THE ESTIMATION OF FLUOTHANE* IN BLOOD†

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A METHOD for the estimation of Fluothane in blood was originally described by Goodall in an appendix to a report on the pharmacology of the drug by Raventos (1). The estimation consisted of the extraction of Fluothane from blood by petroleum ether and it was based on its hydrolysis by means of sodium amoxide, the released halides being estimated nephelometrically as the silver halide.

The method was modified by Duncan (2) who used lithium aluminium hydride as a reducing agent for the extracted Fluothane. He showed that lithium aluminium hydride would reduce Fluothane at room temperature, with the release of 80 per cent of the bromine and 30 per cent of the chlorine.

The following description contains some modifications of Duncan's method, dictated by practice, which simplify the handling process. An attempt is made to assess the accuracy.

Special materials required are: petroleum ether. (A.R.) B.P. 100–120 C.; silver nitrate solution, 0.01 N.; sulphuric acid, 50 per cent v/v; anhydrous di-ethyl ether (A.R.); lithium aluminium hydride; ground glass stoppered test-tubes, 10 ml. capacity, 5.0 ml. syringes, recalibrated to deliver known volumes.

Preparation of lithium aluminium hydride solution (Duncan). Lithium aluminium hydride is used as a solution in di-ethyl ether, prepared by refluxing 500 mg. of the powdered reagent with 20 ml. of anhydrous di-ethyl ether for four hours, filtering through a No. 3 sintered glass filter and diluting the filtrate to 100 ml. with ether. The reagent keeps well if stored under nitrogen.

METHOD

Unknown Samples

A series of stoppered tubes is set up, each containing 6 ml. of petroleum ether. The blood samples, of 4 ml., are introduced into the liquid by means of a syringe and needle, care being taken to ensure that no air mixes with the blood. Samples in quadruplicate are used.

Preparation of Solutions for the Calibration Curve

A known solution of Fluothane in petroleum ether is prepared by weighing a graduated 50 ml. flask almost full of petroleum ether. About 200 mg. of Fluothane is dropped in, the flask is re-stoppered, inverted once or twice to absorb the Fluothane vapour in the neck and re-weighed. It is then filled to the mark with petroleum ether. The contents are well mixed and 5 ml. are removed by means

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of a syringe and needle which are first washed out with the solution. This quantity is introduced into a 100 ml. graduated flask, almost full of petroleum ether and more petroleum ether is added to make up to 100 ml. Each ml. of the final solution then contains about 0.2 mg. of Fluothane.

A series of stoppered test-tubes is set up and 4 ml. of plain blood is placed in each with a syringe. Known volumes of petroleum ether and of the petroleum ether and Fluothane mixture, to a total of 6 ml., are added to make concentrations of from 0-1 mg. in 6 ml. of the solvent. These are best prepared in quadruplicate. As the resultant plot of optical density against Fluothane concentration is linear, the zero and two other points are adequate to establish the calibration curve.

Process

The tubes with the known and the unknown samples are treated together for the rest of the process in order to eliminate systematic error.

The tubes are rotated horizontally for thirty minutes held in clips on the rim of a wheel, to allow equilibration of Fluothane between blood and petroleum ether. They are then centrifuged to separate the layers. From each tube 5 ml. of the petroleum ether layer is taken with a syringe and needle and placed in another series of stoppered test-tubes. The second series of tubes must be scrupulously clean and dry, having been washed in distilled water. To each, 1 ml. of lithium aluminium hydride solution is added and the stoppers replaced, the tubes being shaken to effect mixing. The reduction takes place at room temperature and is allowed to proceed for twenty minutes. The excess reagent is then destroyed by the addition of 1 ml. of sulphuric acid; 5 ml. of distilled water is added and the tubes are shaken for one minute to dissolve the released halide. Then, 5 ml. of the aqueous layer is removed to a separate tube by means of a syringe fitted with a long stainless steel needle; 2 ml. of silver nitrate solution added, the tube is inverted once to mix and is placed in a dark cupboard for ten minutes. Shaking, or contamination with petroleum ether at this stage, will cause coagulation of the suspension of silver halide and give scattered results. The optical density to 520 $m\mu$ in a 1 cm. cell of this suspension is recorded. A calibration curve (see Fig. 1) is prepared from the results of the known solutions and the concentrations of Fluothane in the unknown samples are read directly from this. As the final dilution is the same for all the samples, the concentration found requires only to be multiplied by 25 to give the concentration in mg./100 ml.

Assessment of Accuracy

In order to assess the accuracy of the method, standard solutions of Fluothane in blood were prepared as follows.

A very thin glass ampoule was weighed, some Fluothane was introduced into it, and then it was sealed and weighed again. The accuracy of weighing was ± 0.05 mg. The ampoule was placed in a bottle completely full of blood containing some short lengths of glass rod, the volume being determined previously. The bottle was shaken to break the ampoule and the contents thoroughly mixed for

ten minutes. Quadruplicate samples were taken by means of a syringe and needle and treated in the usual way. A calibration curve was prepared at the same time.

FIGURE 1. Vertical axis: optical density to 525 $m\mu$, d_{1cm} ; horizontal axis: concentration, mg./6 ml. petroleum ether.

RESULTS

Figure 1 shows two curves, one showing the results obtained with Fluothane and petroleum ether and the other with Fluothane, petroleum ether and blood. These were processed together as one batch.

They can be expressed as $y = bx$, b being the slope. The slopes, together with those of three other curves of each type are:

Fluothane and petroleum ether

.481
.447
.431
.416

Mean 0.444 ± 0.014

Fluothane, petroleum ether, blood

.356
.344
.306
.294

Mean 0.325 ± 0.015 ($P < 0.001$)

Table I gives the results of eight known samples prepared and estimated as described. The standard deviation of the estimates from the observed values is

TABLE I

Fluothane mg /100 ml blood		
Expected	Estimated	Difference (mg.)
18 45	15 70	-2 75
18 45	16 00	-2 45
18 45	17 85	-0 60
18 45	17 65	-0 80
18 63	18 65	+0 02
18 63	17 75	-0 88
18 63	18 65	+0 02
18 63	19 25	+0 62
19 70	18 75	-0 95
19 70	20 10	+0 40
19 70	19 25	-0 45
19 70	17 50	-2 20
22 20	22 00	-0 20
22 20	21 50	-0 70
22 20	22 00	-0 20
22 20	21 50	-0 70
13 66	12 75	-0 91
13 66	13 75	+0 09
13 66	14 25	+0 59
13 66	12 40	-1 26
15 30	14 37	-1 80
15 30	13 50	-0 93
15 30	14 15	-1 15
15 30	14 37	-0 93
15 55	15 25	-0 30
15 55	16 00	+0 45
15 55	15 40	-0 15
15 55	15 25	-0 30
11 17	8 90	-2 27
11 17	12 37	+1 20
11 17	11 75	+0 58
11 17	12 00	+0 83

± 1.14 mg. This standard deviation is given because a consideration of Figure 2 and Table I shows that, in the concentration range used, the accuracy is approximately the same at the high as at the low concentrations. This is due to the fact that the error appears to lie mainly in the measurement of the reagent which contributes an error independent of the concentration of Fluothane.

DISCUSSION

In early trials the greatest inaccuracy was found to be in the variable extraction rate. If the blood and petroleum ether are shaken together, as recommended by Goodall and Duncan, sufficiently to ensure extraction of the Fluothane, an emulsion frequently forms or bubbles of petroleum ether become coated with some constituent of the blood, making it impossible to effect separation. We found that thirty minutes of slow rotation of the tubes gave the maximum extraction rate and avoided these difficulties.

Goodall and Duncan recommended the use of 4 ml. of petroleum ether for the extraction of 4 ml. of blood, with the removal of 3.5 ml. for the reaction. We found that the number of spoiled samples was then high as we frequently could not avoid drawing some plasma into the syringe in the effort to obtain 3.5 ml.

FIGURE 2. Vertical axis: estimated concentration, mg./100 ml. blood; horizontal axis: expected concentration, mg./100 ml. blood.

To use 6 ml. and to leave 1 ml., makes this easier and yet gives about the same proportion of Fluothane for the reaction.

The reagent alone is responsible for an optical density reading of between 0.07 and 0.09 which is dependent upon the batch of reagent and the volume used in the reaction. Great care has to be exercised in the precise measurement of its volume.

The activity of 1 ml. of lithium aluminium hydride solution is sufficient for the reduction of all the Fluothane likely to be found in 4 ml. of blood. When it was tested with high concentrations of Fluothane in petroleum ether it produced sufficient halide to give silver halide suspensions too dense to be estimated in a 1 cm. cell. The presence of water or plasma in the reaction tubes, by inactivating some of the reagent, produces low results.

The wave-length used by Goodall and Duncan was 520 $m\mu$. There are, however, no interfering absorption bands and the peak at 520 $m\mu$ is not sharp. A simple null reading electrophotometer was used for this work with a filter peaking at 525 $m\mu$.

The results show that there is a significant difference in the slopes of calibration curves prepared with and without blood. This is due to the retention of a quantity of Fluothane by the blood. The eventual equilibrium of Fluothane between blood and petroleum ether probably depends to a large extent upon the temperature at which this takes place because the slope of the calibration curve varies from day to day with any one batch of reagent solution. It,

therefore, introduces considerable error to use a calibration curve prepared without blood. It is necessary to process the samples for the calibration curve and the unknown samples at the same time.

The method is sufficiently accurate for clinical investigational purposes.

SUMMARY

A modification of the methods of Goodall and Duncan for the estimation of Fluothane (2-bromo-2-chloro-1-1-1-trifluoroethane) in blood is presented.

It is shown to be necessary to construct a calibration curve for each batch of samples to be analyzed. For this purpose it is necessary to use known concentrations of Fluothane with plain blood.

The accuracy is assessed and is shown to be sufficient for clinical investigational purposes.

RÉSUMÉ

Nous avons présenté une modification des méthodes de Goodall et Duncan pour le dosage du Fluothane dans le sang.

Il est nécessaire de faire une courbe de calibration pour chaque groupe d'échantillons à analyser. Dans ce but il faut employer des concentrations connues de Fluothane avec du sang total.

La méthode est précise et suffisante pour fin de recherche clinique.

REFERENCES

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