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INCREASE IN CEREBRAL FLUIDS IN RATS AFTER TREATMENT WITH HEXACHLOROPHANE OR TRIETHYLTIN

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Abstract—A single intraperitoneal injection of hexachlorophane into rats produced an increase in water in the brain and spinal cord. The additional fluid has been shown to contain sodium and chloride ions. The effect of hexachlorophane on some cerebral intermediary metabolites was also examined. Twenty-four hours after hexachlorophane administration a small increase in cerebral glucose was seen. A single intraperitoneal injection of triethyltin also produced an increase in cerebral water and glucose. Calculations of the glucose concentration present in the additional cerebral water indicates that the extra fluid is derived from either cerebrospinal fluid or plasma.

It is now well established that triethyltin produces an increase in water in the brain and spinal cord of several species [1–3]. In rats, the increase in cerebral water is influenced by the environmental temperature [4]. The increased water, which contains sodium chloride [1], is located in the white matter [5]. Electron microscopic examination indicates that the water is intramyelinic and causes splitting of myelin at the intraperiod line [6].

Hexachlorophane [2–2′ methylene bis (3,4,6 trichlorophenol)] also produces an oedematous lesion in the brain of several species including man [7–11]. Again, the increased fluid is localised in the white matter and electron microscopic examination indicates that the water is intramyelinic and causes splitting of myelin at the intraperiod [10, 12].

In this study, an increase in cerebral water has been measured after a single intraperitoneal (i.p.) injection of hexachlorophane or triethyltin into rats. The additional fluid has been shown to contain glucose, sodium and chloride suggesting that the fluid resembles an ultrafiltrate of plasma or cerebrospinal fluid and is therefore extracellular, however a conclusive decision as to whether the fluid is intra- or extracellular cannot yet be made. The effect of hexachlorophane on the level of some cerebral intermediary metabolites has also been examined.

METHODS AND MATERIALS

Triethyltin sulphate was prepared from triethyltin hydroxide supplied by the Tin Research Institute, Greenford, Middlesex, England as previously described [13]. Hexachlorophane [2-2′ methylene bis (3,4,6-trichlorophenol)] was a gift from Cooper Technical Bureau, Berkhampstead, Herts., England. Male rats (170 g body wt) of the Porton strain were injected i.p. with either hexachlorophane (78.5 μmole/kg) in dimethylformamide or triethyltin sulphate (40 μmole/kg) in saline, and kept after dosing at an environmental temperature of 20°.

Brain and spinal cord water content was determined by drying to constant weight at 105°. The dried tissue was then extracted with 5% trichloroacetic acid, and the sodium, potassium and chloride ions present in the extract determined. The sodium and potassium ions were measured by flame photometry using a Unicam SP 90, whilst chloride was determined by potentiometric titration using an Eel chloride meter. The tissue chloride values were corrected for the chloride ions present in 5% trichloroacetic acid.

For the determination of cerebral metabolites, the supratentorial portion of the brain was removed using the brain-blowing technique [14]. The frozen brains were rapidly homogenised in ice-cold 1 M $HClO_4$, the protein removed by centrifugation and the acid soluble fraction neutralised to pH 7 with KOH. The potassium perchlorate precipitate was filtered and the filtrate evaporated to dryness at 35°. The dried brain extract was dissolved in water and stored at −20° prior to use. Blood glucose was measured in blood collected from the trunk after decapitation. Whole blood samples were deproteinised by adding ice-cold 0.2 M $HClO_4$ and the precipitate removed by centrifugation. The supernatant was neutralised to pH 7 with KOH, the potassium perchlorate precipitate was removed and the glucose in the supernatant was determined.

The concentration of cerebral ATP and creatine phosphate was measured by the method of Lowry, Passonneau, Hasselberger and Schulz [15]; malate and 2-oxoglutarate by the method of Goldberg, Passonneau and Lowry [16]; glutamate and aspartate by the method of Graham and Aprison [17] and glutamine by the method of Nahorski [18]. Ammonia was assayed as described by Folbergrova, Passonneau, Lowry and Schulz [19]. Glycogen was extracted, degraded enzymatically and assayed as glucose by essentially the method of Nahorski and Rodgers [20]. Citrate was determined by a fluorimetric modification

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Table 1. A comparison of the glucose and electrolyte content in the additional fluid in rat brain and spinal cord after a single dose of hexachlorophane or triethyltin with that present in either cerebrospinal fluid or plasma

Treatment	Tissue component		Concentration found in additional water (m-mole/l)	Concentration present in: CSF (m-mole/l H_2O)	plasma (m-mole/l H_2O)
Hexachlorophane (78.5 μmole/kg i.p. 24 hr before test)	Brain	Na^+	192	145	150
		Cl^-	192	124	109
		glucose	5.58	7.05	10.55
	Spinal cord	Na^+	151	145	150
		Cl^-	104	124	109
Triethyltin (40 μmole/kg i.p. 24 hr before test)	Brain	glucose	4.65	6.25	9.35

The concentration of glucose or electrolytes found in the tissues was calculated by substituting the results from Fig. 2 and Table 2 into the following equation:

Glucose or electrolyte found in additional tissue water (m-mole/l) =

$$\frac{\text{Glucose or electrolyte tissue content (Experimental} - \text{Control) m-mole/kg dry wt}}{\text{Tissue water content (Experimental} - \text{Control) l/kg dry wt}}$$

The concentration of glucose and electrolytes in plasma and cerebrospinal fluid was calculated using the following data. Plasma Na^+ and Cl^- levels were taken as 142 and 106 meq/l plasma [31] and plasma glucose from the values in Table 3, knowing that 80 per cent of glucose in whole blood is in the plasma [32]. Plasma water was taken at 946 g l plasma [33]. Cerebrospinal fluid Na^+ and Cl^- levels were taken as 145 and 124 meq/l water and cerebrospinal fluid glucose as about 67 per cent of that in plasma [34].

of the enzymatic method of Moellering and Gruber [21]. Lactate was measured spectrophotometrically as described by Bergmeyer [22]. D-Glucose was determined in blood and brain extracts using hexokinase and glucose-6-phosphate dehydrogenase [22].

RESULTS AND DISCUSSION

Following a single i.p. injection of hexachlorophane the water content of the brain increases continuously from 3 hr after administration (Fig. 1). At 24 hr, an increase in brain and spinal cord sodium, chloride and water content is found (Fig. 2) with no change

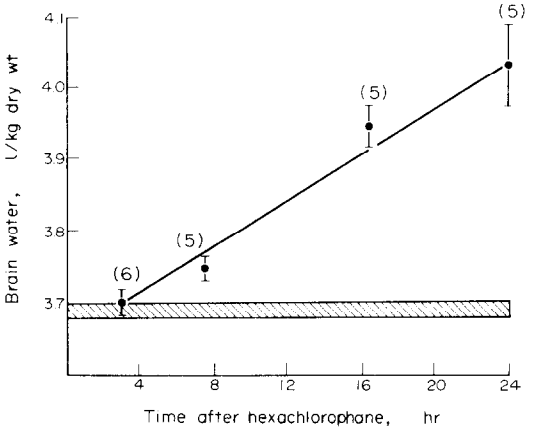

Fig. 1. The increase in brain water after the i.p. injection of hexachlorophane (78.5 μmole/kg body wt). The results for the treated animals are given as the mean (circles) and range (bars). The hatched area is the range of the controls. The number of animals is in brackets.

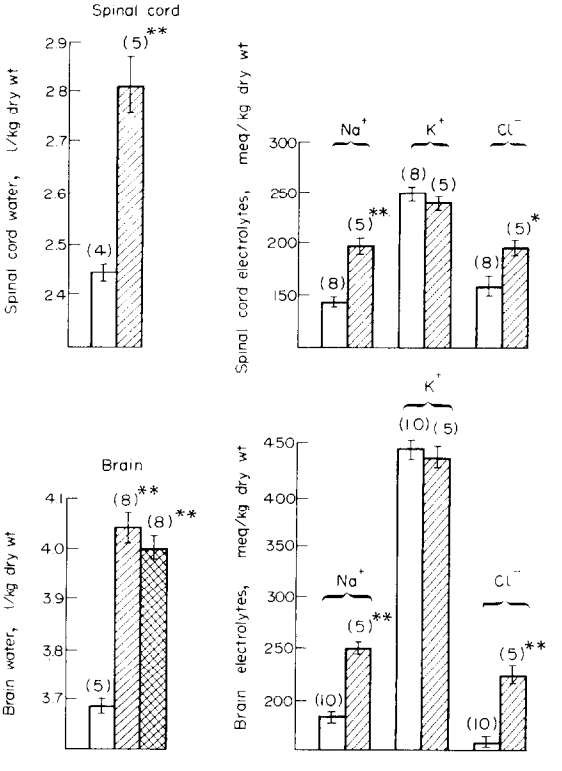

Fig. 2. The increase in brain and spinal cord water and electrolytes 24 hr after a single i.p. injection of hexachlorophane (78.5 μmole/kg) and brain water 24 hr after triethyltin (40 μmole/kg). The plain area is the control and the hatched areas ▨ are hexachlorophane and ▩ triethyltin. The column height represents the mean and the bars the S.E.M. The number of animals is shown in brackets. Significance of difference from appropriate control values $*P < 0.05$, $**P < 0.001$.

Table 2. The effect of hexachlorophane (78.5 μmole/kg) and triethyltin (40 μmole/kg) on the concentration of some cerebral metabolites

Metabolite (μmole/g frozen brain)	Control		Hexachlorophane		Triethyltin	
Glycogen	1.47 ± 0.10	(6)	1.71 ± 0.13	(4)	...	
Glucose	1.51 ± 0.05	(11)	1.91† ± 0.08	(11)	1.85* ± 0.07	(7)
Lactate	1.38 ± 0.16	(13)	1.61 ± 0.16	(8)	1.64 ± 0.30	(6)
ATP	2.25 ± 0.08	(6)	2.34 ± 0.09	(4)	...	
Phosphocreatine	3.83 ± 0.11	(6)	4.00 ± 0.11	(4)	—	
Citrate	0.34 ± 0.01	(6)	0.34 ± 0.01	(4)	—	
2-oxoglutarate	0.18 ± 0.02	(6)	0.13† ± 0.01	(4)	—	
Malate	0.21 ± 0.01	(6)	0.17† ± 0.01	(4)	...	
Aspartate	3.44 ± 0.25	(6)	3.12 ± 0.21	(4)	—	
Glutamate	10.00 ± 0.21	(6)	9.35 ± 0.24	(14)	7.15† ± 0.37	(7)
Glutamine	4.40 ± 0.37	(6)	4.81 ± 0.26	(4)	...	
NH_4^+	0.65 ± 0.03	(4)	0.60 ± 0.04	(4)	—	

Rats were killed 24 hr after a single i.p. injection of hexachlorophane or triethyltin, as described in the methods section. Hexachlorophane results were multiplied by a factor of 1.069 and the triethyltin data by 1.067 to correct for the increased brain water. Results are expressed as Mean ± S.E.M. with the number of determinations in brackets. Test of significance between treated and controls *P <0.01, †P <0.001.

in the potassium content. The exact composition of the extra fluid is not known, but it is possible to calculate the amount of additional fluid present in the oedematous brain and spinal cord and the quantities of sodium and chloride present in it (Table 1); these values were compared with those expected if the additional fluid were derived from the plasma or cerebrospinal fluid (Table 1). These calculations indicate that the extra sodium and chloride present in the additional tissue water may be derived from either the plasma or cerebrospinal fluid.

The concentration of cerebral glucose is significantly increased 24 hr after hexachlorophane (Table 2, see also [23]) and 24 hr after triethyltin administration (Table 2). Both these compounds produce an increase in brain water (Fig. 2) and if the extra fluid is derived from the plasma or cerebrospinal fluid this may be the source of the extra glucose. Hyperglycaemia is seen 24 hr after either hexachlorophane or triethyltin administration (Table 3) with no alteration in the haematocrit values (Table 3). Calculations (Table 1) show that the concentration of glucose in the additional water more closely resembles that calculated to be present in the cerebrospinal fluid than in plasma, but a conclusive decision as to whether the additional fluid is derived from the cerebrospinal fluid or plasma cannot yet be made. The increase in brain glucose seen after hexachlorophane or triethyltin administration is small and no increase in the ratio of brain/blood glucose is seen (Table 3). This is unlike the action of some drugs or anaesthetics, e.g. ethanol [24] and phenobarbitone [16, 25] where a very large increase in brain glucose is seen with a corresponding increase in the brain/blood glucose ratio.

A significant decrease in batch 2-oxoglutarate and malate was found after hexachlorophane (Table 2) which suggested that the redox state of the brain may have changed. However, both glutamate and aspartate were slightly depressed and calculations of the mitochondrial $[NAD^+]/[NADH]$ ratio from the glutamate dehydrogenase reaction as described by Williamson, Lund and Krebs [26] showed no change in the redox state. This finding agrees with that of Harris *et al.*, [23], who could detect no changes in redox or phosphorylation states in the brain 24 hr after a single oral dose of hexachlorophane. That cerebral glutamate is lowered after triethyltin (Table 2) has been known for some time [27] and it has also been shown that cerebral alanine and γ-aminobutyric acid are lowered by triethyltin.

Hexachlorophane is known to uncouple oxidative phosphorylation of isolated rat liver and brain mitochondria [28–30]. In this study changes indicative of uncoupling, i.e. lowering of cerebral ATP and phosphocreatine, or alteration of the redox state were

Table 3. Blood glucose concentration, haematocrit values and brain/blood glucose ratios for rats treated with hexachlorophane (78.5 μmole/kg) or triethyltin (40 μmole/kg)

	Control		Hexachlorophane		Triethyltin	
Blood glucose (μmole/ml whole blood)	5.04 ± 0.14	(8)	7.00* ± 0.44	(9)	6.33* ± 0.24	(9)
Haematocrit (% red blood cells)	42.3 ± 0.5	(8)	43.9 ± 1.0	(9)	42.6 ± 0.7	(9)
Brain/blood glucose	0.30 ± 0.01		0.27 ± 0.02		0.29 ± 0.02	

Blood glucose and haematocrit values were measured in blood collected from severed neck vessels following decapitation of rats, 24 hr after a single i.p. dose. Brain/blood glucose ratios were calculated using the mean brain glucose values in Table 2, and the mean blood glucose values in this Table. The results are expressed as mean ± S.E.M. with the number of observations in parentheses. Significant difference between treated and control group *P <0.001.

not seen 24 hr after hexachlorophane administration. Caldwell *et al.* has shown that the uncoupling action of hexachlorophane is an early response to poisoning [29].

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