Studies on the Site and Mechanism of Action of Phenformin

I. Evidence for Significant "Nonperipheral" Effects of Phenformin on Glucose Metabolism in Normal Subjects

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SUMMARY

Oral and intravenous glucose tolerance tests were performed in ten normal subjects. Phenformin pretreatment flattened the glucose and insulin response to oral glucose loading but did not significantly alter the response to intravenous glucose. It is suggested that these results can best be explained by an inhibitory effect of the drug on the rate of intestinal glucose absorption. DIABETES *19*:45-49, 1970.

Although phenformin has been used extensively for the treatment of diabetes mellitus, its site and mechanism of action are controversial. Early studies with this compound revealed that it could produce hypoglycemia in certain intact or eviscerated laboratory animals[1] and increase the uptake of glucose by isolated muscle.[2] Such findings led to the hypothesis that phenformin lowers the blood sugar level of patients with diabetes mellitus by direct effects on peripheral glucose metabolism. The validity of this "peripheral hypothesis" as it applies to the site of action of phenformin in man may be seriously questioned for the following reasons: (1) the amounts of phenformin required to produce hypoglycemia in laboratory animals (expressed as mg. of drug per kg. of body weight) are considerably higher than the doses employed for the treatment of human subjects, and (2) the concentrations of phenformin required to increase glucose uptake in vitro have generally been orders of magnitude higher than those which can be accounted for in peripheral tissues after oral administration.

The evidence that low concentrations of phenformin and related biguanides may alter glucose metabolism by adipose tissues in vitro is, at best, confusing. Daweke and Bach[3] proposed that biguanides enhance glucose oxidation by adipose tissue in the presence of insulin. Jangaard and associates[4] found that biguanides inhibited glucose oxidation but enhanced glucose uptake by adipose tissue. Söling and coworkers[5] found that phenformin depressed both the uptake and oxidation of glucose by adipose tissue in the presence of insulin. We tend to agree with the latter authors who suggested that "at the moment nothing supports the hypothesis that the normal blood-sugar-lowering effect of biguanides results from an action of biguanides on adipose tissue."

Phenformin has been shown to accumulate in the liver and gastrointestinal tract of the rat.[6] For this reason work in our laboratory has been directed toward studying the possible effects of phenformin in these tissues.

A portion of the drug reaching the liver is hydroxylated to an inactive metabolite, N_1-p-hydroxy-β-phenethylbiguanide. Rat liver hydroxylates phenformin to a much greater extent than does guinea pig liver.[7] This may explain the observation that phenformin effectively inhibits hepatic gluconeogenesis in the fasted guinea pig whereas the rat is comparatively resistant to this action.[8]

The gastrointestinal tract of the rat is quite sensitive to phenformin. Studies from this laboratory show that the active transport of glucose is inhibited in everted sacs of intestine from rats pretreated with phenformin.[9]

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In an attempt to evaluate the gastrointestinal tract as a possible site of action of phenformin in man, the following study was designed. Oral and intravenous glucose tolerance tests were performed in normal subjects with and without phenformin and the results compared.

METHODS

Six normal males and four normal females who were between twenty-two and forty-two years of age and within 10 per cent of their ideal body weight were subjected to the following tests:

1. *Oral glucose tolerance tests.* Heparinized venous blood was obtained before and one-half, one, two and three hours after the administration of 75 gm. of glucose by mouth. Plasma from these samples was analyzed for glucose and insulin. On another day, each subject received 150 mg. of phenformin hydrochloride by mouth forty-five minutes prior to the administration of glucose.

2. *Intravenous glucose tolerance tests.* Twenty-five grams of glucose as a 50 per cent solution was administered intravenously over a three-minute period. Heparinized samples were obtained from an indwelling needle placed in the contralateral brachial vein before and every five minutes after the beginning of the glucose injection for a period of forty minutes. Plasma glucose was determined on all samples and plasma insulin levels were determined on the 0, 5, 10, 20 and 40-minute samples. On another day, 150 mg. of phenformin hydrochloride was given by mouth ninety minutes prior to the administration of glucose.

All tests were performed at approximately the same hour in the morning after a twelve to sixteen-hour fast. All subjects were recumbent during the intravenous tests and for thirty to forty-five minutes prior to the tests whereas activity was not restricted during the oral tests. The order in which tests were performed was alternated in successive patients and consecutive tests in a given patient were performed at intervals of not less than seventy-two hours.

Plasma glucose was measured by the Hoffman method[10] on the AutoAnalyzer. Plasma immunoreactive insulin was measured by the method of Grodsky and Forsham.[11] The intravenous glucose tolerance tests are expressed as the rate of disappearance of total plasma glucose (K-value).[12] Plasma insulin is expressed both as absolute plasma concentrations at the specified times and as the "estimated insulin output," representing the area circumscribed by the insulin concentrations above fasting levels for the entire period of the tests.

RESULTS

Oral glucose tolerance tests

The effects of phenformin on oral glucose tolerance are shown in figure 1.

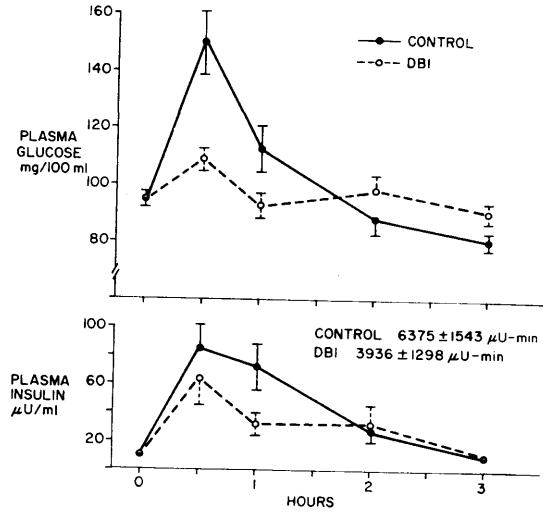

FIG. 1. Effect of phenformin (DBI) on plasma glucose and insulin responses during standard (75 gm.) oral glucose tolerance test in ten normal subjects. Values indicate mean ± S.E.M.

Fasting plasma glucose levels were not altered by phenformin pretreatment. The hyperglycemic response to glucose at one-half hour and one hour was markedly diminished by the drug ($p < 0.005$ and $p < 0.05$, respectively). At two and three hours after glucose administration, glucose levels were somewhat higher when phenformin had been given ($p > 0.1$ and $p < 0.05$, respectively). The mean glucose levels at three hours were lower than the fasting levels in both the control and experimental tests (mean decrement = 13 mg./100 ml. and 3 mg./100 ml., respectively).

The plasma insulin levels were lower after phenformin pretreatment. By a t test analysis of group means, insulin levels were significantly lower at one hour ($p < 0.05$). The estimated insulin output was lower after phenformin (see figure 1). This difference was significant when analyzed as paired differences (decrement after phenformin 2,439 ± 720 μU.-min., $p < 0.01$).

Intravenous glucose tolerance tests

The effects of phenformin on intravenous glucose tolerance are shown in figure 2.

Phenformin did not significantly alter the fasting glucose level, the rate of disappearance of glucose (K-value) or the insulin response during these tests. The

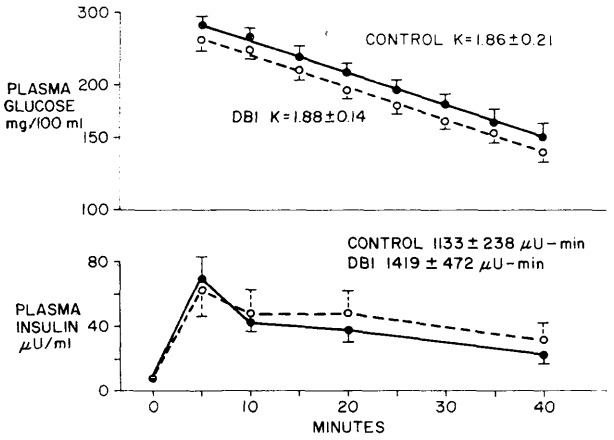

FIG. 2. Effect of phenformin (DBI) on plasma glucose and insulin responses during rapid (25 gm.) intravenous glucose tolerance test in ten normal subjects. Values indicate mean ± S.E.M.

only change, of questionable significance, was a small downward displacement of the glucose response curves after phenformin.

DISCUSSION

Phenformin increases tolerance to orally administered glucose while decreasing the output of endogenous insulin. If this response to phenformin were due to increased peripheral utilization of glucose or to decreased hepatic gluconeogenesis, similar changes should occur following intravenous glucose loading. However, phenformin did not alter tolerance to intravenously administered glucose. The lack of such a change strongly argues against significant peripheral actions of the drug and may also suggest that the liver is not a major site of action in normal man. These findings can best be explained by an inhibitory effect of phenformin on the rate of intestinal glucose absorption.

It may not be justifiable to exclude hepatic actions at our present state of knowledge since the precise relationship between hepatic glucose balance and the glycemic response to glucose loading is not completely clear. Sandler and associates[13] found a greater fall in blood sugar produced by alcohol in fasting, obese patients after phenformin had been given nine to twenty days. They suggested that alcohol and phenformin were acting by different mechanisms to inhibit gluconeogenesis. Conversely, neither Kreisberg[14] nor Searle and coworkers[15] could find evidence that gluconeogenesis was decreased in human subjects by therapeutic doses of phenformin. Subtle alterations of hepatic glucose balance could conceivably be more apparent after oral glucose, since by this route, the entire load would reach the liver immediately after absorption. The absence of fasting hypoglycemia would suggest that the drug does not decrease hepatic glucose output in the postabsorptive state, although it is possible that such an effect would be masked by mechanisms which are operative under such conditions to maintain the "normal" level of fasting blood glucose.

Grodsky and associates[16] found that the magnitude of glucose-induced hyperinsulinism in obese and diabetic subjects was decreased by phenformin. They observed that the effect of the drug on blood glucose levels was more pronounced after oral glucose loading than after giving glucose intravenously. The present report extends these observations to include nonobese, nondiabetic subjects. However, these authors did observe a reduced insulin response to intravenously administered glucose in three obese nondiabetic subjects following phenformin. The present study indicates that no such effect occurs in normal subjects.

Pereira et al.[17] have studied the effects of phenformin on normal subjects. They determined blood glucose response to oral glucose, intravenous insulin, and oral phenformin singly and in all possible combinations. Statistically significant effects of phenformin were found only when the drug was used in combination with oral glucose loading. The authors suggested that their findings were compatible with peripheral actions of phenformin, assuming that neither the absorption of the glucose load nor the endogenous insulin response were altered by the drug. The findings in the present report and those previously cited cast serious doubt on these assumptions.

While this study was in progress, Czyzyk and associates[18] reported data suggesting that phenformin and butylbiguanide may impede the rate of intestinal glucose absorption. The findings in the present study confirm and extend their observations.

The rate of intestinal glucose absorption in man has not been a topic of intensive investigation. It may be that the rapid absorption of dietary carbohydrate is undesirable. The prolonged and perhaps excessive ingestion of refined sugars (as opposed to starch) may lead to elevations of serum cholesterol[19] and triglyceride[20] levels. Several authors have noted a positive correlation between sugar intake and the development of occlusive arteriosclerotic disease.[21-23]

Dietary sugar itself may be considered a diabetogenic factor. Higher levels of plasma glucose and insulin are seen in normal subjects after glucose ingestion than after an isocaloric load of starch.[24] Glucose tolerance is

somewhat decreased in normal subjects whose previous carbohydrate intake has been mainly in the form of sucrose as compared to those who have received a "bread" diet.[25]

Genetic diabetes has been described by Neel[26] as a "thrifty genotype." It is possible that individuals with such a syndrome (if it exists) would manifest "tachy-absorption" of nutrients from the alimentary tract. We are aware of a single report which would indicate that the rate of intestinal glucose absorption is greater in diabetic subjects than in nondiabetics.[27]

Many patients who have had gastric surgery exhibit hyperinsulinism and reactive hypoglycemia, presumably a result of accelerated absorption of nutrients from the alimentary tract.[28] These patients may develop a form of diabetes as evidenced by an impairment in the rate of utilization of intravenous glucose.[29] Diminished glucose tolerance[30] and elevations of serum lipids[31] are seen in normal subjects receiving a "gorging" diet as compared to an isocaloric "nibbling" diet. These abnormalities may be due in part to the rapid absorption of nutrients following the "gorging" meal.

A variety of compounds have been shown to inhibit the absorption of glucose from the intestine.[32-35] The effects of such compounds on the diabetic state have not been systematically studied.

ACKNOWLEDGMENT

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Dietary Control in Animal Experiments

In any type of animal experiment, it should be self-evident that the results are no better than the treatments given the experimental animals. In experiments testing the effect of a dietary treatment on any biological process, the appropriate control diet, in theory, should differ from the experimental diet only in the nutrient material under study. Despite the obviousness of such a statement, there are still far too many papers in which the "normal" or "control group" is fed an unpurified commercial laboratory diet while the experimental group is fed a casein-purified carbohydrate diet.

In cases such as this, it is obvious that some of the differences may be related to the over-all difference in diet composition, rather than to the specific nutrient under study. This criticism is not removed even if a white purified diet is colored green to approximate the commercial laboratory diet (B. Ahluwalia, G. Pincus, and R. T. Holman (*J. Nutrition* 92:205, 1967). Such a paper on EFA deficiency and its effects on the reproductive organs of male rabbits is in contrast to the excellent dietary control used in a recent study of EFA deficiency and testes lipid composition in the rat (J. G. Bieri, K. E. Mason, and E. L. Prival (*J. Nutrition* 97:163, 1969).

Another unfortunate dietary situation occurs when the experimental diet in a deficiency study is deficient in other nutrients, as well as the one under study. This situation is apt to arise when an investigator, who is unaware of the nutrient requirements of a given species, purchases a "deficient purified diet" from a commercial supplier. This appears to have been the situation in the report of S. D. Rockoff, A. Bravo, H. Kaye, and R. P. Spencer (*Calcified Tissue Res.* 3:17, 1969). In this paper on the effect of calcium deficiency on the fractional redistribution of cardiac output to bone, the deficient diet used was a calcium-deficient test diet. The salt mixture used did lack calcium, but also lacked copper and zinc, two other elements involved in bone metabolism (E. A. Underwood, *Trace Elements. Academic Press, New York, 1962*). The rats presumably were kept in galvanized cages, but they were given calcium-free distilled water to drink. The rats fed this diet were also compared with a control group fed another commercial laboratory diet.

The results indicated that, in the growing rats fed the Ca-deficient diet, there was an early increase in the fraction of cardiac output perfusing the capillary bed of the bone, and that this increase occurred during the same general time interval as did detectable, significant alterations in the density, dry weight, and per cent ash of the femurs. The authors pointed out that this, in turn, raised the question of whether increased blood flow in

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