streaked on the surface of agar inseminated with *Sarcina lutea* and containing P152 in excess of minimum inhibiting concentration of the latter, in a modified Gots test for penicillinase(3). Colonies of Sarcina developed around the surface growth of all the penicillinase producing strains of *Staphylococcus aureus* in these plates in the same manner as in similar plates in which penicillins V and G were used, indicating that all 3 of these penicillins are at least qualitatively similar in their susceptibility to penicillinase activity.

Conclusions. Potassium 6-(*a*-phenoxypropionamido) penicillinate and potassium phenoxymethyl penicillin are absorbed in essentially the same manner in normal men and produce comparable levels of antibacterial activity in the serum. Both of these penicillins are qualitatively similar to penicillin G in their susceptibility to penicillinase produced by *Staphylococcus aureus.*

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B_{12} Metabolism and Multiple Sclerosis.* (25451)

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Previous reports(1-5) indicate that the metabolic pattern as well as urinary and serum levels of Vit. B_{12} may differ according to the disease state in question, such as pernicious anemia(6-8), liver disease(9-11), myelogenous leukemia(12-13), diabetes mellitus (14-15) and others. Since Vit. B_{12} has been implicated in metabolism of proteins, carbohydrates, fats and nucleic acids, it is not too surprising that alterations in its disposition by the body in diverse disease states have been observed. Vit. B_{12} has been also implicated with multiple sclerosis (MS) because of the beneficial effect of Vit. B_{12} in preventing and even reversing the demyelinizing effects of pernicious anemia. For this reason, treatment of MS with large doses of Vit. B_{12} has been investigated in many clinics with variable results(16-18). It is, therefore, of interest to ascertain (1) whether there are biochemical signs of Vit. B_{12} deficiency in subjects with MS by measuring Vit. B_{12} serum levels and glutathione (GSH) contents in the erythrocytes and (2) to determine whether such patients can handle orally or parenterally administered Vit. B_{12} as the clinically healthy subjects.

Materials and methods. The Vit. B_{12} containing $Cobalt^{60}$ had a specific activity of approximately 800 μc/mg for urinary excretion studies. Preparation of "resting" cells of *Lactobacillus leichmannii* ATCC 4799 has been described(9). *Serum.* Whole blood was obtained by venipuncture from fasting patients. Serum obtained by centrifugation was stored in frozen state until assayed. Serum Vit. B_{12} levels were determined according to the method of Skeggs, *et al.*(20). *Vit. B_{12} absorption test.* To estimate absorption of orally administered Vit. B_{12}, the Schilling procedure(6) was modified. The details have been published elsewhere(19). *Glutathione in erythrocytes.* Determination of GSH in red blood cells was carried out in duplicate aliquots of 0.5 ml of blood cell suspension by the nitroprusside test as modified by Grunert and Phillips(21-22). *Urinary excretion test.* To determine amount of injected Vit. B_{12} re-

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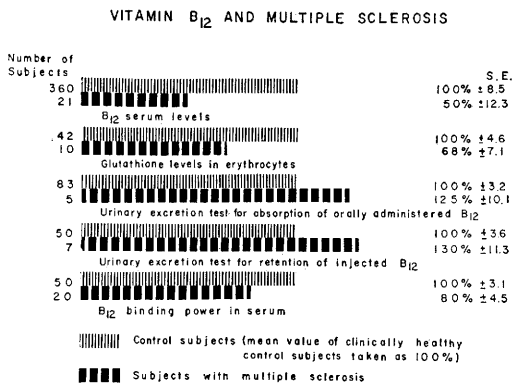

FIG. 1.

tained by tissues, the procedure of Lang, *et al.* (23) was used. All patients here described were diagnosed by several neurologists and followed up in a University setting. These diagnoses were established for years and no recent cases were included. Except for 2 or 3, all were ambulatory patients and none were acutely ill hospitalized patients. All showed progressive disease but no acute exacerbation.

Results. Fig. 1 summarizes results of Vit. B_{12} serum levels, urinary excretion test for both absorption of orally administered and retention of intramuscularly administered Vit. B_{12} serum Vit. B_{12} binding capacity, and finally GSH contents of erythrocytes. In presenting our results, the mean value of clinically healthy control subjects was assigned a level of 100% and results obtained from studies on MS patients were compared percentage-wise to this level.

Serum Vit. B_{12} concentrations as obtained by microbiological assay technic employing *L. leichmannii* ATCC 4797, demonstrated that the MS group had a significantly lowered mean value (50% of normal group). The range of these serum Vit. B_{12} contents was 220 $\mu\mu$g/ml to <50 $\mu\mu$g/ml. Ten of 21 cases had levels well within our range for pernicious anemia patients (50 $\mu\mu$g/ml), whereas some were within normal range.

Since severe Vit. B_{12} deficiency with such low Vit. B_{12} serum levels is often accompanied by marked diminution in the GSH content of red blood cell(22), the latter level was likewise quantitated. The observed data suggest that the MS group has a diminished GSH content of erythrocytes (68% of normal group). This method indicates concentration of sulfhydryl groups, and since GSH is responsible for almost all of the SH of the red blood cell, the measurement is essentially specific for this compound.

To determine whether Vit. B_{12} deficiency is due to poor absorption of orally administered Vit. B_{12}, the urinary excretion test was performed. In the third pair of bars, comparisons are made of urinary excretion results for absorption of orally administered Vit. B_{12} between MS patients and clinically healthy control subjects. The procedure employed followed the directions of Schilling(6) for estimation of orally absorbed Vit. B_{12}. The data indicate that there was greater radioactivity in urine of MS patients than in urine of the control group. However, when ability to retain injected $Co^{60}B_{12}$ was measured, it was observed that the MS group excreted considerably more of injected Vit. B_{12} as quantitated by urinary excretion of this vitamin. The next 2 sets of bars summarize this finding. The last set of bars summarizes data which indicate that serum of MS patients bind somewhat less Vit. B_{12} than the control group (100% vs. 80%). The procedure, following microbial absorption technic, quantitates residual binding capacity of serum for Vit. B_{12}.

In view of data indicating increased excretion of Vit. B_{12} by MS subjects when administered Vit. B_{12} intramuscularly, studies were initiated to compare excretion patterns of MS patients upon administration of Vit. B_{12} in aqueous solution or as a repository. To this end, 5 MS patients (Group A) were treated with Vit. B_{12} (1000 μg), whereas 8 MS patients (Group B) were treated with Depinar (500 μg). Group A excreted 600 $\pm$ 80 μg of Vit. B_{12} in 24 hrs, whereas Group B only 25.1 $\pm$ 2.5 μg in similar period. When such patients (Groups A) were administered Vit. B_{12} at 500 μg level, approximately one-half of 1000 μg excretion level was observed. It is readily observed that the MS patients receiving "Depinar" excreted a fraction of Vit. B_{12} activity of the group receiving Vit. B_{12} (cyanocobalamin) (Table I).

TABLE I. Urinary Excretion of MS Patients Administered Vit. B_{12} and "Depinar" Intramuscularly.

Group	Treatment	No.	Total B_{12} activity in 24 hr urine (μg)
A	Vit. B_{12} (1000 μg)	5	660 $\pm$ 80 *
B	"Depinar" (500 μg)	8	25.1 $\pm$ 2.5

* Stand. error of mean.

Discussion. Vit. B_{12} has been associated with MS since Booth and co-workers attempted to treat this disease with massive doses of Vit. B_{12}. Levin(17) and Leveboullet(18) have likewise explored Vit. B_{12} as an aid in management of MS. Results have been variable and difficult to evaluate because of spontaneous remissions characteristic of this disease. Vit. B_{12} has great efficacy in preventing and even reversing the demyelinizing effect of pernicious anemia. It is perhaps this dramatic therapeutic property of Vit. B_{12} that has led to investigations in metabolism of this vitamin in MS. Our study demonstrates possible biochemical signs of Vit. B_{12} deficiency as indicated by low Vit. B_{12} serum levels. It is also evident that MS patients as a group have diminished GSH contents of the red blood cell. Ling and Chow(22) found decreased level of non-protein sulphydryl groups in erythrocytes of animals fed diets deficient in Vit. B_{12}. Patients with pernicious anemia had marked diminutions of soluble sulphydryl compounds in blood, which increased upon administration of Vit. B_{12}. The changes observed in blood sulphydryl levels were primarily due to those in concentration of GSH. Since these patients were not administered Vit. B_{12}, and many of their serum B_{12} levels indicated deficiencies, further work will determine whether Vit. B_{12} corrects this diminution in sulphydryl compounds in blood. Decreases in blood GSH may also occur in certain conditions other than pernicious anemia, such as potassium or sodium deficiency(24), diabetes, and some chronic infections. Whether the decreased GSH level observed in the MS group is due to a derangement in productive capacity of the soluble sulphydryl compounds or to an increased destruction due to oxidation or conjugation with metabolites produced during the course of MS is not known. The deficiency is not due to poor absorption of orally administered Vit. B_{12}, but to poor tissue retention so that absorbed Vit. B_{12} is readily excreted and, therefore, unavailable for metabolic use. Since parenterally administered cyanocobalamin in an aqueous solution is rapidly excreted in urine even by non-multiple sclerotic subjects, search was made for a preparation containing Vit. B_{12}, with repository effects. It was found that intramuscular administration of Vit. B_{12} in the form of Vit. B_{12}-tannate derivative, "Depinar," resulted in marked retention of this vitamin as measured by urinary excretion. Additional studies(25) on both control human adults and rats demonstrated that "Depinar" administered intramuscularly resulted in marked elevation of serum Vit. B_{12} levels which persisted over several weeks. It was likewise observed that rats administered "Depinar" tagged with Co^{60} Vit. B_{12} had elevated radioactivity in the organs. These results indicated that when Vit. B_{12} was provided in depot form, it would be excreted at a slower rate and thus provide a continuous source of Vit. B_{12} for metabolic functions.

Future studies should indicate whether Vit. B_{12} supplied in the form of "Depinar" will increase low blood levels of B_{12} in several MS patients as observed in a normal group for prolonged periods of time.

Summary. Data have been presented to suggest that MS patients as a group do not metabolize Vit. B_{12} and GSH, quite in the same quantitative manner as a control group. Their average Vit. B_{12} serum level is lower, as is their ability to retain the vitamin when administered intramuscularly, although they absorb adequately. Average GSH content of the red blood cell was reduced in the MS group. When Depinar, a Vit. B_{12}-tannate derivative, was administered to such patients, Vit. B_{12} was excreted much more slowly in urine than aqueous Vit. B_{12}.

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Effect of Inhibitors of Oxidative Phosphorylation on Biosynthesis of Cholesterol and Precursors by Liver Homogenates.* (25452)

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Essentially whole homogenates of rat liver readily convert mevalonic acid (MVA) into non-saponifiable material (NSF) and cholesterol(1). This synthetic capacity is ordinarily retained during a control preincubation period but preincubation with RNAse abolishes capacity to convert MVA to NSF(2). RNAse-treated homogenates, in contrast to controls, are devoid of ATP but homogenates pretreated with RNAse and heated liver extract from which ATP has been removed by anion exchange chromatography maintain a level of ATP and convert MVA to NSF(3,4, 5). In the liver homogenate system studied the amount of added MVA incorporated into NSF appears to be directly proportional to the level of ATP existing at the time of MVA addition. Whole homogenates of liver convert MVA to NSF under aerobic but not under anaerobic conditions(6). Biosynthesis of NSF occurs under anaerobic conditions, however, if the homogenate is first centrifuged at intermediate speed to remove "tissue fragments" relatively rich in ATPase activity. The foregoing observations emphasize the significance of systems concerned with the generation of ATP in the biosynthesis of NSF and cholesterol. ATP is known to be specifically required in the phosphorylation of MVA, the phosphorylation of MVA-5-phosphate and the concerted decarboxylation and dehydration of MVA-5-pyrophosphate(7). Oxidative phosphorylation is the primary source of ATP in aerobic homogenates of liver. Accordingly some inhibitors of oxidative phosphorylation have been studied for the extent to which they indirectly inhibit biosynthesis of NSF and cholesterol and the data

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