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Seizure threshold and hexafluorodiethyl ether in brain tissue*

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DURING studies[1] of the relationship of amino acids and cerebral excitability, it became necessary to measure the content in brain of the volatile convulsant agent, hexafluorodiethyl ether. This material produces convulsions in low concentration so that a sensitive method was important. A literature survey of procedures for analysis of volatile substances in tissue revealed most to be elaborate, time-consuming, requiring special equipment[2–4] and chiefly useful for the analysis of respiratory gases.[5] This report presents a simple quantitative method for the measurement of this volatile compound in brain tissue.

It has been reported previously[1,6] that successive convulsions produced by exposure to hexafluorodiethyl ether reduce the seizure threshold on subsequent determinations. Hypoxic brain damage during the convulsion or enhanced penetration of the convulsant agent into brain have been discussed as explanations for this phenomenon. To clarify this problem, we have measured brain concentration of hexafluorodiethyl ether in animals subjected to prior convulsions.

The chemicals used were *N*-hexane (Fisher Chemicals), b.p. 68°, and hexafluorodiethyl ether (Ohio Chemical Co.), b.p. 63°.

Animals. Male albino rats, 100–150 g (Camm Research, Inc.) were exposed to the convulsant agent in an airtight chamber of 10·8 l. in volume. The hexafluorodiethyl ether was infused into the chamber at a constant rate of 40 μl/min and volatilized from the filter paper.[1]

Analytical procedure. Gas chromatography was performed with a Beckman GC-5 instrument with an electron capture detector. The column was stainless steel, 6 ft in length and $\frac{1}{8}$ in. dia., packed with 3% SE-30 on chromosorb P. The gas flow was helium with 10 ml/min as carrier and 60 ml/min discharged to the detector. The temperatures used were: flash vaporization inlet, 130°; column, 55°; detector line, 160°; and detector 200°. Polarizing voltage and CO_2 were adjusted to peak background current. Attenuation was sufficient to obtain a peak height between 10 and 50 per cent of full-scale deflection. At the end of the exposure period to the convulsant agent noted in each experiment and as rapidly as possible, the animal was removed from the chamber, decapitated and the head was immediately placed in liquid nitrogen for 10–15 sec. The calvarium was opened and the chilled whole brain (cerebral hemispheres, cerebellum and brain stem immediately caudal to the cerebellum) was immediately removed and placed in a precooled 10-ml test tube and sealed with a soft rubber cap.† The test tube with contents was immersed in a water bath at 80° for 3 min. The tube was then transferred to an ice bath and, after 10 min, 500 μl hexane was injected through the stopper by means of a Hamilton syringe. The stopper was removed and 1-μl samples of hexane solution were withdrawn by syringe for gas chromatography.

As a control for these manipulations, varying concentrations of hexafluorodiethyl ether to produce a final dilution from 15·5 to 77·5 $\mu\mu$M were introduced into the stoppered test tubes in 50 μl of hexane. These samples were carried through all the steps outlined above and were diluted to a final vol. of 500 μl with hexane. The samples were analyzed by gas chromatography and the results were compared with standard solutions of known concentrations of hexafluorodiethyl ether dissolved in hexane employed as the reference standard. When the experimental brain tissues were analyzed, reference standards of hexafluorodiethyl ether of equivalent concentrations were analyzed before and after each series of tissue samples.

EXPERIMENTS AND RESULTS

A representative relation of peak height to concentration of hexafluorodiethyl ether in hexane is shown in Fig. 1. Virtually identical results were obtained in the presence or absence of brain tissue.

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† Becton-Dickinson and Co.; Vacutainer No. 4710.

Peak height and concentration are linearly related for a sufficiently wide range (approximately 15·5 to 77·5 $\mu\mu$M) to permit ready analyses. The quantity of hexane used for final dilution may be varied to fit this concentration range. Concentrations below 15 $\mu\mu$M were measured by using a smaller volume of hexane. Recovery of hexafluorodiethyl ether from the brain of normal male albino rats

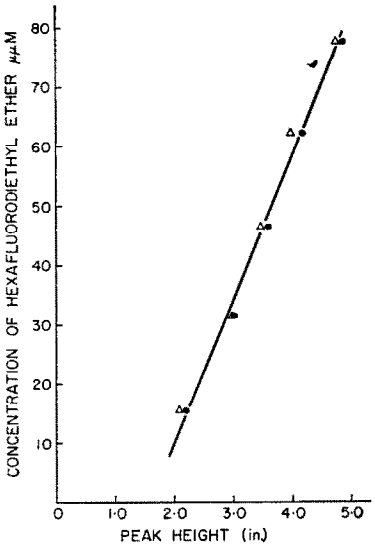

FIG. 1. Peak height as a function of concentration for hexafluorodiethyl ether, directly dissolved in hexane or volatilized prior to addition of hexane. △, Direct dilution; ●, Vaporized.

and similar animals with three previous convulsions is related to time of exposure to the convulsant agent in Table 1. The rate of penetration was similar for both groups; variation between animals was small and the amount of convulsant agent per brain was consistent with time of exposure.

TABLE 1. BRAIN CONCENTRATION OF HEXAFLUORODIETHYL ETHER AFTER INHALATION

Exposure time (sec)	Concentration ($\mu\mu$M/g wet wt.)*	
	Control	3 Previous exposures
100	9·9 ± 1·0	9·6 ± 1·1
150	16·4 ± 1·7	17·0 ± 0·5
200	37·6 ± 1·4	36·9 ± 1·5

* Values are given ± S.E.M. N = 5 for each group.

In order to investigate the effect of previous convulsions on penetration of hexafluorodiethy ether into brain tissue, 32 male albino rats were divided into four equal groups. Group I had no convulsions, group II was convulsed once, group III twice and group IV, three times prior to exposure of all animals to the convulsant agent for a fixed period of time and determination of total brain hexafluorodiethyl ether. The convulsions were produced at 3-day intervals in each group and the time to the first appearance of a myoclonic jerk was recorded (Table 2). It is apparent that the seizure

threshold is inversely related to the number of previous convulsions induced. Three days after group IV was convulsed for the last time, all of the animals were exposed singly to the convulsant agent for 200 sec and the brain concentration of hexafluorodiethyl ether was determined (Table 2). It is apparent that no significant difference exists in penetration of the convulsant agent into brain tissue among any of the groups.

TABLE 2. SEIZURE THRESHOLD AND BRAIN PENETRATION OF HEXAFLUORODIETHYL ETHER IN RELATION TO PREVIOUS CONVULSIONS*

Group	No. of previous convulsions	Seizure threshold (sec)	Hexafluorodiethyl ether ($\mu\mu$M/g wet wt.)
I	0		39·9 ± 1·5
II	1	225 ± 8	39·5 ± 1·3
III	2	199 ± 9	27·0 ± 1·0
IV	3	190 ± 7	36·9 ± 1·5

* All values are given ± S.E.M.

DISCUSSION

The procedure described has several advantages. Time and manipulations are minimal and the partially defatted tissue can be used for further analyses if desired. Gas chromatographic analysis is versatile in that solvents, column packing, temperature and type of detector can be varied to accommodate a variety of similar substances. Application of the procedure to similar agents of high volatility, soluble in organic solvents, should be quite feasible.

With our technique of administering the hexafluorodiethyl ether, the brain concentration approximately doubles between 150 sec of exposure and 200 sec of exposure. This would be equivalent to a rate of 1·8 $\mu\mu$M per 10 sec of exposure for the animals with different seizure thresholds related to previous convulsions. Since the animals with three previous convulsions had a seizure threshold that was 35 sec lower than those animals with one previous convulsion, it is reasonable to assume that the animals with the lower seizure threshold should have had a brain concentration of hexafluorodiethyl ether approximately 6·3 $\mu\mu$M greater than those with the higher seizure threshold, if the difference in threshold were related to penetration of the convulsant agent into brain. No such difference was obtained and it is concluded that the variation in seizure threshold is related to altered cerebral excitability. It appears most reasonable to presume that this altered cerebral excitability is related to another factor such as hypoxic brain damage, although no direct support of this mechanism is offered by these results.

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