Toxicological Studies on Carbutamide

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In 1942, Janbon[1] first observed the hypoglycemic action of certain sulfonamides when he was investigating the antibacterial action of sulfanilamido-isopropylthiadiazoles. Loubatières[2] showed that the compound was inactive in depancreatized dogs and postulated that it brought about hypoglycemia in normal animals by stimulating insulin secretion. In 1955, Achelis and Hardebeck[3] and others [4, 5] reported on the hypoglycemic action of *p*-aminophenylsulfonyl butyl carbamide, carbutamide or BZ-55, in normal rabbits, in normal man and in some diabetic patients. Inasmuch as carbutamide has continued to lower the blood sugar of certain diabetics, long-term toxicity studies have been pursued.

METHODS

Acute toxicity. Albino mice, weighing 14 to 18 gm., were starved overnight prior to injection. For intravenous studies, a 10 per cent solution was prepared with a minimum of Na_2CO_3 (*p*H 8.2). A 20 per cent suspension in sesame oil was used for intraperitoneal injection and a 20 per cent suspension in 5 per cent acacia for the subcutaneous and oral tests. Starved albino rats, weighing 80 to 120 gm., were employed for the acute toxicity studies, a 20 per cent solution in Na_2CO_3 being used intravenously and a 40 per cent suspension in acacia for oral administration. All animals were observed for one week and deaths or any signs of toxicity recorded. The median lethal doses (LD_{50}) were calculated by the method of Bliss.[6]

Blood analyses. Blood sugar values were determined by the Hagedorn-Jensen method [7] and the blood carbutamide concentrations by a modified Bratton-Marshall procedure.[8]

Subacute toxicity. Seven albino New Zealand rabbits, weighing between 2.2 and 2.7 kg., were given daily doses of 1,000 mg. per kg. by stomach tube until each succumbed. A freshly prepared 25 per cent suspension in 5 per cent acacia was employed.

Chronic toxicity: rats. Essentially the same method reported previously from these laboratories was used.[9] Fifty-five female Harlan rats, weighing between 70 and 90 gm., were studied. Ten rats were fed a diet containing 1 per cent carbutamide and ten a diet with 2 per cent carbutamide; five rats on normal diet served as controls. Later, three groups of ten rats each were placed on 0.5 per cent, 0.25 per cent and normal diet respectively.

Chronic toxicity: dogs. Eighteen dogs, weighing from 5.3 to 10.0 kg., received daily doses of 12.5 to 500 mg. per kg. by capsule. Blood samples for various analyses were taken from the jugular vein and urine was obtained by catheterization.

Chronic toxicity: monkeys. A 15 per cent suspension of carbutamide was administered daily to eleven Rhesus monkeys by stomach tube. Blood samples for counts and analyses were taken from the cubital vein. All animals on chronic studies were submitted for necropsy at death or after sacrifice.

RESULTS

Acute toxicity. The doses used, number of deaths, and the calculated LD_{50}'s after intravenous, subcutaneous, intraperitoneal and oral administration to albino mice are shown in table 1. Similar data obtained from rats following intravenous and oral doses are also included.

After intravenous injections, mice had convulsions within one minute, followed by prostration and death in three to five minutes. Intraperitoneal median lethal doses of carbutamide produced deaths, after twenty-four to forty-eight hours, whereas larger doses killed in eighteen hours. Some deaths were recorded within twenty-four hours after subcutaneous or oral administration, while others occurred two to four days later. Rats responded similarly to mice to intravenous injections, and died from two to five days after oral administration. Some reduction in the blood sugar values was found; deaths were not attributable solely to hypoglycemia, as blood sugar values in rats were 104 to 122 mg. per 100 cc. just before death. Blood carbutamide concentrations were greater than 100 mg. per 100 cc. at the same time.

Subacute toxicity: rabbits. Three rabbits succumbed after three daily doses of 1,000 mg. per kg. Necropsy revealed hemopneumothorax caused by mechanical damage during administration. The other four rabbits survived 13, 38, 48 and 68 doses, respectively. All gained weight during the drug regimen, but died from pulmonary edema provoked by the entrance of the stomach tube into the trachea. Blood sugar values fell from

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around 100 mg. per 100 cc. to about 75 or 85 following the dose and returned to normal before the next dose. Blood carbutamide rose to around 40 mg. per 100 cc. about two hours after dosing and returned to a trough of less than 1 mg. per 100 cc. just before the next dose. To a large extent, the drug was present in the blood in the free form. In some rabbits conjugated carbutamide was found, but it rarely represented more than 20 per cent of the total present in the blood.

Chronic toxicity: *rats*. The effects on the growth curves of rats fed diets containing 0.25, 0.5 and 1.0 per cent of carbutamide are compared with those of control rats in figure 1. At the end of ten months all rats on the 0.25 per cent diet survived and remained on test. Two fed the 0.5 per cent diet died after injuries received while being restrained in a holder during blood sampling. No abnormalities were seen at autopsy. Two rats in the 1.0 per cent group died after 33 and 121 days, the former from malnutrition and central necrosis of the liver, the latter from malnutrition, bronchiectasis and crystalluria. Eight remained on test in the 0.5 per cent group after ten months and eight were still on test in 1.0 per cent group after fourteen months. Rats fed the diet containing 2 per cent carbutamide died after 29, 75, 84, 103, 117, 118, 131, 161, 191 and 205 days. Malnutrition was apparent in all and three showed crystalluria as well. Slight hypertrophy of the thyroid was also evident in about half of these animals.

Blood carbutamide and blood sugar values, determined on pooled samples from five rats in each group, are found in table 2. The carbutamide concentrations were greater than 70 mg. per 100 cc. blood in rats on the 2 per cent diet. No marked hypoglycemia has been found in rats in the chronic studies.

Chronic toxicity: *dogs*. Carbutamide has proved to be more toxic to dogs than to other species. The results are summarized in table 3. All dogs that received either 12.5 or 25 mg. per kg. daily remain on test; those on higher doses have succumbed. Necropsies revealed degranulation of the beta cells of the pancreas, hypertrophy of the thyroid and erosions of the gastric mucosa in the three dogs on 50 mg. per kg. Daily doses of 100 mg. per kg. produced the same pathological changes and in addition caused fatty metamorphosis of the liver in two of the three. Higher doses produced quicker deaths with marked hypertrophy of the thyroids and bleeding into the gastrointestinal tract.

Biweekly blood and urine analyses were made. They showed a fall in erythrocyte and leukocyte counts and a reduction in hematocrit and hemoglobin values in dogs on toxic doses. No changes in clot retraction or

TABLE 1

Acute toxicity of carbutamide in mice and rats

Species	Mode of administration	Dose gm. per kg.	No. died / No. used	$LD_{50} \pm$ S.E. gm. per kg.
Mouse	I.V.	1.25	0/10	1.92±0.08
		1.60	2/10	
		2.0	5/10	
	I.P.	2.0	2/10	2.10±0.04
		2.25	9/10	
		2.50	10/10	
	S.C.	2.0	0/10	2.64±0.11
		2.5	4/10	
		3.0	8/10	
	Oral	2.75	0/10	3.46±0.16
		3.30	6/10	
		4.0	7/10	
Rat	I.V.	0.8	0/5	0.98±0.04
		1.0	3/5	
		1.25	5/5	
	Oral	8.0	0/5	10.31±0.70
		10.0	3/5	
		12.5	4/5	

TABLE 2

Blood carbutamide and blood sugar levels in rats after being fed diets for nine months

Per cent drug in diet	Carbutamide mg. per 100 cc.	Blood sugar mg. per 100 cc.
0.25	10	97
0.5	15	104
1.0	32	124
Controls	—	114

TABLE 3

Chronic toxicity in dogs of daily oral carbutamide

Daily dose	Status	Effect on body weight	Blood level Trough	Blood level Peak
mg. per kg.			mg. per 100 cc.	mg. per 100 cc.
12.5	3 survive after 157 doses	Gain		
25	3 survive after 157 doses	Gain	4-6	10-14
50	Deaths after 28, 43 and 73 doses	Loss	4-12 25-46 (Terminal)	
100	Deaths after 32, 50 and 92 doses	Loss	20-40	69-100
250	Deaths after 22, 24 and 24 doses	Loss	39-60	95-111
500	Deaths after 8, 10 and 10 doses	Loss	—	—

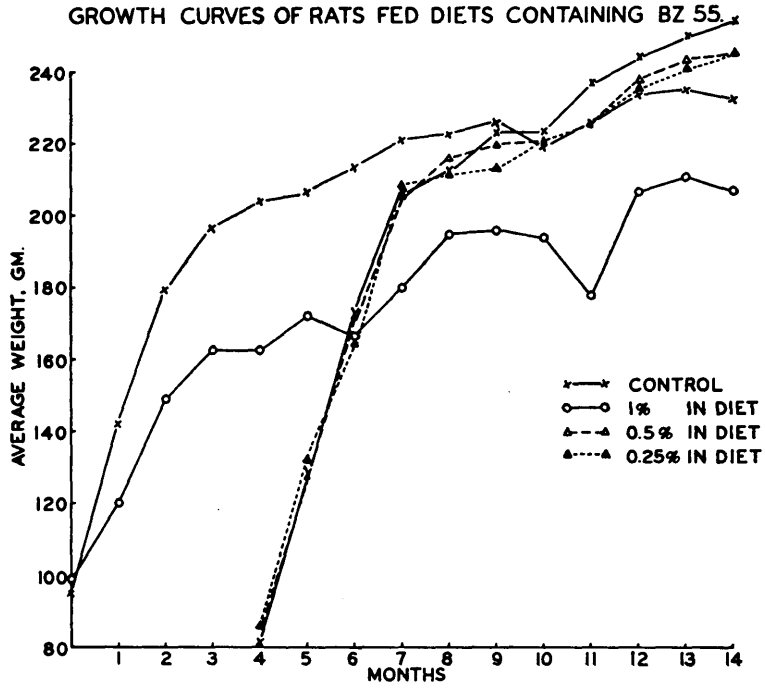

FIG. 1. Growth in rats being fed normal diet containing various per cents of BZ-55.

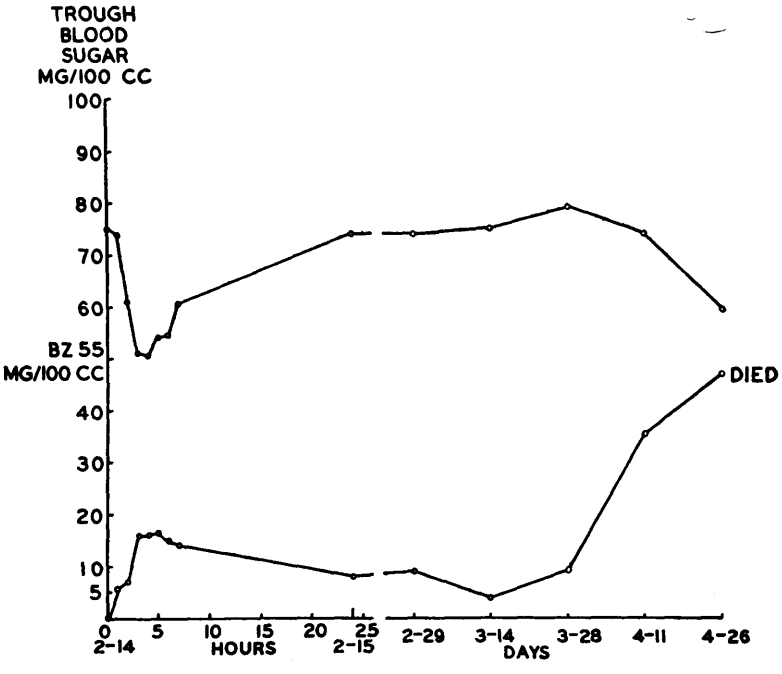

FIG. 2. Typical blood BZ-55 and blood sugar curves in a dog.

whole blood clotting times were noted. In some instances nonprotein nitrogen values rose just before death. No proteinuria or glycosuria was found.

Blood carbutamide and blood sugar curves were determined at repeated intervals. Those found in dog No. 6989 after the first dose on February 14 are plotted in figure 2. Repeated doses were well tolerated through March 28 and trough values of carbutamide — i.e., values found just before the next dose—remained low. Thereafter, however, carbutamide trough concentrations rose and remained within the toxic range during the two weeks preceding death. Figure 3 indicates that the blood sugar continued to fall after daily doses; the response obtained after the 157th dose tended to be similar to that which followed the initial dose.

Chronic toxicity: monkeys. Rhesus monkeys tolerated

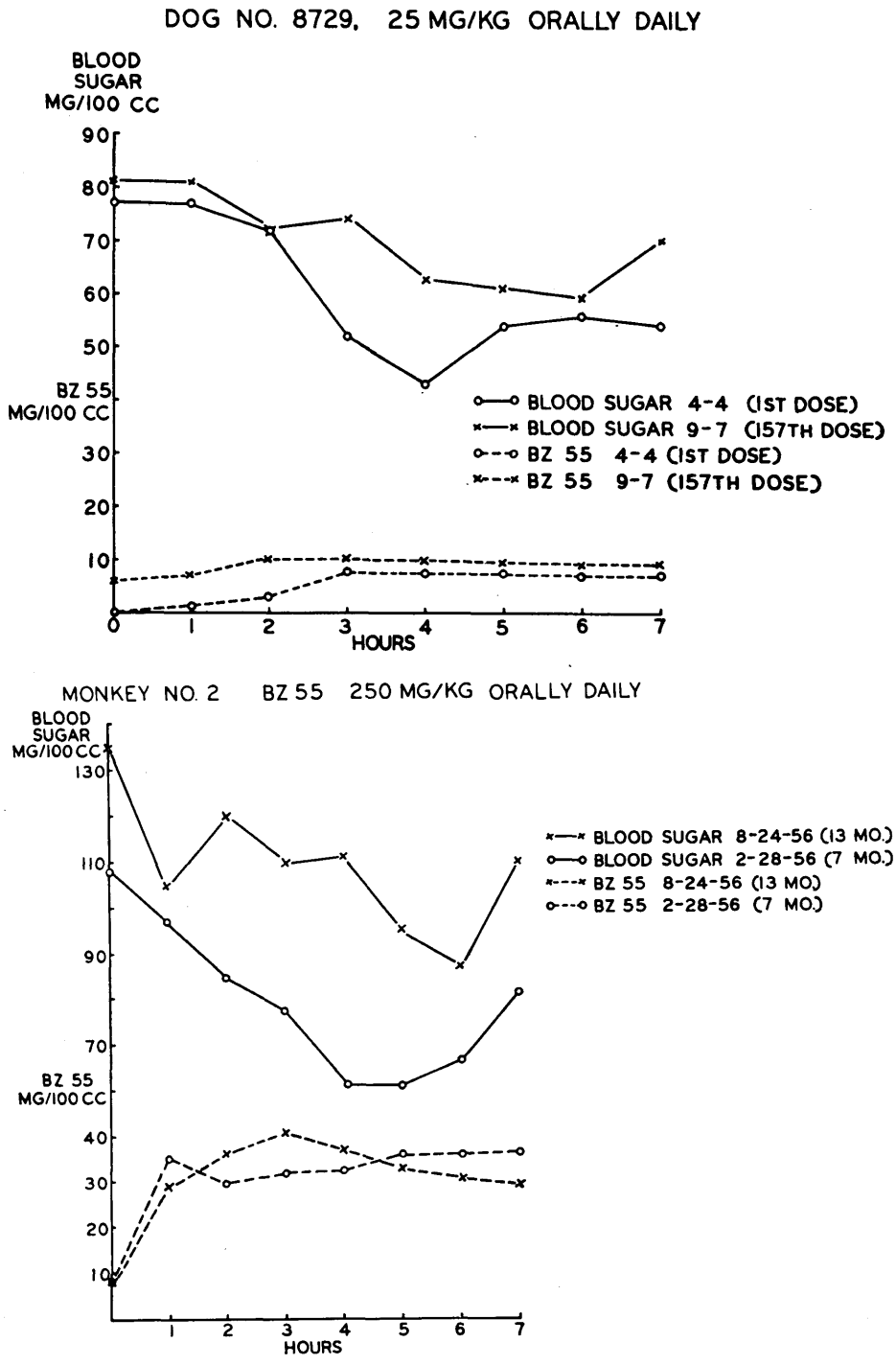

FIG. 3. Blood sugar curve and blood value of BZ-55 in response to the 157th dose of the drug in a dog.

FIG. 4. Response of blood sugar and drug concentration in a monkey after a long daily dosage of BZ-55.

large doses of carbutamide for an extended period of time (table 4). Seven are alive and remain on test. Four monkeys succumbed after repeated doses of 500 mg. per kg. daily. Pulmonary edema and hydrothorax were found in all with no other visceral damage evident.

The typical blood sugar response in monkeys is shown in figure 4, the fall obtained after thirteen months of daily treatments being about as great as that found six months earlier. Blood carbutamide curves also determined after seven and thirteen months were practically superimposable, which shows a lack of accumulation of carbutamide in these monkeys.

TABLE 4

Chronic toxicity in monkeys of daily oral carbutamide

Daily dose	Status	Effect on body weight	Blood level Trough	Blood level Peak
mg. per kg.			mg. per 100 cc.	mg. per 100 cc.
100	3 survive after 203 doses	Gain	2.0-6.0	18-29
250	3 survive after 287 doses and 1 after 421 doses	Minimal	6-18	40-50
500	Deaths after 8, 9, 25 and 73 doses	Loss	45	82

Frequent blood analyses revealed no marked changes in erythrocyte, leukocyte or differential counts, or hematocrit and hemoglobin levels, or whole blood clotting and clot retraction times, or in nonprotein nitrogen values.

DISCUSSION

Subacute and chronic toxicity studies have demonstrated marked differences among various species. Rabbits cleared carbutamide from the blood stream rapidly so that trough levels remained very low after repeated doses of 1,000 mg. per kg. Monkeys tolerated daily doses as large as 250 mg. per kg. for from 41 to 60 weeks. Here again the blood carbutamide trough levels were low and little or no conjugated carbutamide was found in the blood. Dogs, however, succumbed after doses of 50 mg. per kg. daily. As long as the blood carbutamide trough level remained below 10 mg. per 100 cc. the dogs gained weight and appeared normal. When the concentrations began to rise and clearance was reduced, toxic signs developed and death followed. Marshall et al.[10] showed that dogs do not acetylate sulfa drugs as do rabbits and men. Although, carbutamide is not conjugated to a large extent by rabbits, monkeys or man, it would appear that the detoxification mechanism in the dog differs from that in those species and that, when the rate of absorption exceeds the rate of destruction, toxic effects are produced.

SUMMARY

1. Carbutamide had a low order of toxicity in laboratory animals following single doses by various routes.

2. Rats, rabbits and monkeys tolerated large daily doses for an extended period of time. Dogs, however, survived doses only one-tenth the size of those given monkeys.

3. Rabbits and monkeys cleared carbutamide from the blood stream so that trough values remained low. Dogs appeared to lose the ability to detoxify carbutamide quickly.

4. Rabbits, dogs and monkeys continued to show a fall in blood sugar after repeated doses.

5. No visceral or hematopoietic damage attributable to the drug was found in rabbits or monkeys. Rats on high concentrations showed some crystalluria, malnutrition and hypertrophy of the thyroid. Dogs that received toxic doses had degranulation of the beta cells of the pancreas, hypertrophy of the thyroid and erosions of the gastric mucosa with a reduction in hematocrit and hemoglobin values, and a lowering of erythrocyte and leukocyte counts.

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COMMENT

DR. PAUL HARRIS (*Indianapolis*): Pathologic changes in the animals listed in Dr. Anderson's tables have not been impressive. Three rabbits died as a result of trauma following three daily doses of BZ-55; four others died of pulmonary edema following more prolonged daily dosage. No changes were seen in the other viscera.

Two rats given BZ-55 in the diet at a level of 0.5 per cent died of accidental causes and showed no lesions attributable to the drug. Two rats receiving the compound at a level of 1 per cent in the diet died; one showed malnutrition and necrosis of some cells in the center of nearly every liver lobule; the other also showed malnutrition and some crystals in the kidney pelvis Malnutrition was apparent in all of ten rats that died after receiving the drug in the diet at a level of 2

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

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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

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