Special Article

Basic Clinical Metabolic Studies with Phenformin

T. S. Danowski, M.D., Pittsburgh, Moderator

Stanley S. Bergen, Jr., M.D.
New York City

James W. Craig, M.D.
Cleveland

Stefan S. Fajans, M.D.
Ann Arbor

Leonard L. Madison, M.D.
Dallas

Thomas G. Skillman, M.D.
Columbus

Robert E. Tranquada, M.D.
Los Angeles

MODERATOR DANOWSKI, (*Pittsburgh*): I would like to suggest to the panel that we first consider the effects of DBI, if any, on the degradation of insulin, whether it be endogenous or exogenous. Could we disregard this as a possible mechanism of DBI action?

THOMAS G. SKILLMAN, M.D., (*Columbus*): We believe that altered degradation could not account for the hypoglycemic effect of DBI, but decreases in the rate of insulin degradation under the influence of DBI have been reported.

MODERATOR DANOWSKI: On the other hand there are data which indicate that DBI does not alter degradation of insulin in the in vitro systems. We are left, therefore, with some uncertainty. Let us ask Dr. Bergen to comment further on his studies of adrenocortical function in patients receiving DBI.

STANLEY S. BERGEN, JR., M.D., (*New York City*): I think our studies indicate that DBI exerts no gross effects upon adrenocortical function as reflected in the urinary levels of 17-hydroxycorticosteroids and 17-ketosteroids. This type of evidence excludes, of course, the possibility that the observed hypoglycemic effects of DBI were attributable to hypoadrenocorticism. If anything, there was an increased response to DBI, particularly in one patient, following the administration of ACTH.

MODERATOR DANOWSKI: It is conceivable, is it not, that there might have been changes in the production of endogenous ACTH in your patients?

DR. BERGEN: There certainly might, but all our base-line levels were normal. If there was an actual block of pituitary-adrenocortical activity, I would expect the urinary steroids to be at least low normal.

MODERATOR DANOWSKI: I would like to turn your attention now to the DBI-thyroid data. These indicated, you will recall, that DBI did not alter the serum protein bound iodine nor the net trapping of I^{131}. The trend toward decreased trapping which was evident did not prove to be statistically significant. I would like to ask Dr. Skillman, however, whether these findings entirely exclude changes in the levels of circulating hormones and the trapping of iodine?

DR. SKILLMAN: We have considered these possibilities. At times diabetics have a defect in their alpha globulin, i.e., in the thyroxine binding globulin. We too wonder if protein-bound iodine values are always directly reflective of thyroid physiology and thyroid hormone metabolism. Also, insofar as the I^{131} trapping is concerned, our studies do not exclude the possibility of transient early inhibition of iodine uptake which is compensated for later on.

MODERATOR DANOWSKI: Does anyone have any data on epinephrine tolerances with and without DBI? These might prove enlightening because of the increase in lactic acid induced by this hormone.

DR. SKILLMAN: We studied three patients before and after DBI with standard epinephrine tolerances, measuring blood sugar at twenty-minute intervals for an hour. The rise was the same with and without DBI. We did not measure lactate.

MODERATOR DANOWSKI: I would now like to ask Dr. Craig and Dr. Fajans to comment further on their lactate and pyruvate data. You will recall that both of them expressed the view that the rises in these products of glycolysis represented increased production or diminished disposal. Theoretically, there are three other

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 6, 1959.

possible explanations for the observed increases in the levels of lactate and pyruvate: Both increased production and diminished disposal could occur; production and disposal could both be decreased with the latter predominant; and finally, both increased production and increased disposal could be present with the former predominant. Thus in one of Dr. Craig's studies there was suggestion that both the production and the disposal of lactic acid were increased by DBI.

JAMES W. CRAIG, M.D., (*Cleveland*): We have a problem in how to express the rate of disappearance of lactate. One can speak of the fractional rate of disappearance in terms of increase above the fasting level, or one can speak of the rate of disappearance in terms of the total elevation of lactate.

If one does it in terms of the increase above the fasting level, the fractional rates of disappearance after sodium lactate was administered were similar with and without DBI, although the total rise was somewhat greater under the influence of DBI. If one takes into account the higher absolute level, then this is a relatively slower rate of disappearance of lactate.

STEFAN S. FAJANS, M.D., (*Ann Arbor*): As far as the question of increased production versus decreased removal, or any number of combinations of these two factors, one can only speak of the algebraic sum or the net effect in the studies that we have done. We found that there was a decreased disappearance of pyruvate and of lactate from blood. I think when we say decreased disappearance, this takes into consideration the possibilities you have mentioned.

MODERATOR DANOWSKI: Dr. Craig, do you think that lactate production is increased?

DR. CRAIG: I do not think from our data that we are able to make a decision on that particular point. I think there is an alteration in equilibrium, and it is increased in that sense.

ROBERT E. TRANQUADA, M.D., (*Los Angeles*): I would like to add that our data suggest that there is both increased production of lactate peripheral to the liver and increased uptake in the liver.

MODERATOR DANOWSKI: Where is the lactate being produced?

DR. TRANQUADA: We did not measure venous levels, but we presume the lactate is produced by the peripheral tissues and that increased uptake by the liver prevents arterial levels from rising.

DR. CRAIG: With repeated administration of the drug in larger doses, we do find elevations of lactate levels in arterial blood.

MODERATOR DANOWSKI: Let us now turn to the subject of gluconeogenesis. Dr. Madison, how about the extrahepatic production of glucose?

LEONARD L. MADISON, M.D., (*Dallas*): The only significant source of extrahepatic glucose is, as far as I know, the kidney and gluconeogenesis in that organ only occurs under unusual circumstances.

MODERATOR DANOWSKI: Here is one question that I am sure one of you can answer: Does DBI potentiate exogenous insulin?

DR. FAJANS: If this were true, one should find a change in insulin sensitivity and in glucose tolerance. We could not find any such changes, and we therefore feel that DBI does not potentiate the activity of insulin.

DR. MADISON: We have some evidence of a preliminary nature which shows that when we infuse exogenous insulin at a very slow rate, we observe no change in arteriovenous glucose difference. When insulin is given at the same rate with DBI there is apparently a widening of the glucose arteriovenous difference that may indicate potentiation of insulin action.

DR. FAJANS: I do not believe that just because insulin was necessary for the effect of DBI to be manifested, that insulin activity was potentiated. One has to differentiate between the permissive action of a hormone and actual participation of a hormone.

DR. BERGEN: We had a forty-six-year-old patient who had been subjected to a pancreatectomy one year earlier. He was on a high caloric intake, and forty units of long-acting and twenty units of short-acting insulin a day. On this regimen he was spilling 30 to 40 gm. of glucose in twenty-four hours. When we began to add DBI, we got to a point where we were administering over 300 mg. of DBI and had reduced the insulin dosage to ten units. The twenty-four-hour urine then contained 9.7 gm. of glucose. The patient developed anorexia on this dosage and his caloric intake could no longer be maintained.

This indicates that DBI was able to replace a certain amount of insulin, and decrease the carbohydrate wastage in urine. Whether this is potentiation or just straightforward replacement of a certain number of units of insulin, we are unable to say.

MODERATOR DANOWSKI: I would not believe this observation answers the fundamental question. The findings are compatible with an action which is similar to that of insulin or compatible with potentiation. Therefore, it is still moot.

DR. TRANQUADA: We had a similar experience with a twenty-year-old male who required seventy-five units of NPH and twenty-five units of Regular Insulin. On this regimen the spillage of glucose was 10 to 30

gm. per day. We began treating him with DBI and within a two-week period had increased the DBI to 300 mg. a day and decreased his insulin to 25 U. per day. At this stage his urine was sugar-free for four consecutive days, and the blood sugar level was around 80 mg. per 100 ml.

On the day we stopped his insulin he went into severe acidosis with elevation of the blood sugar level to over 300 mg. per 100 ml. He had to be given insulin to get him out of that acidotic episode.

MODERATOR DANOWSKI: Someone asked about the concentration of DBI in the gastric juices of patients.

MAX MILLER, M.D., (*Cleveland*): When labeled DBI was administered in large amounts, the label appeared in the gastric juice. These were studies in animals.

MODERATOR DANOWSKI: What was the label?

DR. MILLER: Carbon 14.

MODERATOR DANOWSKI: Unfortunately, labels have a life of their own. If you label with iodide, the localization of the label does not necessarily indicate what happened to the compound. It indicates what happens to the iodide.

Is there any localization of DBI in the rest of the intestinal tract? Does anyone know?

ARNE WICK, PH.D., (*San Diego*): I would say that most of it is in the gastric juice. While I am on my feet, may I make a statement on this question of lactate. I would like to put in a plea for caution in the interpretation of lactate studies. I think Dr. Miller will bear me out. Lactate is one of the most difficult things to study, although one can measure it easily in the blood. The reason it is difficult to study is that it is immediately converted to CO_2 after injection.

I would also like to speak on the point of tissue sources of glucose. No one has demonstrated that glucose will diffuse out of muscle cells. As a matter of fact, no one has demonstrated free sugar inside the cell under normal conditions. Now, if a diaphragm is exposed to sugar at 2,000 mg. per cent, which I think is worthless, you can demonstrate free sugar. You can also produce free sugar in the kidney only under hyperglycemic conditions. We did that several years ago.

MODERATOR DANOWSKI: The point that you make about lactate is a good one, and yet I was impressed by the narrow range of responses that Dr. Craig and Dr. Fajans observed in their patients, suggesting that perhaps even though the limitation you cite is present, that it is of equal degree with and without DBI.

Here is another question from a member of the audience: Does DBI increase ketone formation in the total diabetic not given insulin?

DR. TRANQUADA: The patient I was referring to spilled increasing amounts of ketones in the urine only when we discontinued insulin not because we gave DBI.

DR. SKILLMAN: Our studies in rabbits suggest that ketosis is not produced by DBI.

MODERATOR DANOWSKI: Is the panel now ready to express itself on the mechanism or mechanisms of action of DBI? Is the hypoglycemic effect related to a decreased output of glucose by the liver?

DR. TRANQUADA: No, I don't think we can draw that conclusion from what we have seen thus far and I think that is as far as we can go.

DR. MADISON: I can only say again what I said before, it doesn't seem to be working on the periphery in the muscle. It may work on fat which we didn't study and it may work on the liver. From Dr. Tranquada's data, it doesn't appear to work on the liver.

DR. MADISON: I would like to speak further on the subject of lactate. When man is in the postabsorptive state, most of the glucose that is utilized by the muscles is utilized through anaerobic metabolism. If I am correct, 60 to 70 per cent of the glucose thus utilized ends in lactate production. There is nothing inherently wrong with that. Such a process can produce energy if the lactate is then metabolized. I believe this is exactly what does happen. Since the lactate is not lost in the urine, it must be metabolized somewhere. There are two ways in which glucose can be used by the diaphragm. Under the influence of insulin, it may go to glycogen. DBI does not appear to stimulate this pathway. On the other hand, when glucose is present at higher levels, this amount utilized is increased and lactic acid is formed. It may be that DBI facilitates this pathway.

MODERATOR DANOWSKI: Perhaps the reason why the importance of anaerobic glycolysis tends to be minimized is that from the point of view of energy production the Krebs cycle is much more effective. Yet as Dr. Madison points out, anaerobic glycolysis is an effective way of producing compounds which can then be utilized in what may well ultimately be energy-producing reactions.

DR. TRANQUADA: But if this is the fact, one would have to utilize more glucose through the glycolytic cycle and the glucose has to come from somewhere, probably the liver, and the two theories, decrease of glucose output and increase in anaerobic glycolysis, are not compatible.

MODERATOR DANOWSKI: You are saying that anaerobic glycolysis is a relatively inefficient mechanism compared to the Krebs cycle in terms of energy production and therefore would require larger amounts

of glucose. What then would be the source of this glucose?

DR. MILLER: It could be glucose that would otherwise be lost in urine.

DR. FAJANS: In one of our patients on DBI the urinary glucose was reduced from 160 gm. down to 30 gm.

MODERATOR DANOWSKI: Logically, we should now proceed to a discussion of possible effects of DBI on the Krebs cycle.

DR. CRAIG: We have studied citric acid, measuring the blood concentrations and the twenty-four-hour urinary excretion. We have not been able to demonstrate increases in citric acid excretion or citric acid levels, although I think there are animal studies in which increases have been observed.

MODERATOR DANOWSKI: Dr. Ungar, you have done some work in animals on this point.

GEORGES UNGAR, M.D., Sc.D., (*New York City*): DBI does have a striking effect on the Krebs cycle when given in large dosages to animals. The detailed changes were discussed, as you know, this morning.

MODERATOR DANOWSKI: Are any of you troubled by the fact that an increase in anaerobic glycolysis might be undesirable?

DR. TRANQUADA: It might be discussed on slightly different terms. It is conceivable that the operations of the Krebs cycle are reduced in the muscle and not in the liver. I don't know whether this is a valid consideration, but it is a possibility that has to be considered.

QUESTION FROM THE AUDIENCE: Dr. Fajans cited a patient in whom the urinary glucose fell and the blood sugar fell following DBI but the ketone levels rose. Isn't that an indication that the DBI was stimulating another pathway of metabolism leading to ketone production?

DR. FAJANS: I don't think we can say that there was any evidence of increased ketone production produced by DBI. I agree with Dr. Skillman's previous statement that the ketosis resulted from withdrawal of insulin.

A MEMBER OF THE AUDIENCE: I'd like to comment on this point. If the diet is low in calories and carbohydrate, the patient will show ketonuria. If the diet is increased, the ketonuria disappears. This is considered to be a starvation effect and not a product of DBI itself.

QUESTION FROM THE AUDIENCE: Doesn't it suggest that a patient on DBI needs more carbohydrate than a patient on insulin only?

MODERATOR DANOWSKI: This takes us back to Dr. Tranquada's idea and is a very large question. I think that diabetics are going to get more carbohydrate in the future, because they are getting too much fat at present; but there is no evidence that DBI increases the requirement for carbohydrate.

QUESTION FROM THE AUDIENCE: The usual ketone level is a little over 1 mg. per cent in the blood of the nondiabetic. In diabetic patients regarded as being in good control on insulin, the ketone level is two or three times higher, i.e., between 2 and 3 mg. per cent.

In patients treated with DBI, the blood ketone level rises three- or fourfold above the basal values.

Do we have any clues why DBI does not work in nondiabetic subjects with endogenous insulin?

MODERATOR DANOWSKI: An excellent question. I'd say it is because you cannot improve upon success.

DR. SKILLMAN: I do not have an answer, but I can add another question. We have not seen hypoglycemia develop with DBI as it does with the sulfonylureas and one might wonder about a built-in glucostat whereby DBI might be more effective at higher concentrations of blood sugar and less effective at lower concentrations.

MODERATOR DANOWSKI: How large have the doses of DBI been in the studies in nondiabetic humans?

DR. SKILLMAN: We have given up to 400 mg. and obtained slight decreases in blood sugar.

DR. FAJANS: We gave up to 400 mg., too, and as you saw observed a slight decrease. This is a minimal change which is not statistically significant. Dr. Madison made a very significant observation, if I understood him correctly; diabetic patients with fasting blood sugar in the normal range did have a hypoglycemic response and the normals did not. Did I understand you correctly?

DR. MADISON: Yes, we encountered such a patient. We didn't see a diabetic or nondiabetic on DBI go below 70 mg. per cent.

DR. MILLER: I am led to believe that no one has seen hypoglycemia.

MODERATOR DANOWSKI: Dr. Ungar, I hope that you will comment upon hypoglycemia following large dosages of DBI given to animals.

DR. UNGAR: I believe it is purely a quantitative matter. I have some evidence from studies in monkeys. In normal monkeys you do not get any hypoglycemia with DBI dosages below 5 mg. per kg. of body weight. At dosages above 8 mg. per kg. you have a drop of 8 per cent in the level of blood sugar.

On the other hand, we have seen one diabetic monkey whose diabetes was perfectly controlled by 1 to 2 mg. of DBI per kg.

I believe that if humans were given DBI at a level corresponding to 5 mg. per kg., more hypoglycemia would be observed.

DR. FAJANS: The dosage used in our normal subjects extended up to 5.7 mg. per kg. but a hypoglycemic effect was not observed.

DR. MILLER: Did the monkey respond to DBI at 5 mg. per kilo?

DR. UNGAR: Yes, and he was controlled.

DR. FAJANS: A person with diabetes may, and I would like to underline with a red pencil the word "may," have an abnormality in the removal of pyruvate. This has been referred to in several papers as a possibility. If this should be so, and if DBI interferes with the removal of pyruvate, we would anticipate a greater effect of DBI in diabetic subjects than in normal ones.

DR. MADISON: If you can't get rid of pyruvate and if you block it more, it would mean that the blood sugar would rise and not fall.

DR. CRAIG: May I ask Dr. Fajans a question in regard to this postulated theory in pyruvate. Have you tested the effect of DBI on a patient with Cushing's syndrome, since these may be more marked than in an ordinary diabetic?

DR. FAJANS: No, we have not.

MODERATOR DANOWSKI: I am afraid that in these at times lengthy discussions we have not settled many of the problems posed by ourselves and by the audience. It might be well to summarize the consensus on some of these.

Though the evidence is not entirely consistent, the hypoglycemic action of DBI does not appear to result from a decrease in the degradation of endogenous or exogenous insulin.

DBI does not potentiate the action of exogenous insulin judging from insulin tolerance tests and from glucose tolerance tests in subjects with some presumed residual production of insulin. Similarly there appear to be no striking or persistent effects of DBI upon adrenocortical or thyroid function nor upon epinephrine-induced hyperglycemia.

The accumulation of lactate and pyruvate under the influence of DBI is definite. Though alternative explanations might be offered, this accumulation could be the result of both increased production and blockade of disposal of glycolytic products via the Krebs cycle.

Evidence is available that, if some insulin is available so that anaerobic glycolysis is occurring, DBI accelerates the operation of the hexosemonophosphate shunt. Though some concern has been expressed concerning the possible harmful effect of acceleration of anaerobic glycolysis, none has been observed to date. It would appear that the drug accelerates the metabolic disposal of glucose and in the diabetic but not the nondiabetic lowers blood sugar levels. Though much is yet to be learned about its mechanism of action, it is already clear that DBI is a prototype of a new group of agents which will add much to our understanding of intermediary metabolism and ultimately lead to more adequate regulation of diabetes mellitus.

Small differences in caloric expenditure which may represent a great accumulation of body fat over a period of time are difficult to demonstrate with statistical assurance. It must always be remembered that measurements upon a relatively small group or with few tests cannot be concluded to be the same simply because they are not statistically different. It is unfortunate that the measurements in this study could not have been repeated, to define the apparent differences between groups and meals with more assurance.

There was shown to be a significant positive correlation between the fasting blood glucose and the degree of deviation from normal weight patients. The correlation, however, was not high ($r = .58$). There was also some indication of a more rapid rise in blood glucose and perhaps a greater decrease, to below fasting levels, in the overweight group after a high carbohydrate meal than in the other groups. Fasting blood pyruvic acid concentrations also tended to be high in the overweight group.

The measurements of various serum lipids failed to reveal significant differences in the . . . groups. These included total serum lipids and cholesterol, various lipoprotein fractions, and chylomicron counts. Large individual differences within each of the groups, as would be expected from the literature (J. W. Gofman et al., *Circulation* 14:691, 1956) were found for these serum constituents. The fasting chylomicron counts tended to be high in the obese group.

However, as long as differences can be shown, new studies suggest themselves. There can be little doubt that the obesity problem is largely one which relates to the disposition of energy. Many of the observations which have been recorded require much more extensive documentation. From *Nutrition Reviews,* Vol. 15, No. 6, p. 165, June 1957.