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INCREASE IN CEREBRAL FLUID IN RATS AFTER TREATMENT WITH TRIETHYLTIN

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Abstract—After intravenous injection of triethyltinsulphate into rats the increase in cerebral water is influenced by the environmental temperature. If at 5° or 19° there is a delay of 12 hr before the brain water rises but if at 34° it rises from the time of injection. The sensitivity to triethyltin of the adenosine triphosphatase stimulated by Na^+ and K^+ has been reexamined. From both *in vitro* and *in vivo* experiments it is concluded that this enzyme is not directly involved in the increase in cerebral fluid.

It is now well established that salts of triethyltin produce an increase in water in the brain and spinal cord of several species [1–3]. The location of the increased water, which contains sodium chloride [1] and probably glucose (Lock, unpublished observations, 1973) is in the white matter [4]. Electron microscopic examination indicates that the water is intramyelinic and causes splitting of myelin at the intraperiod line. Although in its major components the fluid resembles plasma and therefore extracellular fluid, a conclusive decision whether it is intra- or extracellular cannot yet be made.

After the administration of triethyltin sulphate to rats kept at room temperature (15–20°) there is a rapid fall in their body temperature [14]. This fall may be prevented by maintaining the rats in an environmental temperature of 33–34° [15]. The incorporation of [^{32}p]-labelled phosphate to phospholipids is also decreased after the administration of triethyltin to rats kept at low temperature and this is prevented by raising the environmental temperature to 33–34°. In this study the rate of increase in cerebral water has been measured after intravenous (i.v.) injection of triethyltin sulphate into rats which were then kept at different environmental temperatures.

It has been suggested that the genesis of the lesion is the inhibition of the transport of sodium mediated through an adenosinetriphosphatase [5], although the brain enzyme does not seem to be sufficiently sensitive to triethyltin [6]. The enzymic system located in cell membranes is complex [7] and it is known that the sensitivity of this enzyme to at least two substances is determined by the conditions of incubation. For example: ouabain at lower concentrations inhibits the enzyme if it is incubated in the presence of sodium rather than potassium [8], whereas with beryllium the reverse is true [9]. Accordingly the sensitivity of brain microsomal adenosinetriphosphatase has been reexamined with different conditions of preincubation. Also rats have been treated with triethyltin and microsomal fractions have been isolated from the brains when the increase in water is well advanced.

METHODS

Materials. Triethyltin sulphate was prepared from triethyltin hydroxide donated by the Tin Research Institute, Greenford, Middlesex, England using the method previously described [10]. White rats were used of the Porton strain and were injected intravenously in the tail vein with 0·2 ml of the relevant neutralised solution in water triethyltin sulphate to give a dose of 10 mg/kg body wt.

Methods. Brain water content was determined by drying at 105° to constant weight. The rats were decapitated and the brains removed rapidly under a hood containing a humid atmosphere to reduce evaporation.

Microsomal fractions from rat brain were isolated as previously described [11]. The fraction was finally sedimented at 105,000 *g*/20 min after a preliminary sedimentation of the material at 30,000 *g* for 5 min. The speed for this preliminary sedimentation was not critical, since the specific activity of the adenosine-triphosphatase in the fraction so obtained at either 20,000 *g*, 30,000 *g*, or 40,000 *g* was not greatly different between the three preparations.

The adenosinetriphosphatase of microsomal preparations from rat brain was determined as described previously [11] and was linear with respect to enzyme concentration. All assays were carried out in duplicate or triplicate for 10 min at 37°. The medium contained $MgCl_2$ (2 mM), EDTA–Tris, (1 mM) Tris–TrisCl (40 mM, pH 7·4) NaCl (150 mM), KCl (15 mM) and ATP–Tris (5 mM).

For *in vitro* experiments the microsomal preparations were preincubated for 15 min with 100 μM triethyltin under several conditions in the absence of Na^+ and K^+, and in the presence of Na^+, K^+ or Na^+

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and K^+. The enzyme was then assayed adding ATP and the missing alkali metal salts. Protein was determined by the method of Robinson and Hodgson [12] as modified later [13]. The protein pellet was defatted with neutral ethanol–ether (55:45) before solution in NaOH.

RESULTS

Brain water

We have compared the increase of brain water in rats kept after dosing with triethyltin at the three environmental temperatures of 5°, 19° and 34°. The results are given in Fig. 1. At an environmental temperature of 19° and 5° there is a delay of 12 hr before the brain water begins to rise but from 12 to 24 hr it rises continuously. In contrast when the environmental temperature is 34° the brain water increases continuously from the time of injection.

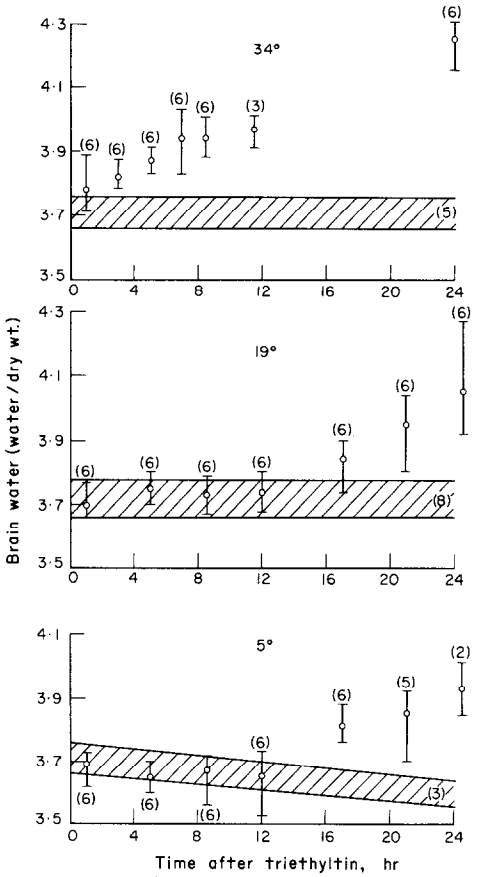

Fig. 1. The increase in brain water after the i.v. injection of triethyltin sulphate (10 mg/kg body wt). The rats were 150–170 g and male. The results for the treated animals are given as the mean (open circles) and range (bars). The hatched area is the range of the controls, which were killed after various times at the various environmental temperatures. The numbers of animals are in brackets. Except for a slight decrease in those kept at 5° their brain water was constant over the 24 hr period.

Adenosinetriphosphatase of the microsomal fraction from rat brain

In vitro *experiments.* Preincubation of the microsomal fraction with triethyltin in the absence of Na^+ and K^+ (see Methods) gave the highest degree of inhibition. This maximum inhibition was 54 per cent, whereas the minimum (that in the presence of Na^+) was 35 per cent. Further experiments (Table 1) using the most effective condition (in the absence of Na^+ or K^+) show that 20 min incubation gives maximum inhibition. The enzyme activity stimulated by Na^+ and K^+ is more inhibited than that determined in the absence of Na^+ and K^+ (Mg^{2+} stimulated adenosine triphosphatase). Higher concentrations than 20 μM triethyltin are required for inhibition of the most sensitive enzymic activity using the most effective time and condition of preincubation.

In vivo *experiments.* Rats were injected intravenously with triethyltin sulphate and then kept at an environmental temperature of 34°. The animals were killed and a microsomal fraction was isolated from their brains at times when an increase in brain water would have occurred (Fig. 1) It is clear from the results in Table 2 that there is no decrease in adenosinetriphosphatase activity due to triethyltin treatment.

DISCUSSION

The experiments measuring the increase in brain water showed that environmental temperatures influence the course of events following treatment with triethyltin. When the temperature of the animal was allowed to fall (environmental temperature 19° and 5°) then there was a delay in the accumulation of water of about 12 hr. When the temperature of the rat does not fall (at an environmental temperature of 34° [15]) then water accumulated from the time of injection. The reason for this phenomenon is not known but whatever it is, the definition of the conditions of an increase in brain water should allow a detailed examination (electron microscopic or physiological) of the development of the lesion.

The enzyme thought to be involved in the transport of sodium across membranes (adenosine triphosphatase dependent upon the presence of Na^+ and K^+) is inhibited by triethyltin (Table 1, see ref. 6). Using conditions giving maximal inhibition *in vitro* (preincubation for 20 min in the absence of Na^+ and K^+) it is clear that to produce a substantial inhibition concentrations of triethyltin approaching 100 μM are required. The overall concentration of triethyltin in rat brain after 10 mg/kg triethyltin sulphate is approx. 20 μM [16–17], and there is no evidence of gross differences in the distribution of triethyltin in rat brain [17]. Isolation of a microsomal fraction from brains of animals treated with triethyltin failed to demonstrate any reduction in adenosine triphosphatase activity.

Table 1. Effect of concentration of triethyltin and time of preparation on rat brain microsomal adenosinetriphosphatase

	nmoles P/mg protein per min					
	(A)		(B)		Difference (B-A)	
Time of preincubation (min ± 100 μM TET)	−TET	+TET	−TET	+TET	−TET	+TET
0	210	160	490	330	280	170
10	220	160	470	300	250	140
20	190	120	430	210	240	90
30	230	120	460	230	230	110

Concn of TET (μM for 20 min)	(A)	(B)	Difference (B-A)
0	220	490	270
20	170	390	220
100	170	250	80
500	140	160	20

Microsomes were preincubated with triethyltin (TET) in the absence of Na^+ and K^+ at 37°. The enzyme was assayed by the addition of either ATP (A) or ATP + Na^+ + K^+ (B). The difference between these two determinations is the activity stimulated by Na^+ and K^+.

Table 2. Adenosine triphosphatase of a microsomal fraction from brain of rats injected with triethyltin

Experimental conditions	No. of rats	nmoles P/mg protein/min (A)	(B)	(B-A)
Control (room temp.)	11	282 ± 26	562 ± 36	279 ± 12
Control (34°)	1	322	525	203
Control (34°, 10 hr without food)	3	297 ± 34	522 ± 28	225 ± 7
Control (34°, 24 hr without food)	3	261 ± 9	512 ± 14	251 ± 11
Control (mean of all at 34°)	7	285 ± 16	518 ± 12	233 ± 8
Experimental (34°, 8 hr)	3	258 ± 11	495 ± 19	237 ± 8
Experimental (34°, 24 hr)	3	324 ± 15	575 ± 25	251 ± 12
Experimental (mean)	6	291 ± 17	535 ± 23	244 ± 7

Male rats (150–170 g) were injected i.v. with triethyltin sulphate (10 mg/kg body wt) and kept for 8 hr and 24 hr at an environmental temperature of 34°. After isolation of the microsomal fraction, enzyme activity was determined by adding ATP (A), or ATP + Na^+ + K^+ (B). The difference between these two (B–A) is the enzyme activity dependent upon Na^+ and K^+. Since the rats injected with triethyltin do not eat, the controls were also deprived of food. The results are given as mean ± S.E.

Although the dilution during isolation of the fraction may cause dissociation of triethyltin from enzymes, the negative finding does remove other possibilities which might occur *in vivo*, e.g. inhibition of enzyme synthesis. Although there are many unknowns about the cellular distribution it seems unlikely on the basis of the present evidence that inhibition of the sodium transport enzyme could be implicated directly in the increase in water in the brains of animals poisoned with triethyltin.

REFERENCES

1. P. N. Magee, H. B. Stoner and J. M. Barnes, *J. Path. Bact.* **73,** 107 (1957).
2. R. M. Torack, R. D. Terry and H. M. Zimmerman, *Am. J. Path.* **36,** 273 (1960).
3. L. C. Scheinberg, *J. Neuropath. exp. Neurol.* **25,** 202 (1966).
4. A. Hirano, H. M. Zimmerman and S. Levine, *J. Neuropath. exp. Neurol.* **27,** 571 (1968).
5. R. M. Torack. *Am. J. Path.* **46,** 245 (1965).
6. W. N. Aldridge and B. W. Street, *Biochem. J.* **91,** 287 (1964).
7. R. Whittam and K. P. Wheeler, *Ann. Rev. Physiol.* **32,** 21 (1970).
8. R. L. Post, C. R. Merritt, C. R. Kinsolving and C. D. Albright, *J. biol. Chem.* **235,** 1796 (1960).
9. G. Toda, T. Hashimoto, T. Asakura and S. Minakami, *Biochim. biophys. Acta* **135,** 570 (1967).
10. W. N. Aldridge and J. E. Cremer, *Biochem. J.* **61,** 406 (1955).
11. W. N. Aldridge, *Biochem. J.* **83,** 527 (1962).
12. H. W. Robinson and C. G. Hogden, *J. biol. Chem.* **135,** 707 (1940).
13. W. N. Aldridge, *Biochem. J.* **67,** 423 (1957).

14. H. B. Stoner and C. J. Threlfall, *Biochem. J.* **69,** 376 (1958).
15. M. S. Rose and W. N. Aldridge, *J. Neurochem.* **13,** 103 (1966).
16. J. E. Cremer, *Biochem. J.* **67,** 87 (1957).
17. M. S. Rose and W. N. Aldridge, *Biochem. J.* **106,** 821 (1968).