ficiency and to a lesser degree in vit. B_{12} deficiency(4,5). In folic acid deficiency the functional form of the vitamin necessary to accept the formimino group of FIGLU is absent or not available, and thus FIGLU is excreted in the urine. It may be reasoned that an increased demand for glycine would increase the folic acid requirement for glycine synthesis and thus decrease that available for FIGLU metabolism, resulting in an increased excretion of FIGLU. The decreased urinary FIGLU with benzoic acid supplementation remains unexplained.

The increase in FIGLU excretion, due to the serine supplementation in the absence of folic acid and with or without the presence of benzoic acid, has also been observed in this laboratory as well as by Brown *et al*(17).

Summary. The effects of folic acid and vit. B_{12} on the toxicity of benzoic acid and on excretion of hippuric acid were studied. Benzoic acid at levels of 2 to 5% produced high mortality and the toxicity was not decreased by folic acid or serine supplementation. Detoxification of benzoic acid was not affected or only slightly reduced in folic acid deficiency. Further supplementation of the benzoic acid diets with DL-serine had no effect on hippuric acid excretion with or without folic acid. Vitamin B_{12} deficiency resulted in a greater reduction of hippuric acid excretion than did folic acid deficiency when the rats were given a test dose of benzoic acid by injection. The inclusion of benzoic acid in the diet decreased urinary excretion of FIGLU in folic acid-deficient rats. On the other hand, serine supplementation increased FIGLU excretion in the vitamin-deficient animals with or without added benzoic acid.

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Cerebrospinal Fluid Binding Capacity for Cyano- and Hydroxocobalamin. (30686)

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In 1960 it was demonstrated that hydroxocobalamin, when injected into dogs, resulted in a higher serum level of vit. B_{12} than did cyanocobalamin(11). Several authors soon reported that this applied also to humans(3, 5,8,9). Chosy, Killander and Schilling(1) found that the *in vitro* binding capacity in serum is considerably higher for hydroxocobalamin than for cyanocobalamin, while in the gastric juice it is approximately equal. Glass *et al*(4) demonstrated a greater retention of injected hydroxo- than of cyanocobalamin in muscles.

Recently, Hertz, Kristensen and Hoff-Jør-

TABLE I. CSF Binding Capacity for Cyano- and Hydroxocobalamin.
pg ($=10^{-12}$g) per ml in a mixture of 0.8 ml CSF and 0.2 ml solution containing 300 pg cyano- or hydroxocobalamin.

	a.	b.	c.	d.	e.
	Euglena gracilis, Z strain			*E. coli*	Dialysis
No.	Total B_{12}	Free B_{12}	Bound B_{12}	Bound B_{12}	Bound B_{12}
			Cyanocobalamin		
1	318	280	38	60	110
2	357	330	27	81	160
3	335	320	15	90	205
4	328	320	8	78	210
5	320	310	10	72	195
6	331	300	31	102	210
Avg	332	310	22	81	182
			Hydroxocobalamin		
1	318	190	128	84	180
2	357	210	147	141	220
3	335	190	145	207	290
4	328	230	98	144	265
5	320	230	90	114	240
6	331	250	81	114	270
Avg	332	217	115	134	244

gensen(6), using a dialysis technique exactly like that used in the present study, found a greater binding capacity *in vitro* for hydroxo- than for cyanocobalamin in human serum. At the same time, however, hydroxocobalamin in aqueous solution was found to dialyze more slowly than cyanocobalamin through cellulose membranes, but this difference was not sufficiently marked to afford the sole explanation of the difference in the serum binding of the two substances.

Meyer, Bertcher and Mulzac(10) found that the ratio of vit. B_{12} binding capacity to protein concentration was higher for cerebrospinal fluid(CSF) than for serum, and Worm-Petersen(12) has reported that although vit. B_{12} is bound in the CSF so that the vitamin cannot be removed by dialysis, a large portion of the vitamin may be utilized by the vit. B_{12}-utilizing microorganism *Euglena gracilis, Z strain,* which cannot utilize any part of the vit. B_{12} bound in the serum. Chromatography on DEAE-cellulose indicates that the binding in the CSF is to α- as well as β-globulins, but mainly to the latter, while in the serum the vitamin is bound exclusively to α-globulins(2,13).

Thus, considering that the binding capacity for hydroxo- and cyanocobalamin varies from one biological medium to another, it is important to compare the binding capacity in the CSF too. To this end, the author used the methods previously employed by Hoff-Jørgensen and Worm-Petersen for determination of vit. B_{12} capacity in serum.

Material. Six samples of CSF from patients without known diseases involving alterations in the composition of the CSF were used. The protein concentration in all the samples was within normal limits. As all the experiments were performed on each of the 6 CSF specimens, the available quantity of CSF limited the extent of the experiments.

Methods. Storage, addition of cyano- and hydroxocobalamin, measurement of vit. B_{12} content, and determination of binding capacity were done as described previously(7). However, no investigations were done using *L. leichmanii.* 0.8 ml CSF was mixed with 0.2 ml saline solution containing 300 pg (1 picogram $= 10^{-12}$ g) hydroxo- or cyanocobalamin, respectively.

Results and discussion. Table I gives results of the determination of the binding of hydroxo- and cyanocobalamin, respectively, to 6 CSF specimens by the *Euglena gracilis, Z strain, E. coli,* and dialysis methods. As has previously been found with serum, the 3 methods also give different results with CSF. Upon addition of 300 pg per ml the binding capacity for hydroxocobalamin was only questionably higher than for cyanoco-

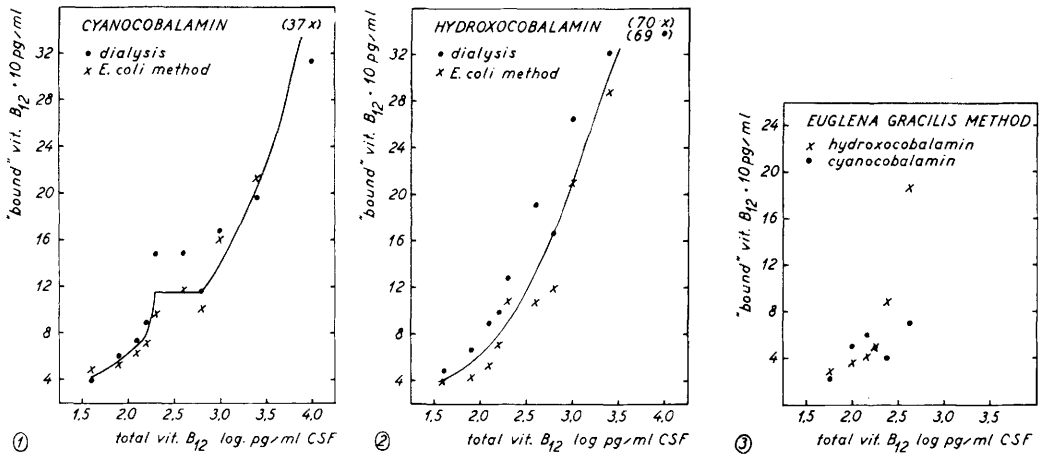

FIG. 1-3. Relationship between total and "bound" vitamin B_{12} as determined by 3 methods. Each point represents average of determinations on the same 6 CSF samples.

balamin, although the added quantity was 15 times the normal vit. B_{12} content of the CSF. The *E. coli* and the dialysis methods showed no major difference in the measured values. Thus, the differing ability of the substances to pass through the dialysis membrane was not manifest in these experiments. In accordance with previous findings with serum, the *Euglena gracilis, Z strain,* method showed a lower binding capacity for cyanocobalamin than the other methods. It is notable, therefore, that the binding capacity for hydroxocobalamin determined by this method corresponded to the results obtained with the *E. coli* and dialysis methods.

Fig. 1-3 present the correlation between total concentration of hydroxo- and cyanocobalamin, respectively, and binding capacity after addition of increasing quantities. The previously described(7) triphasic binding curve for cyanocobalamin in the serum, determined by *E. coli* and dialysis, was also found in the CSF, as the curve reaches a plateau at a vit. B_{12} concentration which is approx. 1/10 of that of the concentration at which the plateau was found in serum. In other words, the 3 methods revealed good conformity between the binding of cyanocobalamin in serum and in CSF.

No such plateau appears in the binding curve for hydroxocobalamin in the CSF. Upon addition of more than 300 pg per ml, the binding capacity for hydroxocobalamin was higher than for cyanocobalamin, and this difference increased with the amount added. With the *Euglena gracilis, Z strain,* method, too, the binding capacity for hydroxocobalamin increased upon addition of more than 200 pg per ml, while that of cyanocobalamin remained constant despite an increase in the added amounts. This is in accurate agreement with previous findings on the serum(7).

In conclusion, it may be deduced primarily that in the CSF, too, hydroxocobalamin is bound in larger quantities than cyanocobalamin, but not until the added amounts are 10-15 times the normal vit. B_{12} content of the CSF. Secondarily, it may be deduced that hydroxocobalamin is bound, entirely or partially, in a different way than cyanocobalamin to the CSF. It is not available to *Euglena gracilis, Z strain,* to an essentially greater extent than to *E. coli,* or to a greater extent than to which it is dialyzable. This is in contrast to the findings with cyanocobalamin. The results with cyanocobalamin correspond to our findings in serum.

The constant maximum binding capacity for cyanocobalamin, regardless of the quantity added if exceeding approximately 200 pg per ml, determined by *Euglena gracilis, Z strain,* must represent the utilization of all available bindings to which cyanocobalamin can be bound, by a type of binding which cannot be opened by the microorganism *Euglena gracilis, Z strain.* In contrast, hydroxocobalamin is bound in increasing quantities with bindings which cannot be opened by the

microorganisms, and it is bound in greater quantities than cyanocobalamin, as evidenced by all 3 methods. The explanation is presumably that this substance can utilize other possibilities of binding. The fact that the binding capacity of hydroxocobalamin exceeds that of cyanocobalamin only upon addition of large quantities may be taken to support the assumption that hydroxocobalamin is primarily bound to the same proteins as cyanocobalamin, and that, when these bindings have been exhausted, hydroxocobalamin has further binding possibilities.

In other words, it is possible that hydroxocobalamin is bound partially to other protein groups than those which can be blocked by cyanocobalamin.

Summary. Using the same methods as in previous studies on the measurement of the vit. B_{12} binding capacity in serum, the author has now investigated the binding capacity for cyano- and hydroxocobalamin in CSF by two microbiological methods and a dialysis technique. The findings were as follows: 1. There is no difference between the binding pattern in the CSF and serum for cyanocobalamin. Its binding capacity was approximately 1/10 of that found in serum. 2. Upon addition of more than 10-15 times the normal vit. B_{12} content of the CSF, or 200-300 pg per ml and more, the binding capacity was found to be higher for hydroxocobalamin than for cyanocobalamin. 3. As the binding pattern for large quantities of hydroxocobalamin differs from that for the corresponding quantities of cyanocobalamin, it is concluded that the increased binding capacity for hydroxocobalamin may be due to a partial binding of this substance to proteins different from those to which cyanocobalamin is bound.

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Reduction of Glutamic Pyruvic Transaminase in Pyridoxine Deficiency in Liver Disease.* (30687)

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Serum glutamic pyruvic transaminase (GPT) activity increases markedly in patients with virus- or drug-induced hepatic necrosis(1,2,3). In contrast, there may be little or no increase of GPT with necrosis in liver disease of the alcoholic, even in the presence of jaundice, fever,

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