators.

Table 3 records the lack of effect of U-2043 on the blood sugar level of depancreatized cats. The first five animals were given only saline as controls for the treated series. When the irregularities of these blood glucose figures are surveyed it seems clear that there is no significant effect of a single dose of U-2043 on the blood glucose under these conditions. In addition to the responses to a single dose (table 3) two depancreatized cats were treated with U-2043 (100 mg. per kg. intraperitoneally twice daily) and no insulin. Both died in diabetic acidosis in three to four days after having the same amount of glycosuria as control animals. Three depancreatized cats have been treated with insulin and U-2043. No potentiation of the action of insulin could be observed in terms of the daily glycosuria on a constant diet, but the severe and labile diabetes of this species would obscure any small effect. Better results have been obtained in the dog by Sirek and Sirek.[6]

It is now recognized that pancreatectomy means not only the removal of insulin but the reaction of the pituitary:adrenal system to the loss of insulin. This is accompanied by variable degrees of insulin resistance, the exact nature of which is not well understood. Such insulin resistance might well inhibit or antagonize a drug with a weak insulin-like action; it might also obscure a weakly hypoglycemic action of some other kind. For this reason the sulfonylureas were tested in Houssay animals. Houssay cats lack insulin but, unlike simply depancreatized cats, are extremely sensitive to insulin, so that 0.25 to 0.5 unit may cause a hypoglycemic reaction. The results of the tests in Houssay cats are presented in table 4 in which eight tests in four

TABLE 1

Alloxan diabetic rats fed hypoglycemic sulfonamides

Rat number	Food per day gm.	Urinary glucose[1] Control gm. per day	Urinary glucose[1] Treatment gm. per day	U-2043 per day mg.	Change in weight Control gm.	Change in weight Treatment gm.
5	20	3.9 (5)	4.5 (4)	10[2]	0	0
5	20	6.2 (8)	6.1 (9)	20[2]	+10	+10
5	20	6.2 (8)	6.6 (10)	20	—	0
5	20	6.2 (8)	6.4 (10)	40	—	+5
13	15	3.4 (7)	3.1 (13)	20	0	+5
13	15	3.5 (9)	3.1 (9)	40	+10	+10
16	15	6.2 (8)	5.2 (13)	20	−10	−10
16	20	6.6 (9)	7.0 (9)	20	+10	+10
16	20	6.6 (9)	7.2 (10)	40	—	+5
25	20	6.4 (8)	5.0 (14)	20	+10	0
25	20	4.7 (10)	5.3 (10)	40	+10	0
Averages		5.4±0.4	5.4±0.4			

[1] Days of periods are given in parentheses.
[2] These animals received BZ-55 instead of U-2043.

TABLE 2

Responses of normal cats to U-2043

Cat number	Anesthesia	U-2043 per kg. mg.	Administered[1]	Blood glucose at hours: 0	2	4	6	8	Fall in blood sugar
				mg. per 100 ml.					
15	Yes	0	IP	96	101	110	115	95	0
34	No	0	IP	72	76	74	79	77	0
36	No	0	IP	69	63	64	69	75	6
36	No	10	IP	73	56	67	67	—	17
34	No	20	IP	110	65	50	70	—	60
13	Yes	100	IP	72	41	40	27	43	45
15	Yes	100	IP	78	22	20	20	34	58
16	Yes	100	IP	84	21	31	27	27	63
17	Yes	100	IP	105	83	115	106	113	22
18	Yes	100	IP	67	34	40	27	30	40
25	Yes	100	S	105	52	62	137	107	53
26	Yes	100	S	109	79	72	95	97	37
Average of treated cats				89	50	55	64	64	44

[1] IP = intraperitoneally; S = by stomach tube.

TABLE 3

Effect of sulfonylureas in depancreatized cats

Cat number	Days after depancreatizing	Anesthesia	U-2043 per kg. mg.	Blood glucose at hours: 0	2	4	6	8	Maximum decrease
				mg. per 100 ml.					
31	1	Yes	0	289	243	249	252	282	46
38	2	Yes	0	262	233	222	193		69
34	2	No	0	277	241	247	246		36
53	2	No	0	295	305	315	289		6
53	3	No	0	270	243	276	279		27
		Averages		279	253	262	252		37
19	2	Yes	100	209	232	251	264	253	0
19	3(a)	Yes	100	301	197	250	260	279	104
20	2	Yes	100	302	242	248	241	252	60
24	3	Yes	100	315	287	288	257	248	67
8	3	Yes	100	228	225	218	202	187	41
		Averages		271	237	251	245	244	54

(a) Fourteen days after operation but three days after last insulin.

TABLE 4

Effect of sulfonylureas in Houssay cats

Cat. number	Days after: Hypox.	Depan.	Drug A=U-2043 B=BZ-55 mg. per kg.	Blood glucose at hours 0	2	4	6
				mg. per 100 ml.			
12	24	10	A–100	162	125	77	–
33	13	4	A–10	212	220	202	203
33	15	6	A–20	246	246	226	194
33[1]	20	11	A–20	319	322	315	302
33[1]	21	12	A–50	380	350	365	360
45	42	4	B–50	36	36	34	27
47	30	3	B–50	208	131	77	51
47	36	9	B–50	236	197	128	89
	Averages			225	203	178	175
29	20	5	None	38	36	27	–
33	12	3	"	206	186	154	134
33	14	5	"	224	230	217	202
33[1]	19	10	"	182	197	176	175
47	33	6	"	223	221	182	165
47	40	13	"	130	88	43	44
47	42	15	"	158	98	62	61
	Averages			166	151	123	130

[1] This animal was receiving 10 mg. of cortisone daily at the time of this test.

animals are compared to seven control curves in three animals. The average curve of each group does not show maximum fall since this occurred at different time intervals. The mean maximum fall in the treated series was 61 compared to 50 mg. per 100 ml. in the control series, an insignificant difference. It is clear that there is no effect of these sulfonylureas on the blood sugar in Houssay animals. Hypoglycemogenic compounds fail to act in the absence of insulin even in test animals that are extremely sensitive to insulin. This is strong evidence for the concept that these drugs have, of themselves, "none" of the action of insulin.

The administration of sulfonylureas in doses of 50 or 100 mg. per kg. intraperitoneally had no effect on the daily excretion of glucose by Houssay animals on a constant diet, but without insulin.

If these drugs acted by stimulating the secretion or potentiating the action of endogenous insulin, they might well be more effective in hypophysectomized animals, in which insulin sensitivity exists in the presence of an intact pancreas. Table 5 shows the results of a number of tests in hypophysectomized cats. The control tests show the occasional occurrence of slight spontaneous hypoglycemia, which is expected after this operation. The treated series shows an irregular response to the drug which is, however, quite significant on the average (mean decrease in mg. per 100 ml.=24-7=17; t=3.6). When one recalls that the initial blood sugar level is about 20 mg. per 100 ml. less than it is in normal animals (table 2), it is probable that the hypophysectomized cat has an essentially normal response to these drugs. There was no suggestion of increased sensitivity to hypoglycemia. From many tests on hypophysectomized cats in the past it is safe to say that if these drugs increased the secretion of insulin, the amount of insulin so added to the animals was certainly less than one unit, the amount which consistently causes a severe hypoglycemic reaction in hypophysectomized cats.

These results in hypophysectomized cats are unexpected in view of the report by Levine[7] of a greatly enhanced effect of BZ-55 after adrenalectomy in the rat. In a single adrenalectomized cat, tested with 50 mg. U-2043 per kg., repeated hypoglycemia and death occurred in spite of the administration of glucose. Hypophysectomy and adrenalectomy would be described as fairly similar in their effect on insulin sensitivity. However, in their effect on the response to sulfonylureas these operations are utterly different. This might well provide the background for future study.

DISCUSSION

The hypoglycemic response of normal animals to two sulfonylureas has been confirmed in the cat. The failure of depancreatized cats and of alloxan diabetic rats to react to the drugs confirms other reports. The results following pancreatectomy show that sulfonylureas do not act in the absence of insulin. This has been emphasized by the lack of action in Houssay animals which are very sensitive to insulin. The failure of alloxan diabetic rats to respond and the absence of

TABLE 5

Effect of sulfonylureas in hypophysectomized cats

Cat number	Days after hypox.	Drug[1] A=U-2043 B=BZ-55 mg. per kg.	Blood glucose at hours 0	2	4	6	8	Maximum decrease
			mg. per 100 ml.					
42	14	0	67	75	65	75	75	2
42	21	0	67	61	67	70	70	6
45	11	0	82	90	90	85		0
45	24	0	63	65	56	50		13
47	8	0	67	67	71	67		0
47	14	0	46	41	34	45		12
49	9	0	62	58	62	63		4
50	7	0	69	69	70	63		6
50	12	0	75	84	70	45		30
51	6	0	66	65	61	68		5
51	11	0	84	82	89	85		2
54	5	0	77	92	69	78		8
	Averages		69	70	67	66		7
42	11	A–50	95	47	57	60	63	48
42	16	A–50	62	51	36	51	70	26
45	10	A–50	56	23	19	18	19	37
45	11	A–50	39	39	45	58	54	0
45	23	A–50	67	38	31	44	42	36
47	12	A–50	55	77	66	59		0
49	7	A–20	79	62	54	57		25
49	10	A–100	65	36	32	32		33
50	8	A–100	59	35	32	80		17
50	5	B–50	77	72	71	66		11
51	4	B–50	77	59	57	64		20
51	7	B–100	67	36	51	52		31
	Averages		67	48	46	53		24

[1] Intraperitoneal administration: no anesthesia.

an increased sensitivity of hypophysectomized cats to the hypoglycemic action of these drugs are noteworthy. These results mean that the islands, which almost certainly produce some insulin in both types of animal, do not measurably increase their output of insulin when sulfonylureas are given. It is perhaps possible that the tendency to lowered blood sugar levels counteracts or offsets this stimulation although it does not do so in normal animals. In any case, no appreciable secretion of insulin occurs in the diabetic rat which needs it or in the hypophysectomized cat which ought to reveal the presence of extra insulin because of its characteristic sensitivity.

If these drugs had no effect on the secretion of insulin but enhanced or potentiated the insulin in the blood or tissues, one or both of these test animals ought to show it. These results, as well as clinical studies in which the patient's dose of insulin sometimes can and sometimes cannot be reduced, suggest that something more than the presence of insulin is needed to provide the conditions in which the drugs act. This thought is further supported by the unexpected difference between the response of hypophysectomized and that of adrenalectomized animals. De Bodo and Sinkoff[11] describe "the greater insulin sensitivity of the hypophysectomized dog . . . compared with that of the adrenalectomized dog." If this applies to other species, adrenalectomized cats or rats are not more sensitive to insulin than their hypophysectomized counterparts, but in our experiments differ directly in their response to sulfonylureas. Such a difference might aid in the elucidation of the action of these drugs in the future. At present one cannot tell whether the difference between hypophysectomized and adrenalectomized animals resides in the liver, the islands or other tissues. The evidence that sulfonylureas do not appreciably alter insulin secretion must in the long run be viewed with results such as those of Ashworth and Haist[6] who reported growth of the islands during prolonged treatment of rats with BZ-55.

SUMMARY

The effect of BZ-55 (carbutamide) and U-2043 (Orinase^{R}) has been observed on the blood sugar level and on experimental diabetes of certain types. Normal cats responded to these drugs with a fall in the blood sugar level. There was no demonstrable effect on the diabetes of alloxan diabetic rats or depancreatized cats untreated with insulin. There was no apparent effect

on the diabetes of three depancreatized cats treated with insulin, but the cat is not recommended for this type of study. The drugs failed to exert an effect on the blood glucose or daily glycosuria of hypophysectomized-depancreatized cats in spite of their known sensitivity to insulin. Hypophysectomized cats have an approximately normal response of the blood sugar in contrast to the great sensitivity of adrenalectomized animals to these drugs. The experiments indicate that these sulfonylureas have little or no effect on the secretion or potentiation of insulin unless this be the mechanism of its action in the adrenalectomized animal.

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Effect of Carbutamide on the Insulin Content of the Dog Pancreas

Mary A. Root, Ph.D., Indianapolis*

One of the mechanisms of action postulated for the hypoglycemic effects of the group of arylsulfonylurea compounds of which carbutamide† is a member, is a stimulation of the β-cells of the pancreas to secrete, or produce and secrete, more insulin. If this is the manner in which such compounds lower blood glucose levels, two possibilities exist as to the final result of such therapy. The islets of Langerhans may be stimulated to produce more or larger β-cells or the existing cells may be stimulated to secrete more insulin per unit of time. If this enhancement of insulin production can be maintained throughout many years, the stimulation would be beneficial to the mild diabetic. If the stimulation causes oversecretion of the cells and consequent exhaustion, however, a patient with mild diabetes might conceivably become a severe diabetic, a far from desirable effect.

In order to discover the effect of carbutamide on the insulin content of the pancreas, dogs were treated with the drug for various periods of time, killed, the pancreas extracted and the extract assayed for insulin content.

METHODS

Dogs, caged separately, were maintained in air-conditioned quarters and allowed water ad libitum. In experiment 1 the animals were fed Friskies dog food ad libitum and the daily consumption was measured. In experiments 2 and 3 the dogs were regulated on a single daily feeding of Pard dog food in an amount that would be eaten within the twenty-four-hour period (usually within one hour of feeding) and would produce a slow weight gain.

The dogs were weighed every other day. Placebo capsules containing acacia were given to control dogs in experiment 1 but not in experiments 2 and 3. Carbutamide was administered twice daily by mouth. The doses varied in the different experiments. Blood glucose

* Pharmacologist, Lilly Research Laboratories, Indianapolis, Indiana.

† Carbutamide, p-aminophenylsulfonyl butylcarbamide, is also known as BZ-55, and in Europe, by the trade names Nadisan and Invenol.