TISSUE LEVELS OF TRICHLOROETHYLENE AFTER ACUTE OR CHRONIC EXPOSURE

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TRICHLOROETHYLENE was used as an anesthetic agent as early as 1911 in Germany, and through a long history of clinical trial and error was in rather common use by the end of World War II (1). As an anesthetic agent it has the obvious advantages of noninflammability, cheapness and effectiveness in small doses. It has also proved effective in obstetrical analgesia and in the treatment of trigeminal neuralgia (1). Trichloroethylene is used extensively in industrial processes as a metal degreaser, in oil, fat and resin extractions, as a solvent for many organic materials and as an insecticide and dry cleaner. Many reports of acute and chronic intoxication during industrial use of trichloroethylene have been noted (2). Most of these cases significantly do not present the syndrome of kidney and liver damage usually associated with chloroform, tetrachloroethylene and carbon tetrachloride poisoning. Trichloroethylene, thus, appears to be primarily a neurotoxic substance.

For the past few years this laboratory has carried out extensive studies concerning toxicity of trichloroethylene during acute and chronic exposure. This report deals mainly with the uptake of trichloroethylene by the tissues and organs of acutely or chronically exposed dogs. The histologic and metabolic studies will be presented shortly.

EXPERIMENTAL

Methods.—Dogs weighing between ten and twenty kilograms and in good health at the beginning of the experimental period were exposed in a gassing chamber of our own design (fig. 1). The two separate chambers were designed for full visibility so that neurological symptoms appearing in the animal during exposure could easily be observed and recorded. The air intake and volatilization equipment is shown in figure 2. With careful control of the powerstat voltage setting and rate of air flow, easily maintainable concentrations of trichloroethylene from about one hundred parts per million to saturation were obtained. Trichloroethylene concentration was measured by pulling known quantities of chamber air through absorption tubes containing trimethylpentane. The concentration of trichloroethylene in the solution was

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then measured by reading the absorption in a Beckman DU spectrophotometer at 245 mμ and comparing with a previously constructed standard curve. Samples of air removed from different sections of the chamber gave levels of trichloroethylene concentration which did not

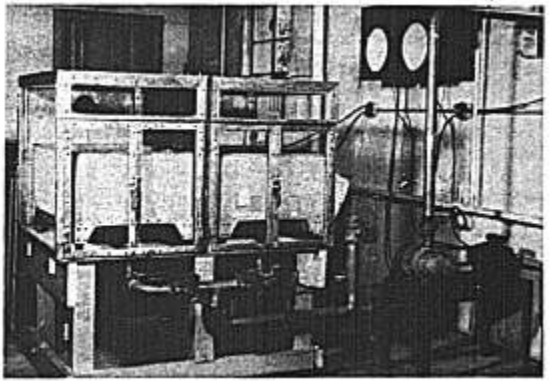

FIG. 1. Gassing chambers and auxiliary equipment—front view.

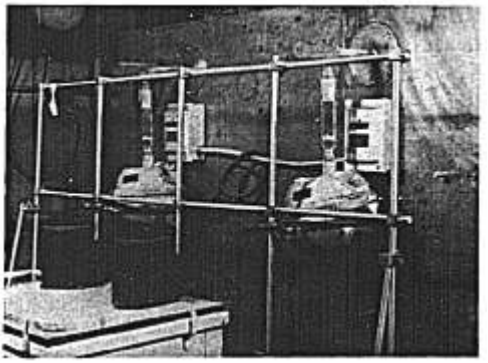

FIG. 2. Gassing chambers, rear view, showing volatilization equipment.

vary by more than ± 5 per cent, five to ten minutes after the proper rate of boiling of the trichloroethylene had been established. Chamber concentrations were measured while animals were undergoing intoxication. Room air samples were taken at weekly intervals as a check

against possible chronic intoxication of the staff. Recordable values of trichloroethylene were never obtained in room air.

At the end of the experimental period, the animal was killed by exposure to fatal levels of trichloroethylene or by intravenous or intracardiac injection of secobarbital or thiopental. Cardiac blood and cerebral spinal fluid via cisternal tap were removed for immediate extraction, and the tissues removed were stored at —85 F. until ready for processing.

The trichloroethylene concentration was determined colorimetrically after the compound was extracted from the various tissues. The extractor is a modified version of the Truhaut (3) and Fabre (4) unit. It has been modified in this laboratory so that the entire unit, with the exception of the silica gel bottle, can be mounted on a single support, making the apparatus more compact and easier to manipulate. The air flow was regulated to permit approximately three bubbles of air per second to enter the extracting tube which is equal to approximately twenty liters of air per hour. The extractions were usually carried out for a period of two and one-half hours.

After the extraction procedure was completed the resultant pyridine solution of trichloroethylene was refrigerated while preparations were made for the colorimetric determination of trichloroethylene. The measurement was based on the modified Fujiwara alkaline pyridine color reaction (5) as described by Fabre (4) and Truhaut (3). The specimens from the two bubblers were run in duplicate, and the trichloroethylene concentration was calculated from a previously constructed standard curve.

Results

Before the experimental extractions were run, known amounts of trichloroethylene were added to a few different tissues obtained from normal dogs, and extracted as was planned for the tissues of exposed animals. In no case did the extraction procedure allow for 100 per cent recovery. Increasing the time and rate of extraction did not help and was not practical. For this reason and also since different tissues gave different recoveries, all tissues to be studied were obtained from normal unexposed animals and subjected to extraction after addition of known quantities of trichloroethylene. The amount of trichloroethylene to be added to each tissue was determined by first extracting the tissues of an acutely exposed animal. This was done to avoid any errors in extraction due to the possible dependence of the rate of extraction on tissue concentration. From these values three ranges of addition to normal tissues were established. Tissues of experimental animals showing over 8.0 mg. per cent trichloroethylene (uncorrected) received 182 μg. of trichloroethylene per 2 Gm. of tissue (wet weight), when the normal tissue was used for control

TABLE 1

EXTRACTION OF TRICHLOROETHYLENE FROM TISSUE TO WHICH KNOWN QUANTITIES OF TRICHLOROETHYLENE WERE ADDED

Tissue	Per Cent Recovery of Trichloroethylene*
Adrenal	85.0±0.4 (5)
Blood	86.8±0.0 (5)
Brain	92.7±2.0 (5)
Fat	74.9±2.2 (4)
Heart	88.8±3.0 (4)
Kidney	79.2±1.5 (9)
Liver	81.5±1.1 (8)
Lung	75.8±0.8 (4)
Muscle	66.5±0.7 (5)
Pancreas	71.6±1.0 (5)
Spinal cord	79.2±0.2 (5)
Spinal fluid (human)	65.9±1.7 (4)
Spleen	84.7±1.2 (7)
Thyroid	78.8±0.5 (4)

* Recovery ± standard error.

TABLE 2

SUMMARY OF EXPERIMENTAL PROCEDURES

Dog Number and Type of Exposure	Number of Exposures	Total Time in Chamber	Trichloroethylene Concentration (p.p.m.)	Termination
12 Acute	1	25 minutes	20,000*	Died in chamber.
15 Acute	1	25 minutes	20,680	Died 10 minutes after removal from chamber. Killed with intracardiac thiopental.
16 Acute	1	29 minutes	19,380	Died in chamber.
17 Acute	1	25 minutes	12,390	Died 10 minutes after removal from chamber. Killed with intracardiac thiopental.
20 Acute	1	35 minutes	15,000	Died in chamber.
25 Acute	3 (daily)	68 minutes	20,000+†	Exposed acutely on 3 successive days—killed in chamber last day.
14 Chronic-Acute	141	99.3 hours	7,000	Died in chamber from acute exposure of 29,710 p.p.m.
21 Chronic-Acute	158	48.5 hours	7,000	Last exposure at approx. 20,000 p.p.m.* for 35 minutes. Killed immediately after exposure with intravenous secobarbital.
19 Chronic	150	89.6 hours	7,000	Expired in kennel 2 hours after last exposure. Examined immediately after death.
22 Chronic	205	126.4 hours	7,000	Killed 24 hours after last exposure—intravenous secobarbital.
24 Chronic	289	219.1 hours	7,000	Killed 24 hours after last exposure—intravenous secobarbital.

* Approximate value based on powerstat setting and air flow through chamber.
† Probably close to saturation point.

TABLE 3

TRICHLOROETHYLENE RECOVERED FROM TISSUE

(Corrected—in milligrams per cent wet weight)

Animal Number	Mode of Exposure	Adrenal	Blood	Brain	Fat	Heart	Kidney	Liver
12	Acute	22.4	72.5	17.0	17.9	8.6	1.6	27.0
15	Acute	6.24	46.0	15.1	14.7	5.0	8.2	9.6
16	Acute	—	52.7	19.7	—	5.4	5.8	38.8
17	Acute	—	22.3	—	4.8	4.2	3.6	10.8
20	Acute	22.5	28.4	8.2	70.4	18.9	3.2	9.2
25	Acute ×3	13.8	50.0	20.9	70.5	13.9	17.5	49.4
14	Chronic-Acute	60.6	46.1	—	—	7.5	21.1	20.6
21	Chronic-Acute	23.1	50.6	23.6	22.1	12.9	5.3	9.7
19	Chronic	—	9.6	2.7	30.7	1.2	1.0	3.2
22	Chronic	0.94	0.13	0.22	14.4	0.11	0.13	0.12
24	Chronic	1.06	0.25	0.22	6.5	0.11	0.25	0.25

Animal Number	Mode of Exposure	Lung	Muscle	Pancreas	Spinal Cord	Cerebro Spinal Fluid	Spleen	Thyroid
12	Acute	2.8	2.7	—	8.8	—	0.71	—
15	Acute	2.2	—	3.2	—	3.8	3.9	2.0
16	Acute	0.92	0.15	9.8	—	1.5	1.2	6.6
17	Acute	0.92	3.3	6.4	—	0.61	5.4	—
20	Acute	0.40	5.1	14.1	—	1.7	1.3	3.9
25	Acute ×3	10.4	9.3	43.8	28.3	—	5.1	14.1
14	Chronic-Acute	2.0	—	8.1	—	0.15	—	5.8
21	Chronic-Acute	1.3	3.8	16.0	—	1.8	8.5	7.4
19	Chronic	0.53	4.1	2.5	—	0.15	0.71	1.1
22	Chronic	0.26	0.45	<0.05	0.13	0.15	<0.05	<0.05
24	Chronic	0.13	0.30	0.28	0.13	0.15	0.12	0.63

extraction. Material in the 4–8 mg. per cent range received 72.8 μg., and all tissues showing less than 4 mg. per cent in the exposed animal received 36.4 μg. of trichloroethylene. Table 1 gives the per cent extraction for all tissues studied.

The initial phase of the study, at the time of writing, has been carried out with 11 dogs. The type of exposure, total time in the chamber, trichloroethylene concentration and method of killing for each animal are given in table 2. The corrected values of the amount of trichloroethylene extracted from the tissues of the experimental animals are presented in table 3. Correction was made using the arithmetic mean of the control extractions. Deviation from the mean can be calculated using the standard error shown with each control value.

DISCUSSION

One of the most interesting aspects of these experiments is the observation that the two and one-half hour hot air extraction does not allow complete recovery of trichloroethylene. The range of recovery

in the control extractions was found to vary from 92.7 ± 2.0 per cent in brain tissue to 65.9 ± 1.7 per cent in spinal fluid. Truhaut (3) stated that he was able to recover a minimum of 94 per cent of the trichloroethylene added to blood, but he does not indicate any attempt to run control studies on other tissues. Extraction techniques employed in this investigation are similar except for the use, by Truhaut, of physiological saline rather than distilled water. In his blood extractions Truhaut added sodium fluoride apparently to inhibit blood coagulation. Many extractions run with and without sodium fluoride in this study showed this compound to be without effect on the extractable quantities. Truhaut also used octyl alcohol or a 10 per cent suspension of calcium stearate in vaseline as an antifoam agent.

There are at least two possible reasons for the inability to recover all trichloroethylene added to tissues. Either (1) the procedure is simply not capable of removing all the material present in the time allotted, or (2) the trichloroethylene is undergoing chemical change and is either not being extracted as such, or else is removed along with the original compound, but in a form no longer capable of reacting with alkaline pyridine. To test the first hypothesis, and also to test the possibility that the octanol might be trapping some of the trichloroethylene, 182 μg. of trichloroethylene was added to 10 ml. of water containing 0.04 ml. of octanol. In a series of experiments the material was extracted for two to four hours. Recovery varied from 99.4 to 100.7 per cent. Although this eliminates the octanol it does not preclude the possibility that fat or fat-like substances in the tissue preparations might be involved. That we are probably not dealing with such a simple situation can be seen by recalling that brain tissue with a lipid content of approximately 50 per cent (6) was easiest to extract, while spinal fluid, which is normally a fat-free ultrafiltrate of blood, showed the poorest recovery of trichloroethylene. It is more likely, therefore, that in the presence of the warm tissue extracts, some of the trichloroethylene is being transformed either to some non-extractable material or else to some substance (s) which can be extracted but does not react with alkaline pyridine.

The recovery of only 66 per cent of added trichloroethylene from human spinal fluid is rather remarkable. Since normal spinal fluid has a total average protein concentration of 28 mg. per cent (7), enzyme activity, if involved, must be very powerful. On the other hand the possibility of a nonenzymatic catalytic process cannot be eliminated. This problem is under study at the present time.

The metabolic transformations of trichloroethylene have been studied by Butler (8) and others. Butler found that two hours following a one-hour inhalation of trichloroethylene, free trichloroethanol and trichloroacetic acid were found in the plasma and trichloroethanol glucuronide in the urine of the exposed dogs. Fabre and Truhaut (9) have observed the formation of trichloroacetic acid by tissue brei of

spleen, lung, kidney, liver and brain. The tissues were incubated for one-half to eight hours at 37 C. with 220 μg. of trichloroethylene per milliliter of brei. These metabolic products of trichloroethylene oxidation are not detected by our procedure.

The time relationship between the injection or inhalation of trichloroethylene and the initial appearance of metabolites in the tissues has not been investigated. However, in a study of the absorption and metabolic fate of chloral hydrate and trichloroethanol in the dog, Marshall and Owens (10) found a plasma trichloroacetic acid level of 0.7 mg. per cent five minutes after the oral administration of a 15.9 mg./kg. dose of chloral hydrate. This is of interest because of Butler's theory (8) that chloral hydrate is an intermediate in the metabolic transformation of trichloroethylene to trichloroethanol and trichloroacetic acid. In any case it is possible that some of the trichloroethylene added to the tissue suspensions was transformed into nonextractable trichloroacetic acid and trichloroethanol. Bray and co-workers (11) have studied the dechlorination by tissue extracts of organic chloro-compounds which are either aliphatic or have the chloride attached to an aliphatic side chain. Although they did not investigate trichloroethylene, they found a great liberation of chloride ions from compounds like chloral hydrate and trichloroacetamide in the presence of liver extracts. On the other hand the retention of trichloroethylene in tissues long after exposure has been reported by several workers. Fujiwara (5) detected trichloroethylene in guinea pigs killed after receiving 0.015 ml./kg. of body weight and stored in a cold room for seven weeks. Barrett, Cunningham, and Johnston (12) detected the substance in fat, muscle, and heart of a dog killed 66 hours after exposure, whereas Gasq (13) found traces in tissues of guinea pigs killed eighteen hours after exposure. In our work we find that chronically exposed animals killed twenty-four hours after the last exposure show measurable levels of trichloroethylene in almost all the tissues analyzed.

We are at present investigating this problem in the light of possible enzymatic activity during aeration of the tissues. However, it is still difficult to account for the peculiarly low recovery of trichloroethylene from spinal fluid unless one postulates the presence of a very active catalyst present in the fluid in extremely small quantities.

An examination of the data in table 3 will indicate how difficult it is to establish "average" values for the trichloroethylene levels in the tissues of dogs even under very similar conditions of treatment. Many uncontrollable factors, such as variable respiratory and circulatory rates and differences in physical constitution, make the task difficult. It was hoped that with a carefully controlled study of a few animals some information might be garnered concerning the "physiological" levels of trichloroethylene in the various tissues during narcosis and the relationship between type of exposure and the

concentration of the solvent in the various tissues of a given animal and in the same tissue or organ of different animals. An unexpected feature of the results was the very low values obtained with the lung tissue of almost all the animals. Since the lung is the organ of transfer of the solvent to the blood stream, one would expect higher concentrations than those observed. While it may be true that the rapid exchange of gas at the alveolar membrane might prevent the accumulation of large quantities of trichloroethylene, there is some evidence that the very low values found in the present study are a result of some active enzymatic mechanism. Acute animals 15 and 17 did not die in the chamber but were killed ten minutes after removal to a trichloroethylene free atmosphere. Acute animals 12 and 16 died in the chamber and were examined immediately. Inspection of the data shows practically no difference between the average concentration of trichloroethylene extracted from the lung tissue of the animals. If simple "blowoff" was involved, ten minutes of respiration in a solvent free atmosphere should have reduced the lung concentration to an insignificant (< .05 mg. per cent) quantity. Moreover, the rather high level of trichloroethylene found in the lung of dog 25 which was exposed acutely on three successive days and killed in the chamber on the third day suggests the inability of the lung to lose trichloroethylene when its enzymatic mechanism is overloaded. Again, if "blowoff" only was concerned, twenty-four hours between exposure should certainly be enough time to bring the trichloroethylene level in the lung down to a very low value.

Summary

The concentration of trichloroethylene found in various tissues and organs of dogs after acute, chronic, and chronic-acute exposure has been presented.

Known quantities of trichloroethylene are not completely recoverable when added to normal tissues and extracted in the same manner as the tissues from animals exposed to trichloroethylene. The data suggests that this is probably due to the transformation of the solvent during extraction to a nonextractable material or to some substance (s) which can be extracted but does not react with alkaline pyridine, or due to both mechanisms. The low recovery from human spinal fluid suggests the presence in this material of some very powerful enzymatic or non-enzymatic catalyst.

The low concentrations of trichloroethylene found in the lungs of all but one experimental animal suggest an active metabolic process along with normal alveolar diffusion.

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REFERENCES

1. Oestlere, G.: Trichloroethylene Anaesthesia. Edinburgh, E. & S. Livingstone, 1953.
2. Browning, E.: Toxicity of Industrial Organic Solvents. New York, Chemical Publishing Co., 1953.
3. Truhaut, R.: Toxicology of Trichloroethylene; Establishment of Method for its Determination in Atmosphere and in Biological Media, Ann. pharm. fr. 9: 175, 1951.
4. Fabre, R., and Truhaut, R.: Toxicology of Trichloroethylene; Determination in Biological Media, Brit. J. Indust. Med. 8: 275 (Oct.) 1951.
5. Fujiwara, K.: New Reaction for Detection of Chloroform, Sitzungsb. nat. ges. Rostock 6: 33, 1916.
6. Brante, G.: Studies on Lipids in Nervous System with Special Reference to Quantitative Chemical Determination and Topical Distribution, Acta physiol. scandinav. (supp. 63) 18: 1–189, i-xxviii, 1949.
7. Merrett, H. H., and Fremont-Smith, F.: Cerebrospinal Fluid. Philadelphia, W. B. Saunders Company, 1937.
8. Butler, T. C.: Metabolic Transformations of Trichloroethylene, J. Pharmacol. & Exper. Therap. 97: 84 (Sept.) 1949.
9. Fabre, R., and Truhaut, R.: Toxicology of Trichloroethylene; Results of Experimental Animal Studies, Brit. J. Indust. Med., 9: 39 (Jan.) 1952.
10. Marshall, E. K., Jr., and Owens, A. H., Jr.: Absorption, Excretion and Metabolic Fate of Chloral Hydrate and Trichloroethanol, Bull. Johns Hopkins Hosp. 95: 1 (July) 1954.
11. Bray, H. G., Thorpe, W. V., and Vallance, D. K.: Liberation of Chloride Ions from Organic Chloro Compounds by Tissue Extracts, Biochem. J. 51: 193 (June) 1952.
12. Barrett, H. M., Cunningham, J. G., and Johnston, J. H.: Study of Fate in Organism of Some Chlorinated Hydrocarbons, J. Indust. Hyg. & Toxicol. 21: 479 (Dec.) 1939.
13. Gasq, M.: A Study of the Toxicology of the Chlorinated Acetylene Solvents. Bordeaux, Delurns, 1936. (From quotations by von Oettingen, W. F.: Halogenated Hydrocarbons: Their Toxicity and Potential Dangers, J. Indust. Hyg. & Toxicol. 19: 349 (Oct.) 1937.)