ESTIMATION OF CHLOROFORM, CARBON TETRACHLORIDE AND TRICHLORETHYLENE IN BLOOD

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There are a number of references in the literature to the determination of organic chloro-compounds in blood and tissues. Nicloux (1906) measured chloroform concentrations in dog's blood during and after anaesthesia by a method which involved distillation with alcohol, hydrolysis of the distillate with alcoholic potash, and determination of the resulting chloride by the Volhard method. Wells (1925) estimated carbon tetrachloride in blood and tissues in the same way, after administration of the compound by mouth. This author reports that a loss of 7–10 per cent. occurs during the preliminary distillation, and that the determination of quantities of carbon tetrachloride less than 1 mg. is very inaccurate. Schtschigol (1933) has found that the above method also gives low results for chloroform. A combustion method for the estimation of carbon tetrachloride in blood has been used by Robbins (1929), but here again the technique is rather laborious and the quantities determined relatively large, i.e. of the order of 1 mg.

The reaction of certain organic halogen compounds with pyridine and alkali to give a coloured complex was first observed by Fujiwara (1914). Cole (1926) and McCollum (1930) used this reaction to determine small amounts of chloroform in aqueous extracts of tissues, but found some difficulty owing to the instability of the coloured complex. Daroga and Pollard (1941), investigating the reaction of chloroform and carbon tetrachloride with pyridine and alkali, have observed that the diluting solvent, the relative concentrations of reagents, and the duration of heating, all influence the final colour. For optimum and reproducible colour development these authors use a reaction mixture containing 10 ml. of pyridine, 5 ml. of 20 per cent. NaOH and 1 ml. of an acetone solution of the chlorhydrocarbon. After heating at 100° C. for five minutes and then cooling thoroughly, the pyridine layer is separated off. Its colour is proportional to the amount of chloro-compound present and is stable for 17–24 hours at room temperature. Quantities of chloroform and carbon tetrachloride as small as 0.02 mg. give a measurable colour under the above conditions.

For the estimation of chloroform, carbon tetrachloride and trichlorethylene in blood, we have applied a similar technique to that used by Daroga and Pollard for the estimation of the first two of these compounds in soil. In the original method, the chlorhydrocarbon is first removed by steam distillation into acetone. About 50 ml. of distillate are collected, and 1 ml. of the aqueous acetone solution is used for the colour development. We have found that the sensitivity of the estimation may be increased as much as 50 times by extraction of the bulky aqueous distillate with a few millilitres of toluene; 1 ml. of the toluene extract is then taken for the colorimetric analysis.

Method

The blood sample is first made up to about 100 ml. with water and the chlorhydrocarbon removed by steam distillation in an all-glass apparatus with a single ground glass joint at the point of attachment of the distillation flask (see fig. 1). Foaming is controlled by the addition of a few drops of tri-*n*-butyl citrate. The receiver is a 50-ml. measuring cylinder with a ground glass neck, and

FIG 1.—Apparatus for estimating chlorhydrocarbons in blood.

A. Distillation flask. B. Washing funnel. C. Toluene. D. Distillate. E. Ice water.

contains a small measured volume of toluene, varying between 1.5 and 5 ml. The receiver is immersed in ice-water. Approximately 40 ml. of distillate are collected and the condenser tube washed down with water from the funnel. The measuring cylinder is then stoppered, shaken and, after settling, the toluene layer is pipetted off. For the final colorimetric analysis, 1 ml. of the toluene extract is added to 10 ml. of pyridine and 5 ml. of 20 per cent. NaOH. After heating at 100° C. for exactly five minutes and then cooling thoroughly, the coloured pyridine layer is immediately separated off, since further contact with alkali causes colour fading, and run into a 20-ml. calibrated measuring cylinder. Under these conditions carbon tetrachloride and chloroform give a purplish-red and trichlorethylene an orange-red pyridine complex. At this stage the coloured solu-

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tion appears turbid but may be cleared by the addition of water to bring the final volume to 15 ml. In a few cases a volume greater than 15 ml. may be reached before the solution is quite clear. Here the final volume is noted and a correction applied in the calculation.

The colour intensity is measured in a Pulfrich photometer using filter S.53. The corresponding concentration is read off from the appropriate calibration curve. Specimen calibration curves are shown in fig. 2.

FIG. 2.—Calibration curves for chloroform, trichloroethylene and carbon tetrachloride.

Extinction coefficient reduced to 0.5 cm. cell length.

Colour is proportional to concentration up to 0.4 mg. of chlorhydrocarbon/ml. of toluene solution, at which point it becomes too intense for convenient measurement. The range given, 0 to 0.2 mg./ml., is that most frequently used. The lower concentrations were actually measured using 2-cm. or 3-cm. cells.

It was found that the colour given by carbon tetrachloride could be nearly doubled by the addition of 1 ml. of acetone to the reaction mixture which already contained 1 ml. of toluene. With chloroform, however, the final colour was decreased by the addition of acetone, while with trichloroethylene the addition of acetone had practically no effect.

It is important that the reagents used should be of A.R. standard and that they should themselves give no colour in the reaction mixture. We have found that it is also advisable to repeat a few points on the calibration curves when a new batch of reagent is used. In the case of pyridine, particularly, it seems that there may be present a trace of some impurity, varying in amount from one batch to another, which modifies slightly the final colour intensity. This effect is apparently uniform at all concentrations of chlorhydrocarbon, so that the calibration points always lie on straight lines.

Results

Varying quantities of carbon tetrachloride, chloroform and trichloroethylene in aqueous or concentrated acetone solution have been added to blood. These, together with the corresponding quantities recovered by

the above method, are shown in Table 1. The sensitivity of the method obviously depends on the amount of blood available for the estimation. A minimum concentration of 0.1 mg. of chlorhydrocarbon/100 ml. of blood can be estimated and a concentration of 0.05 mg./100 ml. detected with a blood sample of 25 ml., 1.5 ml. of toluene being used for the extraction. At higher concentrations, e.g. 10 mg./100 ml., a blood sample of 1 ml. is adequate for the analysis. The size of the blood sample and the amount of toluene used for the extraction may obviously be varied to give a convenient depth of colour for the final measurement.

TABLE 1

RECOVERY OF VARYING AMOUNTS OF CARBON TETRACHLORIDE, TRICHLOROETHYLENE AND CHLOROFORM ADDED TO BLOOD

CARBON TETRACHLORIDE		
Amount added	Amount recovered	Percentage recovery
2.10 mg.	2.07 mg.	98
1.00 "	1.00 "	100
0.525 "	0.535 "	102
0.200 "	0.196 "	98
0.200 "	0.212 "	106
0.100 "	0.103 "	103
0.050 "	0.048 "	96
0.050 "	0.050 "	100
0.030 "	0.029 "	97
0.025 "	0.024 "	95
TRICHLOROETHYLENE		
1.456 mg.	1.500 mg.	103
0.728 "	0.705 "	97
0.364 "	0.360 "	99
0.291 "	0.294 "	101
0.230 "	0.220 "	95
0.146 "	0.142 "	97
0.073 "	0.076 "	104
0.044 "	0.044 "	100
CHLOROFORM		
0.749 mg.	0.720 mg.	96
0.375 "	0.360 "	95
0.375 "	0.365 "	96
0.188 "	0.195 "	104
0.075 "	0.074 "	99
0.094 "	0.095 "	101
0.038 "	0.039 "	103
0.019 "	0.020 "	105

It was found that treatment of blood from normal subjects in the above manner gave a blank reaction, except in a few cases where a faint positive Fujiwara reaction was obtained. This could invariably be explained by the subject's having taken a cough mixture containing chloroform water, or a sedative containing chloral.

Summary

A method has been devised for the measurement of small amounts of chloroform, carbon tetrachloride and trichloroethylene in blood. Quantities of these substances varying between 2 and 0.02 mg. can be recovered from blood with an accuracy within ± 5 per cent.

REFERENCES

- Cole, W. H. (1926). *J. biol. Chem.*, 71, 173.
 Daroga, R. P., and Pollard, A. G. (1941). *J. Soc. chem. Ind.*, 60, 218.
 Fujiwara, K. (1914). *Sitzungsber. u. Abhandl. naturforsch. Ges. Rostock*, 6, 33.
 McCollum, J. L. (1930). *J. Pharm. exp. Ther.*, 40, 305.
 Nicloux, M. (1906). *C.R. Soc. Biol., Paris*, 40, 93.
 Robbins, B. H. (1929). *J. Pharm. exp. Ther.*, 37, 203.
 Schtschigol, M. B. (1933). *Pharm. Zentr.*, 74, 529.
 Wells, H. S. (1925). *J. Pharm. exp. Ther.*, 25, 235.