BODY WATER ESTIMATION IN THE NEWBORN USING TRITIATED WATER*

BY

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Of all the substances used for the estimation *in vivo* of total body water content, the most attractive are the isotopes of hydrogen, since they enter into all the metabolic processes involving water and have no selective secretion or storage.

Deuterium was the first substance used for body water estimation *in vivo* (Hevesy and Hofer, 1934) and is preferable to tritium because it is not radioactive; but assay of deuterium by the falling drop method is difficult and takes time to establish, and other methods, such as the mass spectrometer, require expensive apparatus. The introduction of the liquid scintillation counter has made possible the use of tritium, the radioactive isotope of hydrogen, in safe doses. Imperato, Giovannelli, Battistini, Ghirardini and Ronchetti (1960) used tritium for estimating total body water in older children, but there is no report of its use in the newborn baby. This paper describes the use of tritium in newborn children and the sources of error in the present method.

Method

A standard solution of tritiated water was prepared containing 1.07 μc . tritium per ml. In the laboratory an amount of this standard solution calculated to contain 1.3 μc . per kg. body weight was pipetted into a vial. The vial was taken to the baby in the ward and the dose was injected from a 10 ml. syringe down a fine polythene tube which had been passed into the baby's stomach.

In the normal subject, after administration into the stomach, tritiated water reaches equilibration with the total body water after two hours (Pinson and Langham, 1957), and the tritium activity in urine secreted after equilibration represents the tritium activity in the total body water. The bladder was not catheterized in the babies; urine present in the bladder at the time of administration of tritiated water, and urine secreted during the first two hours while equilibration was taking place would dilute the tritium activity of urine secreted after equilibration was complete. Although all urine passed after the administration of the dose was collected,

the equilibration urine specimen was found to be the second specimen of urine passed more than two hours after the dose.

Urine was sublimated by a modification of the method described by Vaughan and Boling (1961), 6 ml. of the urine sample being transferred to a 100 ml. flask and then frozen to a thin layer lining the inside of the flask by rotating it rapidly in a mixture of acetone and dry ice. The flask was then connected by glass tubing to a 250-ml. flask immersed in a mixture of alcohol and dry ice in a vacuum flask (all joints being well greased with vacuum grease). The apparatus was evacuated by a two-stage high vacuum pump for 40 minutes until the specimen was sublimated (Fig. 1). Then 2.5 ml. of the sublimate were added to 7.5 ml. of a phosphor solution in a special jar for the liquid scintillation counter. This phosphor solution was based on dioxan and contained: diphenyl-oxazole, 7 g.; naphthalene, 50 g.; 1:4-di[2-(5-phenyl-oxazolyl)] benzene 0.050 g.; dioxan 1,000 ml.

Standards and samples were assayed in duplicate in a Panax liquid scintillation counter (type SC/LP) maintained at -2°C . With this particular machine and the cathode tube in it, the most satisfactory E.H.T. voltage was 1,650 with the discriminator bias set at 7 volts and a maximum gain (of 1,000) on the amplifier.

The total body water was estimated according to the formula:

$$V_1 \times C_1 = V_2 \times C_2,$$

where V_1 is the volume injected and C_1 the tritium

FIG. 1.—Apparatus for sublimation of urine.

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activity of the dose, and V_2 is the total exchangeable water and C_s the tritium activity of the sample.

Sources of Error

Pipetting. This causes the largest error in any volumetric estimation. During one estimation of total body water, six pipetting procedures were carried out: preparation of the dose and standard, measurement of a quantity of sample and standard to the scintillation pot and measurement of a quantity of scintillation solution for the sample and standard specimens. The accuracy of pipetting was checked by pipetting known amounts of a solution of radioactive chromium, which were estimated in a well-type scintillation counter. The results were as follows:

Into each of 11 vials 2.5 ml. of a solution of radioactive chromium were pipetted; disintegrations were counted in a Panax well-type scintillation counter incorporating a thallium activated sodium iodide crystal (type USC-B) with an Ecko scaler (type N530F), and each vial was counted for 100 seconds.

The counts ranged from 53,542-54,970 per 100 seconds (mean 54,180, standard deviation $\pm 0.89\%$). The theoretical standard deviation of counting is $\sqrt{54,180} = \pm 0.43\%$. The standard deviation of pipetting alone = $\sqrt{(0.89)^2 - (0.43)^2} = \pm 0.78\%$. Since six pipetting procedures were involved in the method the total predicted pipetting error is $\sqrt{6(0.78)^2} = \pm 1.91\%$.

Administration of Dose. Since the babies were taking very small amounts of fluid it was not possible to dilute the dose in a large amount of fluid. The dose of 2-4 ml. of tritiated water was made up to 5 ml. with water or milk, and injected down the gastric tube. The vial was rinsed with a further 2-3 ml. of water or milk which was then injected down the tube and the tube was blown through with air to empty it, the same syringe being used throughout.

The same procedure was used to add a known quantity of tritium to a 1,000 ml. flask of distilled water. An aliquot from the flask was counted in the usual way. When the tritiated water was delivered from a 10 ml. Luer syringe fitted directly to a wide bored (9 F.G.) aspiration tube there was only a 77% recovery. When the tritiated water was delivered from a 10 ml. syringe fitted with a blunt needle inserted into a fine bored (6 F.G.) feeding tube a 97.5% recovery was obtained.

Sublimation of Urine. Although sublimation of the urine produced an apparently clear fluid,

LIQUID SCINTILLATION OF TRITIUM IN AQUEOUS DIOXAN PHOSPHOR

FIG. 2.—Curve showing counts per minute obtained from varying standard solutions of tritiated water in dioxan phosphor.

recovery of tritium from it was not complete. Known amounts of tritium were added to samples of a normal urine and the samples were then sublimated. Recoveries of tritium were 94.5% and 95%.

Radio-assay. The phosphor mixture based on dioxan was used because it holds more aqueous phase than other phosphor mixtures. It has the disadvantage of having a counting efficiency of about 10% with a high background, but it does give a linear curve with increasing amounts of tritium (Fig. 2). With the low dose of tritium allowed, background and peak sample counts were so similar that variations in background during counting were extremely important. These variations might not always be detected even though numerous background counts were taken during a series of sample counts.

The scintillation pots are specially made from clear glass, but after a standard quantity of tritium had been added to all bottles, the measured activity varied over a range of $\pm 15\%$. Allowance must, therefore, be made in calculations for this quenching effect of each bottle and, if possible, in a series of counts bottles with similar quenching values should be used.

Counting. For each sample, the time taken to record 10,000 counts was measured three times. This should give a standard deviation of $\pm 1\%$, but it was not possible with the equipment used to reduce the standard deviation below $\pm 1.5\%$ for tritium counting.

Since the final calculation involves the subtraction of the background count from two sample counts and the division of one of these results by the other the final theoretical standard deviation becomes $\pm 3\%$.

Total Error

Three types of error were thus found: first, those for which corrections could be made, that is losses of tritium in the administration of the dose and sublimation of the urine, and variations in the quenching effect of scintillation pots. Second, the background variation which was unpredictable and possibly unknown. Third, the predictable errors of pipetting and counting for which no correction could be made.

The predictable errors in pipetting and counting were $\pm 1.9\%$ and $\pm 3\%$. Taken together, these give a statistical standard deviation of $\pm 3.5\%$.

Conclusions

The method used has no value for comparative daily body water estimations in the newborn. F. Puga and J. P. M. Tizard (personal communication) measured the fluid losses in 10 normal full-term babies; in the first 48 hours of life insensible fluid loss was $22.4 (\sigma 1.9)$ g. per kg. birth weight per 24 hours; loss of weight through urine and faeces during the same period is $22.7 (\sigma 6.2)$ g. per kg. body weight per 24 hours. A 3.5 kg. baby will thus lose less than 175 ml. water over

24 hours; with a total body water of approximately 2,625 ml. the experimental error might be 180 ml. over 24 hours. The changes, therefore, could be measured more accurately by weighing.

For the actual measurement of total body water content some form of dilution technique is necessary, and the accuracy of this method could be increased by an increase of the dose from $1.3 \mu\text{C.}$ to $10 \mu\text{C.}$ tritiated water per kg. body weight. This dose would reduce the predictable error to $\pm 2.4\%$ and, more important, it would greatly reduce the unpredictable error of background variation by increasing the ratio of sample to background count.

Summary

A method of estimation of total body water content in the newborn baby is described. Sources of error in the method are discussed. The accuracy achieved is insufficient for the estimation of daily changes in body water content, but by increasing the dose of tritiated water the method has a place for single estimations.

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