

Metabolic Effects of Phenethylbiguanide in Normal Subjects and in Diabetic Patients

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Phenethylbiguanide (formamidinyliminourea or DBI) is a recently developed oral nonsulfonylurea blood sugar-lowering agent.¹ It has been reported that its administration produces hypoglycemia in normal animals^{1,2} and causes a reduction of hyperglycemia and glycosuria in diabetic animals¹ and diabetic patients.^{3,4} Studies related to the mechanism of action of this compound have been reported from work in animals^{2,5} and in isolated tissues.⁵

The experiments described in this report were undertaken in an effort: (1) to ascertain the effects of DBI on the metabolism of carbohydrate, protein and electrolytes, and on adrenal function, and (2) to elucidate possible mechanisms of action of DBI in man. With these objectives in mind we have studied the effect of DBI: (a) upon levels of blood glucose under a variety of testing procedures (fasting, glucose tolerance, glucagon, insulin tolerance); (b) upon metabolic balances; and (c) upon intermediary carbohydrate metabolism in healthy subjects and in diabetic patients.*

SUBJECTS AND METHODS

All subjects for whom metabolic balance studies are reported were admitted to the Metabolism Research Laboratory and were maintained on constant diets. Table 1 summarizes the essential data concerning these subjects and their diets.

The other healthy subjects ingested diets containing 250 gm. of carbohydrate or more, and maintenance calories for at least three days before all tests. The other diabetic patients maintained their usual diets. With the exception of K.L., all patients taking insulin

discontinued its use throughout the experimental periods. In all subjects DBI was given in the amounts indicated in three or four divided doses with meals.

Chemical analyses performed on blood, serum and urine were determined by standard methods previously used in this laboratory.⁸ Venous blood samples for pyruvate⁹ and lactate¹⁰ were collected without veno-occlusion after the subjects had been recumbent for at least one-half hour. Glucose tolerance tests, intravenous insulin tolerance tests and glucagon tests were carried out as previously reported.⁸ Intravenous pyruvate tolerance tests* were performed by administering 10 gm. of sodium pyruvate as a 10 per cent solution over four minutes.

RESULTS

A. Effect of DBI on blood glucose levels.

Fasting blood glucose levels: In ten separate experiments DBI was administered as a single dose of 100 to 400 mg. to three normal fasting subjects. As seen in table 2 there was no decrease in blood glucose levels over a period of four to eight hours whether the subjects had been on a normal diet or after they had eaten a 600-calorie diet for two weeks.

In two of these subjects DBI was given for nine days, and in one of the two for a third course of twelve days. As can be seen from the mean levels during the control, DBI, and recovery periods, the drug had no effect on fasting blood glucose when given in daily dosages up to 250 mg. per day (table 3).

In contrast, administration of DBI caused a fall in the level of the fasting blood glucose in nonketotic stable diabetic patients. In the doses tolerated (up to 250 mg. per day) the blood glucose levels fell to normal values in some but not in all patients (table 4 and figures 1 and 2).

DBI produced no change in the fasting blood glucose level in K.L., a juvenile type of diabetic who was given a constant but suboptimal amount of insulin (table 4). In patient B.S., a tolbutamide-nonresponsive diabetic, withdrawal of 25 units of NPH insulin resulted in a

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*Preliminary reports of these studies have appeared in abstract form.^{6,7}

*Our statement in an abstract that the "pyruvate tolerance test" is a new procedure is incorrect.¹¹⁻¹³

TABLE 1

Subjects			Diabetes				Diet				
Initials	Sex	Age	Wt. kg.	Duration	Type	Insulin requirement, units	Nitrogen gm.	Carbohydrate gm.	Calories	Na mEq.	K mEq.
R.R.*	M	23	70	—	—	—	18.9	313	2932	213	115
J.D.*	M	20	69	—	—	—	18.9	313	2932	213	115
K.W.	M	30	61	25	Labile	35	15.4	198	2140	225	120
C.W.	F	61	60	13	Stable	40-60	14.2	168	1760	109	97
R.B.	F	63	69	3	Stable	35	13.4	182	1670	113	61
B.S.†	M	35	60	12	Stable	25	100 Protein	200	2000	—	—

* Healthy subject. † Studied on medical ward of University Hospital.

TABLE 2
Effect of administration of a single dose of DBI on blood sugar in fasting normal subjects

Dosage mg.	Subjects	F	½	1	1½	2	2½	3	3½	4	5	6	7	8
100	D.M.	86	86	78	87	89	89	85	83	81				
100	R.R.	79	84	74	78	80	77	77	76	77				
200	D.M.	67	71	77	77	79	71	71	77	—				
200	R.R.	90	—	92	—	89	—	85	—	94	86	92		
200	J.D.	87	—	86	—	84	—	81	—	85	88	86		
250	R.R.	86	—	81	—	78	—	74	—	76	75	74	71	78
250	J.D.	83	—	79	—	78	—	76	—	79	73	75	75	74
400	R.R.	88	—	85	—	82	—	85	—	84	85	82	—	—
	Mean	83	80	82	81	82	79	79	79	82	81	82	73	76
250*	R.R.	64	—	66	—	77	—	65	—	66	61	60	58	62
250*	J.D.	62	—	60	—	64	—	61	—	63	61	60	60	64
	Mean	63	—	63	—	71	—	63	—	65	61	60	59	63

*After subjects had eaten a 600 calorie (54 P—64 C—16 F) diet for fourteen days.

TABLE 3
Metabolic effects of DBI in normal subjects

Subject	Period	Days	Mean FBS mg./24 hr.	Mean urinary excretion/24 hours						
				Creatinine	Nitrogen	17-OH	17-KS	Na mEq.	Cl mEq.	K mEq.
R.R.	Control	8	81	1.95	18.1*	10.8	21.8	206	230	92
	DBI ¹	12	81	1.84	18.3†	10.3	20.3	195	218	98
	Recovery	9	86	2.01	17.9‡	9.7	23.8	213	230	99
	Control	12	83	2.15	15.9	10.3	20.8	213	226	103
	DBI ²	9	83	2.07	16.6	13.0	20.8	202	219	100
	J.D.	Control	8	80	2.30	12.2	11.6	19.4	189	204
	DBI ³	9	78	2.01	11.9	10.4	25.9	176	191	75
	Recovery	3	80	2.18	12.4	9.7	25.9	178	185	85

¹ Daily dose in mg.: 100, 125, 150, 200 x 6, 250 x 3.

² Daily dose in mg.: 150, 200, 200, 250, 175, 200 x 3, 100.

³ Daily dose in mg.: 150, 200, 200, 250, 175, 200 x 3, 100.

*Includes stool nitrogen of 1.1 gm.

†Includes stool nitrogen of 1.0 gm.

‡Includes stool nitrogen of 1.1 gm.

rise of the fasting blood sugar from normal values to 291 mg./100 ml. in three days. Urinary excretion of glucose was 167 gm. per twenty-four hours on the third day of insulin withdrawal and there was moderate ketonuria. DBI was administered in a dosage of 150 to 200 mg. per day and produced a decrease in hyperglycemia and glycosuria (to 91 gm.) on the third day. On the fourth day of drug therapy 30 units of Regular Insulin were given over twenty hours because

of persistent ketonuria. Over the next five days levels of fasting blood glucose varied between 135 and 180 mg./100 ml. and urinary excretion of glucose varied between 2.4 and 29 gm. per day. Moderate ketonuria persisted.

Glucose Tolerance Tests: Glucose tolerance tests were performed in ten healthy subjects before and during administration of DBI. As can be seen in figure 4, DBI did not cause a significant change in blood glu-

TABLE 4
Metabolic effects of DBI in diabetic subjects

Subjects	Period	Days	Mean urinary excretion per 24 hours								
			FBS mg./24-hr.	Creatinine gm.	Glucose gm.	Nitrogen gm.	17-OH mg.	17-KS mg.	Na mEq.	Cl mEq.	K mEq.
K.L.*	Control	5	292	1.52	116	15.9	5.9	13.4	239	243	120
	DBI ¹	11	297	1.49	119	15.8	6.5	14.3	193	213	92
	Recovery	5	281	1.72	191	17.3	5.3	13.7	262	286	115
C.W.	Control	8	306	1.49	9.5	14.1	5.7	13.7	114	116	77
	DBI ²	14	165	1.42	2.6	14.0	8.3	12.7	99	108	66
	Recovery	5	270	1.38	7.3	14.1	5.9	10.5	99	111	65
R.B.	Control	8	242	1.20	4.2	9.5	4.1	10.2	137	134	61
	DBI ³	14	88	1.12	1.1	9.2	8.2	10.1	77	95	51
	Recovery	5	176	1.17	1.0	9.3	5.9	10.1	127	127	65

¹ Daily dose in mg.: 100, 175, 200, 250 x 7, 300 x 2.

² Daily dose in mg.: 100 x 2, 125, 175, 200, 150, 175, 200, 200, 225, 250 x 3, 100.

³ Daily dose in mg.: 100, 150, 175 x 8, 100, 125 x 3.

*Maintained on 15 units PZI throughout study.

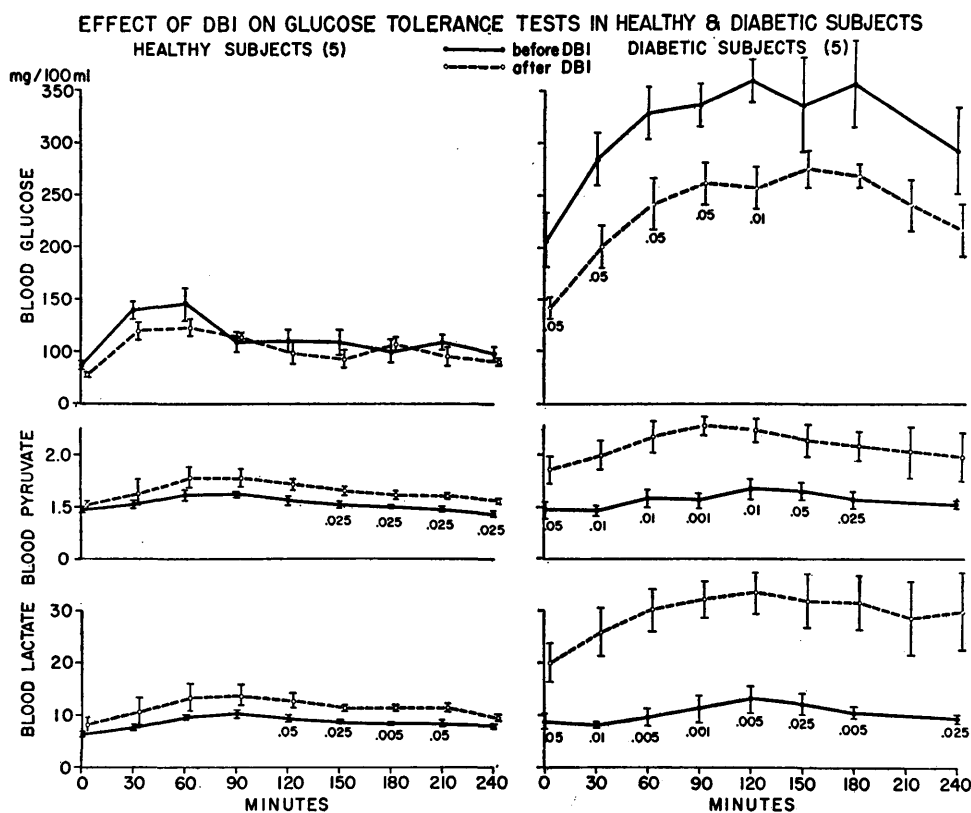


FIG. 1. Effect of DBI on blood levels of glucose, pyruvate and lactate during glucose tolerance tests in healthy subjects and in diabetic patients. The perpendicular bars indicate the standard errors of the means. Where the difference between the means was statistically significant, the *p* value is recorded directly below the two points.

cose levels during the glucose tolerance tests.

Glucose tolerance tests were performed before and during administration of DBI in five diabetic subjects. Although the fasting blood glucose level was lowered significantly by administration of the drug, there was no decrease in the height or duration of the hyperglycemic response after glucose (figure 1).

Glucagon tests: Glucagon tests were performed in three healthy subjects seven times before, and six times after administration of DBI. As can be seen from figure 5, the hyperglycemic response to glucagon was the same or slightly greater after administration of DBI. The somewhat greater response to glucagon was statistically significant only thirty minutes after injection

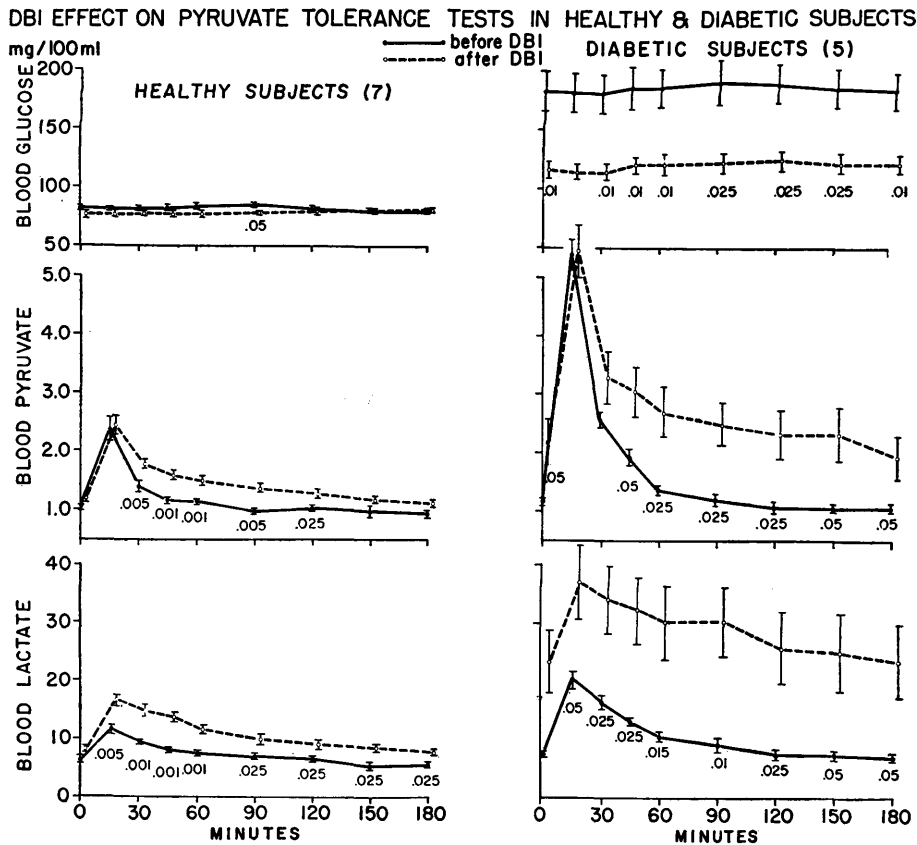


FIG. 2. Effect of DBI on blood levels of glucose, pyruvate and lactate during pyruvate tolerance tests in healthy subjects and in diabetic patients. The perpendicular bars indicate the standard errors of the means. Where the difference between the means was statistically significant, the *p* value is recorded directly below the two points.

of glucagon ($P < 0.05$).

Glucagon tests were performed in two stable diabetic subjects. The hyperglycemic response to glucagon was slightly greater (88 mg. to 199 mg./100 ml.) during DBI therapy than before its administration (242 mg. to 319 mg./100 ml.) in one subject, and was the same in the other patient.

Intravenous insulin tolerance tests: In two healthy subjects intravenous insulin tolerance tests were performed six times as indicated in figure 3. The administration of DBI did not alter insulin sensitivity on one occasion (R.R.), decreased it slightly on another occasion (J.D.) and caused a definite decrease in insulin sensitivity on two other occasions (R.R.). Urinary excretion of 17-hydroxycorticoids was slightly elevated (12.7 mg./24 hrs.) immediately preceding one of these days.

Insulin tolerance tests (0.05 units/kg.) were performed before and during administration of DBI in two diabetic subjects. DBI produced no change in in-

sulin sensitivity although the fasting blood sugar had decreased from hyperglycemic to normal levels.

B. Effect of DBI on metabolic balances and upon renal excretion of steroids.

Three metabolic balance studies were conducted in two healthy subjects (table 3). In these three experiments administration of DBI caused no significant changes from control averages for the mean urinary excretion of nitrogen, 17-ketosteroids and potassium. Excretion of creatinine, sodium and chloride was slightly lower during administration of DBI. There were no changes in serum levels of sodium, potassium, chloride, creatinine, blood urea, and hematocrit. On one occasion mean urinary excretion of 17-hydroxycorticoids was higher during administration of DBI (R.R.).

Metabolic balance studies were performed on three diabetic subjects (table 4) to investigate the relationship of nitrogen excretion to carbohydrate metabolism and to determine whether a decrease in gluconeogenesis from protein could be one of the causes of decreased

EFFECT OF DBI ON INTRAVENOUS INSULIN TOLERANCE TESTS IN NORMAL SUBJECTS
(INSULIN - 0.05 UNITS / Kg)

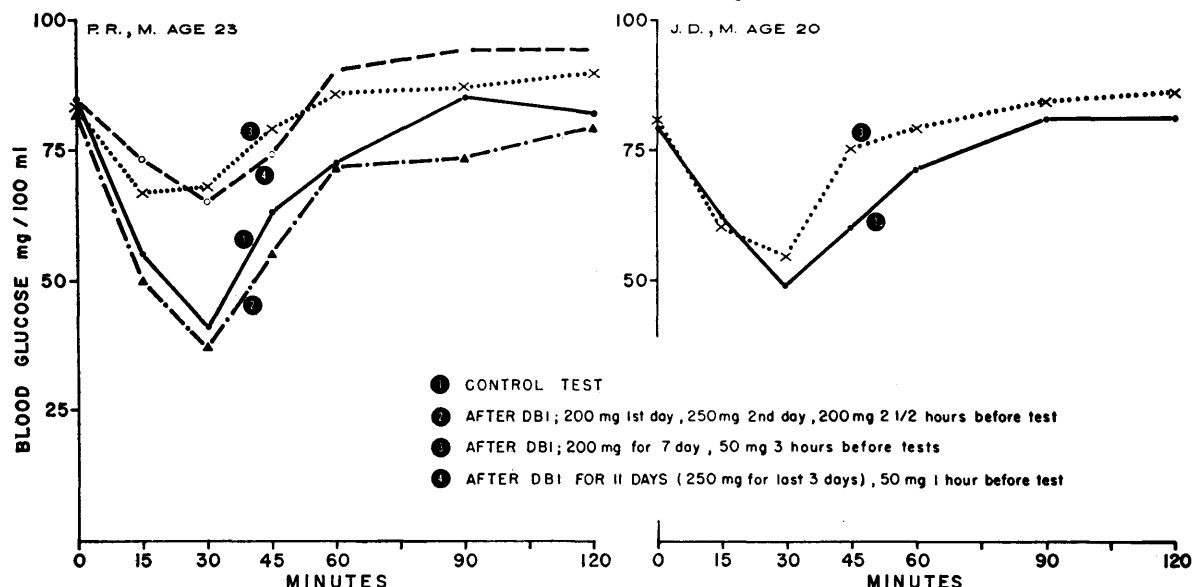


FIG. 3. Effect of DBI on intravenous insulin tolerance test (0.05 units/kg.) in normal subjects.

hyperglycemia and glycosuria following administration of DBI. In subject K.L. mean urinary excretion of glucose and nitrogen was the same during an eleven-day period of DBI administration as during the control period. However, on several days during the second half of the DBI period, glycosuria was significantly diminished. Urinary nitrogen excretion fluctuated in the same direction as glucose excretion. Following discontinuation of DBI there was a progressive increase in glycosuria and an increase in nitrogen excretion. Although it is impossible to be certain of any effect of DBI in this patient, one gains the impression that the drug may have prevented the further intensification of the diabetic state which followed the withdrawal of the drug.

Since in grossly uncontrolled diabetic patients, large, daily, spontaneous fluctuations occur in the quantities of glucose and nitrogen excreted, patients with minimal glycosuria were chosen as subjects for further balance studies. In subject C.W., DBI produced a fall in the fasting blood sugar from 306 to 165 mg./100 ml. As can be seen from table 4 there was no change in urinary nitrogen excretion during the DBI period. In subject R.B. the level of fasting blood sugar fell from 242 to 88 mg./100 ml. while the patient was given DBI. Again there was no significant change in nitrogen excretion.

During administration of DBI to the three patients

on balance study the following were noted: (1) urinary excretion of 17-hydroxycorticoids rose slightly in all, and excretion of 17-ketosteroids remained unchanged; (2) urinary excretion of creatinine remained essentially unchanged but increased in subject K.L. in the recovery period; (3) excretion of sodium and chloride was definitely decreased in subjects K.L. and R.B., and potassium excretion was decreased in subject K.L. and possibly in subject R.B.; and (4) there were no significant changes in serum sodium, chloride, potassium, hematocrit and blood urea nitrogen.

C. Effect of DBI upon the metabolism of pyruvate and lactate.

Blood levels of pyruvate and lactate were measured in the fasting state, during oral glucose tolerance tests and during intravenous pyruvate tolerance tests in normal subjects and in diabetic patients both before and during the administration of DBI. Each glucose tolerance test or pyruvate tolerance test was performed on the same subject before and after administration of 200 mg. of DBI daily for at least two days, an additional dose of 50 mg. being given two to three hours before the beginning of the second test.

In normal subjects DBI caused no change in the fasting levels of pyruvate or lactate. DBI did produce slight increases in blood pyruvate and lactate levels during the glucose tolerance tests and slight but highly significant elevations of these levels during the pyruvate

tolerance tests (figures 1 and 2). The blood glucose levels were not changed by administration of DBI to normal subjects.

In the diabetic subjects DBI caused marked increases in the blood levels of pyruvate and lactate both during fasting and during the glucose tolerance and pyruvate tolerance tests (figures 1 and 2). In these subjects DBI produced marked depression of blood glucose levels. The blood levels of pyruvate and lactate during the first sixty minutes of the pyruvate tolerance tests, before DBI was given, were higher in the diabetic than in the normal subjects (figure 2).

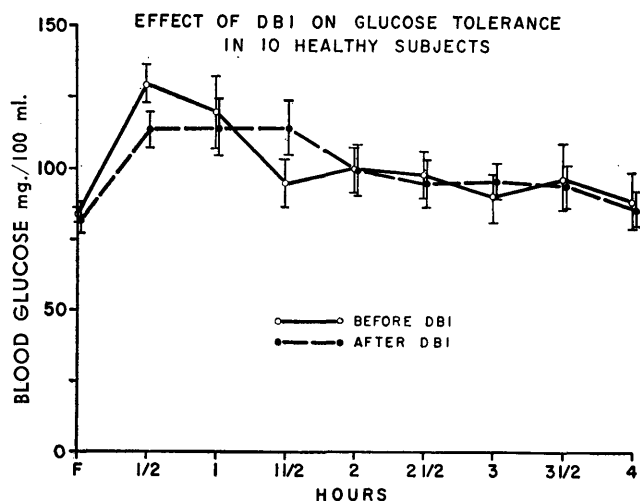


FIG. 4. Effect of DBI on glucose tolerance in ten healthy subjects. DBI was given in a dosage of 200 mg. per day for two days in six experiments, and 300 mg. per day for two days in two experiments. In addition the subjects received 50 to 200 mg. of DBI one to three hours before the second glucose tolerance test.

Urinary lactate¹⁰ was determined in two subjects. In subject R.B. maximum urinary excretion of lactate during the DBI period was 2.86 gm. per 24 hours (0.19 during control period). Blood lactate rose from 7.4 mg./100 ml. in the control to 35.8 mg./100 ml. in the DBI period. In subject B.S., in whom DBI had produced a decrease in glycosuria from 167 gm. to between 29 and 2.5 gm. per day, maximal urinary excretion of lactate was 12.5 gm. per twenty-four hours on the day of minimum glycosuria.

DISCUSSION

As has been reported by others^{3,4} DBI produces marked lowering of the blood glucose level in some diabetic subjects. However, in contrast to the findings in normal animals¹ DBI has no effect on blood glucose of healthy human subjects. There was no significant effect on blood glucose levels up to eight hours after

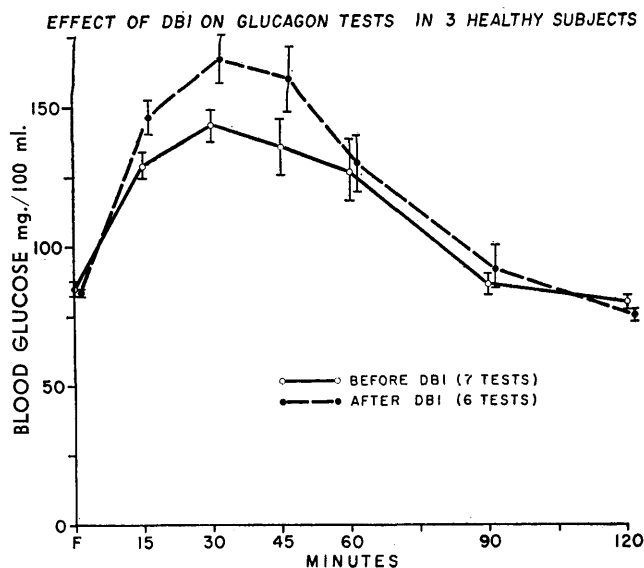


FIG. 5. Effect of DBI on glucagon tests in three healthy subjects. DBI was given in a dose of 200 mg. per day for two days in one experiment and for five to seven days in the other five experiments.

administration of a single dose of DBI in fasting healthy subjects. The dosage used extended up to 5.7 mg./kg. of body weight. In monkeys a single dose of 5 mg./kg. produced hypoglycemia three to five hours after its administration.¹ Even when these subjects had ingested a diet containing 64 gm. of carbohydrate and 600 calories for two weeks DBI produced no measurable hypoglycemic effect. Also, DBI had no effect on fasting blood glucose levels when it was administered for periods up to twelve days.

The data indicate that DBI did not interfere with the hyperglycemic action of glucagon in healthy or in diabetic subjects. This suggests that DBI does not lower blood glucose by decreasing the rate of any of the three enzyme systems (phosphorylase, phosphoglucomutase, or glucose-6-phosphatase) involved in hepatic glycogenolysis. These findings differ from those reported in DBI-treated animals in which glucagon did not increase hepatic glucose output.^{2,5}

In contrast to a previous report³ DBI had no significant effect on glucose tolerance in healthy or in diabetic subjects. DBI did not alter sensitivity to insulin in the normal or in the diabetic subjects when evaluated by the intravenous insulin tolerance test. This suggests that DBI does not exert its hypoglycemic effect by potentiation of insulin activity (endogenous or exogenous). The same conclusion has been reached by a different technic in animal experiments.^{2,5}

Metabolic balance studies in the diabetic patients

showed that decreases in hyperglycemia were not accompanied by decreased excretion of urinary nitrogen. Thus, there is no evidence in man that a decrease in the rate of gluconeogenesis from protein is a contributing factor to the hypoglycemic effect produced by DBI. Such a mechanism has been postulated from studies in animals.⁵

The data indicate that decreased function of the pituitary-adrenal system is not one mode of action of DBI. There were no decreases in renal excretion of 17-hydroxycorticoids or 17-ketosteroids and results of metabolic balance studies did not suggest decreased adrenal cortical activity. On the contrary, during several periods of DBI administration there were increases in the mean excretions of 17-hydroxycorticoids. This may have been related to the anorexia and nausea produced by administration of DBI in these subjects.

The data discussed so far do not reveal the mechanisms by which DBI lowers the concentration of blood glucose in diabetic subjects. They do help to eliminate possibilities which had to be considered.

DBI produces an increase in blood pyruvate and lactate levels during pyruvate tolerance tests as well as during glucose tolerance tests in both healthy and diabetic subjects. This suggests that DBI causes a delay in the utilization of pyruvate. In the diabetic subjects the marked elevations in blood pyruvate and lactate levels were associated with sharp decreases in blood glucose levels. In the healthy subjects, the small increases in blood pyruvate and lactate were not accompanied by changes in blood glucose levels. These data suggest that the hypoglycemic effect of DBI may be quantitatively related to and the result of its effect on pyruvate disposal.

Interpretation of the data in this way can be supported and extended by reference to the work of others upon the effects of DBI in isolated tissues.¹⁴⁻¹⁶ It has been shown that DBI inhibits one or more reactions within the Krebs oxidative cycle, thereby diminishing the uptake of oxygen, and enhancing the breakdown of glucose by anaerobic glycolysis to form pyruvate and lactate. Increased utilization of glucose as a result of decreased utilization of oxygen is known as the Pasteur effect.¹⁷ Reduction of the blood glucose level by such a mechanism in the intact animal or human subject has not been recognized. Nevertheless, our experiments are consistent with the hypothesis that DBI produces its hypoglycemic effect in diabetic patients by inhibiting cellular oxidative reactions, thereby increasing the utilization of glucose by anaerobic metabolism. Further

support for this hypothesis is found in the recent report that acetylsalicylic acid and dinitrophenol, agents which uncouple oxidative phosphorylation, also have a hypoglycemic effect in diabetic subjects.¹⁸

It must be pointed out that our data give no indication of the fate of the DBI-induced accumulation of pyruvate other than that some of it is found in the urine as lactate. Anaerobic degradation of glucose to lactate is an energy-yielding reaction of relatively low magnitude. It must be emphasized that a given decrease of blood sugar induced by DBI does not have the same metabolic implications as one induced by insulin.

The reason for the much greater effect of DBI upon blood pyruvate and lactate levels in the diabetic as compared to the healthy subjects is not apparent from present knowledge. The marked difference suggests that there may have been a pre-existing endogenous block in pyruvate metabolism in the diabetic subjects. The presence of such a biochemical lesion in diabetes has been suspected in the past, but it has not been clearly defined.¹⁹⁻²³ The elevation of the first part of the pyruvate tolerance curve in the diabetic subjects before they were given DBI may also be a reflection of an impairment of pyruvate disposal, but such an interpretation is not warranted on the basis of the present data.

SUMMARY

Administration of phenformin to diabetic subjects produces lowering of the blood glucose level. In sharp contrast to the findings in normal animals, phenformin has no effect on blood glucose in healthy people. Phenformin causes no changes in glucose tolerance or sensitivity to insulin, has no effect on glucagon-induced hyperglycemia, and does not decrease urinary excretion of nitrogen, potassium, 17-hydroxycorticoids and 17-ketosteroids in healthy subjects or in diabetic patients. Phenformin increases blood pyruvate and lactate levels during glucose tolerance tests and during pyruvate tolerance tests. This effect is much greater in diabetic patients than in normal subjects.

Thus, there is no evidence that inhibition of adrenal function, decreased gluconeogenesis from protein, increased insulin activity or decreased glucagon activity are contributing factors to the blood glucose-lowering effect produced by phenformin. The findings suggest that phenformin delays the disappearance of pyruvate and lactate from blood. Comparison of the data from normal and diabetic subjects indicates that the hypoglycemic action of phenformin may be quantitatively related to the effect on pyruvate disposal. These results, together with certain *in vitro* studies by other workers,

suggest that phenformin produces its hypoglycemic effect by causing an increase in anaerobic glycolysis as a result of suppression of cellular oxidation.

SUMMARIO IN INTERLINGUA

Effectos Metabolic de Phenethylbiguanida (DBI) in Subjectos Normal e in Patientes con Diabete

Le administration de DBI a subjectos diabetic produce un reduction del nivello sanguinee de glucosa. Per contrasto marcate con le constatationes in normal animales, DBI ha nulle effecto in le glucosa del sanguine de humanos normal. DBI causa nulle alteration del tolerantia pro glucosa o del sensibilitate pro insulina, ha nulle effecto super le hyperglycemia inducite per glucagon, e non augmenta le excretion urinari de nitrogeno, kalium, 17-hydroxycorticoides, e 17-cetosteroides in subjectos normal o in patientes con diabete. DBI augmenta le nivellos de pyruvato e lactato durante tests de tolerantia pro glucosa e durante tests de tolerantia pro pyruvato. Iste effecto es multo plus grande in patientes diabetic que in subjectos normal.

Assi il existe nulle supporto pro le assertion que le inhibition del function adrenal, un reduce gluconeogenesis ab proteina, un augmento del activitate de insulina, o un reduction del activitate de glucagon es factores contribuyente al effecto glucoso-reductor de DBI. Le constatationes suggere que DBI retarda le disparition de pyruvato e de lactato ab le sanguine. Le comparison de datos ab subjectos normal e diabetic indica que le action hypoglycemic de DBI es forsan relationate quantitativamente al effecto super le elimination de pyruvato. Iste observationes—insimul con le resultados de certe studios in vitro per altere investigadores—suggere que DBI produce su effecto hypoglycemic per causar un augmento del glycolyse anaerobie in consequentia del suppression de oxydation cellular.

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