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ASSAY OF TRITIUM ACTIVITY IN BODY FLUIDS WITH USE OF A LIQUID SCINTILLATION SYSTEM

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Los Alamos, N. M.

BECAUSE of the advantages of tritium water (HTO) as a tracer for ordinary water in studies of water metabolism, fluid balance, and water transfer, tritium is assuming an increasingly important role as a radioactive tracer.[1] The assay of tritium in biologic materials is complicated, however, by its very low beta particle energy ($\bar{E}\beta = 5.69$ kev).

Pinson[2] in his early studies completely distilled the water from body fluids and, by means of a reduction furnace, produced hydrogen and tritium gas. These gases were pumped into an ionization chamber and the current measured with an electrometer. Other techniques have been developed that involve ionization chamber measurements and/or proportional and Geiger counting of a variety of gaseous samples, for example, acetylene, methane, propane, and water vapor.[3]

Hayes and Gould,[4] and Farmer and Berstein[5] later described a liquid scintillation method that was satisfactory for the assay of tritium water and tritium-labeled sterols, but they presented no specific procedures for the assay of biologic fluids. The present study was undertaken to develop a shorter and more reliable method, based on the liquid scintillation principle, for the assay of tritium activity in various body fluids. Okita, Spratt, and LeRoy[6] developed a simplified method for the assay of HTO in human urine which is based on the same principle. Their method, however, differs in some respects from the procedure reported here and appears to have less precision.

MATERIALS AND METHODS

The apparatus for counting the tritium activity was a fast coincidence liquid scintillation counter, designed and constructed at this laboratory,[7] as shown in Fig. 1.† The photomultiplier tubes were encased in a 2-inch lead shield (Fig. 2) and housed in a Deep-freeze unit at a temperature of 0 to 4° C. The counting efficiency varied from 6 to 9 per cent, depending on the quenching properties of the sample system used. Coincidence backgrounds were 70 to 100 c.p.m.

The scintillation solution used in all assays was prepared as follows: 7 Gm. of 2,5-diphenyloxazole (PPO), 50 Gm. of naphthalene‡ (recrystallized from alcohol), and 50 mg. of 1,4-di-[2-(5-phenyloxazolyl)]-benzene (POPOP) made up to 1 liter with p-dioxane.§

From the Los Alamos Scientific Laboratory, University of California, Los Alamos, N. M.
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*Department of Biology, University of New Mexico.
†Analogs of this counting system are now available commercially.
‡Eastman Kodak Company.
§1,4-dioxane (A. R. Grade, Stock No. 2347) Matheson, Coleman and Bell, Norwood, Ohio.

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Human Blood Serum.—One milliliter of fresh serum, which contained a known amount of tritium as HTO, was thoroughly mixed with 2 ml. of a 10 per cent aqueous solution of trichloroacetic acid. After precipitation of the serum protein, the sample was centrifuged. The colorless supernatant was decanted and 1 ml. was pipetted into a special bottle* for counting, which contained 24 ml. of scintillation solution. Samples were allowed to stand for one hour at 2 to 3° C. before counting. Dark adaptation was unnecessary.

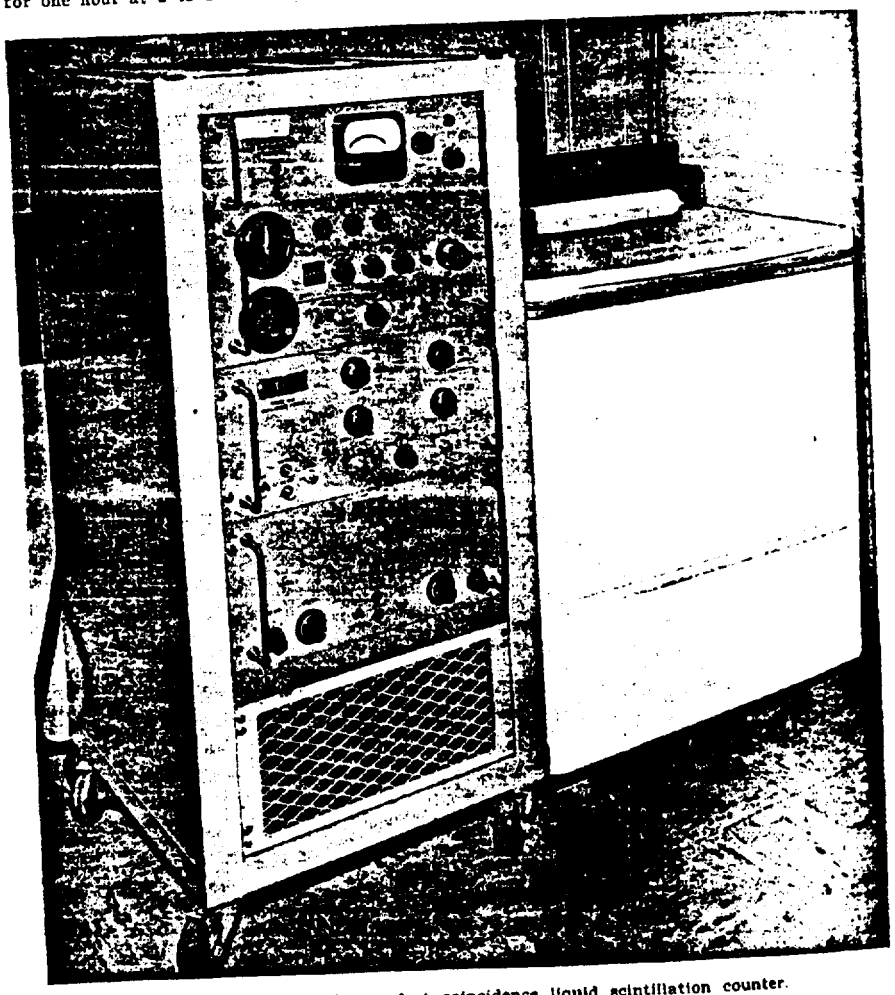

Fig. 1.—The Los Alamos fast coincidence liquid scintillation counter.

A standard containing a known amount of tritium activity and a background blank were prepared from a 1-ml. aliquot of a solution containing one part water to two parts 10 per cent trichloroacetic acid which was added to 24 ml. of scintillation solution in the same manner used for the preparation of samples. The standard was made with HTO and the background blank with distilled water.

*Kimble Opticlear 10-dram vial with polyethylene stopper.

Rat Blood Serum.—On
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Fig. 2.—Counting chamber

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Rat Blood Serum.—One milliliter of rat serum containing HTO was added to 15 ml. of scintillation solution, mixed for one minute, and centrifuged. The colorless supernatant was decanted into a counting bottle; the precipitate was broken up in the centrifuge cone and washed with an additional 9 ml. of scintillation solution and centrifuged again. The second supernatant was added to the first and the combined sample was allowed to cool for one hour before counting.

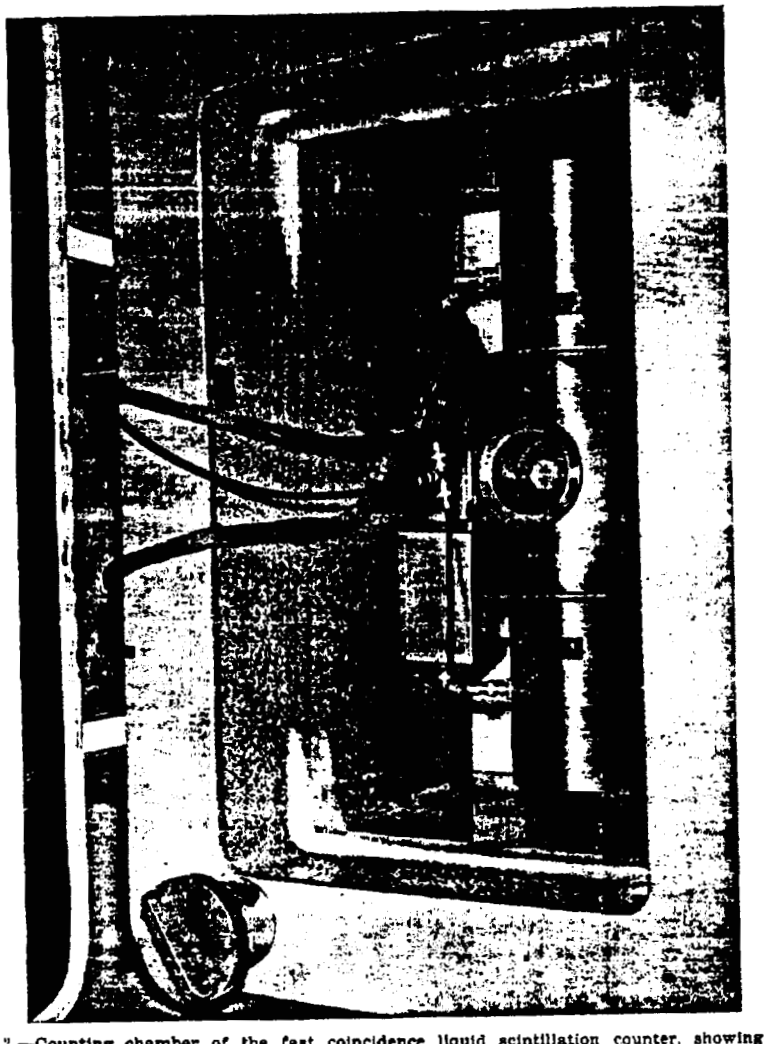

Fig. 2.—Counting chamber of the fast coincidence liquid scintillation counter, showing lead shield, sample holder, and preamplifiers.

The standard and background blank were prepared by the adding of 1 ml. of HTO (or distilled water) to 24 ml. of scintillation solution.

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Rat serum may be assayed for tritium activity in a manner identical to that used for human serum. Addition of trichloroacetic acid is essential for complete recovery in human serum but not for rat serum (see Results and Discussion).

Human and Rat Urine.—Tritiated urine was decolorized with activated charcoal.* The charcoal (0.5 Gm.) was placed in a 2-ml. fine-pore, fritted-glass Buchner funnel, and 2 ml. of urine was drawn through the charcoal bed very rapidly by suction. One milliliter of decolorized urine was transferred to a 40-ml. centrifuge tube and 24 ml. of scintillation solution was added. The resulting turbid solution was centrifuged at 2,500 r.p.m. for 10 minutes and the clear supernatant was transferred to a counting bottle. The sample was cooled for approximately one hour at 2 to 3° C. before counting.

The standard and the background blank were prepared by the addition of, respectively, 1 ml. of charcoal-processed urine and 1 ml. of distilled water to 24 ml. of scintillation solution.

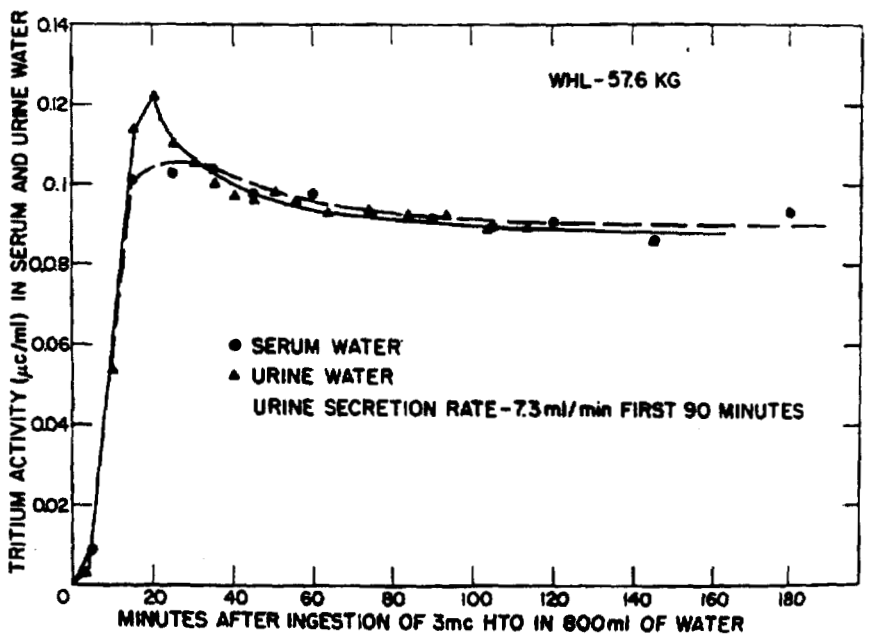

Fig. 3.—Tritium activity in the water of human urine (solid line) and blood serum (interrupted line) as a function of time after the ingestion of HTO.

Application of Methods to the Study of Water Absorption and Distribution in a Human Subject.—In order to test the applicability of the methods, an experiment was designed to determine the rate of absorption and distribution of ingested HTO by a normal human subject. A fasted normal man (weight 126 pounds) ingested 3 mc. of HTO in 800 ml. of water, after which venous blood samples were drawn and urine samples collected every 5 to 10 minutes for approximately two hours. The samples were assayed for tritium by the liquid scintillation method and the results are given in Fig. 3.

RESULTS AND DISCUSSION

As seen in Table I, the methods employed for determining tritium in blood sera of either humans or rats were accurate and reliable. Recovery from

*Fisher, 80 mesh.

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TABLE

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samples tritiated with 7,130 to 770,300 d./m./ml. was slightly over 99 per cent, with an error of less than 2 per cent.

TABLE I. ASSAY OF TRITIUM ACTIVITY IN BLOOD SERUM

NO. OF SAMPLES	D./M./ML. EXPECTED	D./M./ML. OBSERVED (MEAN)	RECOVERY % (MEAN)
		Human	
4	770,300	768,400	99.7
2	113,200	112,000	98.9
2	37.740	38,090	100.9
8		Mean ± S.D. = 99.8 ±	1.0
		Rat	
2	770,300	756,200	98.2
2	192,600	193,300	100.3
2	21,400	21,040	98.3
2*	7,130	7,120	99.8
8		Mean ± S.D. = 99.1 ±	1.2

*Tritium activity assayed by means of the method used for human serum.

The data in Table II show the results of the assays of human and rat urine samples for known amounts of tritium activity. Recovery from samples tritiated with 5,660 to 566,100 d./m./ml. was over 96 per cent, with an error of approximately 3 per cent.

TABLE II. ASSAY OF TRITIUM ACTIVITY IN URINE

NO. OF SAMPLES	D./M./ML. EXPECTED	D./M./ML. OBSERVED (MEAN)	RECOVERY % (MEAN)
		Human	
5	566,100	537,700	95.0
10	50,320	48,440	96.3
8	22,640	22,170	98.1
5	5,660	5,440	98.6
28		Mean ± S.D. = 96.9 ±	2.2
		Rat	
5	226,400	223,100	98.5 ± 3.2
9	22,640	21,790	96.2 ± 2.5

The data in Fig. 3 show the tritium activity in the water of blood serum and urine from a normal male following ingestion of HTO. The values for urine samples were plotted on the abscissa at the midpoint of the collection period, rather than at the end. In the calculation of the tritium activity in terms of microcuries per milliliter of serum and urine water, it was assumed that serum is 92 per cent and urine 98 per cent water. Since recovery of tritium in serum was 3 per cent greater than in urine, a corresponding correction was made in the urine values before they were plotted.

These data show that the method for both urine and blood serum gave water absorption and distribution patterns quite in keeping with the expected normal for water metabolism in man. Both urine and serum reached their peaks in tritium activity within 20 minutes after the ingestion of HTO and it

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would appear that HTO was in equilibrium with all body fluids within 90 minutes. On this basis, it was estimated that the total body water in this individual was approximately 58 per cent of the body weight.

The methods reported here for the assay of tritium in body fluids appear to be simple and reliable. The mean recovery values obtained for tritiated blood serum and urine were at least 97 per cent of the expected values and the error was relatively small (<3 per cent).

Success was encountered in the search for a scintillation solution that would: (1) when mixed with HTO produce a high photon yield, and (2) be completely miscible with water.

Furst, Kallmann, and Brown[8] found recently that the addition of naphthalene to a solution of PPO in a mixture of dioxane and water increased the fluorescence intensity of such a solution. A similar system for the assay of tritium activity in body fluids was tried. Not only was their solute combination a satisfactory one for determination of HTO in aqueous media, but it could be improved by the addition of still another secondary solute, namely, POPOP, thus increasing the efficiency of counting HTO in a small volume (24 ml. of scintillator solution in the presence of 1 ml. of water).

The yellow pigments in human serum (but not present in rat serum) produce low results and must be removed. Charcoal filtration did not remove these pigments but trichloroacetic acid decolorized human serum simultaneously with the precipitation of the serum proteins. Two milliliters of 10 per cent trichloroacetic acid mixed with 1 ml. of tritiated serum, when centrifuged, gave 2.5 ml. of a colorless supernatant. One milliliter of the supernatant, representing one-third milliliter of serum, was counted and values expressed in terms of activity in 1 ml. of serum. Rat serum did not have to be processed with trichloroacetic acid for full recovery, because addition of rat serum to the scintillation solution and subsequent centrifugation resulted in the formation of a clear supernatant fluid that could be decanted from the settled protein precipitate. However, rat serum can be processed in the same manner as human serum. It is important that serum samples be colorless and protein-free.

A number of precautionary steps in the procedure are worth consideration. Since addition of trichloroacetic acid to serum had a quenching effect, proportional to the amount of acid used, standard and blank samples must be prepared with use of the same amount of acid and the same conditions used in the preparation of the samples. Slight turbidity of samples resulted in falsely high and variable recoveries; therefore, care must be taken not to transfer precipitated protein to the counting bottle. Cooling of samples below the freezing point of water should be avoided, since such temperature may cause samples to become turbid. Counting bottles should be of optically clear glass to avoid the possibility of low results from loss of light. Only nonabsorbing stoppers should be used to avoid possible sample contamination.

These methods for the determination of tritium in serum and urine can probably be adapted to the analyses of other body fluids.

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SUMMARY

Simple and rapid methods have been developed for the determination of tritium water (HTO) in mammalian serum and urine. Mean recovery values obtained for tritiated blood serum and urine were at least 97 per cent of the expected values and the error was relatively small (<3 per cent).

In order to obtain consistent and reliable values for tritium activity, we were necessitated to remove proteins and pigments from all samples. In human serum this was accomplished by the use of trichloroacetic acid. Rat serum did not contain enough pigments to make decolorization necessary. Urine was decolorized by filtration through activated charcoal, and cleared urine was added directly to the scintillation solution. After centrifugation and decanting, the clear supernatant solutions were placed in optically clear glass counting bottles, chilled for one hour at 2 to 3° C., and counted.

These procedures were shown to be applicable to the study of water absorption and distribution studies in a normal human using HTO.

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