The transfer of ^{35}S -methionine sulphone across the blood-cerebrospinal fluid barrier¹

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The study of the transfer of amino-acids across the blood-brain and blood cerebrospinal fluid barriers is complicated by the rapid incorporation of amino-acids into the proteins and lipoproteins of the brain. The turnover of methionine in the brain has been demonstrated by the work of Gaitonde and Richter (1955, 1956), Niklas and Oehlert (1956), and Clouet and Richter (1959). In order to study the rate of transfer of amino-acids across the blood cerebrospinal fluid barrier it is necessary to use compounds similar to amino-acids but which do not enter into the metabolic processes of the brain. Koloušek and Babický (1961) investigated the metabolism of methionine sulfoximine and methionine sulphone in rat brain. They found that methionine sulphone was not incorporated in the acid insoluble fraction and concluded that methionine sulphone does not take part in protein synthesis in the brain. Methionine sulphone, therefore, appeared to be an inert amino-acid derivative which might be suitable for studies of blood-cerebrospinal fluid transport.

We report here the preliminary findings of an investigation into the blood-cerebrospinal fluid barrier to methionine sulphone in human subjects undertaken as part of an investigation of the blood-cerebrospinal fluid barrier in mental disorders. In previous work (Coppén, 1960) the rate of transfer of ^{24}Na from blood to cerebrospinal fluid was measured: it was found to be normal in schizophrenic patients but very much slower in patients suffering from a depressive illness.

PATIENTS AND METHODS

Three small groups of patients (13 in all) were selected for testing. The control group consisted of three psychiatrically normal patients undergoing neurological investigations who were subsequently found to have no organic illness of the central nervous system. Five schizophrenic patients and five patients suffering from

organic disease of the nervous system was also tested. No patients had received drugs within one month of the test.

Samples of 1 mc. of ^{35}S -DL-methionine from the Radiochemical Centre, Amersham, were dissolved in 1 to 2 ml. water and diluted to 10 ml. with a solution containing 300 mg. carrier L-methionine. To oxidize methionine to its sulphone 1 ml. H_2O_2 (30%) was added to the tube and the oxidation was completed by heating the tube containing the reaction mixture in a boiling water-bath for one to two hours. The solution was cooled, methionine sulphone was precipitated by adding absolute ethanol, and the precipitate was crystallized twice from ethanol. The purity of the final product was confirmed by two-dimensional chromatography which showed a single ninhydrin-positive spot of methionine sulphone containing more than 95% of the activity originally transferred on the paper.

A stock solution of ^{35}S -methionine sulphone was prepared as described above and was diluted further in a carrier to give a final concentration of $6\text{ }\mu\text{c./10 mg.}$ methionine sulphone/ml.: 5 ml. portions of this solution were sealed into ampoules and sterilized by autoclave. This solution (5 ml.) was injected into an antecubital vein and blood samples were withdrawn at frequent intervals from the opposite limb through an indwelling Gordh needle. No clinical effects were observed following the injection of methionine sulphone. Ten ml. of cerebrospinal fluid was withdrawn by lumbar puncture at either one, two, or four hours after the injection of ^{35}S -methionine sulphone.

A known volume of each sample of whole blood or cerebrospinal fluid (4 to 5 ml.) was deproteinized with 1 ml. 40% trichloroacetic acid (T.C.A.). After vigorous mixing the contents of the tube were centrifuged and the clear supernatant filtrate was filtered by decantation. The precipitate in the tube was washed once with 5 ml. 10% trichloroacetic acid and the washings were combined with the main filtrate. The combined trichloroacetic acid extract was washed four times with ether. The aqueous extract was passed through a column (10 cm.) of Zeo-Karb 225 (H+ form) and the column was washed with 25 ml. of water. Methionine sulphone was eluted from the column with 70 ml. 1N-NH_3 . The washings of the column and the NH_3 eluate were evaporated to dryness and the residue was collected in plastic planchets using a technique described elsewhere (Vrba, Gaitonde, and Richter, 1962). The samples were counted in a nuclear Chicago automatic counter, and since they originated

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FIG. 1

from approximately the same volume of blood and cerebrospinal fluid no absorption correction was necessary.

The counting rates of the cerebrospinal fluid and blood samples were plotted against time (Fig. 1). The rate of entry was expressed as a transfer constant (k_{in}) in the equation:

$$\frac{dC_{c.s.f.}}{dt} = k_{in} C_b - C_{c.s.f.}$$

where $C_{c.s.f.}$ and C_b is the concentration of methionine sulphone in cerebrospinal fluid and blood respectively. The transfer constant was estimated by graphical integration (Davson and Luck, 1959).

RESULTS AND DISCUSSION

The transfer rates of methionine sulphone from blood into cerebrospinal fluid are shown in Table I. The diagnosis, time of taking fluid after the injection of methionine sulphone, and the calculated values for k_{in} have been given. The figures for k_{in} show considerable scatter (range 0.00013 to 0.0031). The schizophrenic patients had slower entry rates than the other patients, but clearly no conclusion can be deduced from such small numbers. The case with spinal block is of interest since it shows that, similarly to Na^{24} (Tubiana, Benda, and Constans 1951; Crow,

1955), methionine sulphone can pass from blood to cerebrospinal fluid independently of flow from the choroid plexus.

All the radioactivity of whole blood was found in compounds soluble in trichloroacetic acid but it was also found that a considerable part of the radioactivity in these extracts was present in compounds other than methionine sulphone. ^{35}S -Methionine sulphone was separated from other ^{35}S -labelled compounds by adsorbing the sulphone on a cation exchange resin followed by elution with ammonia solution. Two-dimensional paper chromatography showed that about 95% of the radioactivity eluted from the resin was present as ^{35}S -methionine sulphone. The washings of the resin contained other ^{35}S -labelled compounds which accounted for 40 to 50% of the total radioactivity in the trichloroacetic acid extracts of blood. In urine the uptake of ^{35}S in these compounds reached 80 to 92% of the total radioactivity. A small amount of this radioactivity was precipitated as benzidine ^{35}S -sulphate but the major fraction of ^{35}S was present in other compounds which were not identified. It is therefore clear that methionine sulphone is not metabolically inert. The amounts of unchanged ^{35}S -methionine sulphone remaining in the blood and cerebrospinal fluid were sufficient for transfer constants to be determined; but the possibility must be considered that the unidentified compounds might compete with methionine sulphone for transfer across the blood cerebrospinal fluid barrier.

TABLE II
ESTIMATED TRANSFER CONSTANT
FOR METHIONINE SULPHONE COMPARED WITH
VALUES FOR OTHER SUBSTANCES

<i>Substance</i>	<i>k_{ta}</i> min ⁻¹	<i>Animal</i>	<i>Reference</i>
Ethyl alcohol	0.225	Rabbit	Davson (1956)
Thiourea	0.0057	Dog	Davson (1956)
³⁵ Cl	0.019	Dog	Davson (1956)
Glucose	0.0087	Rabbit	Davson (1956)
²⁴ Na	0.00062	Man (lumbar fluid)	Coppen (1960)
Tritiated water	0.025	Man (lumbar fluid)	Coppen (1960)
Methionine sulphone	0.00020-0.0031	Man (lumbar fluid)	This paper

TABLE I

TRANSFER RATES OF METHIONINE SULPHONE FROM BLOOD INTO CEREBROSPINAL FLUID

Control Group				Organic Group					Schizophrenic Group			
Age (yr.)	Sex	Time After Lumbar Puncture (hr.)	$k_{in}min^{-1}$	Age (yr.)	Sex	Diagnosis	Time After Lumbar Puncture (hr.)	$k_{in}min^{-1}$	Age (yr.)	Sex	Time After Lumbar Puncture (hr.)	$k_{in}min^{-1}$
68	M	4	0.0019	54	M	Spinal compression	2	0.00079	52	M	1	0.00020
28	M	4	0.0021	36	M	Huntington's chorea	2	0.00092	54	M	1	0.0046
50	M	4	0.00074	18	M	Friedreich's ataxia	2	0.0014	24	M	2	0.00013
				48	F	Spinal compression	4	0.00023	16	M	4	0.00055
				50	M	Cortical atrophy	4	0.0031	41	M	4	0.00043
		Means	0.0016					0.0013				0.00030

The estimates of the transfer constant for methionine sulphone are compared with values calculated for some other substances in Table II.

SUMMARY

The rate of transfer of methionine sulphone was measured from blood to lumbar cerebrospinal fluid in man. This substance was found to enter the cerebrospinal fluid relatively slowly at a rate comparable to that found for sodium ions.

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