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Free and Total Vitamin B_{12} in Cerebrospinal Fluid

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ABSTRACT

Free and total vitamin B_{12} levels in serum and cerebrospinal fluid (CSF) were bioassayed, since there were no available data on the relationship between free and total vitamin B_{12} in CSF or between free vitamin in serum and CSF vitamin B_{12}. The subjects were 43 neurological patients. Serum levels were normal in 40 of 43 patients. Values for free and total vitamin B_{12} in CSF were the same in 42 of 43 patients. Mean CSF vitamin B_{12} was 21 $\mu\mu$g./ml. In 17 cases CSF vitamin B_{12} equalled free vitamin B_{12} level in serum, in 16 cases CSF vitamin B_{12} was lower than the free level in serum, and in 10 cases CSF vitamin B_{12} was higher than the free vitamin level in serum. There was no apparent diagnostic correlation. The findings suggest that vitamin B_{12} is not bound in CSF and that there is some selective control of passage of vitamin B_{12} across the blood-CSF barrier.

ROSS[1] reported in 1950 that in the few cerebrospinal fluids (CSF) assayed for vitamin B_{12}, using *Euglena gracilis* as the test organism, he had found levels of 0-16 micro-micrograms/ml. ($\mu\mu$g./ml.). He also noted that heating CSF to 100° C. did not significantly increase the assay results; in the light of subsequent development of the technique this would indicate that B_{12} in CSF is in the free or uncombined form only.

Three other groups have studied vitamin B_{12} levels in CSF, but none reported the proportion of free to total B_{12}, or the relationship between CSF and serumfree vitamin levels. It was thought that such information might add to the available data on CSF dynamics, and possibly be of significance when extended to the group of demyelinating diseases.

In three papers published between 1958 and 1960, Sobotka and co-workers reported studies on CSF vitamin levels in normals and in patients with multiple sclerosis. Using *Ochromonas malhamensis* bioassay, Sobotka, Baker and Frank[2] found a normal range for B_{12} in CSF of 0-30 $\mu\mu$g./ml. The "normals" were 43 patients undergoing spinal anesthesia for minor surgical conditions. Concomitant serum B_{12} estimations ranged from 300 to 1000 $\mu\mu$g./ml. In 1958 Sobotka, Christoff and Baker[3] reported B_{12} and folic acid levels in CSF in 43 cases of multiple sclerosis. An elevation of CSF vitamin B_{12} was found in 14 of 41 patients. In 1960 Sobotka *et al.*[4] published a further study on CSF vitamin levels in multiple sclerosis. CSF vitamin B_{12} values were given for 41 cases (presumably the same 41 cases reported in 1958) and were elevated to 40-600 $\mu\mu$g./ml. in 14 of 41 patients. Only 21 of these 41 patients had serum B_{12} estimations. Six of the 21 had elevations of serum B_{12} ranging from 1100 to 4800 $\mu\mu$g./ml. Correlation between the B_{12} increase in the sera and CSF was not good. Two of the patients with high serum levels had normal CSF values, one had a slight rise to 40 $\mu\mu$g./ml., two had gross elevations in both fluids, and with the highest CSF level of 600 $\mu\mu$g./ml. the serum B_{12} was only increased to 1100 $\mu\mu$g./ml. An additional 12 cases of multiple sclerosis had CSF vitamin B_{12} levels determined, and three of the 12 had values above normal, ranging from 40-80 $\mu\mu$g./ml. No serum B_{12} values were reported for these additional 12 cases. During the course of this multiple sclerosis investigation the vitamin B_{12} content of CSF was determined in 420 unselected neurological cases. The authors stated that a relatively high percentage of the 420 cases had B_{12} values above the normal range, and that the incidence of high values seemed to be concentrated in patients with multiple sclerosis, but no details were given. In summary, Sobotka and co-workers claimed that the normal range for CSF vitamin B_{12} was 0-30 $\mu\mu$g./ml., and they found values above this normal in 17 of 53 patients with multiple sclerosis.

In 1961 Herbert and Zalusky[5] confirmed the observations of Sobotka's group for B_{12} levels in normal CSF. Using *Euglena gracilis* bioassay, they reported a mean CSF level of 22 $\mu\mu$g./ml., with an average serum value of 546 $\mu\mu$g./ml.; 14 subjects undergoing diagnostic study or spinal anesthesia were studied. They concluded that CSF appears to exclude protein-bound B_{12}, though no values for free and bound B_{12} were reported.

Using *E. coli* assay, Yavorkovsky and Mai[6] determined CSF vitamin B_{12} levels in 41 patients predominantly with neurological disorders. *E. coli* assay is not usually considered sensitive enough to detect B_{12} levels of less than 50 $\mu\mu$g./ml., though the authors claim that with their modification of the method results are reliable down to 20 $\mu\mu$g./ml. In 32 patients levels ranged from 0 to 15 $\mu\mu$g./ml., four were from 15 to 25 $\mu\mu$g./ml. and five patients had values between 51 and 65 $\mu\mu$g./ml. These authors reported a fairly good correlation between increased protein and increased B_{12} in the CSF. This occurred particularly with the meningitides,

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and the authors felt that in cases which did not show this correlation the reason might be that bacteria in CSF had utilized the B_{12}. They also felt that the increase of both protein and vitamin B_{12} in CSF was probably due to increased permeability of the vessels.

Reisner and Weiner[7] reported CSF levels of vitamin B_{12} in two cases before and after an intramuscular injection of 1000 μg. of B_{12}. Since they used *Lactobacillus leichmannii* as the test organism, and this organism is of low sensitivity and not entirely specific for B_{12},[8] their results are not very reliable.

The capacity of CSF to bind radioactive B_{12} (Co^{60}-B_{12}) was studied *in vitro* by Meyer, Bertcher and Mulzac.[9] They investigated 22 subjects with no apparent neurological disorder; specimens were obtained at the time of spinal anesthesia. These authors found that the serum-binding capacity was 2.5 to 3.5 higher than that of CSF, but that in relation to protein content, normal CSF had a higher binding capacity than normal serum. In both fluids the binding capacity increased as the concentration of radiovitamin was raised from 1 mμg. to 100 mμg./ml. of Co^{60}-B_{12}.

Using modern techniques, various workers have shown that CSF contains most of the proteins that have been identified in serum, though not necessarily in exactly the same proportion.[10, 11] Some of the CSF proteins show differences in physicochemical characteristics from their counterparts in serum.[12] The dynamics of CSF formation, circulation and reabsorption are still incompletely understood, but it is no longer possible to consider CSF a simple filtrate or transudate. Lowenthal, Karcher and van Sande[11] studied CSF and cerebral tissue proteins by both paper and agar electrophoresis. In some diseases, such as multiple myeloma, the same abnormal protein may be detected in serum, CSF and urine. Such parallelism has not been demonstrated in multiple sclerosis; on the contrary, abnormal CSF proteins do not have counterparts in the serum. In various encephalitides also, protein patterns in the serum differ markedly from CSF protein patterns. Lowenthal and co-workers have presented evidence to support the hypothesis that some abnormal protein components of CSF in disease originate within the central nervous system rather than in the serum.

In 1960 O'Connor *et al.*[13] reported that the mean serum B_{12} level in 21 patients with multiple sclerosis was 50% of the mean level in clinically healthy control subjects. *Lactobacillus leichmannii* was the test organism used for bioassay. They also stated that patients with multiple sclerosis had impaired ability to retain injected B_{12} in the tissues, although absorption was adequate. Grann and Glass[14] studied serum levels and intestinal absorption of vitamin B_{12} in multiple sclerosis. *Ochromonas malhamensis* was used for bioassay of the serum, and hepatic uptake of radioactive B_{12} for evaluation of intestinal absorption. They found that the majority of multiple sclerosis cases were normal in both respects. The findings of these two groups of investigators are considerably at variance with those of Sobotka's group, who reported a high incidence of high B_{12} levels in both serum and CSF in multiple sclerosis.

From the scattered reports in the literature briefly reviewed above, it seems that there is some ambiguity regarding CSF vitamin B_{12} levels, and the relationship between serum and CSF levels.

SUBJECTS

Owing to the low levels of B_{12}, relatively large amounts of CSF were needed for assay of free and total vitamin. It was seldom possible to obtain sufficient material from a routine spinal puncture, and cases had to be limited to those patients undergoing pneumoencephalography. Consequently, CSF was collected from cases with a variety of neurological diagnoses. Patients suspected to have infected CSF were not considered suitable for study; other than this there was no element of selection. The diagnoses listed in Table I were the final discharge diagnoses; it will be noted that two initially unsuspected encephalitides are included. No normal asymptomatic persons were studied, but in some of the patients no organic lesions were found. Five patients with demyelinating disease are included in the total group of 43 patients. None of the patients in the present series received B_{12} therapy in hospital before the specimens were obtained, nor was there any record of B_{12} therapy prior to admission.

METHOD

The biological method of Mollin and Ross,[8, 15, 16] using the Z strain of *Euglena gracilis*, was employed for the assays. The assay of B_{12} in CSF is essentially the same as the assay of B_{12} in serum. All glassware, pneumoencephalogram equipment, etc. in direct or indirect contact with the CSF specimens were subjected to special B_{12}-decontamination cleaning[17] before use. CSF specimens were centrifuged at 3000 revolutions per minute for five minutes immediately after collection to precipitate any cells, and the supernatant was stored at —20° C. until assayed.

Blood for serum B_{12} determinations was collected immediately before or after the pneumoencephalogram.

Two dilutions for free B_{12} and two for total B_{12} level were included in the first assay of the CSF. Dilutions of 1/8 and 1/16 were commonly used for both free and total assay, using duplicate tubes for each dilution. These dilutions occasionally had to be varied (1/4 to 1/40) in the repeat assay, depending on the values obtained in the initial assay. A 1/4 dilution is satisfactory for CSF assay owing to the low protein concentration in CSF, but in order to conserve material it was not used routinely. For complete B_{12} assay by this method 12-15 ml. of CSF is needed. Levels reported are the mean values for

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TABLE I.—LIST OF CASES, WITH DIAGNOSES AND LABORATORY FINDINGS

Case	Sex and Age	Final discharge diagnosis	Cerebrospinal fluid: RBC/ c.mm.	WBC/ c.mm.	% Polymorphs	% Lymphocytes	Protein mg.%	Colloidal gold curve	Kolmer	Other	Serum B_{12}* µµg./ml. Free	Serum B_{12}* µµg./ml. Total	CSF B_{12} µµg./ml. Free	CSF B_{12} µµg./ml. Total
GROUP 1. Cases with less than 10 µµg./ml. difference between free serum and CSF B_{12}.														
3	F 65	Amyotrophic lateral sclerosis	0	0	—	—	30	—	Neg.		24	242	11	15
4	M 41	Torticollis, traumatic sequela	0	1	—	—	40	—	NSQ		12	310	16	16
5	M 70	Arteriosclerosis of legs, heart and cerebellum	0	2	—	—	20	000000	Neg.	Chlorides 666 mg.%	7	306	6	7
8	M 54	Chronic brain syndrome, memory lapses	0	5	—	—	40	000111	Neg.		36	450	27	34
10	M 47	Cerebral contusion	1	0	—	—	40	—	Neg.		35	204	30	34
15	M 82	Cerebral arteriosclerosis	0	0	—	—	20	—	Neg.		2	56	<2	<4
19	M 23	Idiopathic epilepsy, fractured jaw	0	0	—	—	30	001100	Neg.	Chlorides 667 mg.%	12	383	14	17
22	M 21	Encephalitis (probably viral)	217	66	0	100	40	001210	Neg.		13	420	12	15
24	M 20	Idiopathic epilepsy	1	1	—	—	30	000100	—	Globulin—no increase	17	278	12	13
27	M 50	"Polyneuritis"	2	0	—	—	15	001210	—		3	339	10	10
29	F 37	Viral encephalitis (unlikely to be multiple sclerosis)	2	0	—	—	35	555543	NSQ		19	370	12	12
30	F 33	Tension headaches	3	1	—	—	20	—	—		10	200	12	14
31	M 32	Multiple sclerosis	2	13	16	84	75	444433	Neg.		5	185	5	8
32	F 69	Epilepsy, late onset-? arteriopathic	3	1	—	—	15	—	—		23	324	20	22
33	M 68	Neurosyphilis with vascular insufficiency	4	0	—	—	40	001222	Pos.		9	275	9	10
39	F 35	Multiple sclerosis, gastric ulcer	0	0	—	—	20	001111	NSQ		20	396	21	18
42	M 53	Chronic lead poisoning, acute arthritis right knee	0	2	—	—	30	001221	Neg.		38	448	28	34
Group 2. Cases in which free serum B_{12} was 10 or more than 10 µµg./ml. higher than CSF B_{12}														
6	F 35	Cerebral atrophy NYD, psychoneurosis	1	0	—	—	40	000110	Neg.	Pandy—slight increase	85	379	32	29
7	F 37	Migraine	0	0	—	—	20	—	—	Pandy—no increase	26	167	12	13
9	M 45	Temporal lobe epilepsy	0	0	—	—	35	—	Neg.		54	364	23	23
11	M 32	Psychoneurosis, tension state, stress erythropoiesis	0	8	0	100	20	000110	Neg.		43	326	28	28
13	F 37	Migraine headaches, anxiety state	0	0	—	—	20	—	—		34	243	22	23
14	M 53	Paraparesis, unknown etiology	2	7	—	—	30	000111	—	Globulin—no increase	37	402	22	24
16	M 34	Latent syphilis, rheumatic heart disease with mitral stenosis, chronic otitis media, anemia	0	0	—	—	NSQ	001100	Neg.	Chlorides 696 mg.% Sugar 53 mg.%	30	392	10	14
17	M 62	Parkinson's syndrome, cortical atrophy	2	0	—	—	40	—	—		51	451	21	23
20	F 47	Cortical atrophy, minor epilepsy	0	1	—	—	20	—	Neg.		114	385	22	23
25	F 38	Hyperostosis frontalis interna	3	16	21	79	30	000110	Neg.		75	281	29	30
26	M 13	Ataxia, cause undetermined	0	3	—	—	20	001110	—		32	436	16	21
34	M 45	Cerebral thrombosis, mild	0	0	—	—	15	—	Neg.		62	295	13	13
35	F 36	Syringomyelia, chronic anxiety state	4	2	—	—	30	—	Neg.		46	366	13	16
38	M 41	Toxic radiculoneuritis, Guillain-Barré syndrome**	0	1	—	—	150	442233	Prozoning	Sugar 105 mg.%	142	801	26	50
40	M 59	Cerebral atrophy, duodenal ulcer	2	3	—	—	20	000010	—		89	243	26	25
43	M 41	Ageusia, NYD	2	2	—	—	30	001210	Neg.	Globulin—no increase	55	500	18	17
Group 3. Cases in which CSF B_{12} was 10 or more than 10 µµg./ml. higher than free serum B_{12}														
1	F 62	Cerebellar ataxia (arteriosclerotic), osteoarthritis cervical spine	0	3	—	—	30	—	—		5	156	15	15
2	F 74	Focal seizures, left temporal area lesion	0	5	—	—	25	—	Neg.		9	355	17	20
12	F 64	Arteriosclerotic cerebrovascular disease, bronchiectasis	0	0	—	—	40	000110	Neg.		4	214	17	14
18	M 63	Cerebral contusion, alcoholism, subacute subdural	3	1	—	—	70	—	Neg.		10	350	33	23
21	F 47	Multiple sclerosis	3	0	—	—	30	444433	NSQ	Globulin—negative	24	238	38	38
23	F 79	Hypertensive cardiovascular disease, cerebrovascular accident	12	2	—	—	80	—	—		8	467	26	27
28	M 19	Suspect brain tumour, or low grade encephalitis, or idiopathic epilepsy	6	28	12	88	20	001100	Neg.		8	507	25	26
36	F 17	Focal epileptic seizure NYD	8	5	—	—	40	000100	Neg.	Pandy—negative	13	471	23	24
37	F 37	Chronic anxiety state	0	0	—	—	20	001110	—		7	380	16	20
41	M 60	Astasia abasia, cervical and lumbar osteoarthritis, essential hypertension	377	0	—	—	30	000110	—	Globulin—no increase	12	349	34	38

*Normal serum B_{12} levels for this laboratory are: Free B_{12} = less than 100 µµg./ml.
Total B_{12} = 100 - 700 µµg./ml.

**Demyelination proved post mortem.

the four assays (eight tubes), i.e. two initial and two recheck assays for both free and total B_{12}. Other details of the assay technique have already been published.[17]

Cell counts, protein estimations, colloidal gold curves, etc. were done in the routine hospital laboratory.

RESULTS

Free and total serum and CSF values for B_{12}, diagnoses and other pertinent data are presented in Table I.

Sera

In 40 of the 43 cases the serum levels for both free and total B_{12} were normal. The mean free serum level in these 40 cases was 28 $\mu\mu$g./ml., and the mean total level was 337 $\mu\mu$g./ml. Case 15 in Group 1 had low B_{12} levels in the serum, almost pathognomonic of pernicious anemia or subacute combined degeneration of the cord, but he had no physical signs or laboratory evidence of either condition. Case 38 in Group 2 had slightly increased serum levels of free and total B_{12}; this specimen was obtained one month before death and the increased values may be a reflection of beginning terminal liver dysfunction. Case 20 in Group 2 had a slight increase in free serum B_{12} with a normal total level.

Cerebrospinal Fluid

The free and total B_{12} levels in CSF were essentially the same in 42 of 43 cases. The measured differences ranged from 0 to 10 $\mu\mu$g./ml. with a mean value of 2 $\mu\mu$g./ml. Subsequent references to the level of B_{12} in CSF will mean free or total, whichever is the higher value. The details of CSF free and total levels are shown in Table I. The one exception was Case 38 in Group 2, in which the total level of B_{12} in CSF was double the free value.

The B_{12} level in CSF ranged from 0-50 $\mu\mu$g./ml. with a mean value of 21 $\mu\mu$g./ml. for 43 cases. Only one patient had no detectable B_{12} in CSF.

The cases in Table I are divided into three main groups, depending on whether the CSF levels were equal to, less than, or greater than the free B_{12} level in the serum.

Group 1 comprised 17 patients in whom there was less than 10 $\mu\mu$g./ml. difference between the free serum and CSF levels of B_{12}. The mean free level in the serum was 16.8 and in the CSF 16.6 $\mu\mu$g./ml. The measured values in the CSF in this group ranged from 0-34 $\mu\mu$g./ml.

Group 2 comprised 16 patients in whom the free B_{12} level in the serum was 10 or more than 10 $\mu\mu$g./ml. higher than the B_{12} level in CSF. The mean value for free B_{12} in serum was 61 $\mu\mu$g./ml. CSF level of B_{12} ranged from 13-50 $\mu\mu$g./ml., with a mean value of 24 $\mu\mu$g./ml. Expressed as percentages, CSF levels were from 20% to 68% of the free serum levels.

TABLE II.—DIAGNOSES AND FREE SERUM AND CSF LEVELS OF B_{12}

	Group 1	*Group 2*	*Group 3*
	B_{12} in CSF = free B_{12} in serum	*B_{12} in CSF less than free B_{12} in serum*	*B_{12} in CSF more than free B_{12} in serum*
Diagnosis	*No. of cases*	*No. of cases*	*No. of cases*
No organic lesion Cases 7, 11, 13, 30, 37	1	3	1
Arteriosclerosis Cases 1, 5, 12, 15, 17?, 40?.....	2	2	2
Epilepsy Cases 2, 9, 19, 20, 24, 32, 36.....	3	2	2
Demyelinating disease Cases 21, 27, 31, 38, 39.....	3	1	1
Infections Cases 22, 29.....	2		
Hemorrhage Cases 10, 18, 23, 34.....	1	1	2
Miscellaneous Cases 3, 4, 6, 8, 14, 16, 25, 26, 28, 33, 35, 41, 42, 43.....	5	7	2
Totals.....	17	16	10

Group 3 comprised 10 patients in whom the CSF level was 10 or more than 10 $\mu\mu$g./ml. higher than the free serum B_{12} level. The CSF values ranged from 15-38 $\mu\mu$g./ml., with a mean value of 26 $\mu\mu$g./ml. The mean free level in the serum was 10 $\mu\mu$g./ml.

CSF Protein and B_{12} Levels

The mean CSF protein level for 42 cases was 34 mg. %. Group 1 cases had a mean CSF protein level of 32 mg. % with a mean B_{12} level of 17 $\mu\mu$g./ml. Group 2 cases had a mean protein level of 35 mg. % with a mean B_{12} level of 24 $\mu\mu$g./ml. Group 3 cases had a mean protein level of 38.5 mg. % with a mean B_{12} level of 26 $\mu\mu$g./ml. It is doubtful if these differences are of any significance. Cases 18, 23, 31 and 38, which had above normal levels of CSF protein, had a mean B_{12} level in CSF of 30 $\mu\mu$g./ml.

Vitamin B_{12} Levels Related to Diagnoses

Table II subdivides the cases into broad diagnostic categories, and notes whether they are in Groups 1, 2 or 3 with reference to B_{12} serum and CSF correlation. There are too few cases to draw conclusions, but it would appear that there is no correlation.

DISCUSSION

Euglena gracilis is a very sensitive test organism; in our hands vitamin B_{12} in as little as 0.25 $\mu\mu$g./ml. can usually be detected, and 0.5 $\mu\mu$g./ml. regularly. Blank control tubes and standard tubes containing various dilutions of B_{12} are included in every assay batch. The six control tubes usually have a mean optical density reading between 3 and 4. The weakest dilution of B_{12} included in the standard tubes is 0.25 $\mu\mu$g./ml., and the mean optical density readings for the six tubes of this dilution range

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from 5.5 to 6.5. These mean values have always shown good differentiation; very rarely one of the 0.25 μμg./ml. standard tubes may be as low as the highest blank control tube. Ross[15] states that with the Euglena test organism as little as 1 μμg./ml. of B_{12} can be detected, but that in serum it is not practicable to measure less than 10 μμg./ml., as serum present in greater concentration than 1 in 8 coagulates with heating to 100° C. and may be inhibitory. This limitation should not apply to free serum assays, in which the tubes are only heated to 56° C. Ross states also that CSF can be set up for assay in ¼ concentration because of its low protein content. Although the 10 μμg./ml. limit of sensitivity probably does not apply to free serum assays or CSF assays, we selected the 10 μμg./ml. difference between free serum and CSF vitamin B_{12} levels to subdivide our cases, as this is the generally accepted level of sensitivity.

The overall mean level of 21 μμg./ml. of B_{12} in CSF in the present miscellaneous group of patients is almost identical with the mean of 22 μμg./ml. reported by Herbert and Zalusky[5] for normal individuals. Only eight of the present series of 43 patients had CSF levels of B_{12} above the normal range of 0-30 μμg./ml. determined by Sobotka and co-workers;[2] only one of five patients with demyelinating disease was above 30 μμg./ml.

The present authors are unable to confirm Sobotka and co-workers' report[4] of high serum and CSF levels of B_{12} in multiple sclerosis, or O'Connor and co-workers'[13] findings of low serum B_{12} values in this disease. The number of patients with demyelinating disease in the present series is small, but seven of eight above normal levels of CSF B_{12} were in patients who did not have demyelinating disease. Sobotka and co-workers' values, for both serum and CSF levels of B_{12}, appear excessively high when compared with the results of other investigators, yet they were using a test organism considered to be the most specific.[8] It is not stated whether any of their patients were receiving B_{12} therapy, or had done so in the recent past.

In normal serum, B_{12} is bound to alpha-globulin, though other serum globulins are involved when serum B_{12} levels are increased in some disease states. Since high molecular weight α_2-macroglobulins have been detected in CSF,[12] one cannot postulate that serum-bound B_{12} is too large a particle to pass the blood-brain or blood-CSF barrier. Lowenthal and his group[11] did not report finding any macroglobulins in cerebral tissue. Free B_{12}, with a molecular weight of approximately 1360, should be able to circulate freely between serum and CSF, but free serum and CSF levels cannot be correlated. If, as reported by Meyer's group,[9] the binding capacity of CSF in relation to protein content is greater than the binding capacity of serum, one would expect that total B_{12} in CSF would be higher than free B_{12}, but only in Case 38 was there any evidence of B_{12} binding in CSF. The results of this study suggest that (a) there is no bound B_{12} in CSF in the majority of cases and (b) there must be some selective control of B_{12} passage at the blood-CSF barrier.

For the 10 cases in Group 3, in which the B_{12} level in the CSF was higher than the free level in the serum, no reasonable explanation was discernible. Two of the 10 cases had increased CSF protein, but so did two cases in the other groups.

While the measured differences between free B_{12} in serum and B_{12} in CSF may be arithmetically significant, in the majority of cases (28, or 65%) the difference was less than 16 μμg./ml. and is not likely of any biological significance. In a further seven cases (16%) the difference was less than 31 μμg./ml., of doubtful biological significance. The remaining eight cases (19%), all from Group 2 with higher free levels in the serum, had differences ranging from 31-92 μμg./ml., probably biologically significant. On the other hand, there are no data on what is the minimal requirement, if any, of B_{12} in the CSF.

SUMMARY AND CONCLUSIONS

Free and total levels of vitamin B_{12} were assayed in serum and cerebrospinal fluid (CSF) in 43 patients from the neurological service. CSF levels of vitamin B_{12} ranged from 0-50 μμg./ml. with a mean value of 21 μμg./ml. Only one patient had no detectable B_{12} in CSF. Free and total levels of vitamin B_{12} in CSF were the same in 42 patients. In 40% of the cases the vitamin B_{12} level in CSF was the same as the free level in the serum, in 37% the CSF level was lower than the free level in the serum, and in the remaining 23% the CSF level was higher than the free level in serum. There were three patients with multiple sclerosis. In two the serum and CSF levels were normal; the third case had a slight elevation of vitamin B_{12} in the CSF though serum levels were normal. Of two other patients with demyelinating disease, one had normal serum and CSF levels, and the other had slight elevation of free and total serum vitamin B_{12} and a total CSF level double the free level, suggesting some binding of vitamin B_{12} in CSF.

The findings suggest that there is some selective control of passage of vitamin B_{12} across the blood-CSF barrier. While the measured differences between free serum and CSF vitamin B_{12} may be arithmetically significant, a biological significance is considered possible in only 19%. In this limited number of cases no diagnostic correlation was apparent.

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Trial of Amino-oxyacetic Acid, an Anticonvulsant

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ALTHOUGH the overall outlook for control of seizures in children is satisfactory, there remains a small number of patients resistant to all forms of drug therapy. It is toward this group that any new forms of therapy will first be directed.

The present trial of a new anticonvulsant was carried out on children, and was stimulated by the desire to determine whether the drug under investigation had any effect on the syndrome of infantile massive spasms with mental deterioration. This is a well-defined clinical entity consisting of a generalized muscular jerk with flexion of the trunk and limbs, lasting only a matter of seconds and beginning usually in the first year of life. The characteristic electroencephalographic (EEG) pattern associated with these massive spasms has been called "hypsarrhythmia", and the spells themselves have been variously termed "massive myoclonic jerks", "jack-knife", "salaam", and "lightning" spasms. In perhaps half the cases the cause is diffuse cerebral damage from perinatal birth trauma or anoxia, whereas in the remainder the cause is thought to be developmental or idiopathic in origin. In some instances the spasms can be controlled by the use of corticosteroids, but in the great majority mental deterioration occurs even though the spasms are stopped. For this reason, it is a matter of some concern to find a form of medication which prevents this unfortunate outcome.

There is another group of children with seizure disorders in which some degree of mental deterioration may occur. In this group also, control of the seizures is difficult. These children tend to be rather older than those with massive spasms and present with akinetic or myoclonic minor motor seizures. There is no characteristic EEG pattern associated with these seizures.

ABSTRACT

It has been shown experimentally that the drug amino-oxyacetic acid (AOA) can raise the level of gamma aminobutyric acid (GABA) in the brain. Since GABA is a powerful neuronal inhibitor it seemed worth while to assess the value of AOA as an anticonvulsant.

This drug was given to 23 infants and children, all but one of whom were resistant to usual anticonvulsant medication. The types of seizure patterns were classed as major (including focal) and minor (akinetic, myoclonic and hypsarrhythmic) and the patients were followed for up to one year. Of eight children with major seizures, five were improved; of eight with minor seizures, three were improved; and of six with hypsarrhythmia, none were improved. One patient with phenylketonuria and minor seizures was improved.

It is concluded that this approach to anticonvulsant therapy is worth pursuing and that the drug may also find some use in the treatment of phenylketonuria and of seizures due to vitamin B_6 dependency.

PHARMACOLOGY

In 1950 it was found that gamma aminobutyric acid (GABA) was present in considerable quantities in mammalian brain, and it was later observed that extracts from mammalian central nervous systems could modify the effects of stimuli on the neurones of crayfish.[1] Subsequent experiments showed that this effect was due mainly to the GABA content of mammalian brain.

Animal experiments showed that GABA protects against convulsions induced by thiosemicarbazide and isoniazid, and that these convulsions are associated with a decrease in the amount of GABA which

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Since this paper was submitted, the Upjohn Company has suspended manufacture of the drug U-7524. Despite the fact that this drug may not be available commercially in the future, the authors feel that the results obtained in children are of sufficient interest to justify recording.