Effect of Biguanides on Intestinal Absorption of Glucose

Artur Czyzyk, M.D., January Tawecki, M.D., Jan Sadowski, M.D., Irena Ponikowska, M.B., and Zdistawa Szczepanik, Pharm. M., Warsaw, Poland

SUMMARY

In a group of fifty-eight patients with mild maturity onset diabetes, the double oral glucose loading test after Staub-Traugott, the intravenous glucose loading test with calculation of the glucose disappearance rates and intraduodenal glucose loading test were performed before and after phenethylbiguanide administration. The drug produced no changes in the intravenous glucose loading test, whereas it caused a significant lowering of the glycemia curves obtained in oral and intraduodenal loading tests. In order to complement these investigations, double fistula of the initial and distal segment of the small intestine were made in six dogs and perfusion of the intestine performed in situ with a glucose solution. Previous administration of butylbiguanide produced a marked flattening of the glycemia curve, and the recovery of glucose from the distal segment of the small intestine was more than doubled.

Suppression of intestinal glucose absorption by biguanide derivatives, demonstrated in the present study, could be explained by specific action of these compounds on the small intestine wall. Biguanide derivatives, accumulated in intestinal cells in higher concentrations than in other tissues, could impair glucose utilization by suppressing the synthesis of ATP required for active absorption of glucose. DIABETES *17*:492-98, August, 1968.

Soon after the introduction of biguanide derivatives, attention was called to the occurrence of abdominal discomfort in diabetic patients treated with these drugs.[1] This fact suggested that biguanide derivatives might in some way affect the absorption of glucose in the small intestine. Investigations performed so far have failed to elucidate this point, however. Biro et al.[2] observed that phenethylbiguanide decreased considerably glucose absorption in the small intestine of the rat. According to Creutzfeldt et al.[3] as well as Förster et al.,[4] butylbiguanide induced the foregoing effect to a slight degree only, whereas it markedly delayed evacuation of glucose from the stomach into the duodenum. Consequently, an indirect effect on intestinal absorption of glucose, consisting in an impairment of the passage from the stomach to the subsequent parts of the alimentary tract, could be involved.

The present paper describes the effect of previous phenethylbiguanide administration on the course of glycemia after intravenous, oral and intraduodenal glucose loading in diabetic patients. In addition, the effect of butylbiguanide on the course of glucose perfusion of the small intestine was evaluated in dogs with a double fistula of the proximal and distal segments of the small intestine.

MATERIALS AND METHODS

The material comprised fifty-eight patients with mild maturity onset diabetes; in the majority of cases, diet was the only treatment applied. In five patients treated with tolbutamide, the drug was discontinued one week prior to the first test. The following glucose loading tests were performed:

1. Double oral loading with 50 gm. glucose after Staub-Traugott, administered at an interval of 90 min. Blood sugar levels were determined at fasting and then at intervals of 30 min., over 240 min. (ten patients).

2. Rapid intravenous loading with 0.33 gm. glucose/kg. body weight (0.66 ml. of 50 per cent glucose solution). Blood sugar levels were determined at fasting and then at intervals of 10 min. over 60 min.; glucose assimilation coefficient K was calculated after Conard[5] (nine patients).

3. Rapid intravenous glucose loading, as described above, performed 90 min. after oral ingestion of 50 gm. glucose. In this double (oral and intravenous) glucose loading test, blood sugar levels were determined at fasting and then at 30-min. intervals, over 90 min. after oral glucose ingestion. Then the levels were determined at intervals of 10 min. over 60 min. after rapid intravenous injection of 50 per cent glucose solution. Glu-

From the Second Department of Medicine, Central Clinical Hospital, 137 Wolowska, Warsaw, and from the Second Department of Surgery, Warsaw Medical School, Warsaw, Poland.

cose assimilation coefficient K was also calculated (nine patients).

4. Intraduodenal glucose loading. After introduction of the probe into the duodenum under X-ray control, a drip of 250 ml. of 20 per cent glucose solution was administered over 15 min. No side effects occurred at this infusion rate. Blood sugar levels were measured at fasting and then at 15-min. intervals, over 180 min. from the beginning of glucose drip (thirty patients).

All glucose loading tests were performed twice in the same subjects, at an interval of about one week. The second loading tests were performed after previous phenethylbiguanide administration. In groups 1, 2, and 3, the patients were given 150 mg. phenethylbiguanide daily for three days. On the fourth day the whole daily dose was taken in the morning, one hour before the second glucose loading. Group 4 was divided into three subgroups, each comprising ten subjects and differing with regard to the dosage of phenethylbiguanide administered prior to the second intraduodenal glucose infusion. In subgroup *a* the drug was given as a single dose of 150 mg. one hour prior to glucose administration. In subgroup *b* 100 mg. were given daily for three days, and 100 mg. were administered on the fourth day, one hour prior to glucose loading. In subgroup *c* the regime was the same as in subgroup *b*, except for the magnitude of the daily dose which amounted to 150 mg. These doses had induced vomiting in two patients, and the latter were not included in the present tabulation. Nearly one half of the patients complained of discrete side effects, e.g., bad taste and heavy feeling in the epigastrium.

Intraintestinal glucose solution infusions were carried out in dogs with previously made double fistulae of the proximal and distal parts of the small intestine. The principle, according to which the fistulae were made, is schematically presented in figure 1. This type of fistula permits perfusion of the small intestine in situ and at the same time it allows of free passage of intestinal contents as well as prevent losses of intestinal juice in the intervals between the consecutive experiments. Tests were performed in six dogs weighing 9 to 12 kg. After surgery and healing of the operation wound, the unanesthetized animal was immobilized on a stand. Two probes of a length exceeding that of the intestinal segment with a centripetal course of the peristaltic wave were introduced into both fistulae. After the determination of the initial blood sugar level, a drip of 10 per cent glucose solution amounting to 20 ml. per kg. of body weight (2.0 gm. glucose per kg. of body weight)

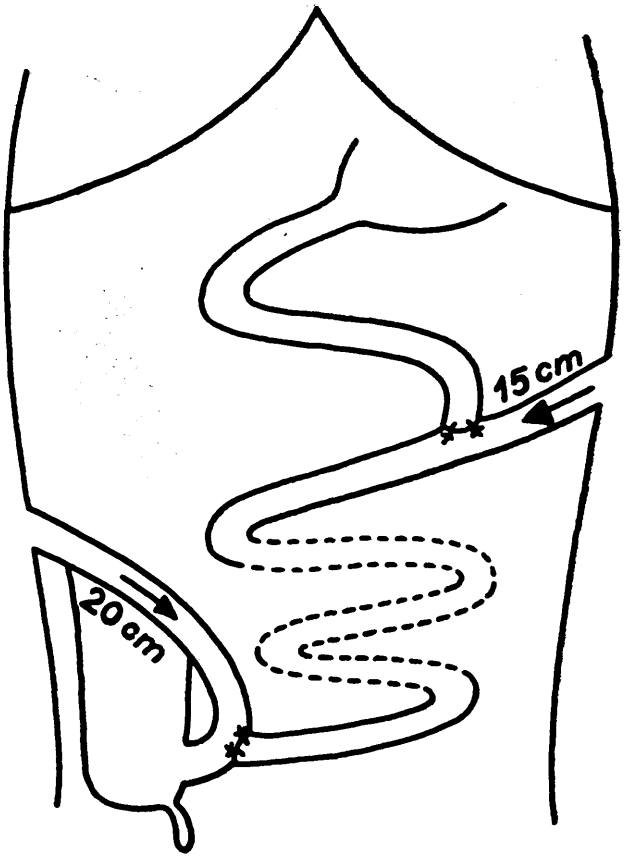

FIG. 1. Scheme of double fistula of the initial and distal part of the small intestine (T-system) in dog. Arrows indicate the direction of intestinal peristalsis. This system of fistulae permits isolated perfusion of the small intestine in situ in unanesthetized dog, whereas in the intervals between experiments it allows of free internal passage of small intestine contents and prevents losses of intestinal juice.

was administered into the proximal segment of the small intestine for 15 min. Blood sugar levels were determined at 15-min. intervals over 165 min. from the beginning of the drip. At the same time, intestinal juice was collected over three hours through the probe located in the distal segment of the small intestine, and its glucose content was determined. The experiment was repeated three days later. This time, one hour prior to intraintestine glucose drip, butylbiguanide was administered through the probe over 15 min. into the intestine. The drug was given in a dose of 10 mg./kg. of body weight, the required amount having been dissolved in 50 ml. of saline.

True glucose was determined after King and Garner[6] in the capillary blood of humans and venous blood of dogs. Mean values and standard errors were calculated for the respective groups.

RESULTS

Oral and intravenous glucose loading

In the group of ten patients with mild diabetes, the course of glycemia observed after double loading with 50 gm. glucose was typical for this form of the disease; mean values of the first and second peak of the glycemia curve were 230 and 210 mg. per 100 ml. respectively (figure 2). This glycemia curve was markedly modified due to previous administration of phenethylbiguanide. The course of the individual curves and of the mean one was distinctly lowered, and the differences in blood sugar levels at 30 and 60 min. were statistically significant. It is noteworthy, however, that notwithstanding the clear-cut flattening of the curve, its two-peak character and marked decrease in glycemia towards the end of the test were preserved. These findings suggest that the modification of glycemia course cannot be explained by impairment of stomach evacuation alone.

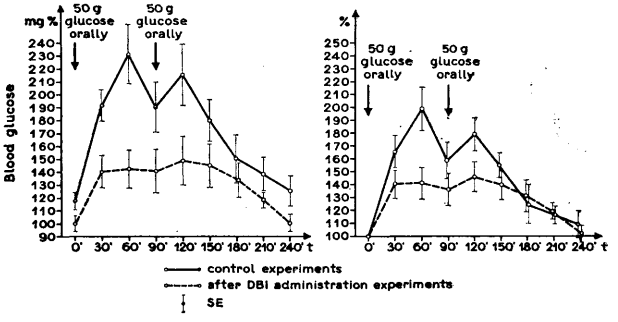

FIG. 2. Mean glycemia curves after double oral glucose loading in ten patients with mild diabetes, prior to and after phenethylbiguanide administration in a daily dose of 150 mg. lasting over three days. On the day of the second test, the patients took the whole daily dose of the drug one hour prior to glucose ingestion. Differences in the glycemia values in the 30th and 60th min. of both curves were significant ($p < 0.02$ and $p < 0.01$, respectively).

In contrast to the oral glucose loading test, that involving intravenous loading failed to be affected by previous phenethylbiguanide administration, when evaluated on the basis of the K value. This applies to the K values determined before and 90 min. after oral glucose ingestion. At this time there is presumed to be increased insulin secretion, which could account for its somewhat higher mean K values (table 1). The difference between the effects of previous phenethylbiguanide administration upon the course of the oral and intravenous glucose loading tests is clearly expressed in the double (oral and intravenous) glucose loading test. In contrast to oral ingestion of glucose which induces a pronounced flattening of the glycemia curve, as in the Staub-Traugott test, the mean glycemia curve obtained after rapid intravenous glucose injection exhibits a similar course in both groups of tests (figure 3).

TABLE 1

The course of intravenous glucose loading in phenethylbiguanide-treated diabetics

	Coefficient of glucose assimilation	
	On fasting n = 9	90 min. after oral administration of 50 gm. glucose n = 9
Control	0.80±0.11	1.31±0.10
Phenethylbiguanide treatment	0.75±0.07	1.24±0.11

Intraduodenal glucose loading

This test was designed to eliminate the possible effect of impaired stomach evacuation upon glucose absorption in the small intestine. The highest sugar levels were noted 30 min. after the beginning of the intraduodenal glucose drip; then they gradually decreased and fell to the initial values after a lapse of three hours from the beginning of the test. In all three subgroups, previous phenethylbiguanide administration produced a flattening of the glycemia curve. The degree to which the glycemia curve was modified was related to the magnitude of the dosage. When phenethylbiguanide had been administered as a single dose or in smaller doses, the difference between the mean glycemia curves showed a lower level of significance than in the case of higher doses, such as had been given in groups 1, 2, 3. As in the case of oral glucose administration, the greatest difference in the course of the glycemia curves was observed in the initial stage of the test, whereas towards its end the course of the mean glycemia curves became more similar (figure 4, 5 and 6).

Glucose drip into the small intestine of the dog

In all six dogs receiving a glucose drip into the small intestine, previous butylbiguanide administration caused a decrease in the blood sugar values as compared with control tests. At the same time, glucose recovery from the distal segment of the small intestine was distinctly increased in five dogs (table 2). In control tests, the highest values were noted after a lapse of 30 to 45 min. from the beginning of the drip, whereas they fell to the initial values after 150 min. from the drip. Previous intraintestinal administration of butylbiguanide in a dose of 10 mg./kg. of body weight induced a flattening of the glycemia curve. This effect was particularly prominent in the initial stage of the

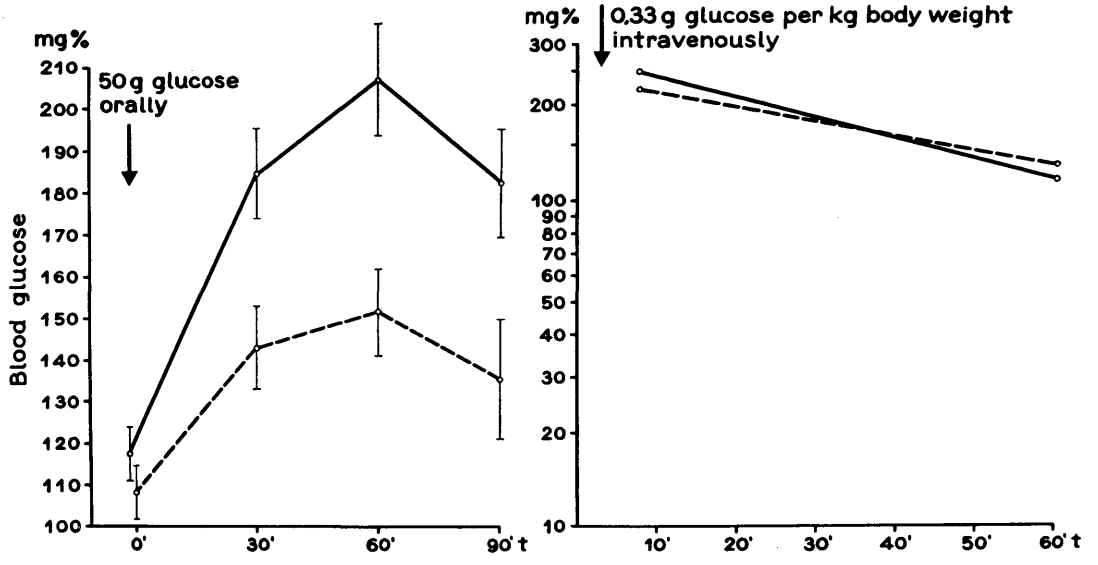

FIG. 3. Mean glycemia curves after double (oral and intravenous) glucose loading in nine patients with mild diabetes, prior to and after phenethylbiguanide administration in a daily dose of 150 mg. lasting over three days. On the day of the second test, the patients took the whole daily dose of the drug one hour prior to glucose ingestion. In the first part of the test, beside the mean glycemia values, the standard errors are also given. Differences in the glycemia values were significant in the 30th, 60th and 90th min. of the test ($p = 0.02$; $p < 0.02$ and $p < 0.05$, respectively).

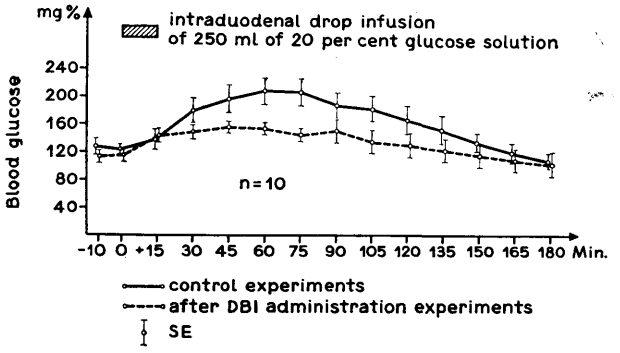

FIG. 4. Mean glycemia curves after intraduodenal drip amounting to 250 ml. of 20 per cent glucose solution, in ten patients with mild diabetes, prior to and after single administration of 150 mg. phenethylbiguanide given one hour before the beginning of the intraduodenal drip. Differences in the glycemia values of both curves were significant in the 60th and 75th min., counting from the beginning of the drip ($p < 0.05$ and $p < 0.02$, respectively).

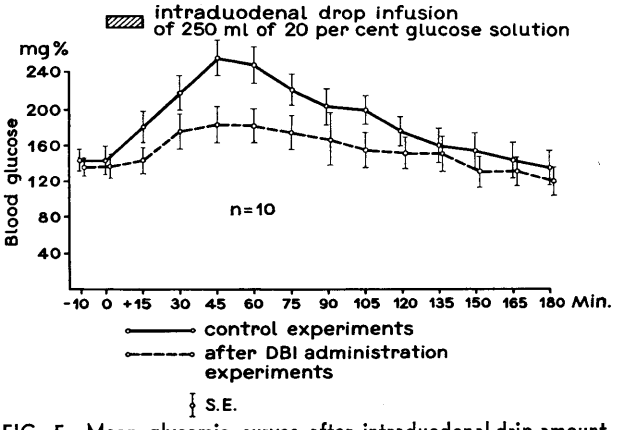

FIG. 5. Mean glycemia curves after intraduodenal drip amounting to 250 ml. of 20 per cent glucose solution in ten patients with mild diabetes, prior to and after phenethylbiguanide administration in a daily dose of 100 mg. lasting over three days. On the day of the second test, the patients took 100 mg. phenethylbiguanide one hour prior to the beginning of the drip. Differences in the glycemia values of both curves were significant in the 45th and 60th min. counting from the beginning of the drip ($p < 0.05$ and $p < 0.05$, respectively).

test, corresponding to intensified glucose absorption, and was reflected by a high peak of the glycemia curve in control tests. Notwithstanding the above mentioned flattening, the mean glycemia curve preserved its characteristic shape, with a clearly outlined peak during the first hour of the test and distinct lowering after two hours (figure 7). After previous butylbiguanide administration, mean glucose recovery from the distal segment of the small intestine was over two times higher than in the control tests. That is, the dose of glucose administered as intraintestinal drip was 18 to 24 gm.; consequently, mean glucose recovery from the distal segment of the small intestine amounted in the control tests to about 25 per cent and after previous butylbiguanide administration to about 50 per cent of the dose given. According to these results, the flattening of

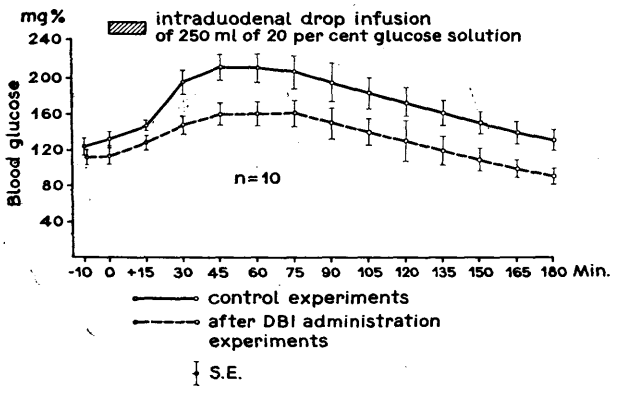

FIG. 6. Mean glycemia curves after intraduodenal drip amounting to 250 ml. of 20 per cent glucose solution, in ten patients with mild diabetes, prior to and after phenethylbiguanide administration in a daily dose of 150 mg. lasting over three days. On the day of the second test, the patients took 150 mg. phenethylbiguanide one hour prior to the beginning of the drip. Differences in the glycemia values of both curves were significant in the 15th, 30th, 45th, 60th, 90th, 150th, 165th and 180th min., counting from the beginning of the drip ($p = 0.05$; $p = 0.01$; $p = 0.02$; $p = 0.05$; $p < 0.05$; $p = 0.05$; $p < 0.05$ and $p < 0.02$, respectively).

the glycemia curve after intraintestinal glucose drip, observed in the case of previous butylbiguanide administration, is not related to the disturbances in small intestine peristalsis but results from a distinct impairment of the intestinal absorption of this sugar.

DISCUSSION

In the first reports dealing with the effect of biguanide derivatives upon the course of the oral glucose tolerance test, no mention was made of the considerable modification of the glycemia curve due to application of these drugs; critical examination of these results showed, however, that the mean glycemia curves obtained after glucose loading, in the case of simultaneous administration of biguanide derivative, were lower than those of the control tests. Owing to the small amount of data, these results cannot be duly evaluated.[7-9] Gutsche[10] has investigated the effect of butylbiguanide upon the course of glycemia after double oral glucose loading, according to Staub-Traugott, in a group of twenty-one patients with maturity onset diabetes. He observed a mean decrease in glycemia by 12 per cent, without, however, any modification of the shape of the curve, which remained typical for diabetes. A comparison of the investigations of Gutsche with the present experiments, shows that this author's patients had higher blood sugar values. Moreover, all diabetic patients had been grouped together, regardless of the results of butylbiguanide treatment. The finding of a distinct flattening of the glycemia curve after double oral glucose administration observed in six normal subjects after previous daily administration of 150 to 200 mg. butylbiguanide, over fourteen days, is also noteworthy.[10] Pereira et al.[11] have recently observed in a group of fifteen normal subjects a pronounced flattening of the glycemia curve in the glucose tolerance test, after a single dose of 200 mg. phenethylbiguanide; the difference was maximum at 60 min. of the test.

These observations are in good agreement with the present results. The preservation of the two-peak character of the glycemia curve in spite of its lowered course after double oral glucose loading, as well as the distinct flattening of the glycemia curves in the case of intraduodenal glucose administration, indicates that an impairment of stomach evacuation cannot exert a decisive effect upon the modification of the glycemia curve, observed after oral administration of glucose and phenethylbiguanide. Furthermore, experiments in dogs with isolated perfusion of the small intestine in situ with a glucose solution disclosed evidence of a butylbiguanide-induced impairment of glucose absorption.

The decrease in intestinal absorption of glucose and perhaps of some other food constituents effected by

TABLE 2

Glycemia curves and glucose recovery after intraintestinal glucose infusion in dogs before and after butylbiguanide administration

No.	No. of dog	Glycemia (mg. per 100 ml.) in control experiments												
		—15	0	15	30	45	60	75	90	105	120	135	150	165
1.	256/6	76	83	123	127	139	130	119	102	119	123	116	99	92
2.	236/6	80	86	156	214	206	154	83	74	72	76	66	72	66
3.	309/6	81	77	126	177	167	154	147	122	113	107	94	80	80
4.	449/6	81	82	84	129	174	142	142	134	142	119	93	74	56
5.	832/6	80	80	140	186	162	140	126	112	92	84	73	78	72
6.	136/7	71	73	92	100	114	89	87	84	75	72	67	57	45
Mean values		78	80	120	155	160	135	117	104	102	97	85	77	68

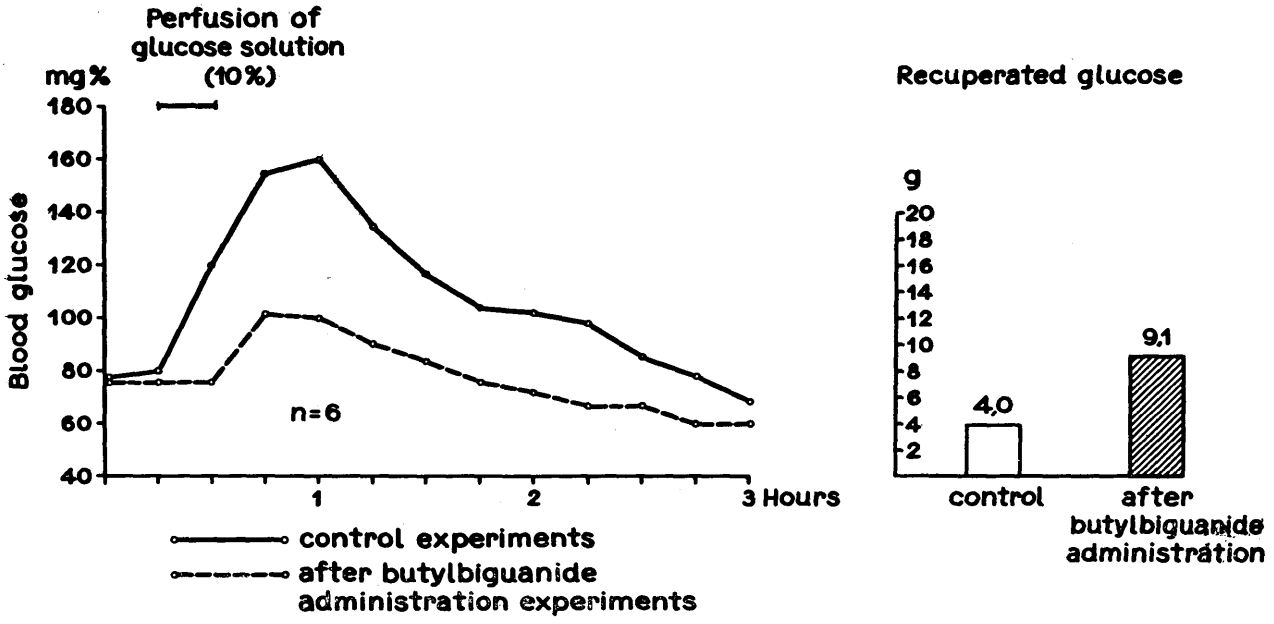

FIG. 7. Mean glycemia curves and mean values of glucose recovered from the distal segment of the small intestine, in six dogs, after intraintestinal drip of 10 per cent glucose solution in the amount of 20 ml./kg. body weight, prior to and after butylbiguanide administration as a single dose of 10 mg./kg. of body weight given one hour before the beginning of the glucose drip.

biguanide derivatives is not surprising and may be related to the metabolic effects of these drugs. The transport of glucose from the small intestine lumen to the portal vein system across the intestinal barrier is well recognized as an active process related, among other factors, to the presence of ATP in the intestinal epithelium cells.[12] It is conceivable that biguanide derivatives affect cytochrome oxidase,[13,14] succinic oxidase, or some other stages of the tricarboxylic acid cycle,[15] and impair the transfer of high-energy bonds to ADP,[16,17] thus decreasing the supply of ATP in the intestinal epithelium cells. These effects of biguanide derivatives had been observed at drug concentrations considerably exceeding those occurring in blood plasma after therapeutic doses.[18] But studies of C-14-labeled biguanide derivatives have shown that these drugs, administered orally and parenterally, accumulate in the intestinal wall and at considerably higher concentrations than in other tissues.[19,20] This tropism of biguanide derivatives for the cells of the small intestine wall suggests that the processes of inhibition of tissue respiration, leading to ATP deficiency, may occur in the intestinal epithelium cells after administration of therapeutic doses of the discussed compounds.

It is not known to what extent the effect of biguanide derivatives on intestinal absorption of glucose could contribute to their therapeutic action in diabetes. It is possible that their effect in diabetes associated with

TABLE 2 (*continued*)

Glycemia (mg. per 100 ml.) in experiments involving butylbiguanide administration															Glucose recovery (gm.)	
—75	—60	—15	0	15	30	45	60	75	90	105	120	135	150	165	Control	After butyl-biguanide
58	61	68	62	79	83	86	80	74	67	70	71	73	74	74	12.9	19.0
82	84	75	75	118	118	112	97	103	96	84	84	74	54	47	7.1	13.4
78	79	70	76	78	97	88	81	78	81	75	63	53	51	54	0.02	0.4
84	78	79	63	68	113	129	116	74	58	61	58	53	48	62	0.8	5.3
79	83	90	96	112	115	100	88	92	81	70	62	70	66	60	2.35	4.2
72	73	81	83	98	86	84	76	82	80	73	68	72	64	60	1.15	12.6
76	76	77	76	76	102	100	90	84	77	72	67	66	60	60	4.04	9.1

obesity[21] could be related to impairment of intestinal absorption of glucose.

REFERENCES

[1] Pomeranze, J., and Gadek, R. J.: Nineteen-month clinical experience with a new hypoglycemic drug. *In* Diabetes Mellitus, Oberdisse, K., and Jahnke, K., Eds., III Kongress der Internationalen Diabetes Federation. Stuttgart, Georg Thieme Verlag, 1959, p. 440-43.

[2] Biro, L., Banyasz, T., Kovacs, M. B., and Bajor, M.: Die Wirkung des Phenyläthylbiguanids auf die Glukoseresorption. Klin. Wschr. *39*:760-62, 1961.

[3] Creutzfeldt, W., Söling, H. D., Moench, A., Rauch, E., and Bol, M.: Die Wirkung von N_1-n-Butylbiguanid (W 37) and N_1-B-Phenyläthylbiguanid (W 32) auf den Alloxan- und Phlorrizin-Diabetes und die intestinale Glukoseabsorption von Ratten. Naunyn Schmiedeberg's Arch. Pharm. Exp. Path. *244*: 31-47, 1962.

[4] Förster, H., Hager, E. and Mehnert, H.: Der Einfluss von Butylbiguanid in Tierversuch auf die Resorption von Glucose und Fructose. Arzneimittelforschung *15*:1340-44, 1965.

[5] Conard, V.: Mesure de l'assimilation du glucose. Bases théoriques et applications cliniques. Bruxelles, Acta Med. Belg. Ed., 1955.

[6] King, E. J.: Microanalysis in Medical Biochemistry, London, J. A. Churchill Ltd., 1957.

[7] Butterfield, W. J., Fry Kelsey I., and Whichelow, M. J.: The hypoglycemic action of phenformin. Studies in diabetics after short-term therapy. Lancet *2*:563-67, 1961.

[8] Schilling, I.: Uber die orale Behandlung des Diabetes mellitus mit Biguaniden.. I und II Mitteilung. Z. Ges. Inn. Med. *14*:705-11, 753-59, 1959.

[9] Fajans, S. S., Moorhouse, J. A., Doorenbos, H., Louis, L. H., and Conn, J. W.: Metabolic effects of phenethylbiguanide in normal subjects and diabetic patients. Diabetes *9*:194-201.

[10] Gutsche, H.: Beeinflussung des Staub-Traugott-Effektes bei Biguanidbehandlung. *In* Internationales Biguanid-Symposium, Stuttgart, Georg Thieme Verlag, 1960, p. 102-110.

[11] Pereira, V. G., Wajchenberg, B. L., and Shnaider, J.: Mechanism of action of phenethylbiguanide in normal subjects. Diabetes *16*:302-05, 1967.

[12] Crane, R. K.: Intestinal absorption of sugars. Physiol. Rev. *40*:789-825, 1960.

[13] Williams, R. H., Tyberghein, J. M., Hyde, P. M., and Nielsen, R. L.: Studies related to the hypoglycemic action of phenethylbiguanide. Metabolism 6:311-19, 1957.

[14] Steiner, D. F., and Williams, R. H.: Respiratory inhibition and hypoglycemia by biguanide and decamethylenediguanidine. Biochim. Biophys. Acta 30:329-40, 1958.

[15] Wick, A. N., Larson, E. R., and Serif, G. S.: Site of action of phenethylbiguanide, a hypoglycemic compound. J. Biol. Chem. *233*:296-98, 1958.

[16] Hollunger, G.: Guanidines and oxidative phosphorylations. Acta Pharmacol. *11*:1-84, 1955.

[17] Kruger, F. A., Skillman, T. G., Hamwi, G. J., Grubbs, R. C., and Danforth, N.: The mechanism of action of hypoglycemic guanidine derivatives. Diabetes 9:170-79, 1960.

[18] Tranquada, R. E.: The mechanism of action of phenethylbiguanide. 4e Congrès de la Fédération Internationale de Diabète, Genève, Médecine et Hygiène, 1961, p. 716-17.

[19] Wick, A. N., Stewart, Ch. J., and Serif, G. S.: Tissue distribution of C-14-labeled betaphenethylbiguanide. Diabetes 9:163-66, 1960.

[20] Cohen, Y., and Costerousse, O.: Etude autoradiographique chez la souris d'un antidiabétique oral, le NN Diméthylbiguanide marqué au carbone 14. Thérapie *16*:109-20, 1961.

[21] Grabowska, M. J., and Skrok, T.: Zastosowanie dibotyny w leczeniu cukrzycy skojarzonej z otyloscia. Wiad. Lek. *18*:1777-81, 1965.