TRICHLORETHYLENE: ABSORPTION, ELIMINATION AND METABOLISM

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In a recent paper from this laboratory (Stewart and Witts, 1944) the clinical picture of chronic carbon tetrachloride intoxication was described. As a result of this experience it seemed desirable to devise quantitative methods for measurement of the degree of exposure to chloro-compounds of this type. Such methods would be of value not only in industrial medicine but also in therapeutics, as, for example, in studying the action and side effects of chloro-compounds used as anaesthetics and parasiticides. Subsequently a paper was published describing the estimation of trichlorethylene, chloroform and carbon tetrachloride in the blood in man (Habgood and Powell, 1945). The present paper, which is the third in the series, is a detailed study of the absorption and elimination of trichlorethylene. Trichlorethylene was chosen because we were able to study human subjects to whom it was given as an anaesthetic. The results have nevertheless a wider application, since trichlorethylene (Trilene, Westrosol, Crawshawpol) is used in industry in large quantities as a degreaser and a dry cleaning agent. Compared with tetrachlorethane and carbon tetrachloride, its toxicity is low (Hamilton, 1943) and some of the ill-effects which it is reported to produce in the nervous system can possibly be attributed to impurities, chiefly dichloroacetylene, in the commercial preparation (Hunter, 1944).

Although trichlorethylene was selected for study largely on the grounds of convenience and accessibility of cases, the results we have obtained have a special interest in view of the curious chemical reaction involved in the metabolism of trichlorethylene. Barrett and Johnston (1939) have found that 5-8 per cent. of the trichlorethylene absorbed by dogs during anaesthesia could be recovered from the urine as trichloroacetic acid. The urine of human subjects who had been exposed to trichlorethylene vapour also gave chemical reactions indicative of the presence of trichloroacetic acid, although the latter was not actually isolated (Barrett, Cunningham and Johnston, 1939). In the following investigation, trichlorethylene concentrations have been determined in blood during anaesthesia, and an attempt has been made to follow the elimination of trichlorethylene and its metabolite by analyses of blood, urine and expired air. We have been able to demonstrate that in man also trichlorethylene is in part metabolized to trichloroacetic acid. This phenomenon is of considerable chemical and toxicological interest. It is also probable that it could be applied as the basis of a simple urinary test for undue exposure to trichlorethylene in industry.

Methods

Blood

For the determination of trichlorethylene in blood (Habgood and Powell, 1945), the chlorhydrocarbon was first removed by steam distillation. The aqueous distillate was then extracted with toluene, and an aliquot of the toluene extract treated with pyridine and alkali. An orange-red colour was thus developed, the intensity of which was proportional to the amount of trichlorethylene present. Analyses of blood specimens, obtained at intervals after the anaesthetic was stopped, indicated the rate of elimination of trichlorethylene from the blood stream.

When blood obtained 24 hours, and later, after anaesthesia

was treated in the above manner, the steam distillate gave a faint but definite purplish-pink colour, quite different from the orange red given by trichlorethylene. Urine collected at this time gave a similar reaction. The same purplish-pink colour was obtained on heating trichloroacetic acid or chloroform with pyridine, alkali and toluene. The substance present in the blood after trichlorethylene anaesthesia could not, however, be removed by a current of air at room temperature (*v.i.*). This observation, together with the findings of Barrett, Cunningham and Johnston, suggested that it was trichloroacetic acid or some closely related substance. While an attempt was being made to isolate trichloroacetic acid or a derivative from urine (*v.i.*), the following procedure was adopted for the simultaneous determination of trichlorethylene and trichloroacetic acid in blood.

The trichlorethylene was first removed by passing a stream of air through blood diluted with 7 times its volume of water in the apparatus shown in fig. 1; 5-10 ml. of blood

FIG 1.—Apparatus for estimating trichlorethylene and trichloroacetic acid in blood.

A. Sintered glass plate. B. Blood. C. Toluene. D. Flow-meter. E. Ice water. G. Glass beads.

were used for the analysis, and a few drops of tri-*n*-butyl citrate added to prevent excessive frothing. This substance was shown not to interfere with the final colour reaction. Air was then drawn through for 30 minutes at 100 ml./minute, and for 30 minutes at 200 ml./minute, the trichlorethylene thus removed being absorbed in 2-5 ml. of toluene.

For the colorimetric analysis, 1 ml. of the toluene solution was added to 10 ml. of pyridine and 5 ml. of 20 per cent. sodium hydroxide. The mixture was then heated and the resulting colour measured as before.

For the determination of trichloroacetic acid, or trichloroacetate, 8 ml. of the diluted blood from which trichlorethylene had been removed were treated with 1 ml. of 10 per cent. sodium tungstate and 1 ml. of 2/3-*N*-sulphuric acid; 5 ml. of the protein-free filtrate were then added to a mixture of 10 ml. of pyridine, 1.2 ml. of saturated aqueous sodium hydroxide and 1 ml. of toluene. On heating at 100° C. for 5 minutes, a purplish-pink colour developed, and this was measured as before.

Trichlorethylene and trichloroacetic acid added to the same blood sample could be recovered in this way with an accuracy of ± 5 per cent.

Expired Air

Trichlorethylene in expired air was determined after absorption in toluene. In carrying out this analysis, it is essential that the absorption apparatus should have a low resistance to normal breathing. Absorption bottles with sintered glass discs were found to be too resistant, so bottles were made with inlet tubes terminating in perforated flattened bulbs. It was found that 92 per cent. absorption of trichlorethylene from air containing up to 3.5 mg./litre could be effected by passage through three absorption bottles of the above type connected in parallel, at a total rate of 1 litre/minute. The patient breathed into a mask which was fitted with an inspiratory and expiratory valve; from the latter a breathing tube led to the absorption train. A side branch in this tube, which permitted the greater part of the expired gases to escape into the atmosphere, was controlled by a screw clip. The latter was adjusted so that roughly one-eighth of the expired air passed through the bottles, each of which contained 30 ml. of toluene. The tube from mask to bottles was of polythene, which does not absorb chlorohydrocarbons as readily as does rubber. After passing through the absorbers, the air was collected in a Haldane bag and its volume subsequently measured. The volume of air passing through the absorbers varied between 15 and 40 litres—depending on the amount of trichlorethylene expected.

Urine

No attempt was made to determine trichlorethylene in urine—the amount excreted by this route is probably very small and would require troublesome precautions for accurate measurement. For the determination of trichloroacetic acid in urine, it was generally convenient to add 0.5 or 1 ml. of urine to 10 ml. of pyridine, 5 ml. of 20 per cent. sodium hydroxide and 1 ml. of toluene. When the trichloroacetic acid concentration is very low, i.e. less than 2 mg./100 ml., 5 ml. of urine can be used for the estimation, together with 10 ml. of pyridine, 1.2 ml. of saturated aqueous sodium hydroxide and 1 ml. of toluene. The colour interference produced by urinary pigments is negligible when 1 ml. of urine is taken for the analysis, and very slight with 5 ml. of urine.

Proof of the Presence of Trichloroacetic Acid in Urine after Trichlorethylene Anaesthesia

Eight litres of urine, collected from three patients during the period 24–72 hours after anaesthesia with trichlorethylene, were made strongly acid with hydrochloric acid, and extracted four times with 1 litre of ether. After evaporating to a bulk of 100 ml., the highly pigmented ether extract was boiled with charcoal to remove the colour. At this stage a 20 per cent. loss of trichloroacetic acid occurred. This was shown by adding a small quantity of ether extract before and after charcoal treatment to a measured volume of water, the ether being blown off and an aliquot of the residue taken for analysis with pyridine and alkali. To half of the colourless ether solution of trichloroacetic acid the calculated amount of piperazine was added. After stirring thoroughly and adding a large excess of ether, the white precipitate formed was filtered off, washed with ether and crystallized from glacial acetic acid. The product thus obtained melted sharply at 121.0° C., and no depression of melting-point was observed after mixing with an authentic specimen of the piperazinium salt of trichloroacetic acid.

The remaining half of the ethereal solution was extracted with the calculated amount of dilute sodium hydroxide necessary to form the sodium salt of trichloroacetic acid. The alkali extract was then evaporated to dryness. The resulting white solid considered to be mainly sodium trichloroacetate was heated with aniline and sodium hydroxide, when the characteristic carbonylamine smell was detected. This was proof of the presence of chloroform formed by decomposition of the trichloroacetate.

Results

Trichlorethylene concentrations in venous blood have been determined in 12 patients during anaesthesia, blood samples being collected after the patient had received 1.5–2.5 volumes per cent. trichlorethylene from an Oxford vaporizer for at least half an hour. The concentrations found varied between 6.5 and 12.5 mg./100 ml. Arterial and venous blood specimens obtained at the same time from a patient who had received an

average of 2 per cent. trichlorethylene for an hour contained 9.8 and 9.9 mg./100 ml. respectively. In five cases, blood samples were collected during a short period of deep anaesthesia associated with rapid shallow breathing, and here the trichlorethylene concentrations were in the region of 10.0–12.5 mg./100 ml. In another patient with slight hyperpnoea, from whom both arterial and venous blood specimens were obtained, it is of interest to note that, although the level in venous blood was only 6.5 mg./100 ml., the arterial concentration was 10.8 mg./100 ml.

Results obtained by analysis of blood, urine and expired air from two patients are given in fig. 2. Less

Patient I.—2.5–3 per cent. trichlorethylene for 1 hour 20 minutes.

FIG. 2.

Patient II.—1.5–2 per cent. trichlorethylene for 20 minutes.

- A. Trichlorethylene concentrations in blood (mg/100 ml.).
- B. Trichlorethylene concentrations in expired air (mg./litre).
- C. Trichloroacetic acid concentrations in blood (mg./100 ml.).
- D. Trichloroacetic acid in urine. Daily excretion (mg.).

complete data from two other cases, in which urine collection was not satisfactory, are summarized in Table 1.

It was found that trichlorethylene disappeared very rapidly from the blood, the concentration falling below 1.0 mg. per cent. in 3 hours and to 0.1 mg. per cent. in 24 hours. The amount of trichlorethylene in the expired air showed the same steep decline. Meanwhile a non-volatile substance giving a pink colour with pyridine and alkali, similar to that given by trichloroacetic acid, appeared in blood and urine. The concentration of this compound, which has been expressed in terms of trichloroacetic acid (*v.i.*), reaches a maximum in about two days and seems to depend roughly on the amount of trichlorethylene inhaled. Thus, in the first patient, where 2.5–3 per cent. trichlorethylene was administered for nearly 1½ hours, a maximum concentration of 8.6 mg./100 ml. was reached, and in spite of a high urinary excretion this level was maintained for four days, after which it slowly decreased. In three other cases where considerably less trichlorethylene was given, maximum concentrations of trichloroacetic acid were lower, in the region of 5–6 mg./100 ml. Here again the trichloroacetic acid concentration decreased very slowly, being still rather more than 50 per cent. of the maximum concentration six days after this was attained.

TABLE 1
BLOOD TRICHLOROETHYLENE AND TRICHLOROACETIC ACID CONCENTRATIONS AFTER ANAESTHESIA

		Trichlorethylene (mg./100 ml. blood)	Trichloroacetic acid (mg./100 ml. blood)
Patient III 4 per cent. for 25 mins.	End of anaesthesia	8.1	—
	Expired air 2.8 mg./litre.		
	5 hours	0.27	0.3
	24 "	0.1	4.5
	Expired air 0.035 mg./litre.		
	4 days	—	3.0
	6 "	—	2.5
Patient IV 1–1½ per cent for 1 hour	8 "	—	2.1
	12 "	—	2.0
	End of anaesthesia	8.1	0.3
	3 hours	0.7	0.5
	6 "	0.3	0.9
	24 "	0.1	4.8
	2 days	—	5.5
	5 "	—	5.0
	8 "	—	3.5

The daily excretion of trichloroacetic acid in the urine ran roughly parallel with the blood concentration. In patient I, the average excretion during the first six days was 500 mg. This had fallen to 200 mg. on the eighth day and to 100 mg. on the tenth day. The total amount excreted during the 10-day period was 3.7 g. In the other three patients, who received much less trichlorethylene, the amount of trichloroacetic acid excreted was correspondingly smaller. In patient II, the total amount in the urine during the 7-day period following anaesthesia was only 0.5 g. In patients III and IV, the total amount excreted during the same period was approximately 1 g.

Discussion

Van Dessel (1923) has found the concentration of chloroform in the blood of human subjects during anaesthesia to vary between 15 and 25 mg./100 ml. Although the anaesthetic potency of chloroform and trichlorethylene, as derived from the atmospheric concentrations causing deep narcosis in animals in 30 minutes (Haggard and Henderson, 1943), is found to be about

equal, the anaesthetic concentrations obtained here for trichlorethylene are markedly lower than those given for chloroform. The above definition of anaesthetic potency does not, however, take into consideration the relative solubilities of chloroform and trichlorethylene in blood, which is obviously of great relevance in this connexion. It is interesting to note that the rapid shallow breathing observed in several of the patients during anaesthesia was associated with a blood trichlorethylene concentration of about 10 mg./100 ml. With regard to the elimination of trichlorethylene from the body, it is evident from the analyses of expired air that trichlorethylene, as such, virtually ceases to be excreted via the lungs in 48 hours. The rate of disappearance from the blood stream is comparable with that found for chloroform (Nicloux, 1906), falling to about 10 per cent. of the initial value in 3 hours, and to 5 per cent. in 6 hours.

It has also been shown here that a non-volatile organic chloro-compound is present in blood and urine for relatively long periods after anaesthesia. This compound gives a colour with pyridine and alkali indistinguishable from that given by trichloroacetic acid. Taking into consideration the isolation of a derivative of trichloroacetic acid from urine, and of a sodium salt which readily gives a carbylamine reaction with aniline and sodium hydroxide, it seems that trichloroacetic acid does appear in blood and urine as a product of metabolism of trichlorethylene. At the pH of blood, trichloroacetic acid would obviously exist as a salt. It would be rather difficult to prove that trichloroacetic acid is the only metabolic product, although this appears to be likely. The chloro-compound in urine cannot be extracted with ether from an alkaline solution, but only if the urine is made acid, which indicates that it is essentially completely acidic in character. It seems very unlikely that a mixture of chlorinated organic acids is present, since a pure specimen of the piperazinium salt of trichloroacetic acid was obtained from the ether extract of urine without difficulty.

In all the cases studied, the trichloroacetic concentration in blood increases to a maximum about 48 hours after anaesthesia, and then decreases very slowly. In patient I, the maximum concentration is maintained for several days. It seems probable that trichlorethylene is not metabolized in the blood stream, and to explain the relatively slow appearance of trichloroacetic acid in the circulation it is tentatively suggested that the level is built up by diffusion from some organ where fixation and metabolism of trichlorethylene has taken place.

It is possible that routine blood and urine tests for trichloroacetic acid might be used to control harmful exposure to trichlorethylene in industry. The excretion of trichloroacetic acid is very slow, and a cumulative effect might be produced by small but frequent exposures to trichlorethylene vapour. There are no experimental data on this point, but such data could easily be obtained. At the same time, it must be remembered that a similar colour reaction is given by chloroform and chloral, which may have been taken medicinally.

With regard to the toxicity of salts of trichloroacetic acid, it is interesting to note that Liebreich (1869) injected sodium trichloracetate intravenously into human subjects, with production of slight anaesthesia. We have found that intravenous injection of 400 mg. of sodium trichloracetate produced a slight decrease of blood pressure in a cat previously anaesthetized with phenobarbitone. A blood sample taken 20 minutes after the injection contained 47 mg. of trichloracetate per 100 ml. Injection of 320 mg. of sodium trichloracetate into an anaesthetized rabbit also produced little effect, the blood concentration being 33 mg./100 ml. 15 minutes after the injection. This would suggest that the toxicity of trichloracetate at the concentrations found in the blood after trichlorethylene anaesthesia is negligible.

Smaller doses have proved equally harmless in man. We have made several observations on the rate of excretion of sodium trichloracetate by human subjects after intravenous injection of 2–3 g. of the salt. Blood samples were collected at intervals over a period of 7–10 days after the injection. The total urinary output

was also collected and analysed over the same period. It was found that in the first 36 hours after the injection the blood concentration had fallen to 50 per cent. of the initial value. This fall was partly accounted for by diffusion into the tissues, since only 15–20 per cent. of the total trichloracetate injected had appeared in the urine during this period. After this, the blood concentration decreased very slowly, at a rate comparable with that found in the four patients after trichlorethylene anaesthesia. Seven days after the trichloracetate was injected, blood concentrations of 2–4 mg./100 ml. were still found.

The mechanism of conversion of trichlorethylene into trichloroacetic acid, with the possibility of toxic intermediary compounds, remains a matter for conjecture. The conversion implies either a migration of chlorine from one carbon atom to the other, or an addition of hydrochloric acid to the trichlorethylene molecule. It is interesting to note that chloral may be produced from trichlorethylene *in vitro* via the unstable trichlorethylene oxide CCl_2CHCl (British patent 523,555; 1940),

and trichloroacetic acid might be formed in the same way. It would be remarkable if this channel of metabolism could be followed with so little evidence of ill-effect. It is true that a number of cases has recently been reported in which cranial nerve palsies and encephalitis have followed the use of trichlorethylene as an anaesthetic, but these all appear to have occurred under circumstances in which dichloroacetylene might have been expected to be produced through passage of the trichlorethylene through the soda lime used for absorption of carbon dioxide (McAuley, 1943; Carden, 1944; Humphrey and McClelland, 1944). There is no evidence that the trichloroacetate produced in the metabolism of trichlorethylene or its precursors are toxic, but the possibility seems worthy of further exploration.

Summary

Trichlorethylene concentrations in the blood of 12 patients during anaesthesia ranged between 6.5 and 12.5 mg./100 ml. Part of the trichlorethylene absorbed is converted into trichloroacetic acid, and derivatives of this compound have been prepared from urine.

The elimination of trichlorethylene and its metabolite from the body has been studied by analysis of blood, urine and expired air in four patients after anaesthesia.

Intravenous injections of sodium trichloroacetate into animals and human subjects have produced no ill-effects; this salt is excreted rather slowly after intravenous injection.

The possible relation of these findings to the toxic hazards of trichlorethylene and the detection of undue exposure in industry are briefly discussed.

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