Comparison of McN-3495 [*N*-(1-methyl-2-pyrrolidinylidene)-*N'*-phenyl-1-pyrrolidinecarboximidamide], A New, Orally Effective, Hypoglycemic Agent, with the Biguanides

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SUMMARY

McN-3495 [chemical name: *N*-(1-methyl-2-pyrrolidinylidene)-*N'*-phenyl-1-pyrrolidinecarboximidamide], a new, oral, effective hypoglycemic agent that is structurally distinct from the biguanides, has been shown to be mechanistically distinct from the biguanides and to lack comparable side effects in animals. The biguanides (phenformin, buformin, metformin), which were active when glucose was given orally to fasted rats and dogs, failed to lower fasting blood glucose or improve glucose tolerance when the glucose was administered parenterally, thus suggesting that biguanides inhibit intestinal glucose absorption. McN-3495, however, lowered the fasting concentrations of glucose and increased glucose disappearance whether the glucose was administered orally or parenterally. Furthermore, intestinal glucose transport, measured by use of the everted sac technique, was inhibited after oral pretreatment of rats with buformin and phenformin but not with McN-3495. However, both phenformin and McN-3495 inhibited glucose transport when added directly in vitro, although McN-3495 did so at higher concentrations. The reasons for the differing actions of McN-3495 and the biguanides on intestinal transport in vivo and in vitro are discussed. New models for biguanide-induced lactacidemia using normal and streptozotocin-diabetic rats were developed. In addition, a number of the gastrointestinal side effects of phenformin use were observed in animal experiments. McN-3495, at doses above projected therapeutic doses, however, did not produce lactacidemia or comparable gastrointestinal side effects. Thus, McN-3495 is a novel oral hypoglycemic agent, probably safer than phenformin, that could provide better control of blood glucose in diabetic patients. DIABETES *27*:868-76, August, 1978.

McN-3495 [chemical name: *N*-(1-methyl-2-pyrrolidinylidene)- *N'*- phenyl-1- pyrrolidinecarboximidamide] is a new, orally effective, hypoglycemic agent that is structurally distinct from the biguanides. Data in the preceding paper (Tutwiler, G. F., Kirsch, T., and Bridi, G.: A pharmacologic profile of McN-3495 [*N*-(1-methyl-2- pyrrolidinylidene)- *N'*-phenyl-1- pyrrolidinecarboximidamide], a new, orally effective, hypoglycemic agent. DIABETES *27*:856-67, 1978) suggested that McN-3495 might also be mechanistically distinct from the biguanides since it could lower the blood glucose of fasting dogs or rats and could improve the tolerance to subcutaneously administered glucose in fasted diabetic and nondiabetic rats.

The exact mechanism by which the hypoglycemic biguanides lower blood glucose in man is still unknown. Recent animal and human studies[1-4] have indicated that the primary effect of moderate doses of the biguanides is an inhibition of intestinal glucose

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absorption. The results reported here give additional evidence in animals that the biguanides inhibit intestinal glucose transport. Also, evidence is presented that McN-3495 does not produce a similar effect. Comparative data on lactate changes and gastrointestinal side effects are also presented.

METHODS

Glucose Tolerance Tests in the Dog

Tests consisted of (a) a control glucose-tolerance test conducted on day 1 and (b) an experimental glucose-tolerance test on day 2 at various times after oral administration of compounds in 0.5 per cent methylcellulose. The oral glucose load was 1.75 gm. per kilogram body weight and all dogs were fasted for 18 hours. For intravenous glucose-tolerance test (IVGTT), the glucose was given as a rapid (two-minute) intravenous injection of 0.5–1 gm. per kilogram body weight as a 70 per cent sterile solution. Blood samples were taken before and at 4, 8, 12, 16, 20, 30, and 60 minutes after glucose administration. For most IVGTT studies, two consecutive IVGTTs were done on the same day separated by 60 minutes. The first IVGTT was started 60 minutes after vehicle was given and the second started 60 minutes after the drug was given.

A straight-line semilogarithmic plot of plasma glucose concentration versus time was obtained at from four to 20, 30, or 40 minutes after the injection of glucose. The rate of disappearance of plasma glucose, defined as KD (per cent glucose disappearance per minute), was obtained with a calculator programmed to yield a linear correlation between time and the natural logarithm of the plasma glucose. The regression lines generated from data collected in each dog before (vehicle) and after drug dosing were compared by student's *t*-test.

Details of the nutritional state of the beagle dogs and glucose analysis were described in the preceding paper. Three different salt forms of this compound were used. They were the tartrate (McN-3495-48), the iodide (McN-3495-15), and, for in vitro studies, the sulfate. All doses were calculated as the free base.

Glucose Tolerance Tests in the Rat

This procedure was described in detail in the preceding article in this journal.

Intestinal Transport

The intestinal transport of glucose was studied using the everted sac preparation of Wilson and Wiseman.[5] The entire small intestine of male Sprague-Dawley rats (180–210 gm.), fasted for 48 hours and killed by cervical dislocation, was rinsed thoroughly in situ with 0.9 per cent NaCl at 37° C. Three sacs of everted proximal ileum (each about 60 mm. in length) were prepared from each rat for in vitro studies (using a randomized distribution of sacs from several animals), and two or three sacs were prepared from animals that had been predosed with drugs (drugs given per os by mixing in 1 ml. of 0.5 per cent methylcellulose). Each sac was filled with 0.6 ml. Krebs-Ringer bicarbonate buffer, pH 7.4, containing 200 mg. per 100 ml. glucose. The outer, mucosal, incubation medium consisted of 10 ml. of the buffer containing the same concentration of glucose. When test compounds were added directly in vitro, they were added to the outer buffer solution. The sacs were incubated for one hour at 37° C. at 120 cycles per minute, with O_2 (95 per cent) and CO_2 (5 per cent) being delivered continuously to each flask by a hypodermic needle inserted through a serum cap. Glucose was determined by the glucose-oxidase procedure (Glucostat, Worthington Biochemicals). Without correction for small changes in volume in the serosal fluid or for the decrease in glucose concentration in the mucosal medium, the mean increase in the glucose concentration of the serosal fluid (in milligrams per 100 ml.) of the control and treated sacs were calculated and compared by the student's *t*-test. For each sac in the treatment group, the per cent inhibition was also calculated by comparison with the mean increase of glucose concentration for the controls.

Lactate Studies

For the studies using intraperitoneal administration, the drugs or vehicle were given to 24-hour-fasted male rats in 0.5 per cent methylcellulose at 24, 18, and two hours before bleeding. For repeated-dose oral studies, all animals were dosed with drug or vehicle at 72, 65, 48, 45, 41, 24, 21, 18, and 2 hours before obtaining blood samples. Body weight, food intake, urine output, and water intake were checked for abnormalities after three, six, and nine doses.

Streptozotocin-diabetic rats were prepared and treated as specified in the preceding paper. Blood samples were drawn by cardiac puncture into heparinized tubes and placed on ice, and duplicate 1-ml. samples were then taken and immediately deproteinized with 2 ml. of cold 0.6 N $HClO_4$. Duplicate deproteinized samples were taken for spectrophotometric determination[6,7] of L(+)-lactate by rabbit-muscle lactate dehydrogenase (Boehringer-Mannheim).

RESULTS

Glucose Tolerance Tests in the Dog

Phenformin and McN-3495 were compared in fasted dogs for their ability to improve the tolerance to intravenously administered glucose. A summary of these results is given in table 1. An example of the testing results with McN-3495 is shown in figure 1. Phenformin did not significantly increase the glucose disappearance rate or lower the fasting blood glucose, whereas McN-3495 both lowered the fasting blood glucose and increased the glucose disappearance rate in beagle dogs.

Phenformin (at 17 and 25 mg. per kilogram, per os) did lower, however, the peak plasma glucose concentrations after oral glucose administration to fasted dogs. These results are shown in table 2. Fasting plasma glucose levels were not lowered. Phenformin appears to be a less potent hypoglycemic agent than McN-3495 since it failed to improve glucose tolerance at 10 mg. per kilogram, per os. Statistically significant lowerings of plasma glucose of fasting dogs have been found (see preceding article) with doses of McN-3495 as low as 5 mg. per kilogram, per os. Lowered fasting plasma glucose and improved oral glucose tolerance were also found in dogs (two per dose) given 34, 25, 15, 10, and 5 mg. per kilogram, per os, but not 1 mg. per kilogram, per os, of McN-3495 before oral glucose administration.

Glucose Tolerance Tests in the Rat

The above findings in dogs were consistent with our findings in rats reported in the preceding article in this journal. Oral administration of McN-3495 but *not* phenformin was found to improve rat tolerance to parenterally administered glucose. These results are summarized in table 3 along with our findings using

TABLE 1

Effect of phenformin and McN-3495 on intravenous glucose tolerance in the dog

Treatment	Dose (mg./kg., p.o.)	Pretreatment (hr.)	KD (% Decrease of blood glucose per minute) Control	After treatment	P†	% Lowering of fasting blood glucose
Phenformin	50	1	3.6	3.4	>0.5	0
	50	0.5	4.2	4.2	>0.5	0
	50*	0.5	6.3	6.9	>0.5	17
	25	1	4.3	4.3	>0.5	0
	25	1	4.5	4.3	<0.2	0
	25	0.5	3.8	4.8	>0.2	9
McN-3495-15	25	1	4.2	5.7	<0.01	44
	15	1	6.4	9.6	<0.05	48
	15	1	6.9	9.8	<0.01	42
	10	1	5.1	5.7	>0.5	21
McN-3495-48	25	1	3.1	4.9	<0.001	27
	25	1	2.5	5.3	<0.001	25
	25	1	3.3	4.8	<0.01	35
	25	2	3.4	4.6	<0.01	11
	25	0.5	4.9	11.8	<0.001	13

*Sixty minutes after dosing, the dogs became emetic and ataxic and, at 120 minutes, hyperglycemic with respiratory depression, resulting in death.

†Slopes of regression lines generated from data collected in each dog before and after drug loading were compared by *t*-test.

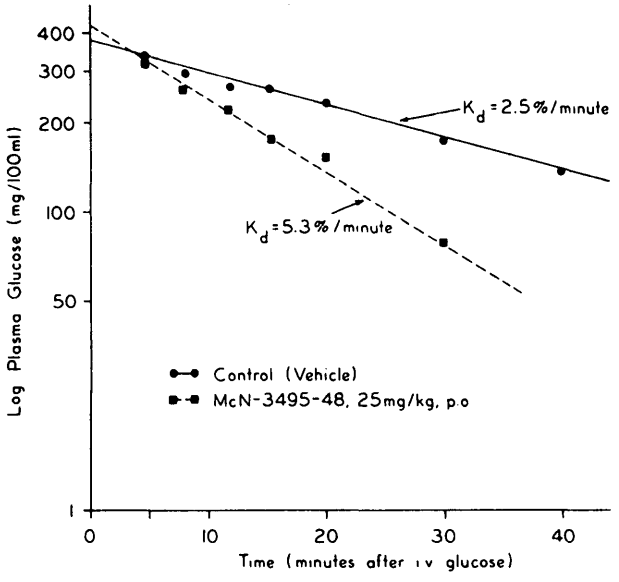

FIG. 1. Effect of McN-3495-48 on the disappearance of glucose of a fasted dog subjected to an intravenous glucose-tolerance test. (Drug or vehicle was given one hour before the test.)

TABLE 2

Effect of phenformin on oral glucose tolerance in the dog

Experiment number	Treatment	Dose (mg./kg., p.o.)	Plasma glucose (mg./100 ml.) at minutes after oral glucose 0	30	60	90	120	150	180
1	Vehicle	—	113	196	180	91	102	115	115
	Phenformin	25*	104	141	121	116	103	104	108
2	Vehicle	—	116	172	148	98	112	110	118
	Phenformin	25*	117	117	170	144	127	118	117
3	Vehicle	—	110	152	154	90	105	112	120
	Phenformin	25*	107	131	120	120	102	113	106
4	Vehicle	—	108	201	255	115	114	113	118
	Phenformin	17†	116	133	150	151	119	116	116
5	Vehicle	—	110	154	151	148	103	114	115
	Phenformin	10†	112	143	132	151	124	151	113
6	Vehicle	—	108	131	129	90	107	115	111
	Phenformin	10*	109	136	122	76	105	110	108

*Treated half an hour before test.
†Treated one hour before test.

the biguanides, buformin and metformin, and the biguanide-like compound, MK-270. It should be noted that phenformin also failed to improve tolerance to parenterally administered glucose when given intraperitoneally at maximally allowable doses in this species. However, at comparable or lower doses, all these biguanides and MK-270 were found, in our laboratory, to be active in rats when the glucose is administered orally instead of parenterally. An example of this data using phenformin is shown in figure 2.

Glucose Absorption in Isolated Rat Intestine

In everted intestinal sacs obtained from rats after pretreatment with buformin and phenformin, the glucose transport was inhibited. Pretreatment with McN-3495-48, on the other hand, did not significantly inhibit, in three separate experiments, glucose transport at hypoglycemic doses. The results of a typical experiment are shown in figure 3. The animals had been sacrificed two hours after dosing for these studies because phenformin has been reported[2,8] to produce maximal inhibition of glucose transport under these conditions, and the hypoglycemic effect of McN-3495 is also maximal. In other studies using shorter and longer pretreatment times with McN-3495, intestinal

TABLE 3

Effects of McN-3495 and the biguanides on parenteral glucose tolerance in the rat

Generic name	Drug Dose (mg./kg.)	Route	Pretreatment time (hr.)	Glucose route	Active	Inactive
Phenformin	100	p.o.	1	s.c.		x
	150	p.o.	2	s.c.		x
	200	p.o.	2	s.c.		x
	300	p.o.	2	s.c.		x
	100	i.p.	2	s.c.		x
	100	i.p.	1	s.c.		x
	150*	i.p.	1	s.c.		x
Buformin	250	p.o.	1	s.c.		x
Metformin	750	p.o.	1	s.c.		x
MK-270	240	p.o.	2	s.c.	x (slight)	
	160	p.o.	2	s.c.		x
	100	p.o.	2	s.c.		x
McN-3495-48	100	p.o.	1	s.c.	x	
	10	p.o.	1	s.c.	x	
	5	p.o.	1	s.c.	x	
	1	p.o.	1	s.c.		x

*Caused death of all rats by four hours after dosing.

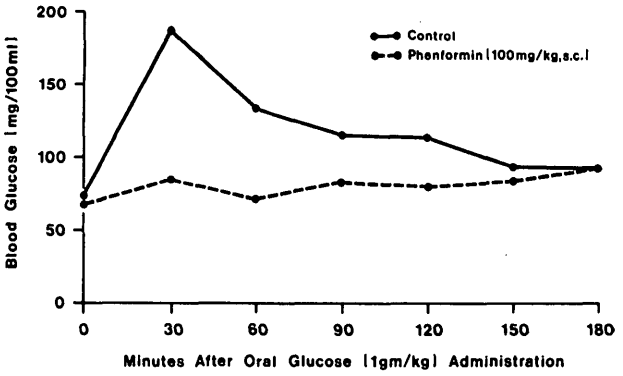

FIG. 2. Effect of phenformin on oral glucose tolerance in the rat. [Phenformin (100 mg./kg., subcutaneously) and vehicle were given to four rats each at 60 minutes before oral glucose administration. The blood glucose values shown are the mean results.]

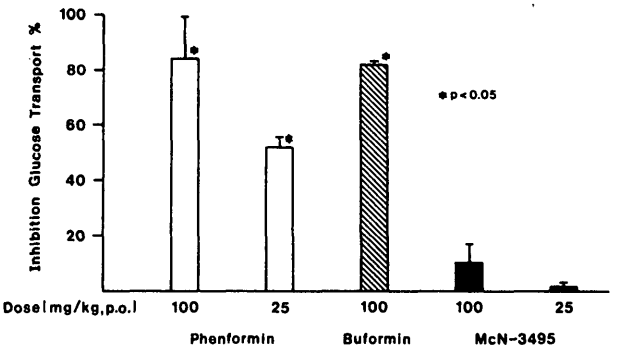

FIG. 3. Inhibition of glucose transport in intestinal sacs of rats pretreated with phenformin, buformin, and McN-3495-48. [Drug doses or vehicle were administered to three or four rats per dose group two hours before preparation of the everted intestinal sacs (three sacs per rat). Inhibition of glucose transport was calculated from the difference observed between tissues obtained from drug-treated and vehicle-treated rats.]

transport also was not inhibited.

When McN-3495 or phenformin was added directly to the incubation medium using the everted intestinal sacs, a concentration-dependent inhibition of glucose transport was observed. These results are seen in table 4.

Effect of Phenformin and McN-3495 on Blood Lactic Acid Concentrations in Normal and Diabetic Rats

Phenformin was found, in six separate experiments, to induce elevations of the blood lactic acid concentrations in fasted rats when given intraperitoneally (22 and 44 mg. per kilogram, intraperitoneally). McN-3495, on the other hand, did not raise lactic acid levels. The results of one experiment are shown in figure 4. Fasting blood glucose levels were lowered an average of 42 per cent with doses of McN-3495, while the fasting blood glucose levels in the phenformin-treated animals did not differ significantly from the controls. Synthalin A, at 10 mg. per kilogram intraperitoneally, an early hypoglycemic guanidine,[9] and the biguanides buformin (44 mg. per kilogram, per os) and metformin (44 mg. per kilogram, intraperitoneally) were also found to increase blood lactic acid in this model without altering blood glucose. These results are shown in table 5.

Blood lactate levels were also found to be elevated when rats were repeatedly dosed orally with phenformin over a 72-hour period (three times a day) (table 6). Rats dosed with McN-3495 did not show signifi-

TABLE 4

Inhibition of intestinal glucose transport by McN-3495 and phenformin in vitro

Experiment number	Compound	Concentration (mM)	Number of sacs	% Inhibition* ± S.E.M.
1	Phenformin	1.0	4	52 ± 10‡
		0.1	4	12 ± 11
	McN-3495	1.0	4	27 ± 16
		0.1	4	10 ± 15
2	Phenformin	0.9	4	62 ± 8§
		0.6	4	31 ± 6§
		0.3	4	25 ± 8†
	McN-3495	0.9	4	30 ± 13†
		0.6	4	5 ± 2
		0.3	4	13 ± 6
3	Phenformin	10.0	4	72 ± 4§
		1.0	4	38 ± 12†
	McN-3495	10.0	4	77 ± 10§
		1.0	4	15 ± 5

*Per cent inhibition of glucose transport compared with control glucose transport determined using sacs from the same animal (paired comparison). Significance determined by Student's *t*-test: †$P < 0.05$, ‡$P < 0.01$, §$P < 0.001$.

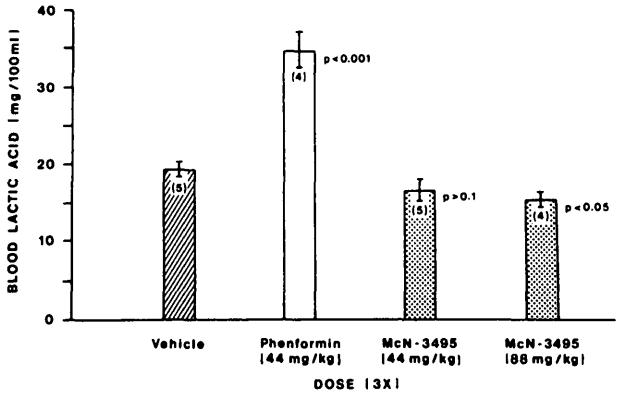

FIG. 4. Effects of intraperitoneal administration of phenformin and McN-3495 on blood lactic acid concentrations of fasted nondiabetic rats. (Numbers in parentheses are the numbers of rats studied.)

cant elevations. Also during experiment 3 (table 6), the effects of McN-3495 and phenformin on body weight, food and water intake, urine output, and blood glucose were measured (table 7). McN-3495 at 50 and 75 mg. per kilogram, per os, lowered both the fed blood glucose concentrations measured after three (11 to 16 per cent) and six doses (11 to 23 per cent) and lowered the fasted blood glucose levels (31 per cent) after nine doses. While lowering glucose, McN-3495 did not adversely affect the other parameters measured. Phenformin, on the other hand, produced anorexia, weight loss, and diarrhea.

Shown in table 8 is a typical example of our testing results in streptozotocin-diabetic rats fasted for five hours. Phenformin increased blood lactic acid levels, while McN-3495 did not. Blood glucose was lowered in all drug-treated groups in these rats fed ad libitum before drug administration. In other studies, slight elevations of blood lactic acid levels were also noted in streptozotocin-diabetic rats fasted for 21 hours and treated with phenformin (50–100 mg. per kilogram, per os) but not with McN-3495 (50 mg. per kilogram, per os).

DISCUSSION

Various mechanisms have been proposed to explain the therapeutic action of phenformin,[10-13] none of which has been satisfactorily proved in man. While

TABLE 5

Effects of buformin, metformin, and synthalin A on blood glucose and lactate concentrations of fasted rats

Treatment	Dose 3 × (mg./kg., i.p.)	Blood glucose* (mg./dl.)	Blood lactate (mg./dl.)	% Increase
Vehicle	0	117 ± 5†	6.6 ± 0.2†	
Buformin	44	119 ± 3	20.7 ± 2.5§	214
Metformin	44	115 ± 4	8.3 ± 0.5‡	25
Metformin	88	121 ± 11	9.5 ± 0.9‡	44
Vehicle	0	82 ± 2	12.8 ± 1.3	
Synthalin A	10	87 ± 4	32.9 ± 4.7‡	165

*Measured on duplicate blood samples taken when rats were killed by decapitation; for experiment with synthalin A, blood samples for glucose analysis were taken from the tail vein before killing.

†Mean ± S.E.M. Significance determined by student's *t*-test: ‡$P < 0.01$, §$P < 0.001$.

TABLE 6

Effects of repeated oral administration of phenformin and McN-3495-15 on lactic acid in normal rat blood

Experiment number	Treatment	Dose (mg./kg., p.o.)	No. of rats	Blood lactic acid (mg. %) Mean ± S.E.M.	P (Student's *t*-test)	% Change from controls
1	Control	—	4	21.3 ± 0.6		
	Phenformin	75	4	24.8 ± 1.5	<0.05	17
	Phenformin	100	4	36.5 ± 5.6	<0.05	72
2	Control	—	4	10.2 ± 2		
	Phenformin	75	3	19.7 ± 1	<0.02	92
	Phenformin	100	3	22.5 ± 3	<0.05	120
3	Control	—	4	11.0 ± 1		
	Phenformin	75	4	15.8 ± 0.3	<0.01	44
	Phenformin	100	4	16.0 ± 1	<0.05	46
	McN-3495-15	50	4	12.6 ± 1	<0.2	15
	McN-3495-15	75	4	9.9 ± 1	<0.2	0

TABLE 7

Effects of repeated oral administration of phenformin and McN-3495-15 on body weight, food and water intake, urine output, and blood glucose of nondiabetic rats

Treatment	Dose* (mg./kg., p.o.)	Mean % of controls† Body wt. increase	Food intake	H_2O intake	Urine output	Blood glucose
		After Three Doses				
McN-3495-15	75		83	73	74	89
McN-3495-15	50		80	104	108	84
Phenformin	75		74	72	72	84
Phenformin	100		55	55	66	80
		After Six Doses				
McN-3495-15	75	101	84	87	92	77
McN-3495-15	50	101	87	160	97	89
Phenformin	75	95	70	167	77	90
Phenformin	100	0‡	48	133	87	84
		After Nine Doses—Fasted for Previous 24 Hours				
McN-3495-15	75	100§	—	168	74	69
McN-3495-15	50	100§	—	79	74	69
Phenformin	75	0§	—	279	211	83
Phenformin	100	0§	—	279	216	86

*Four rats studied per dose group. All rats were fed ad libitum and then fasted 24 hours before being killed.

†For each parameter, the mean results for all drug treatment groups were divided by the mean results for the vehicle-treated animals and expressed as a percentage.

‡All lost weight.

§Taken after eight doses, phenformin-treated rats lost a mean of 4 gm. per rat.

comparing the pharmacologic activity of McN-3495 with that of the biguanides, we obtained results with phenformin that were consistent with only one of these proposed mechanisms, that is, that the biguanides inhibit alimentary glucose absorption. The biguanides failed to lower the blood glucose of nondiabetic or streptozotocin-diabetic rats fasted 24 hours. Also, as summarized in table 3, all the biguanides that were active in fasted rats when glucose was given orally failed to improve glucose tolerance when the glucose was administered parenterally.

Similar findings were also made using nondiabetic dogs. First, phenformin (table 2) failed to lower fasting glucose levels in dogs at doses that caused a flattening of oral glucose tolerance curves. In addition, the glucose lowering seen in the oral glucose tolerance tests is somewhat deceiving. Experiments 2 to 5 in table 2 demonstrate that, even though phenformin-treated dogs have low plasma glucose levels 30 minutes after glucose administration, the plasma glucose levels at later times are actually elevated. This kind of glucose-tolerance profile is generally interpreted to

TABLE 8

Effects of McN-3495 and phenformin on blood glucose and lactic acid of streptozotocin-diabetic rats

[Rats were dosed with drugs or vehicle and food was taken away five hours before bleeding by cardiac puncture for measurement of blood lactic acid. All rats were bled from the tail vein before dosing and at two and four hours after dosing for evaluation of the blood glucose–lowering activity of these compounds. Because of the variation in diabetic animal blood glucose, the per cent change of blood glucose from the pretreatment fasting blood glucose was calculated for each animal. Then the mean per cent change was calculated for each group. Tolbutamide (300 mg./kg., p.o.) was also given to four rats (predose blood glucose 404 to 438 mg./dl.) in this study. It lowered blood glucose 4 ± 5 per cent and 5 ± 7 per cent at two and four hours after dosing, respectively.]

Treatment	N	Range of blood glucose before dosing (mg./dl.)	% Change of blood glucose at hours after dosing Two	Four	Blood lactate (mg./dl.) Mean ± S.E.M.
Vehicle	4	404-449	7 ± 1*	13 ± 1	9.3 ± 0.7
McN-3495 (100 mg./kg., p.o.)	4	426-516	16 ± 4†	20 ± 2†	10.7 ± 1.1
Phenformin (200 mg./kg., p.o.)	4	359-471	12 ± 2	18 ± 2†	50.1 ± 4.4†

*Mean ± S.E.M.

†Significantly different ($P < 0.05$) from vehicle-treated rats.

indicate delayed stomach emptying or delayed absorption of glucose from the gastrointestinal tract.

Czyzyk et al.[1] and Hollobaugh et al.[4] pointed out that, in man, biguanides cause flattening of the glucose curve only after oral glucose administration but not after intravenous injection. Similar findings were reported here using dogs. Phenformin, at doses that flattened the glucose curves after oral glucose administration to dogs, did not increase the disappearance rate when the glucose was administered intravenously. Certainly there are differences in the metabolism of phenformin in rats, dogs, and man,[13] which leave in doubt the relevance of the above findings to the therapeutic action of phenformin in man. However, the finding that McN-3495 lowered the fasting blood glucose and improved glucose disappearance in rat and dogs whether the glucose was administered orally or parenterally suggests that different mechanisms exist for the hypoglycemic actions of McN-3495 and phenformin.

Kruger and co-workers[8] and Lorch,[2] using the everted sac technique, demonstrated an inhibition of intestinal glucose transport after oral administration of biguanides to normal and diabetic rats. The results in figure 3 confirm their findings and show that intestinal glucose transport was not inhibited when everted intestinal sacs from rats treated orally with McN-3495 were incubated in vitro at doses far above the minimum, effective, hypoglycemic dose.

Biguanides have also been reported to inhibit sugar transport when added directly to the medium in which everted sacs of rat[2] or hamster[14] intestine are incubated in vitro. These findings were confirmed here (table 4), and it was demonstrated that McN-3495 can also inhibit at high concentrations. The concentrations of phenformin used for these in vitro studies are high compared with the plasma levels achieved after a therapeutic oral dose of biguanides.[15] It has been shown, however, that the biguanides accumulate selectively in the gastrointestinal tract of rats, mice, and men.[16-18] Consequently, the intestinal concentrations used for these in vitro studies may well be achievable after an oral hypoglycemic dose of biguanides in animals. Therefore, even though inhibition of glucose transport was also seen with McN-3495 in vitro, the difference of its effects from those of the biguanides after pretreatment of rats may reflect either the ability of biguanides but not McN-3495 (P. O'Neill, McNeil Laboratories, private communication) to accumulate in the gastrointestinal tract or the lower potency of McN-3495 when it was added directly in vitro.

In addition to the above mechanistic differences, McN-3495 also may offer an improvement over phenformin treatment since it was found *not* to produce lactacidemia in normal and diabetic rats under conditions where biguanide-induced elevations were quite apparent. Lactate elevations after phenformin treatment using different experimental conditions have been reported for normal and alloxan-treated rats[22] and several other animal species.[22-24] Similar to these findings, we find that blood lactate concentrations can be elevated in phenformin-treated animals regardless of whether blood glucose concentrations are altered. Furthermore, we reported in the preceding paper that repeated administration of high doses of McN-3495 (60 mg. per kilogram per day) did not perturb the blood lactate concentrations of dogs. All the above findings suggest that use of McN-3495 may not be hampered by inappropriate lactic acidosis, which is the most serious drawback of phenformin treatment in patients who have cardiovascular and renal disease.[10,11,13,19-21]

The maximal dose of phenformin that can be used in man is limited by the side effects of anorexia, nausea, vomiting, diarrhea, malaise, weight loss, and abdominal pain. A number of these side effects of phenformin use were seen in our animal experiments. Phenformin, at admittedly high and nontherapeutic doses, produced anorexia with weight loss and diarrhea in rats. Nausea and vomiting were repeatedly observed when phenformin was administered to dogs (30 to 50 per cent of our experiments). McN-3495, on the other hand, at doses far above the minimum effective doses in animals, did not produce anorexia, diarrhea, or vomiting in any of our acute or chronic dog or rat studies. All our studies taken together suggest that McN-3495 is a safe oral hypoglycemic agent that deserves investigation in man as a potential therapeutic drug for diabetic hyperglycemia.

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