Carbohydrate Metabolism and Capillary Basement-membrane Thickness in Children

II. Longitudinal Studies

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SUMMARY

Longitudinal biochemical and histologic studies were carried out in 11 children receiving oral hypoglycemic agents. There were five "suspected" diabetics (with evidence of glucose intolerance but without repeated fasting hyperglycemia) and six diabetics.

Mean fasting plasma glucose (FPG) values showed no significant change during treatment with phenformin alone. The mean FPG decreased significantly within six to 10 weeks after addition of tolazamide to the regimen, but the decrease was not sustained during long-term observation (seven months to four years). Glucose disappearance rate (K) generally increased as FPG decreased, but the number of observations was smaller, and mean values showed no significant change. Mean values for fasting plasma insulin and for peak insulin response to intravenously administered glucose did not change significantly.

Changes in capillary basement-membrane thickness (BMT) were found to be statistically significant in a number of individual instances. Decreasing BMTs were associated with increasing Ks and vice versa. A similar trend was apparent among other patients, in whom individual changes in BMT were not statistically significant. The pooled data were therefore subjected to chi-square analysis; K and average BMT were found to change in opposite directions ($P < 0.001$); a similar relationship held for K and minimum BMT ($P < 0.05$). But K and BMT did not correlate well as regards magnitude of change. Influences of phenformin and tolazamide on these changes could not be evaluated.

Both BMT and K may be influenced by many complex variables, but the present findings indicate that glucose tolerance and BMT have a close interdependence. DIABETES *25*:661-66, August, 1976.

Until insulin therapy became available, coma was the leading cause of death among diabetics. With progress in management of diabetes itself, vascular complications have replaced coma as the leading cause of death. There is a strong correlation between cardiovascular complications and duration of clinically recognized diabetes.[1] It is not known whether improvement in carbohydrate tolerance has any effect on the cardiovascular complications, but the question is of evident interest.

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The earliest recognized structural alteration of the microcirculation in diabetes mellitus is an increase in capillary basement-membrane thickness (BMT).[2-6] It is not clear whether increased BMT is related to clinical cardiovascular disease; but BMT examined in muscle biopsies provides a readily measured histologic variable for comparison with metabolic status.

The present communication describes longitudinal biochemical and morphologic studies in a group of children with carbohydrate intolerance but without ketoacidosis, who were treated first with phenformin alone and then with phenformin and tolazamide in combination. The report addresses one primary question: Is improvement (or deterioration) in carbohydrate tolerance associated with any consistent change in BMT? The role of the drugs in influencing the changes could not be determined as such.

PATIENTS

Fifteen patients were examined longitudinally with two or more intravenous glucose tolerance tests (IVGTTs) and muscle biopsies. Clinical data were summarized in the preceding paper.[7] Four of these children (15, 20, 22, and 31) did not receive oral hypoglycemic agents. Their courses were described earlier. This communication reports longitudinal studies obtained in the other 11 children, who entered a special protocol and received oral hypoglycemic agents. Five of these children (14, 16, 17, 18, and 19) were classified as "suspected" diabetics; the other six (25, 26, 27, 33, 36, and 39) were classified as diabetics.[7] All patients were free of visible retinovascular abnormalities. Most of them had been referred to our clinic for inclusion in this study. Informed consent to the study was obtained in each instance.*

PROCEDURE

Before therapy was begun, IVGTTs were performed in all patients, one or more oral glucose tolerance tests (OGTTs) were carried out in those patients whose diagnosis was not readily obvious, and muscle biopsies were obtained in all patients except patient 18. Patient 18 was studied before approval of muscle biopsies by our human experimentation committee. After initial (baseline) studies were obtained, phenformin HCl (DBI-TD) was begun in a dosage of 50 mg. twice a day and continued as the only medication for a period that varied from two to 52 weeks; in patients who had gastrointestinal complaints the dose was reduced to 50 mg. daily. In most instances, studies were repeated before adding tolazamide (Tolinase), 2 to 4 mg./kg. of body weight once a day, to the regimen. Most patients were studied again six to 10 weeks after initiation of tolazamide. Thereafter, patients

*After the UGDP report on the effect of oral hypoglycemic agents, all patients and their parents were interviewed and informed about the UGDP conclusions. The FDA current drug information of October, 1970, and the American Diabetes Association letter of October 27, 1970, were read by parents and were fully discussed with them. They were allowed to discontinue the medication under our supervision whenever they desired.

TABLE 1

Changes in FPG, K value, and insulin response to intravenous glucose loading during the administration of oral hypoglycemic agents

Pt.	Pretreatment						Phenformin						Phenformin & Tolazamide (Short-term)					
			Insulin			Duration			Insulin			Duration			Insulin			
	FPG mg.%	K	F μU./ml.	Max. μU./ml.	min.	wk.	FPG mg.%	K	F μU./ml.	Max. μU./ml.	min.	wk.	FPG mg.%	K	F μU./ml.	Max. μU./ml.	min.	
							"Suspected" Diabetics											
14	85	1.27	6	48	2.5	2						8	77	1.61	13	40	2.5	
16	68	1.15	100	388	5	6	78	2.01	79	492	2.5	6	59	2.61	68	506	5	
17	106	1.82	25	80	5	14	69	1.11	20	104	2.5							
18	84	0.80	27	32	2.5	52	144	0.84	33	55	20	6	65	0.72	19	53	2.5	
19	95	1.03	66	200	2.5	6	94	1.80	25	200	2.5	6	54	3.15	20	43	2.5	
M_S	71						96						64					
							Diabetics											
25	161	0.33				6	91	0.60	43	46	40	8	114	0.72	43	44	50	
26	325	0.66	7	15	20	3						6	89	0.88				
27	169	0.82	55	49	20	26	281	0.53	28	36	40							
33	129	0.69	52	71	20	27	132	0.59	42	59	10	10	98	0.68	18	25	2.5	
36	173	0.98	54	102	20	38	154	0.82	75	112	20	6	113	0.86	79	139	10	
39	151	1.90				6	80	1.70	27	137	30	6	66	2.10	32	198	2.5	
M_d	185						148						96					
M_t	141						125						82					

M_S = mean value, "suspected" diabetics.
M_d = mean value, diabetics.
M_t = mean value, total study group.
Pt = patient; FPG = fasting plasma glucose; F = fasting, Max. = maximum response; wk = week.

Differences in FPG: pretreatment vs. phenformin, nonsignificant; pretreatment vs. short-term phenformin-tolazamide, $P < 0.01$; phenformin vs. short-term phenformin-tolazamide, $P < 0.05$; short-term vs. long-term phenformin-tolazamide, $P < 0.01$; pretreatment vs. long-term phenformin-tolazamide, nonsignificant (statistics apply to total study group in all instances).

consenting to continue on the protocol were studied once a year or at the time the medications were discontinued, but patients were seen more frequently for clinical assessment.

The follow-up studies consisted of an IVGTT and a muscle biopsy. The methods used for the measurement of glucose, insulin, growth hormone, and BMT were described previously.[7] Statistical methods used were cited earlier;[7] in addition, changes in BMT in relation to changes in K value were examined by chi-square analysis.[8]

RESULTS

Metabolic Studies

The results obtained from serial fasting plasma glucoses (FPGs) and IVGTTs are shown in table 1. Pretreatment values, discussed earlier, are included for ease of comparison.

The mean FPG for the study group as a whole did not change significantly during treatment with phenformin alone. Addition of tolazamide to the regimen was followed within six to 10 weeks by a significant decrease in mean FPG. This decrease was not sustained during long-term treatment; in fact, the mean FPG eventually returned to a value that was not significantly different from the pretreatment value. When the mean FPGs for the diabetic and the "suspected" diabetic groups were examined separately, similar trends were evident, but statistically significant differences were not demonstrable on these smaller data bases. Mean K values generally increased as FPG decreased, but no statistically significant differences were found either for the study group as a whole or for the diabetic and the "suspected" diabetic groups considered separately. It should be evident from the table that there were fewer K values than FPG values available for statistical analysis. Indeed, FPGs were more abundant than any other biochemical or morphologic data obtained in this study. This may account, in part, for the finding of statistically significant changes in mean FPG but not in other mean values.

TABLE 1 (*cont.*)

Changes in FPG, K value, and insulin response to intravenous glucose loading during the administration of oral hypoglycemic agents

Pt.	Phenformin & Tolazamide (Long-term)					
	Duration	FPG	K	Insulin F	Insulin Max.	
	wk.	mg.%		μU./ml.	μU./ml.	min.
14	84	88	1.75	14	32	2.5
16	82	98	1.90	103	708	2.5
17						
18	104	63	0.90	16	52	5
"	156	68	1.26	6	33	5
19	116	88	1.90	16	171	2.5
M_S		81				
25	22	397				
26	68	320				
27	55	234	0.46	26	31	20
33						
36						
39	43	84	1.33	20	229	2.5
M_d		259				
M_t		160				

M_S = mean value, "suspected" diabetics.
M_d = mean value, diabetics.
M_t = mean value, total study group.
Pt = patient; FPG = fasting plasma glucose; F = fasting; Max. = maximum response; wk = week.

Differences in FPG: short-term vs. long-term phenformin-tolazamide, $P < 0.01$; pretreatment vs. long-term phenformin-tolazamide, nonsignificant (statistics apply to total study group in all instances).

There were no statistically significant changes in mean fasting insulin values or in mean peak insulin responses for the group as a whole or for either of the two subgroups of children. In considering the courses followed by individual patients it is of some interest to note that, in general, children who exhibited a considerable rise in plasma insulin during their initial IVGTTs maintained fairly good carbohydrate tolerance throughout the period of observation, and did well clinically. In contrast, four of the diabetics (25, 26, 27, and 33) eventually became insulin-dependent, i.e., exogenous insulin became necessary for control of glycosuria or for adequate weight gain. Of these four patients, one (25) had previously been treated with insulin; the remainder had shown little or no rise in plasma insulin during their initial IVGTTs.

Plasma growth hormone responses, not shown in the table, were essentially unchanged after treatment with oral hypoglycemic agents.

Capillary BMT During Administration of Oral Hypoglycemic Agents

Absolute values for ABMT and MBMT, respectively, are plotted against time in figures 1 and 2. It is evident that BMT increased in some instances and decreased in others. Mean values during each treatment period were determined for the study group as a whole and for the diabetic and "suspected" diabetic groups separately. In no case were statistically sig-

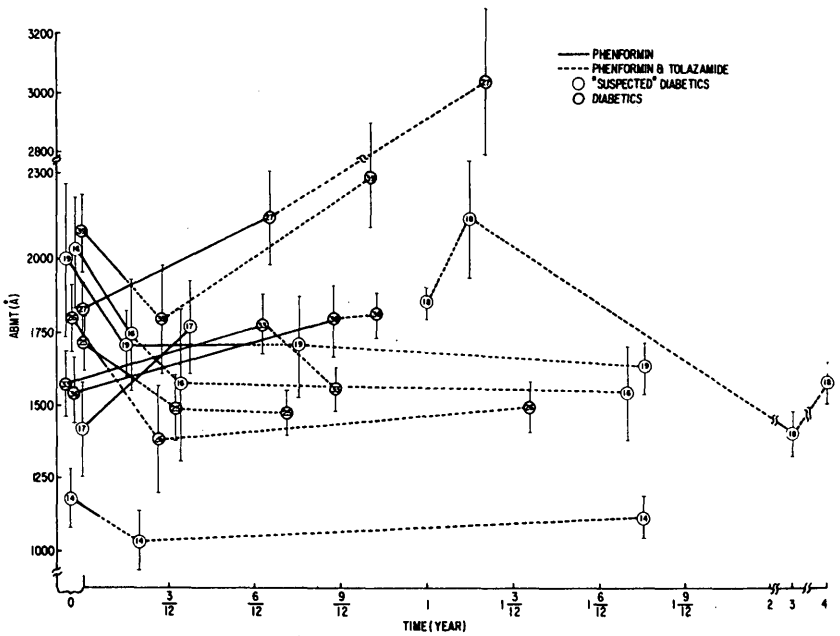

FIGURE 1

Changes in ABMT during the administration of oral hypoglycemic agents. I = S.E.M.

nificant differences found. BMT changes occurring in each patient were also examined, and some suggestive trends were evident. Patients with persistently low or declining K values and high FPGs tended to have increasing ABMT, while patients with decreasing ABMT generally had increasing K values. In a number of individual instances the changes in ABMT were statistically significant.

MBMT correlated closely with ABMT (r = 0.93), and changes in MBMT, with a few exceptions, paralleled those in ABMT.

Because the data suggested an interdependence between changes in BMT and changes in K value, the relationship between these variables was analyzed further. Figure 3 expresses changes in ABMT as per cent increase or decrease from pretreatment values and displays them as functions of per cent increase or decrease in K value. Patient 18 is excluded from the figure because she was not biopsied before treatment. Clustering of the data into the right lower and left upper quadrants indicates an inverse relationship between direction of change in ABMT and direction of

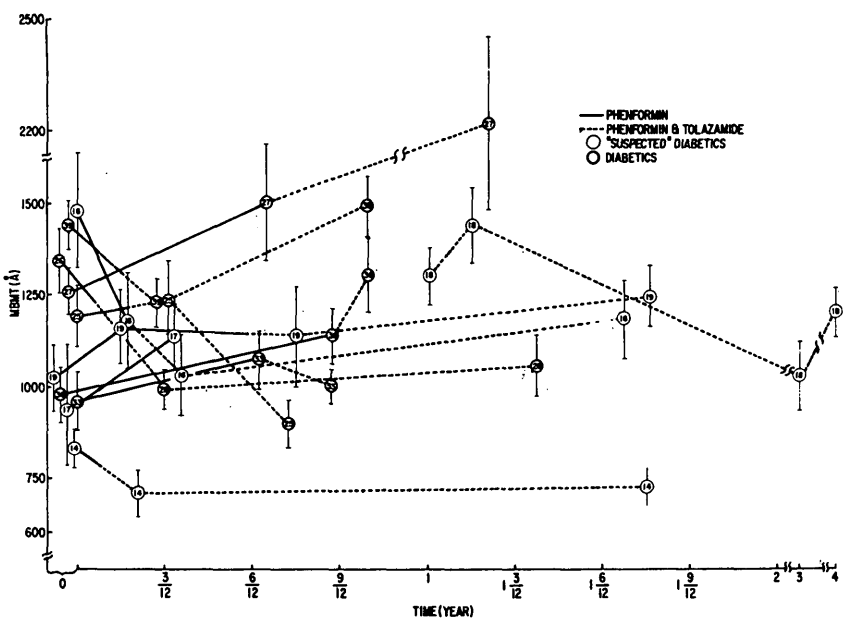

FIGURE 2

Changes in MBMT during the administration of oral hypoglycemic agents.

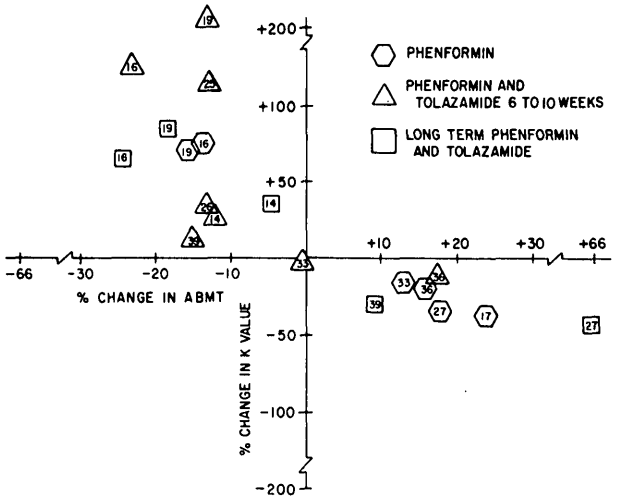

FIG. 3. Changes in ABMT as a function of changes in K value. Decreases from and increases over each pretreatment value are expressed as percentages of that value.

change in K value. This impression is confirmed by chi-square analysis ($P < 0.001$). Similar treatment of MBMT data reveals a comparable but less striking relationship, with $P < 0.05$.

Despite the strong interdependence between K value and BMT as regards direction of change, these variables did not exhibit a high correlation as regards magnitude of change: for per cent change in K value and in ABMT, $r = 0.69$; for per cent change in K value and in MBMT, $r = 0.40$.

The analysis of the relationship shown in figure 3 has, of course, nothing to do with the statistical significance of any individual change in K value or in ABMT; but it may be noted parenthetically that most of the ABMT changes shown exceed the differences to be expected as a result of technical variation, i.e., in the majority of instances the changes exceeded 13.5 per cent.[7]

Other Findings (Not Shown in Table or Figures)

Adverse responses (gastrointestinal complaints and symptoms suggestive of hypoglycemia) were minor and responded readily to reduction in dosage.

No retinovascular or cardiovascular abnormalities were detected on physical examination.

DISCUSSION

The present communication describes a longitudinal study of carbohydrate metabolism and capillary BMT in a group of children with evidence of glucose intolerance. A previous report from this laboratory[7] presented cross-sectional data obtained from a larger number of children, some of whom had no evidence of glucose intolerance. As noted in that communication, the present state of information does not yet permit definitive classification of all the findings in such cross-sectional studies as "normal" or "abnormal." Similarly, biologic significance cannot as yet be assigned to changes observed in longitudinal studies like the present one; only statistical significance can be determined. As more information comes to light, study of statistical significance should help to elucidate biologic significance.

Improvement in carbohydrate metabolism and insulin release after treatment with oral hypoglycemic agents has been reported by others in children and in adults.[9-13] The usefulness of such treatment could not be determined in the present study, since the larger placebo-controlled design required for this purpose did not prove feasible (publication of the UGDP results had the effect of restricting the number of subjects willing to be studied). Changes in metabolic status during treatment with phenformin alone were quite variable. Mean FPG decreased significantly after addition of tolazamide to the regimen, but this change was not sustained during long-term observation. Changes in clinical status were variable: children who had initially exhibited relatively mild manifestations of insulin deficiency continued to do well and did not become insulin-dependent. In contrast, the four diabetics who had more severe manifestations of insulin deficiency at the beginning of the study did become insulin-dependent. The courses observed in these patients may well represent the natural history of the disease[14] rather than the effect of the drugs.[9-13]

The concomitant changes observed in BMT were statistically significant in a number of individual instances. But both increases and decreases were observed, and mean values for the diabetic group and for the "suspected" diabetic group showed no significant change. Camerini-Davalos et al.[15] have reported a cross-sectional study in which patients treated with oral hypoglycemic agents were found to have lower BMT values than untreated patients. Their conclusions are provocative and suggest a need for further investigation. The present longitudinal study leaves unresolved the question of possible effects of such drugs on BMT.

The finding that BMT and K value change in opposite directions (figure 3) is intriguing. The biologic significance of the changes observed is unknown, but the striking interdependence demonstrated suggests that longitudinal studies will be more effective than

purely cross-sectional studies in elucidating the relationship between metabolic and histologic changes. As to possible cause-and-effect relationships between changes in glucose tolerance and changes in BMT, proposed mechanisms must as yet be speculative. Muir[16] has suggested that the disturbed carbohydrate metabolism of diabetes may produce "abnormal amounts of a reactive intermediate which might form cross-links with collagen hastening its 'aging' and reducing its rate of breakdown. . . ." Spiro[17] has proposed that increased glucose availability in a tissue not requiring insulin for glucose utilization may favor overproduction of basement membrane. He notes that diabetic basement membrane has an increased content of hydroxylysine-linked disaccharide units and that alloxan-diabetic animals have increased concentrations of the specific glucosyltransferase operative in assembly of such units.

The relationship between BMT and carbohydrate tolerance may be a complex one, since each of these variables may be influenced by a number of genetic and environmental factors. It may be pertinent in this connection to consider, for example, the course followed by patient 26. This child had exhibited a decrease in ABMT and improvement in glucose tolerance during the first weeks of treatment with oral hypoglycemic agents. As treatment was continued, marked hyperglycemia and glycosuria recurred, but the ABMT value remained low. It may be noted that this ABMT value was excluded from figure 3 because the clinical status of the patient precluded repetition of an IVGTT.

The findings in the present study are compatible with the suggestion of Williamson[3] that carbohydrate intolerance may be a prerequisite for increased BMT. In addition, it appears that BMT decreases as carbohydrate tolerance improves.

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