

Concentration of Thiocyanate by the Choroid Plexus of the Rabbit *in vitro*. (27389)

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There exists in the choroid plexus a mechanism capable of the uphill transport of iodide ions, similar to that known for the thyroid and for certain other glands(1). It was of some interest to know whether thiocyanate, which behaves as though it were a competitive inhibitor of iodide transport, might itself be concentrated by the plexus as it is known to be by stomach mucosa(2), salivary glands in certain species(3), and, though not in the thyroid taken as a whole(4), in the colloid of that organ(5).

The experiments to be described demonstrate that thiocyanate is concentrated by the choroid plexus of the rabbit *in vitro*.

Materials and methods. Rabbits weighing 2-3 kg were killed by insufflation of air into an aural vein followed by the opening of the great vessels in the thorax. The choroid plexus of each lateral ventricle and of the fourth ventricle was rapidly removed and placed in iced Tyrode's solution. Each plexus was gently blotted and weighed before incubation.

The incubation fluid was Tyrode's solution prepared according to the direction of Becker (6). Carbon dioxide and oxygen were bubbled through the fluid for 10 minutes immediately before its use. To the fluid was added potassium thiocyanate, ordinarily in a concentration of 5×10^{-6} M. The thiocyanate contained S^{35} ; the initial specific activity was 11 mc/mM.[†]

Incubations were usually carried out at 25°C for 40 minutes. The tissue was then removed from its bath, blotted and lysed in .1 ml 2 N NaOH. The volume was adjusted to 2 ml and a 1 ml portion was placed in a copper planchet and dried. Counts of this and of incubation fluid were made in a continuous-flow counter. No corrections for self absorption were necessary. From the counts

of the tissue and of the medium the ratio of concentrations of S^{35} in tissue (T) and medium (M) was calculated.

$$\frac{T}{M} = \frac{\text{Counts/mg tissue}}{\text{Counts}/\mu\text{l medium}}$$

The radioactivity of the thiocyanate standard and that of tissue which had accumulated thiocyanate were compared by paper chromatography. For this the tissue was incubated in presence of 5×15^{-6} M thiocyanate, homogenized in a small amount of water, and the entire sample spotted on Whatman No. 1 filter paper. N-butanol NH_3 was employed as the solvent. The strips were scanned for radioactivity and were also cut into 1 cm lengths and counted in a flow counter.

Results. The results of incubation of choroid plexus in media containing several concentrations of thiocyanate and a variety of inhibitors are shown in Table I. At low concentrations of thiocyanate, the accumulation in the plexus was approximately proportional to the concentration, T/M ratios averaging around 3. The highest ratio observed for a single plexus was 7. No significant difference was found between tissue taken from the lateral ventricle and that from the fourth ventricle.

The accumulation of SCN^- was inhibited in the presence of perchlorate, fluoroborate and iodide, presumably on a competitive basis, and by fluoride, cyanide, 2,4 dinitrophenol or in oxygen deficient media.

The radioactivity accumulated in the plexus was shown by paper chromatography to be almost entirely in the form of thiocyanate (Fig. 1). A small peak at the origin representing less than 10% of the activity is considered to be sulfate. Upon chromatography of the thiocyanate solution there was relatively less radioactivity at the origin, and there was additionally a concentration of radioactivity at an Rf of approximately .9.

Discussion. Thiocyanate is taken up un-

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† Obtained from Tracerlab, Waltham, Mass.

TABLE I. Concentration of Thiocyanate by Choroid Plexus.

Conc. inhibitors		T/M values at various SCN ⁻ concentrations in incubation fluid			
		5 × 10 ⁻⁷ M SCN ⁻	5 × 10 ⁻⁶ M SCN ⁻	5 × 10 ⁻⁵ M SCN ⁻	10 ⁻⁴ M SCN ⁻
None		2.78 ± .15 (18)	3.16 ± .2 (28)	1.8 ± .24 (14)	.7 ± .04 (4)
ClO ₄ ⁻	10 ⁻⁵ M		.88 ± .04 (6)		
BF ₄ ⁻	10 ⁻⁵ M		1.4 ± .06 (4)		
I ⁻	10 ⁻⁴ M		1.03 ± .02 (4)		
CN ⁻	10 ⁻³ M		1.48 ± .14 (4)		
F ⁻	10 ⁻² M		.99 ± .13 (4)		
2,4 DNP	5 × 10 ⁻⁴ M		2.05 ± .24 (8)		
Digoxin	2.7 × 10 ⁻⁶ M		1.24 ± .05 (4)		

All values are mean ± S.E. of mean.

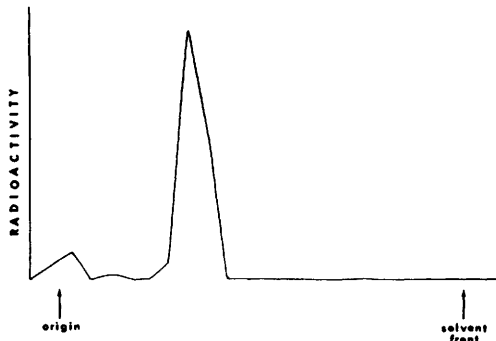


FIG. 1. Distribution of radioactivity is shown along a paper strip employed for ascending chromatography of tissue homogenate after incubation in the presence of 5×10^{-6} M SCN⁻ containing S³⁵. Solvent was N-butanol NH₃. One cm lengths of the strip were cut and counted in a flow counter.

changed in the choroid plexus of the rabbit *in vitro* against a gradient of concentration. The accumulation is proportional to the concentration of thiocyanate only at low concentrations, is inhibited by several monovalent inorganic anions considered along with thiocyanate to be competitive for iodide transport, and is inhibited also by certain metabolic poisons and by oxygen deficiency. Thus, the ordinary criteria for an active transport process are met and thiocyanate appears to share the iodide carrier of the choroid plexus.

The deficiencies in the cerebrospinal fluid of I⁻ and SCN⁻, which may thus be accounted for, were suggested on general grounds by Brown-Grant(7) to be based upon carrier transport of the ions out of the fluid. Such a process was shown by Becker(6) to operate in freeing the aqueous humor of iodide and by Forbes and Becker(8) to be involved in the exit from the aqueous of certain organic

anions. The operation of a carrier similar to the latter was shown by Pappenheimer, Heisey and Jordan(9) to be concerned with the movement of diodrast and PSP out of the cerebrospinal fluid, and it seems likely that this is connected with the capacity of embryonic choroid plexus grown in organ culture to accumulate PSP against a gradient of concentration(10).

The biological significance of an iodide-thiocyanate carrier in the choroid plexus and of the deficiencies of these ions in the cerebrospinal fluid is not known.

Summary. The choroid plexus of the rabbit was incubated *in vitro* in a medium containing S³⁵ C N⁻. The thiocyanate was accumulated in the tissue against a gradient of concentration. The accumulation was impaired by both competitive and metabolic inhibitors and was proportional to the concentration of thiocyanate only at low concentration.

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