vides an index of extent of collagen formation.

Summary. 1. Lysine-2-C^{14} becomes incorporated and hydroxylated in connective tissue incubated in Krebs-Henseleit medium for 12 hours. 2. Specific activity of collagenous and non-collagenous protein in the tissue studied *in vitro* was greater than that in tissue studied *in vivo*. 3. Hydroxylation of labeled lysine appears to occur more effectively in connective tissue of rats given labeled lysine intraperitoneally than in the connective tissue incubated with lysine-2-C^{14}.

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Elevated Vitamin Levels in Cerebrospinal Fluid in Multiple Sclerosis.* (24097)

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Very few studies have been reported on vitamin content of CSF (cerebrospinal fluid) in health and in disease. Since vitamins supply the prosthetic groups of enzymes, observations of their absence, or inability of absorption or transmission to the proper tissues may be forged into tools to elucidate specific metabolic dysfunctions; vice versa their pathological accumulation may be correlated with hyperfunction(1). Cyanocobalamin and folic acid (PGA) are known to provide the enzymatic apparatus for important steps in the synthesis of nucleic acids. Disturbances in distribution of these vitamins may interfere with the transfer of methyl groups and upset the equilibrium between ethanolamine and choline, or between the respective phosphatides cephalin and lecithin(2). These chemical changes in turn may give rise to demyelination, a histological picture of obscure, but undoubtedly enzymatic etiology(3).

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Methods. The quantities of these vitamins in body fluids, tissues and organs are of such minute order of magnitude that conventional methods of chemical analysis cannot be used, but one has to resort to microbiological assay (4). The analysis of PGA offers particular intricacies, since PGA occurs conjugated to variable extent with peptides of variable molecular weight, requiring enzymatic deconjugation previous to microbioassay; the enzymatic material which must be introduced is itself a source of folic acid which considerably complicates the analysis. We have described (5) a microbioassay, using a thermophilic bacillus which grows rapidly on a simple, purely synthetic medium and which responds equally well to various conjugates as to free folic acid, thus obviating previous enzymatic deconjugation. In the case of Vit. B_{12} preference has hitherto been given to the flagellate *Euglena gracilis* over various strains of *Lactobacillus* used for this purpose, because of its higher sensitivity and specificity(6). However, even *Euglena* shows a wider spectrum than appears desirable, as it responds to certain congeners of Vit. B_{12}, which are ineffective in man and

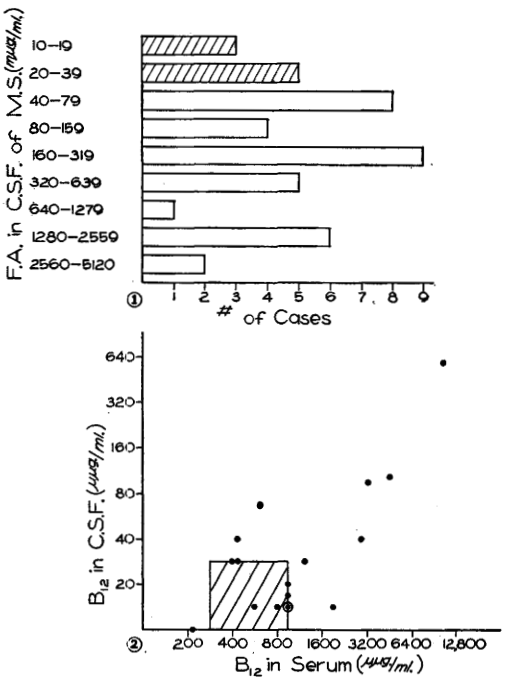

FIG. 1. Folic acid levels in CSF of 43 cases of multiple sclerosis. Normal range 10-30 mμg, hatched staves.

FIG. 2. B_{12} levels in CSF and serum in 20 cases of multiple sclerosis. Normal level in serum 300-1000 μμg/ml. Normal level in CSF 0-30 μμg/ml. Hatched area.

higher animals. We therefore use a test with *Ochromonas malhamensis,* which has been reported to cover those forms of B_{12} to which higher animals respond(7).

Results. We first established the levels of B_{12} and PGA in normal CSF, obtained from a number of non-neurological cases, prior to operative procedures. The normal range of folic acid in CSF is 10-30 mμg/ml; PGA appears to be evenly distributed between serum and CSF.

The figures for B_{12} are 0-30 μμg/ml in CSF as against 300-1000 μμg/ml in blood serum. Vit. B_{12} is known to pass the glomerular membrane without much hindrance, but its rather elevated molecular weight causes considerable difficulty in crossing the blood-brain barrier.

Comparison of vitamin levels in 310 unselected neurological cases shows an unexpectedly high incidence of values above the range observed in normal controls. The patients in our series did not receive vitamin medication. Deviations are found in PGA level of CSF and in B_{12} level of both serum and CSF. Their incidence appears to be concentrated in patients with multiple sclerosis, (MS) comprising a group of 43 cases.

Here PGA was found elevated in CSF in 34 out of 42 cases tested (Fig. 1). The levels of B_{12} in CSF were elevated in 14 out of 41 cases. Amongst these, 9 cases were higher than twice the upper limit of the normal range. The B_{12} values in blood serum of MS patients show a similar incidence of elevated values with 6 out of 20 cases above 1000 μμg/ml and 7 additional cases at 1000 μμg/ml, which is considered the upper limit of the normal range for this vitamin. The values for B_{12} in CSF and serum show fair correlation (Fig. 2).

Discussion. In pernicious anemia administration of folic acid initially benefits the patient clinically, but it seems to mobilize all available stores of B_{12}, primarily in liver and possibly in bone marrow; thus depletion of the organism of B_{12} after a few weeks gives rise to severe neurological symptoms (subacute combined degeneration of the cord). No analogy to this functional antagonism between the 2 vitamins exists in MS. No correlation was found between high PGA and low B_{12} values. In fact 4 cases among the highest B_{12} values in serum and CSF are ranking high among the folic acid results.

Because of the intermittent nature of MS, a single specimen can give us only a cross-sectional view of a surmisedly unstable situation. In view of the possibility that deviations in level of serum and CSF constituents may be detectable only during acute episodes, the observed incidence of elevated values is as high as may be expected and gains considerable significance. High vitamin values in CSF may be involved in the etiology of MS, or may act merely as indicators of changes in permeability of the blood-brain barrier. That this permeability is subject to variations was observed in a collateral study, where very high PGA and B_{12}-levels in CSF of neurological patients dropped abruptly within 5 min. during electric shock therapy, indicating a temporary breakdown of the blood-brain barrier. These observations support the concept that

vitamin B_{12} is involved in various pathological cerebral manifestations(8), which are ultimately based on enzymatic disturbances. Decreases and increases(9) of various hydrolases in degenerating nervous tissue have been reviewed by Cavanagh and Thompson (3). Some of these enzymes (*e.g.* the glycosidase synthesizing nucleosides) as well as transmethylase are interrelated with B_{12} and PGA metabolism.

Summary. The folic acid and Vit. B_{12} content of CSF and B_{12} in serum was studied in more than 300 neurological cases. The PGA levels in CSF in a number of cases were elevated; high values were scattered among a variety of neurological conditions, but were high in four-fifths of cases with MS. The titre of B_{12} in CSF and serum of MS patients was elevated in one-third and reached the upper limit of the normal range in another third of the cases. We assume that increased levels of these vitamins in CSF are not merely an indication of increased permeability of the blood-brain barrier, but of etiological significance for MS.

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Effect of Total Adrenalectomy on Benzol-Induced Leukopenia in Rabbits.* (24098)

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Selling(3) was one of the first investigators to show that following acute benzol poisoning in rabbits there is a prompt fall in circulating white blood count following which, if sepsis and death do not intervene, there is a gradual rise to normal levels after 2-3 weeks. He also noted that, while there was considerable variation in response of various animals to toxic action of the drug, the general response was a fall to low levels following which there was a preliminary rise, followed by a fall, and then again by a secondary rise which gradually reached normal levels. He believed that this phenomenon was probably dependent upon 2 factors: (1) rapidity of recovery of regenerative organs, and (2) amount of toxic material still present in circulating blood.

Weiskotten(4) also noted uniform but complex response of leukopenia in rabbits to acute benzol poisoning and called the phenomenon a "Diphasic Leucopenia." He named the primary fall and rise the "Protophase," the secondary fall and rise the "Deuterophase." He found that the response was not due to antigen-antibody reaction, nor was it due to amount of toxic material present in the circulating blood or delayed absorption of the substance. He also found that in the presence of a pre-existing quiescent infection there was a "lighting up" of the infectious process following injection of benzol and the leucocyte curve behaved in an irregular fashion. The leukocyte count characteristically rose to abnormally high levels despite acute benzol poisoning. Weiskotten and his co-workers were unable to determine the cause of the characteristic diphasic curve. Harkins(2) investigated the effect of nucleotide K96 upon the benzol induced leukopenia and found no significant alterations of the curve. Since a satisfactory explanation for the "diphasic" response has

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