Medical Memoranda

A Simplified Method for the Estimation of Chloroform in Blood

Small amounts of chloroform may readily be estimated by the colorimetric reaction with pyridine and caustic alkali (Fujiwara, 1914; Daroga and Pollard, 1941; Habgood and Powell, 1945). The normal procedure is to recover the chloroform from blood by steam distillation. This is time-consuming and requires ground-glass apparatus, which is now difficult to procure. The present method utilizes Conway's microdiffusion technique (Conway, 1947) and is both simple and rapid; 10 to 20 estimations may be performed in 1½ to 2½ hours, and require only 1 to 2 ml. of blood.

The principle is very simple. Undiluted blood is placed in the outer compartment of the diffusion cell and toluene in the centre compartment. The cell is sealed and, on standing, the chloroform distributes itself so that its vapour pressure in the blood, toluene, and air phases is equal; and, since the Conway cells provide a large liquid surface compared with the volumes used, this occurs rapidly. Now chloroform is some 100 times as soluble in toluene as in blood, so the vapour pressure of chloroform in toluene is 1/100 that in blood; hence with the volumes used here about 96 to 97% of chloroform will be transferred to the toluene at equilibrium. At room temperature (20° C.) the equilibrium is 95% complete in 3 to 4 hours, but at 37° C. the equilibrium is 95% complete in 15 minutes, and 99% complete in 45 minutes if 1 ml. of blood is used; it is 80% complete in 15 minutes and 98% complete in 45 minutes if 2 ml. of blood is used.

REAGENTS

Toluene.—Pure toluene is shaken with concentrated sulphuric acid to remove thiophen, and then twice distilled, the middle fraction boiling at 115° C. being retained.

Pyridine.—Analytical grade or redistilled. It must be quite clear and colourless.

20% Sodium Hydroxide.

Diffusion Unit Fixative.—Five g. of gum tragacanth is ground with 70 ml. of distilled water to a smooth jelly and 25 ml. of glycerol is then added.

METHOD

Toluene 0.8 ml. is pipetted in the centre compartment of a standard Conway unit (obtainable from A. Gallenkamp, Ltd., Finsbury Square. London); 1 or 2 ml. of whole blood collected with suitable precautions (no oil in the syringe; narrow tubes filled to the top, and closed with a cork covered with silver foil) is rapidly pipetted into the outer compartment and quickly

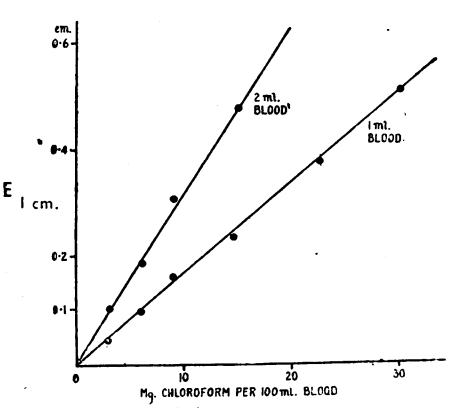

Chart showing that the intensity of the colour obtained (1-cm. cells) increases linearly as the concentration of chloroform in the blood is increased.

spread with the tip of the pipette. The cover, which has been smeared with fixative, is applied and the whole unit placed in an incubator at 37° C. for 50–60 minutes. The unit is then removed from the incubator, uncovered, and 0.5 ml. of toluene from the centre compartment transferred to 5 ml. of pyridine in a test-tube. Now 2.5 ml. of 20% sodium hydroxide is added, the tube is agitated and then placed in a boiling-water bath for precisely 5 minutes. It is then cooled in iced water, and, when cool, 5 ml. of the supernatant pyridine layer is transferred to a clean tube and 1 ml. of water added to remove the turbidity. The resulting purplish-red colour is stable for about one hour.

The colour is measured in a colorimeter with an Ilford micro-3 green filter, setting to zero with a blank. The intensity of absorption is linear with concentration (see Chart). Normally 1-cm. cells are used, but for concentrations below 5 mg. per 100 ml., cells of 2 to 4 cm. should be used if available. The absolute values for the absorption vary somewhat with different batches of pyridine, but owing to the linearity of the absorption curve the slope of the line can readily be checked by running a few standards.

RESULTS

Chloroform in blood can be estimated in concentrations of 0.5 to 35 mg. per 100 ml., the concentrations likely to be found during and following anaesthesia, with an accuracy of ± 5%. The method is applicable without modification to estimations in urine, cerebrospinal fluid, and tissue extracts.

The method has also been applied to the estimation of trichlorethylene. The colour obtained here with pyridine–sodium hydroxide is orange-red, with an extinction approximately one-third that of chloroform. Since the concentration of trichlorethylene found in blood during anaesthesia is only 6 to 12.5 mg. per 100 ml. (Powell, 1945) the colours obtained are rather feeble and the use of 4-cm. cells is essential.

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Similar Obstetric Behaviour in Identical Twins

Two primiparous identical twin patients were confined within three weeks of each other at the same hospital, both having extended breech presentation, failed version, and toxaemia. It may be of interest to publish the histories of these cases, if only as a "curiosity."

CASE HISTORIES

The patients were the only children of their mother and were stated to have been born three to four weeks early, with no history of maternal toxaemia, "about 7 lb. (3.18 kg.) the two," and "both in the same afterbirth." An aunt of their father had had twins and their mother had a brother and sister who were twins.

In the present instance the patients were delivered of their first babies (one each) within three weeks of each other in the autumn of 1945. Both were referred to the hospital antenatal clinic five weeks before term (as calculated) with extended breech presentations, right sacro-anterior, confirmed by *x*-ray examination, each breech being deep in the pelvis. *X*-ray examination also showed normal shapes of the pelvic brim. In each case, a week later, version under general anaesthesia failed. The first started in labour two days and the second nine days after attempted version. Both had uneventful extended breech deliveries with episiotomy, the one baby weighing 4 lb. 14 oz. (2.21 kg.) and the other 4 lb. 2 oz. (1.87 kg.), being three and four weeks premature respectively. The babies went home when 5 lb. (2.27 kg.) in weight, being breast-fed three-hourly, and both mothers had normal puerperia.

When first seen at the clinic both mothers had blood pressures of 140/90. A week later the first was admitted with toxaemia, B.P. 160/100, but no albuminuria; this settled with rest. The second rested at home on acount of a B.P. of 145/100 (after version) and was readmitted a week later in labour, with B.P. 150/105 and albuminuria. Both patients had had scarlet fever at the age of 6 and were of stocky build (height 5 ft. 2 in.—157 cm.), as was their mother. Menses were regular 5/28 in both cases.

At the postnatal clinic (both failed to attend at the routine date !) the blood pressures were 160/90 and 150/100. They had no symptoms nor albuminuria and had each put on 2 stone (12.7 kg.) in weight nine months after delivery. Menses subsequently started again three and five months post partum.

I wish to thank Mr. Bryan L. Jeaffreson, consultant obstetrician, and Dr. James W. Affleck, acting medical superintendent, St. Mary's Infirmary, Leeds, for permission to publish these cases.

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