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SHORT COMMUNICATIONS

Transport of vitamin B_{12} from blood to cerebrospinal fluid

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PRELIMINARY investigations[1] have shown that there is no simple correlation between the concentration of vitamin B_{12} in serum and that in the cerebrospinal fluid (CSF) in human beings. These observations led to investigations of the transport of vitamin B_{12} from blood to CSF. Apparently no earlier publications have appeared on this subject.

Albino rabbits of homogeneous descent and breed were used for the experiments. The animals were anaesthetised with urethan (1·5 g/kg body weight). During the surgical operation the anaesthesia was supplemented with ether. Blood specimens were withdrawn from the femoral vein after bilateral ligation of the renal arteries and veins.

Cyanocobalamin (vitamin B_{12}) (25 μg/kg body weight) was then injected into an ear vein. Thirty minutes later another blood specimen was withdrawn. The experiment was concluded by exsanguination of the animal during which a further blood specimen was taken. Finally the maximum amount of CSF (0·5–2·0 ml) was withdrawn from the cisterne magna with a cannula.

Vitamin B_{12} in serum and CSF was assayed microbiologically by means of the *Euglena gracilis*, *z-strain* method[2] using a standard solution of cyanocobalamin containing 5 $\mu\mu$g/ml. The standard deviation of the assay was $\pm$ 18·0 per cent over the whole range of measurement. In serum both the total and the non-dialysable vitamin B_{12} were assayed, the former after heating at 100 ° for 15 min, the latter after dialysis against two changes of 10 vols of 0·0005 per cent aqueous KCN for 16 hr.

A control group of animals of the same descent and breed as the treated animals were submitted to a similar procedure except that vitamin B_{12} was not injected.

TABLE 1. THE TRANSFER OF VITAMIN B_{12} FROM BLOOD TO CSF, IN RABBITS (EACH LINE REFERS TO A SEPARATE ANIMAL).

Duration of experiment (min)	Total concentration of B_{12} in serum before injection (C_{serum}) (mμg/ml)	Total concentration of B_{12} in CSF (C_{CSF}) (mμg/ml) after injection	$\frac{C_{CSF}}{C_{serum}} \times 100$	Concentration of dialysable B_{12} in serum * after injection ($C_{serum\text{-}dialys.}$) (mμg/ml)	$\frac{C_{CSF}}{(C_{serum\text{-}dialys.})} \times 100$	Total concentration of B_{12} in serum. (mμg/ml)	Total concentration of B_{12} in CSF. (mμg/ml)	$\frac{C_{CSF}}{C_{serum}} \times 100$
30	5·4	0·396	7·7	55·0	0·72	3·0	0·12	4·00
60	8·7	1·78	20·4	91·5	1·95	2·4	0·10	4·16
90	11·0	1·71	15·6	125·1	1·37	2·4	0·08	3·34
90	1·5	0·378	25·2	128·3	0·29	15·0	0·25	1·67
135	3·9	0·279	7·4	9·8	2·93	1·4	0·06	4·30
180	10·2	1·13	11·0	91·5	1·23	4·6	0·10	2·18
180	7·2	0·810	11·2	72·8	1·11	2·8	0·08	2·86
180	3·6	0,347	9·7	49·5	0·70	7·0	0·13	1·86
180	5·1	0·662	13·0	54·8	1·20	3·4	0·14	4·10
						1·6	0·06	3·75
	M: 6·59 S.D.: ±3·29	M: 0·902	M: 14·2		M: 1·07	M: 4·36 S.D.: ±4·05	M: 0·112 S.D.: ±0·056	M: 3·22 S.D.: ±1·01

* Estimated as the difference between total and non-dialysable B_{12}

A comparison of the results in the table for treated and control animals shows that the concentration of vitamin B_{12} in the CSF is significantly higher in the treated group than in the controls. Furthermore considerable amounts of vitamin B_{12} have been transported to the CSF as shown by the fact that the ratio of the vitamin B_{12} level in CSF to the pre-injection serum level is 4 times higher in the treated group than in the control group.

The ratio CSF-B_{12}/dialysable-B_{12} in serum during the experiments is explicitly lower than the ratio CSF-B_{12}/serum-B_{12} in the control group.

Although only the concentration of dialysable vitamin B_{12} is brought into calculations in the treated group,[3] it is evident that equality is not reached since the ratio has not been as high as in the normal controls. (It has not been possible to demonstrate dialysable vitamin B_{12} in the normal rabbit-serum.)

The ratios show considerable variation but without any significant correlation to time as would have been expected. This may be due to variations in the age of the rabbits, since the blood-brain barrier is known to vary with age.[4]

From these experiments no conclusions can be drawn regarding the mechanism of the transport of vitamin B_{12} from blood to CSF, but the results demonstrate that vitamin B_{12} is transported from blood to CSF with relative ease, since, as already mentioned, the concentration of vitamin B_{12} is raised in the CSF 30 min after injection of the vitamin into the blood stream.

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5-Bromo-2′-deoxycytidine (BCDR)–I. Studies on metabolism *in vitro* and in mice

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HALOGENATED pyrimidine derivatives have been known for more than half a century, but investigations of their metabolic activity in mammalian systems have been conducted only recently. Although 5-iodo-2′-deoxyuridine (IUDR) markedly inhibited the growth of experimental neoplasms in mice,[1] the results obtained in man have been only moderately encouraging.[2] Whether this discrepancy may be explained by the more rapid catabolism of IUDR in man,[2, 3] as compared to the mouse,[4] is not yet clear. In both species there is rapid cleavage of IUDR to 5-iodouracil and dehalogenation to uracil.[3, 4]

Because halogenated derivatives of deoxyuridine can lower the threshold of mamalian cells to radiation-injury,[5, 6] the analogous derivatives of 2′-deoxycytidine have been investigated in the hope that these molecular species might have greater metabolic stability, a circumstance which could result in their more extensive incorporation into the acid-soluble nucleotide pools, as well as into deoxyribonucleic acid (DNA). The possible advantage of the use of the deoxycytidine derivatives as chemotherapeutic agents, because of their potentially greater resistance to metabolic degradation, has been discussed elsewhere.[7] Biochemical evidence suggests that the presence of the amino group, in lieu of oxygen in position 4 of the pyrimidine ring, should result in greater metabolic stability.[8, 9] These studies have shown that the enzyme, deoxycytidylate (dCMP) deaminase, present in rapidly growing mammalian tissues, e.g., neoplasms, embryonic tissues, regenerating liver, thymus, and bone marrow, is absent or is suppressed,[8, 9, 10] in most normal tissues. Thus, 5-bromo-2′-deoxycytidine (BCDR)[11] and 5-iodo-2′-deoxycytidine (ICDR)[12] might be expected to exhibit relatively selective action for tumour tissues, since many mouse, rat and human neoplasms are rich in dCMP deaminase. A previous note[13] has demonstrated that ^{3}H-BCDR is incorporated into the DNA of cells as 5-bromo-2′-deoxyuridine 5′-phosphate, replacing an equivalent amount of thymidylic acid, but it is not yet