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THE TOXICITY OF TRICHLORETHYLENE

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Before it came to be used for anaesthetic purposes, trichlorethylene was recognized as one of the safest fat solvents available for industrial use (M.R.C. Report 80, 1937; Hamilton, 1934), and to-day, eight years after the widespread adoption of this drug as a general anaesthetic, the literature contains only two cases of acute yellow atrophy which have been laid to its charge (Herdman, 1945; *Lancet*, 1944). Further investigation of the toxicity of trichlorethylene in the quantities used for anaesthesia has been carried out in the human subject, using the cephalin flocculation test as a measure of liver function (Armstrong, 1947). The results of this study showed that trilene produced less disturbance of liver function than the classical and relatively safe anaesthetic agent, ether. None the less, trichlorethylene is a close chemical relation of the known hepatotoxins chloroform, carbon tetrachloride, and tetrachlorethane, and there has been for long a lingering suspicion that, if enough of this agent were given, liver necrosis might ensue. In fact, in a study of the anthelmintic action of trichlorethylene and other drugs, Barsoum and Saad (1934) state that this, in common with the other agents of the same chemical group, will produce fatty degeneration of the liver when given by mouth to experimental animals. This may, of course, temporarily involve high concentrations in the portal blood and liver. It therefore seemed desirable to carry out a further investigation on the action of trichlorethylene on the liver.

Many experimental studies of the toxicity of trichlorethylene have already been reported, but most of these were directed to the discovery of the dangers of the use of the drug in industry. Accidental narcosis to the point of respiratory arrest is the commonest cause of death in industrial accidents. Several workers (Lehmann, 1911; Lehmann and Schmidt-Kehl, 1936) therefore rate the toxicity of chlorinated hydrocarbons in the order of their anaesthetic potency, and regard trichlorethylene as dangerous because it is a powerful agent in low concentrations. Other studies (Lande *et al.*, 1939) were more concerned with the chronic toxicity of the drug, but only in the amounts in which it might be inhaled by those using it in the course of their work. The only workers who carried the administration of trichlorethylene to the point of anaesthesia on a series of occasions were Krantz and his colleagues (1935). Once anaesthesia had been induced in their animals, however, they made no attempt to maintain it, nor was the concentration of the drug measured in this study. It was the purpose of the present investigation to submit animals repeatedly for as long as possible to the maximum amount of trichlorethylene vapour they would tolerate.

METHODS

For the purposes of this experiment, it was necessary to design an apparatus which would provide a vapour of constant strength for a considerable period. An ordinary Boyle's apparatus with a trilene bottle obviously would not serve. It is well known that the amount of anaesthetic vaporized by the gases in such a machine depends on the rate of flow, the force with which the gases impinge on the liquid, and the temperature of the liquid. Only the first of these variables is controlled in a Boyle's apparatus. As the liquid in the bottle evaporates, its level falls and the force with which the gases impinge on the liquid is reduced. Similarly, because the temperature of the liquid falls as evaporation occurs, the partial pressure of the liquid becomes less and a smaller amount is volatilized by the gaseous stream. It proved difficult to obtain a satisfactory thermostatically controlled heater to place in the trilene bottle, nor did it seem easy to make an adequate apparatus for main-

taining the level of the liquid in the bottle at a constant height. In the earliest experiments, therefore, a known quantity of trichlorethylene was vaporized in a large aspirator of known capacity, the contents of which could be mixed by a small fan. A measured quantity of the liquid was run into a piece of cotton-wool attached to the blades of the fan. As soon as these began to spin, the trichlorethylene was thrown by centrifugal force on to the walls of the aspirator, where it was almost immediately vaporized by the air stream. After the liquid had been vaporized, the fan was not run continuously, but was turned on for five minutes in each quarter-hour. The amount of oxygen taken up and the amount of carbon dioxide produced by 10 mice in 1 hour was insufficient to vitiate the results with an aspirator bottle of 11.5 litres capacity. Later, however, it became necessary to provide a steady flow of gas containing a known amount of trichlorethylene. For this purpose, a pump running at a constant speed with a displacement of about 16 litres a minute was used. It passed air over trichlorethylene in a 250 c.c. conical flask partially immersed in a water bath whose temperature was thermostatically controlled. After about 20 minutes' running a steady temperature gradient was established between the trichlorethylene and the water, and a vapour of constant strength was delivered. Further, only an initial calibration was necessary and thereafter the temperature of the trichlorethylene was an accurate indication of its concentration in the mixture. The rate of evaporation with this apparatus was so slow that only a negligible change in the level of the liquid occurred during one hour's use. To make it easy to obtain mixtures containing small amounts of trichlorethylene vapour, a by-pass for part of the air was added.

In order to calibrate this apparatus, it was necessary to find some accurate method of estimating small quantities of trichlorethylene in air. The Fujiwara reaction, as described by Habgood and Powell (1945), was used, but the results were found to be too readily influenced by minor variations in analytical technique. In addition, considerable difficulty was found in bringing the alkali into contact with the trichlorethylene dissolved in the supernatant toluene layer. An attempt was also made to use Waller's chloroform balance (Macintosh and Mushin, 1946), but it, too, was not sufficiently accurate for the purposes of the experiment. In the end a method originally designed by Dr. E. Falkner Hill for the estimation of chloroform was employed.

The basis of this technique is the direct measurement of the increase in weight which occurs when the air in a flask is replaced by a more dense mixture of trichlorethylene and air. The author found it necessary to make certain modifications in the original technique. The error which was due to difference in temperature between the air outside the flask and the gas within it was eliminated by passing the mixture containing trichlorethylene through a long cooling coil immersed in a large volume of water at room temperature. The calculation, too, was modified to correct for the weight of the air displaced from the flask by the trichlorethylene. The details of the method are as follows:

The special flask was weighed containing ordinary air at room temperature. Air containing trichlorethylene was then blown through it until its weight became constant. After a trial, it was found that five minutes was enough for this purpose. The flask was then weighed again and finally its capacity determined by filling it with water. The details of the calculation, which is based on elementary conceptions of specific gravity, will be found in the Appendix.

Animal Experiments

In the first experiment a batch of 10 mice was exposed for one hour to 1 per cent trichlorethylene vapour in air daily, except on Sundays, for 12 days. Three animals died during the first inhalation of the mixture, but the remainder survived except that during each of the ninth, eleventh, and twelfth exposures one mouse died. During the earlier inhalations, however, animals were sometimes removed from the vapour before the whole hour had elapsed, when they were obviously on the point of respiratory arrest. The next experiment was exactly similar except that 1.5 per cent trichlorethylene was administered and no attempt was made to rescue animals on the point of death until the whole hour had passed. With this concentration, the toxicity of the drug was even more marked and none of the animals survived the eighth inhalation.

Post-mortem examination of these animals revealed no direct evidence of the cause of their deaths, nor did it afford any explanation why those which survived one hour's exposure to trichlorethylene on one day should succumb after exactly the same treatment on the day following. It seemed fairly clear that the immediate cause of the deaths was anoxia. Thus the animals which were not going to survive became cyanotic during the inhalation and ultimately developed gasping respirations which became progressively less frequent until the animal died. A few had typical epileptiform convulsions which were also evidence of anoxia. Finally, a proportion of the animals whose breathing had ceased was resuscitated by artificial respiration. The mechanism of the production of the anoxia was fairly clear. As in the human subject, so in these mice, trichlorethylene caused tachypnoea. Because the drug augments the sensitivity of the pulmonary stretch receptors (Whitteridge and Bülbring, 1946), this increase in respiratory rate occurred at the expense of the depth of the breathing. Presently, the respiratory excursion became so small that the air drawn in probably filled little more than the dead space, and alveolar ventilation was reduced to negligible proportions, with consequent development of progressively increasing anoxaemia.

In view of the facts set out in the last paragraph, it seemed probable that mice would withstand anaesthesia with trichlorethylene very much better in oxygen than in air. To test this thesis, a batch of 10 mice was exposed for one hour as before to 1.2 per cent trichlorethylene vapour in oxygen. At the end of the first inhalation, all the animals were well, but the laboratory attendant found two of them dead in their cage half an hour later. It seemed probable that these animals had suffered asphyxia because they were suddenly removed from an atmosphere rich in oxygen while their respiration was still too shallow to maintain life when they were breathing air. At the end of each inhalation thereafter, therefore, the animals were gradually weaned over a period of about five minutes from oxygen to air. There was no further mortality until the seventh inhalation was about to begin, when two mice were found dead in the cage. A third animal died during the succeeding night. All these animals were found to be suffering from pneumonia at post mortem, and it is, of course, well known that small animals frequently exposed to high concentrations of oxygen may develop this condition. For this reason the experiment was not continued, but it was felt that a sufficient number of animals had survived a large enough dose of trichlorethylene often enough to make it clear that the drug was less toxic when given in oxygen than in air.

As has already been indicated, post-mortem examination of the animals which died during exposure to trichlorethylene revealed no immediate cause of death. Two animals were apparently suffering from subacute nephritis of relatively long standing, but the kidneys of the remainder and the livers of all the animals were healthy on naked-eye examination. Sections were made of the livers and kidneys of the animals, and, apart from the nephritis already noted on naked-eye examination, there was no evidence of tissue destruction in either of these organs which might have been the result of the action of trichlorethylene. There was some disappearance of liver glycogen in the animals which had died during anaesthesia, but in the four which survived the first series of inhalations and were sacrificed later the livers and kidneys were indistinguishable from those of healthy mice. In order to obtain further information on this matter, an additional series of six mice was exposed to 1 per cent trichlorethylene for forty minutes on twelve occasions and their livers and kidneys submitted to a pathologist for examination. Again it was reported that there were no changes which could be ascribed to the toxic action of trichlorethylene on these organs. Finally, in order to make quite certain that the animals used were relatively sensitive to hepatotoxins, a series of ten mice was exposed to 1.0 per cent chloroform on twelve successive occasions. Five died of overdose during the first inhalation, which lasted an hour. On successive days, the remainder were exposed for forty minutes. Two died, one during the third and one during the seventh inhalation, and, at post mortem, both showed the shrunken, fatty liver typical of delayed chloroform poisoning.

SUMMARY

The results of this investigation indicate that trichlorethylene is a potential poison to mice. Further, their sensitivity to the drug seems to increase with repeated exposure. Animals under the influence of trichlorethylene appear to die because the drug increases the rate of their respirations until ventilation becomes inefficient. In the doses used in this study, the agent had no toxic action on the liver or kidneys. The practical importance of the findings of this investigation to clinical anaesthesia are twofold. First, they emphasize the desirability of giving plenty of oxygen to patients who develop tachypnoea as a response to trichlorethylene. Secondly, they confirm the previously made observations that this drug has no serious toxic effects on the parenchyma of the liver and kidneys of healthy mice, even when administered repeatedly in anaesthetic concentrations for an hour.

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Appendix

The details of the calculation for the estimation of trichlorethylene by the method used in this study are:

Let W_1 be the result of the first weighing.
W_2 be the result of the second weighing.

By the principle of Archimedes,

W_1 and W_2 are the absolute weights of the flask and its contents less the weight of air displaced by the flask.

$W_2 - W_1$ = weight of gaseous mixture in the flask − weight of air which the flask could contain.

or Weight of trichlorethylene vapour in the flask + weight of air still in flask − weight of air which the flask could contain.

or Weight of the trichlorethylene vapour − the weight of the air which it has displaced from within the flask. (1)

These two quantities have the same volume,

$$\therefore \frac{\text{Wt. of air displaced by trichlorethylene}}{\text{Density of air}} = \frac{\text{Wt. of trichlorethylene}}{\text{Density of trichlorethylene}}$$

$$\text{i.e., Wt. of air displaced by trichlorethylene} = \frac{\text{Weight of trichlorethylene vapour}}{\text{Density of trichlorethylene vapour}} \times \text{Density of air}$$

Substitute this expression in (1), using symbols D_T and D_A for densities of trichlorethylene and air respectively.

$$W_2 - W_1 = \text{weight of trichlorethylene vapour in the flask} - (\text{weight of trichlorethylene in flask}) \times \frac{D_A}{D_T}$$

$$= \text{weight of trichlorethylene in flask} \left(1 - \frac{D_A}{D_T}\right)$$

$$\therefore \text{Weight of trichlorethylene in flask} = \frac{W_2 - W_1}{\left(1 - \frac{D_A}{D_T}\right)}$$

Now 1 gram molecule of any gas at 0° C. occupies 22.4 litres, approximately.

The molecular weight of trichlorethylene is 131.5.

$$\text{Volume of trichlorethylene in flask} = \frac{\text{Weight of trichlorethylene}}{131.5} \times 22.4 \text{ litres}$$

$$= \frac{22.4(W_2 - W_1)}{131.5\left(1 - \frac{D_A}{D_T}\right)} \text{ at } 0° \text{ C.}$$

If the determination is carried out at room temperature of $t°$ C., the volume obtained from the last equation will require to be corrected by multiplication by $\frac{t + 273}{273}$ (Charles's Law).