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Distribution of Endosulfan in Cat Brain

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Organochlorine insecticides of the cyclodiene type have been of interest for years because of their potential toxicological activities in a wide variety of organisms, including man. Endosulfan, one of the important insecticides of the cyclodiene group, has a wide spread use in the crop protection (MARTIN 1964, MILLER 1965). Studies of its mechanisms of action in insects (BALLSCHMITER and TOELG 1966) and other animal species (SCHOETTGER 1970, ELDON et al. 1970, GUPTA and CHANDRA 1975, GUPTA 1976) have indicated that the nervous system is a major site of activity. ISRAELI et al. (1969) have also reported that workers engaged in the grinding of the endosulfan suffered poisoning characterized by epileptiform symptoms.

The correlation of clinical symptoms of acute endosulfan poisoning with the concentration of endosulfan in brain, spinal cord or plasma is not available in the literature. However, an investigation of the distribution of DDT in the brain of the cat showed that the relative concentration varied both with the brain areas considered and with the time after administration of an intravenous dose (SCHWABE 1965).

The present investigation was undertaken in an attempt to determine the distribution of endosulfan in different brain areas at various times after intravenous administration of a single dose of endosulfan in the cat.

MATERIAL AND METHODS

Adult healthy cats purchased from the local supplier were kept in ITRC animal house one week prior to experiment for acclimatization. A total of 28 cats of either sex (body weight 2.5-4.5 kg) were separated into seven groups of four cats each and were anesthetized with ip injection of sodium pentobarbital (40-60 mg/kg). The tracheas were cannulated and the animals were ventilated with respiratory pump. One group

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served as controls. The cats of the other groups were injected a single dose of 3 mg/kg of endosulfan, dissolved in propylene glycol, through a cannula inserted into a femoral vein and were sacrificed at an interval of 15 min, 30 min, and 1, 2, 4 and 6 hr by giving air directly into the heart. One cat was daily cannulated and sacrificed after a fixed time interval.

At the end of each interval, blood was drawn from the femoral vein in a heparinized tube and plasma was separated. Liver, spinal cord, cerebral cortex, cerebellum and brain stem (medulla and pons) were also removed. All the plasma and tissue samples were immediately frozen for subsequent analysis.

The tissues were homogenized in distilled water, acidified with 5 N HCl and extracted three times each with 10 ml of hexane. The total hexane extract was extracted successively with 10, 5, 5 and 5 ml of acetonitrile. The acetonitrile phase was separated and shaken with water, the resultant one phase system was then extracted three times each with 10 ml of hexane. For extraction of plasma, 1 ml sample was mixed with 5 ml of water, acidified with 5 N HCl and extracted successively with 10, 10, 5 and 5 ml of hexane. The total hexane extract was kept together in a flask. Each hexane extract was cleaned up on a florisil column by using 30 ml of hexane in 5-10 ml aliquots to complete the transfer. Solvents were concentrated to about 5 ml, dried with anhydrous Na_2SO_4 and transferred to 10-ml volumetric flasks with hexane and made to volume.

Analysis was carried out by using a Varian Aerograph, series 2400 GC equipped with a electron capture detector. A 1.8 m x 2 mm glass column packed with 1.5% OV-17 + 1.95% OV-210 on 100/120 mesh Chromosorb W was employed. Carrier gas was nitrogen at 60 ml/min. The injector, detector and column temperatures were maintained at 200°, 200°and 180°C, respectively.

The retention times for compounds analyzed were, α-endosulfan, 3.8 min; β-endosulfan, 6.5 min; endosulfan sulfate, 11.0 min. The recorder response of elution of every compound was a single sharp peak and the height was found to be proportional to the concentration. The identity of the peak for endosulfan and endosulfan sulfate in plasma and tissue was based on its retention time and the absence of these peaks in samples of control animals. Recovery experiments with endosulfan and endosulfan sulfate added to different

TABLE 1

Distribution of endosulfan in lipids of the central nervous system, liver and in plasma of the cat

Tissue	Endosulfan concentration[a] (µg per gram tissue lipid or millilitre plasma) Period after administration of 3 mg/kg endosulfan						
	15 Min	30 Min	1 Hr	2 Hr	4 Hr	6 Hr	Percent lipid in fresh tissue
Cerebral-cortex	64.26±3.34	30.64±2.55	24.36±1.87	14.48±1.42	14.33±1.16	7.51±0.65	8.86±0.24
Cerebellum	61.92±2.93	23.97±3.54	17.57±1.65	12.03±1.17	6.46±0.59	5.79±0.46	11.26±0.32
Brain-stem	23.65±1.76	18.52±1.95	13.51±1.15	9.86±0.57	5.86±0.32	5.14±0.17	17.95±0.49
Spinal-cord	19.28±1.05	12.10±0.85	11.60±0.95	8.18±0.46	8.05±0.36	6.60±0.24	20.41±0.60
Liver	31.38±1.78	19.00±1.78	14.31±1.55	8.86±0.55	6.80±0.28	4.84±0.17	3.41±0.09
Plasma	347.80±10.55	198.40±11.64	153.20±7.44	107.60±6.86	105.60±7.54	86.60±4.83	0.50±0.01

a: Mean of 4 cats ± S.E.

TABLE 2

Endosulfan concentration per unit fresh tissue weight in the central nervous system, liver and plasma of the cat

Tissue	Endosulfan concentration[a] (/ug per gram of fresh tissue or millilitre plasma) Period after administration of 3 mg/kg endosulfan					
	15 Min	30 Min	1 Hr	2 Hr	4 Hr	6 Hr
Cerebral cortex	6.69±0.44	2.72±0.16	2.16±0.18	1.28±0.09	1.27±0.06	0.67±0.02
Cerebellum	5.97±0.58	2.70±0.21	1.98±0.11	1.36±0.14	0.73±0.06	0.65±0.05
Brain stem	4.25±0.39	3.32±0.36	2.43±0.22	1.77±0.12	1.05±0.09	0.92±0.06
Spinal cord	3.94±0.30	2.47±0.20	2.37±0.11	1.67±0.10	1.64±0.12	1.35±0.09
Liver	1.07±0.09	0.65±0.05	0.49±0.04	0.30±0.01	0.23±0.03	0.17±0.01
Plasma	1.74±0.10	0.99±0.07	0.77±0.09	0.54±0.04	0.53±0.04	0.43±0.01

a: Mean of 4 cats ± S.E.

tissues and following the procedure outlined above indicated a recovery of 95.0±9.5% and 90.0 ± 4.5% respectively. The tissue lipid was determined by using the method of FOLCH et al. (1957).

RESULTS

The principal symptoms of endosulfan poisoning were hyperexcitability, tremors and convulsions. These were more intense from 15 min to 1 hr, mild upto 2 hr and had subsided after 4 hr of endosulfan treatment.

The concentrations of endosulfan per gram of CNS lipid were about three times higher in the cerebral cortex and cerebellum than in the spinal cord and brain stem at 15 min (Table 1). Generally the concentration of endosulfan was highest in the lipids of all the brain areas at 15 min and then gradually decreased with time. Thus, two patterns of endosulfan turnover in the CNS lipids were apparent, a relatively fast uptake and release with the maximal concentration at 15 min, and a slower uptake and release with a relatively lower maximal concentration. The first pattern was characteristic of areas with a low percentage of lipid (cerebral cortex and cerebellum) and the second was particularly evident in areas with a high percentage of lipid (spinal cord and brain stem). The endosulfan levels in the liver and whole plasma lipids were also relatively high at 15 min and showed a decline pattern similar to that exhibited by different areas of brain.

When endosulfan concentrations were expressed in terms of wet tissue weight (Table 2) instead of the lipid fraction, the differences between various CNS areas appeared to be much less. For example, at 15 min the endosulfan expressed as concentration in the cerebral cortex lipids was more than three times greater than in the spinal cord lipids, whereas endosulfan per unit fresh tissue was only 30% greater in the cerebral cortex than in the spinal cord.

Also, the pattern of distribution appeared different depending on how concentrations were expressed. Thus, endosulfan concentration, per unit cerebellum lipid was more than three times the concentration in the spinal cord lipids at 15 min and then gradually decreased in both the tissues but after two hours the concentrations were nearly equal because of the decline in cerebellum concentration of the compound (Table 1). On the other hand, the endosulfan

TABLE 3

Concentration of endosulfan sulfate per unit fresh tissue weight in the central nervous system, liver and plasma of the cat

Tissue	Concentration[a] of endosulfan sulfate (µg per gram of fresh tissue or millilitre plasma) Period after administration of 3 mg/kg endosulfan					
	15 Min	30 Min	1 Hr	2 Hr	4 Hr	6 Hr
Cerebral cortex	0.21±0.04	0.74±0.12	1.06±0.09	0.75±0.08	0.70±0.05	0.56±0.04
Cerebellum	0.15±0.03	0.58±0.09	0.82±0.06	0.77±0.05	0.35±0.04	0.32±0.04
Brain stem	0.25±0.07	0.35±0.04	0.55±0.05	0.49±0.02	0.48±0.05	0.35±0.02
Spinal cord	0.32±0.06	0.51±0.07	0.71±0.07	0.56±0.05	0.37±0.02	0.37±0.05
Liver	4.04±0.22	3.04±0.18	1.58±0.12	1.51±0.10	0.55±0.02	0.45±0.09
Plasma	-	-	0.17±0.02	0.21±0.02	0.16±0.01	0.15±0.01

a: Mean of 4 cats ± S.E.

concentration in cerebellum was about double than the spinal cord at 15 min on per unit fresh weight basis. At 30 min interval both exhibited similar values and at all the time intervals studied cerebellum showed a lower value than spinal cord (Table 2).

Among all the time intervals studied the concentration of the metabolite endosulfan sulfate was highest in the liver at 15 min (Table 3) but in the CNS the pattern was different. The concentration was gradually increased upto 1 hr and then subsequently decreased. In liver the concentration of the metabolite is about four times than the parent compound suggesting that the tissue possesses high activity for metabolizing the compound.

DISCUSSION

The present results emphasize that after intravenous administration, endosulfan distributes differentially in the CNS and that this pattern changes with time. Differences in the concentration of endosulfan in the lipids of brain areas and spinal cord result from a rapid uptake and release with a high maximum concentration in areas with a low percentage of lipid i.e. in gray matter and a slower uptake and release with a lower maximum concentration in areas with a high percentage of lipid i.e. in white matter.

The pattern of distribution in fresh tissue shows that, although endosulfan concentration is higher in gray matter initially, it also disappears more rapidly from gray matter than from areas with a high myelin content (compare cerebral cortex and spinal cord Table 2). Thus six hours after administration, endosulfan fresh tissues concentrations are higher in the spinal cord and brain stem than in the cerebral cortex and cerebellum. Since the blood flow is 5-10 times higher in gray matter than in white matter (LANDAU et al. 1955), therefore, the equilibrium between blood and white matter will be slower than between blood and gray matter, hence the slow uptake and release of endosulfan in areas with a high percentage of lipid. The lower maximal endosulfan concentration in white than in gray matter results from the fact that plasma levels fall before equilibration is complete in white matter. It is also possible that endosulfan is poorly soluble in myelin, and hence reaches a low maximal concentration therein.

The rate of formation of endosulfan sulfate appearsto be maximum in the first 30 min (Table 2) and a parallelism existed between decrease in endosulfan

concentration and increase in the concentration of endosulfan sulfate. However, this relationship is not quantitative because of the formation of other metabolites of the compound. Studies of the identification of all the metabolites of the compound are in progress.

The convulsions produced by the dose of endosulfan used were generally more intense between 15 min and 1 hr than at other time intervals suggest that the early symptoms of intoxication primarily reflect the combined effect of the compound and its metabolite in gray matter so appeared with the peak concentration of both the compounds in the cerebral cortex and cerebellum. In the later stages, effects on CNS areas with a high myelin content may predominate.

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