Pharmacological Studies of a New Oral Hypoglycemic Drug. (23163)

GEORGES UNGAR, LOUIS FREEDMAN AND SEYMOUR L. SHAPIRO
Departments of Organic Chemistry and Pharmacology, U. S. Vitamin Corp., N. Y. City.

In the past 25 to 30 years, a number of synthetic hypoglycemic drugs, for oral as well as parenteral use, have been described. None have proven successful in the treatment of diabetes. Slotta and Tschesche(1) reported on the oral hypoglycemic action of some lower alkyl derivatives of biguanides (referred to here as formamidinyliminoureas). A systematic investigation was, therefore, undertaken involving the synthesis and evaluation of approximately 200 new mono- and disubstituted alkyl and aralkyl derivatives of formamidinyliminoureas.

The present report deals with the pharmacological properties of one of these derivatives. N'-beta-phenethylformamidinyliminourea HCl (DBI) which was found to be a highly active oral hypoglycemic agent in both normal and alloxan-diabetic animals.

Method. DBI, a water-soluble, crystalline compound, was given in solution by stomach tube unless otherwise stated. Blood samples were collected from animals fasted for 18 hours. *Blood sugar* was estimated by micromodification of the Folin-Wu method and expressed in mg/100 ml of blood. Glycogen was estimated by the method of Good, Kramer and Somogyi(2). *Diabetes* was produced by administration of alloxan; rabbits received 200 mg kg intravenously, rats 250 mg/kg intraperitoneally and Rhesus monkeys 150 mg kg intravenously. Rabbits and rats were used 5 to 7 days after injection. One monkey in which diabetes developed, reached a stable high blood glucose level 2 weeks after alloxan administration.

Results. Table I summarizes the results obtained in guinea-pigs, rats, rabbits, cats

TABLE I. Action of DBI on Blood Sugar in Normal Animals.

	Dose, mg/kg	Blood sugar, mg/100 ml, hr						No. of animals
		0	1	2	3	5	24	
Guinea-pigs, subcut.	5	74	68	66	69	63		4
	10	88	75	40	25	42		4
	20	85	88	33	17.5†	*		4
	28	82	94		10			4
	33	84	90		16	22		4
	65	79	7		*			4
Guinea-pigs, oral	20	83			80	56	86	4
	25	89			69	39‡	86	15
	30	83	79		62	34†	82	4
	40	75			47	20†		4
	50	93	100		29	17*		6
Rats (CFW strain), oral	50	72			75	65		4
	80	75			70	56		4
	120	68			56	32	66	4
Rabbits, oral	20	80			88	62	85	3
	40	88			66	45	82	4
	60	79			44	28†	76	4
Cats, oral	20	60				52		2
	40	70				39		2
	50	72				16*		2
Rhesus monkeys, oral	2	62			60	57		2
	5	63			53	46		3
	10	59			43	25	62	4
	15	66			36	12	58§	4
	25	58			5	*		2

* All died. No glucose given. † Two died. No glucose given. ‡ Five died. No glucose given. § Two out of 4 animals were given glucose between 5th and 7th hr.

and monkeys. After observing that DBI lowers blood sugar when given subcutaneously to guinea-pigs, further testing was done by oral administration. It is seen that hypoglycemia reached its maximum in 5 hours, and in all cases tested it was back to normal in 24 hours. The species most sensitive to the drug is the Rhesus monkey, followed by guinea-pig, rabbit, cat and rat. Variation was high even within the same species. DBI was tried in dogs, in which it failed to cause hypoglycemia, but produced a complex effect which will be reported later. Death by hypoglycemia occurred in a number of animals of all species investigated. This could be prevented by prolonged administration of glucose.

In alloxan-diabetic animals it was possible to bring the blood sugar to normal levels by appropriate doses of DBI (Table II). The correct dosage was reached by gradual increase in the daily dose, starting with the dose which was 50%-effective in normal animals. When the normal level was attained, the animals were maintained with the effective dose for 3 days in rats and 5 days in rabbits. In 2 cases, where animals required high doses of DBI, they died before normal blood sugar values could be reached. When DBI-treatment was discontinued, the blood sugar values rose slowly and reached pretreatment level in 4 to 7 days.

Fig. 1 shows effect of DBI on the alloxan-diabetic monkey. This animal was treated

TABLE II. Action of DBI on Blood Sugar in Alloxan-Diabetic Animals.

	Initial blood sugar*	Daily dose, mg/kg	Final blood sugar*
Rabbit	232	60	60
	177	50	66
	188	40	75
	418	200	200†
	190	90	95
	189	90	85
	198	100	98
Rat (CFW strain)	194	500	83
	326	1000	102†
	192	400	75
	161	150	82
	320	500	70
	257	1200	76
	265	450	87
	174	100	66

* mg/100 ml.
† Died before normal level could be reached.

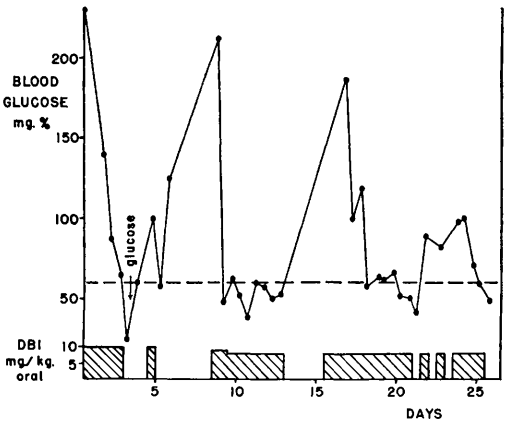

FIG. 1. Effect of oral admin. of DBI on blood glucose in alloxan-diabetic Rhesus monkey. Abscissa, time in days; ordinate, blood sugar levels in mg %. Broken line indicates avg blood glucose in normal monkeys. Dosage indicated at bottom of chart in mg/kg/day given in single oral admin. Blank spaces indicate intervals (usually weekends) when DBI was not admin.

for 26 days with oral administration of 10 mg/kg, later reduced to 9 and 8 mg/kg. Under the influence of treatment the blood sugar dropped from 228 mg % to normal and once to 16 mg %. The hypoglycemia was reversed by administration of glucose. It is seen that suspension of treatment always resulted in hyperglycemia which was promptly corrected by resumption of DBI administration.

The effect of DBI on liver and muscle glycogen is shown in Fig. 2. Five hours after administration there was a slight fall in liver glycogen (31%), not more pronounced than that observed after insulin. Twenty-four hours after treatment both liver and muscle glycogen levels were higher than in the controls. In alloxan-diabetic rats there was a marked glycogen increase in both liver and muscle 5 hours after treatment with DBI. The effect on glycogen, however, should be interpreted with caution because of the extreme variability of glycogen levels in control animals.

Evaluation of toxicity of DBI is made difficult by the fact that the animals die of hypoglycemia before any other toxic effects can be observed. Thus the LD_{min} in mice injected intraperitoneally was 100 mg/kg and the oral LD_{50} in guinea-pigs 40 mg/kg. Experiments

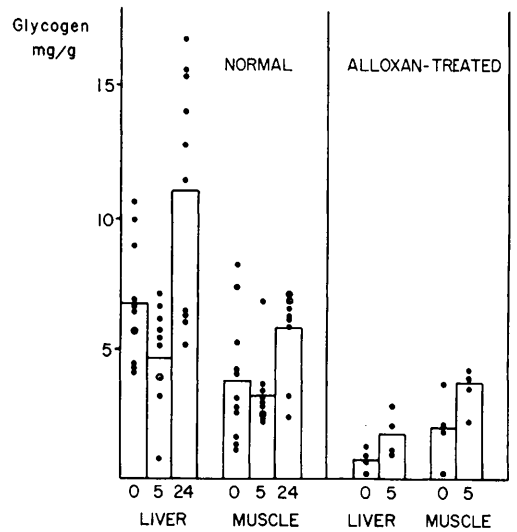

FIG. 2. Liver and muscle glycogen changes after oral admin. of DBI to normal guinea-pigs (20 mg/kg) and alloxan-diabetic rats (500 mg/kg). Abscissa: time in hr after admin. of the drug; ordinate: glycogen in mg/g.

now being conducted show that, under administration of glucose, the animals can tolerate very high doses of DBI. The results obtained in alloxan-treated animals also show that they can tolerate several times the median hypoglycemic dose of DBI without toxic effect. Two alloxan-diabetic animals died without evidence of hypoglycemia (Table II). At the high doses of DBI these animals were receiving, the toxic effect may have been independent of hypoglycemia. Ten guinea-pigs receiving 20 mg/kg of DBI orally for 2 weeks showed no untoward effect on their blood chemistry (other than a fall of blood sugar to 40 to 50 mg %), blood cells and coagulation. Pathological examination of tissues from these guinea-pigs and from a monkey which died as a result of hypoglycemia after a high initial dose of the drug (25 mg/kg), showed no damage in kidney, adrenals, liver, spleen, intestine, heart, pancreas, skeletal muscle, lungs, testes, lymph nodes and thymus.

Outside of its hypoglycemic effect, DBI has no marked acute pharmacological actions; at high doses in rats it has a slight depressive action on spontaneous motor activity and has a mild antidiuretic effect. On intravenous injection into dogs, it causes a transient rise of blood pressure; it has no potentiating or blocking action on epinephrine, acetylcholine or histamine.

Discussion. The hypoglycemic agent described above is different from other orally active drugs hitherto described. Under the conditions of our experiments, it did not cause significant and durable glycogen depletion. Results of pathological examination also show that no histologically detectable liver damage was produced by acute administration of a high dose or prolonged administration of what may be called a therapeutic dose. Unlike the sulfonylurea derivatives recently investigated, DBI was fully active in alloxan-diabetic animals. It is therefore probable that it can act in the absence of insulin. Preliminary experiments, which will be reported, suggest that DBI increases glucose uptake in the isolated rat diaphragm. This would suggest that DBI may act by a mechanism involving peripheral glucose utilization.

Summary. N'-beta-phenethylformamidinyliminourea (DBI), a synthetic substance, administered parenterally or orally, causes hypoglycemia in guinea-pigs, rats, rabbits, cats and Rhesus monkeys. It can also reduce blood sugar in alloxan-diabetic rats, rabbits and monkeys and maintain them at a normal level. DBI does not cause significant changes in glycogen content of liver or muscle. Further work will be necessary to elucidate its mechanism of action and assess its possible clinical usefulness.

1. Slotta, K. H., and Tschesche, R., *Ber. dtsch. chem. Ges.*, 1929, v62 B, 1398.

2. Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, v100, 485.

Received March 12, 1957. P.S.E.B.M., 1957, v95.