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Received July 3, 1957. P.S.E.B.M., 1957, v96.

Diabetes Mellitus and Serum Vitamin B_{12} Concentrations: 333 Patients. (23464)

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Since a role for vit. B_{12} in transmethylation and the transformation of carbohydrate to fat has been invoked(1), it is natural that deviations in the B_{12} metabolic pattern should be looked for in the most obvious defect of carbohydrate metabolism, human diabetes mellitus. It is conceded that patients with diabetes show an accelerated rate of development of cardiovascular degenerative disease, not directly correlated with age of onset, duration, or severity of the diabetes. Vascular disease of the retina, diabetic retinopathy and glomerulosclerosis with or without the Kimmelstiel-Wilson syndrome, are regarded as advanced manifestations of vascular degeneration. Observations correlated with these conditions raise the question whether the relationship is with severe vascular disease independent of diabetes or with diabetes itself.

Methods. Patients from 4 large diabetic clinics* were studied. Single serum samples were obtained from fasting patients and were assayed for their B_{12} content by the *Lactobacillus leichmanii* method(2,3,4). All sera were frozen and held at –20°C until assay and those specimens which were *not frozen* upon receipt were discarded. The diagnosis of diabetic retinitis in the patients studied was accepted only on the basis of an examination by an experienced ophthalmologist within a week of assay. The diagnosis of diabetes in all patients studied was established by history and the requirement of insulin for prolonged periods.

Results. The results are recorded in Tables I to IV. Normal groups for comparison were not obtainable from all institutions for it is not common for normal individuals in any number to be institutionalized. The statistical methods seemingly most appropriate to this study have been employed in the analysis of the data(5,6). Certain variables inherent in such a study as has been carried out, cannot be eliminated. The onset of diabetes mellitus is superimposed upon *pre-existent* physiology and pathology and diabetes in "pure" form in a patient free from abnormalities which, in the absence of diabetes would be regarded as falling within the limits of normal, is rare.

It is clear that where controls were available there was no consistent difference between diabetics and these "normals." Similarly, in the 3 studies where the subjects had been screened for retinopathy there was no apparent difference between patients with and

* The authors wish to express their indebtedness to Dr. Wallace W. Dyer of the Philadelphia General Hospital and Dr. Harry S. Lipscomb of Jefferson Davis Hospital, Houston, Texas, for their kindness in having supplied us with the sera from diabetic patients.

TABLE I. B_{12} Levels ($\mu\mu$g/ml) in Diabetics (PaH).

	♂		♀	
	No retinopathy	Retinopathy	No retinopathy	Retinopathy
Avg B_{12}	640	520	970	980
Range B_{12}	380–970	260–780	580–1440	260–2490
Avg age	68	63	65	57
No. subjects	5	2	4	12

There was no obvious difference between subjects with retinopathy and those without retinopathy. The females had significantly ($P < 0.05$) higher levels than the males. No "normal" controls were available for this study.

without retinal disease. The smallness of numbers raises some question of significance but the numbers present a true reflection of occurrence of retinopathy in the groups studied. There was some suggestion that the female diabetics have slightly higher levels than the males; this did not seem to be true for the normals included as controls. In other studies(3,4) there has been a suggestion that normal females have slightly higher, but not significantly different, levels than males.

Discussion. As pointed out, the question can be raised about the appropriateness of interpreting changes in vit. B_{12} metabolism observed in the presence of diabetic retinitis and glomerulosclerosis as correlating with diabetes rather than with severe vascular damage from any cause. Serum levels have been reported to be higher in diabetics in the presence of diabetic retinitis than in its absence(7). On the other hand, lower concentrations in the presence of severe retinopathy and glomerulosclerosis than in uncomplicated diabetes have been demonstrated(8). It has been implied, rather than established, that retinal vascular damage directly reflects similar systemic vascular damage. This may be so, but not necessarily, as has been pointed out in an excellent pathologic survey that states, ". . . retinal arteriosclerosis occurred more frequently in the absence of than with glomerular arteriosclerosis"(9).

Similar disagreement of observations is recorded with regard to the urinary excretion

TABLE II. B_{12} Serum Levels ($\mu\mu$g/ml) in Diabetics (NSH).

	♂			♀		
	No retinopathy	Retinopathy	Normal	No retinopathy	Retinopathy	Normal
Avg B_{12}	610	310	580	860*	760	570
Range B_{12}	250–870	—	100–1620	400–3400	1520–1020	20–2170
Avg age	49	52	42	60	53	59
No. subjects	6	1	108	14	6	238

* If one high value is omitted, the avg becomes 0.66.

There were too few values to make a valid test for effect of retinopathy. Avg level was higher for females than for males, but difference was not significant statistically ($P = 0.20$).

Avg B_{12} blood level was essentially the same for males and females in the "normal" controls. If the one high value in the diabetic group is omitted, the difference between diabetics and "normal" controls is not significant statistically ($P > 0.10$).

TABLE III. B_{12} Serum Levels ($\mu\mu$g/ml) in Diabetics (PGH).

	♂		♀	
	No retinopathy	Retinopathy	No retinopathy	Retinopathy
Avg B_{12}	480	480	600	630
Range B_{12}	80–1470	220–1010	140–1260	150–1230
Avg age	58	67	54	61
No. subjects	47	5	62	19

There was no obvious difference between subjects with retinopathy and those without retinopathy. The females had significantly ($P = 0.01$) higher levels than the males. No "normal" controls were available for this study.

TABLE IV. B_{12} Blood Levels ($\mu\mu$g/ml) in Diabetics (JDH).

	♂		♀	
	Diabetic	Normal	Diabetic	Normal
Avg B_{12}	650	660	780	780
Range B_{12}	80–1560	260–2100	70–1890	260–3000
Avg age	55	41	56	39
No. subjects	42	40	108	34

The diabetic females showed a tendency for higher levels than the males, but the difference did not reach the customary 5% level of significance ($P = .09$). The subjects were not screened for retinopathy.

In "normal" controls, the apparent sex difference was not significant statistically ($P = 0.21$). There was no obvious difference between diabetics and "normal" controls.

of vit. B_{12}; some investigators state that excretion of vit. B_{12} is *greater* in diabetics with retinopathy than in those without, and *higher* in diabetics than in normals(10). Others have found that there were no differences between such groups of patients(11). This investigation was directed toward determining in a large group of diabetics whether or not there was any difference in the serum B_{12} concentrations of such persons and non-diabetics. Normal values of serum vit. B_{12} have been previously established(3) as averaging 560 $\mu\mu$g/ml (70-1060 $\mu\mu$g/ml). This normal figure conforms with other published work (12) and statistically significant deviations from such normal values have been established in hepatitis(13,14), acute(15) and chronic myologenous leukemia(16,17), in pregnancy(18) and in newborns(4,19). Our study has shown (a) no difference between diabetics and normals, (b) a slight difference between male and female diabetics in favor of the females, and (c) no difference between patients with and without diabetic retinopathy. In face of these observations it is suggested that abnormal serum concentrations of B_{12} or alterations of urinary excretion of this vitamin are more probably related to extensive vascular damage *per se*, than to the metabolic disease, diabetes. The liver is known to be the chief reservoir for vit. B_{12} and the transport of the vitamin in the body has been related to blood proteins. That abnormal liver function can influence serum B_{12} concentrations has been shown(13,14). Since the liver is not uncommonly involved in diabetes, hepatic function may be responsible for some abnormal serum concentrations observed in diabetes.

There have been suggestions that the glucose tolerance test can be altered by B_{12}(20) and that the insulin requirement of diabetics can be altered by administration of the vitamin(21,22). Favorable and unfavorable reports of the benefit of B_{12} therapy for neuropathy in the presence of diabetes have been published. Certainly there has not been the uniformity of response that has been observed in the neuropathic manifestations of vit. B_{12} deficiency states(23,24).

Conclusion. A study of serum vit. B_{12} concentrations has failed to show significant differences between 333 diabetics and 420 normal persons, between 45 diabetics *with* and 138 *without* retinopathy, and only a slight difference between 225 females and 108 males in favor of the females. Within the limits of this study, the serum B_{12} values of diabetic subjects are the same as those for normal individuals. It is suggested that previously observed differences may reflect vascular damage rather than the diabetic state *per se*.

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Received July 8, 1957. P.S.E.B.M., 1957, v96.

Acetoacetate Induced Dehydroascorbic Acid Accumulation in Blood and Tissues and Its Prevention by Glucose-Cyclo-Acetoacetate. (23465)

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Acetoacetate, an intermediary metabolite of lipids found by Nath *et al.*(1,2) to cause an immediate rise in blood glucose and decrease in glucose tolerance, has been reported(3) to cause a sudden increase in vit. C of blood of rats, when doses are relatively small. The higher doses (500 mg/kg or above) were, however, reported to depress such biosynthesis. Acetoacetate has also been reported to deplete GSH of blood of rabbits(4), and administration of the condensation product (Na salt) of glucose and acetoacetate (glucose-cyclo-acetoacetate) prior to injections of acetoacetate has been found to prevent this depletion of GSH and the rise in blood sugar(5). This depletion of GSH has been attributed to the oxidizing action of dehydroascorbic acid formed as a result of acetoacetate injections. The hydrolysed condensation product has been found by the authors(6) to act like GSH in converting dehydroascorbic acid back to ascorbic acid. It can be assumed that glucose-cyclo-acetoacetate exerts its sparing effect on GSH by undergoing enzymatic hydrolysis in the system. The present paper deals with the effect of glucose-cyclo-acetoacetate on accumulation of dehydroascorbic acid in blood and tissues and its excretion in urine as a result of acetoacetate injection in high doses.

Methods. Twenty-four male albino rats, of about 200 g each were divided into 4 groups, A, B, C, and D, of 6 rats each. Group A served as controls while Group B was given daily injection of Na-acetoacetate 500 mg/kg body weight. Group C were given daily 900 mg/kg body weight of condensation product (Na salt) prior to injections of 500 mg/kg of acetoacetate. In Group D, 900 mg/kg of hydrolysed condensation product (hydrolysed by 2N HCl for 10 minutes in boiling water, cooled and neutralized with NaOH to pH 7.2) was administered prior to acetoacetate injections daily. All injections were given intraperitoneally. The 24 hr urine of

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