Scintillation Counter Assay of I^{131} and P^{32} in Blood Volume Studies. (20822)

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(Introduced by B. J. Jandorf.)

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The studies of this report were designed to investigate the use of the sodium iodide crystal scintillation counter to determine simultaneously the volumes of distribution of red cells labeled with P^{32} and albumin labeled with I^{131}. Berson and Yalow(1) have used this combination previously measuring the 2 isotopes with separate counting systems. The procedures used in measuring the circulating red cell mass and the plasma volume simultaneously have been reviewed by Berson and Yalow(1) and by Sjostrand(2). In most of these methods chemical as well as isotope labeling is required and a single isotope is used. In the procedure described in this report, 2 isotopes are injected together and the individual activities analyzed from the same sample of whole blood.

Method. Although the well-type sodium iodide crystal scintillation counter was designed primarily for the assay of "gamma" activity from liquid samples, it is also sensitive to high energy beta emissions that penetrate the light shield covering the crystal. The presently used crystal (Harshaw Chemical Co., Cleveland, Ohio) is sheathed with 0.032 inch of aluminum. From P^{32}, a pure beta emitter (beta max. 1.7 mev), 2 x 10^4 counts per minute per microcurie were obtained. The above mentioned amount of aluminum will absorb approximately 70% of the incident beta rays from the radioactive phosphorus. To avoid the further absorption that would be present with the glass wall of the conventional test tube, a thin walled plastic ("Lusteroid," International Equipment Co., Boston, Mass.) test tube (13 mm I.D.) was used to contain the sample during the counting.

Approximately 5.3 x 10^5 counts per minute per microcurie were obtained from I^{131} with the equipment used. Separation of the activities of the 2 isotopes was obtained by counting twice; once when the test tube was placed within a brass cup (wall 1 mm thick, equivalent to 840 mg/cm^2) and once without the cup. This thickness of brass is sufficient to prevent penetration of the primary P^{32} beta rays, but counts are still obtained through the brass, presumably as a result of secondary Bremsstrahlung X-rays. With the instrumentation used in the studies of this report, the P^{32} counting rate with the cup was reduced to 20% of that for the same sample without the cup, and the I^{131} counting rate with the cup was 95% of that without it. Changes of these "cup factors" were observed when different photomultiplier tubes were used, but no changes were found with a given tube. Factors (f_P and f_I) varying between 15 and 30% were found for the P^{32} counting rates and between 91 and 98% for the I^{131}. Individual activities of the 2 isotopes were calculated by solving the following simultaneous equations:

$$\text{Counts without cup} = I^{131}\text{ counts} + P^{32}\text{ counts}$$
$$\text{Counts with cup} = f_I\, I^{131}\text{ counts} + f_P\, P^{32}\text{ counts}$$

It was found necessary to have high counting rates and to adjust the relative activities so that 2 to 3 times as many counts were obtained from the phosphorus as from the

TABLE I. *In Vitro* Studies.

Diluent	I^{131} (iodinated albumin)			P^{32} (sodium phosphate)		
	Alkaline saline		Blood	Alkaline saline		Blood
	Unmixed*	Mixed*		Unmixed*	Mixed*	
Avg of 10 samples, counts/min.	2993	2927	2963	7927	7793	7470
Coefficient variation	1.9%	2.8%	2.6%	1.0%	1.9%	2.6%
Ratio: Mixed/unmixed		.98	.99		.98	.94

* See text for explanation of unmixed and mixed solutions.

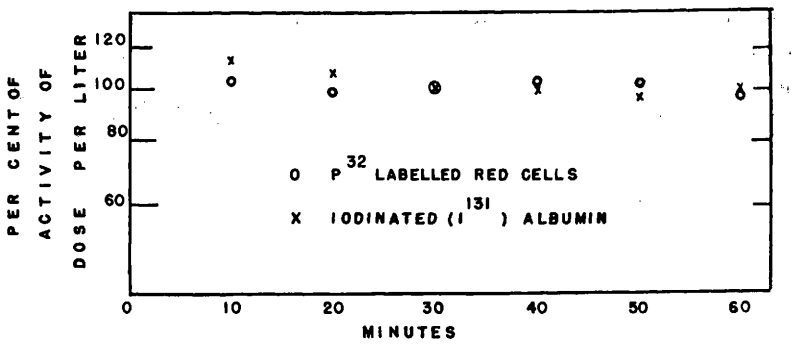

FIG. 1.

iodine. In the present experiments, the average counts were approximately 6,000 counts per minute for the P^{32} and 2,500 counts per minute for the I^{131}. Three-minute counting periods were used and each sample was counted in duplicate. The iodinated (I^{131}) human serum albumin and a sterile buffered sodium phosphate (P^{32}) solution were obtained from Abbott Laboratories, North Chicago, Ill.

In Table I are presented data obtained from an *in vitro* "recovery" study. From a solution containing P^{32} (sodium phosphate) and from a separate one containing I^{131} (iodinated albumin), 1/20 dilutions were made and counted separately (unmixed values, Table I). Equal parts of the 2 solutions were then mixed and recounted at a 1/10 dilution, diluents being saline containing 0.2% NaOH and whole heparinized blood. These samples were counted both with and without the cup and the separate activities of the 2 isotopes were calculated (mixed values, Table I). The observed lower counts from whole blood, as compared to saline, are considered due to the difference in the density of the 2 solutions; blood 1.057 g/cm^3, alkaline saline 1.012 g/cm^3. Two specific problems were encountered in the handling of these radioactive agents. First, with high dilutions of the iodinated albumin it was noticed that as much as 20% of the activity appeared to be "lost". Attraction of the protein to the wall of the glassware was considered responsible and the effect was not observed if amounts of carrier albumin, in the form of a few ml of plasma, were added to the primary dilution. This precaution was suggested by Dr. C. Leewallen of the Brookhaven National Laboratory. Secondly, it was observed that the P^{32} activity in the counting tubes appeared to increase, when counted on successive days, if neutral water or saline solutions were used. This effect was explained by postulating adherence to the wall of the plastic tube, thus changing the geometry so that less of the beta energy was absorbed in the water and a higher counting efficiency resulted. The phenomenon was not observed when the diluent was whole blood or alkalinized saline.

In vivo dilution measurements. The results of a preliminary experiment describing the blood dilution values of P^{32} tagged red cells and of iodinated (I^{131}) human serum albumin in a 10 kg dog are plotted in Fig. 1. The dog red cells were labeled in the manner described by Hahn and Hevesy(3) and as modified by Berson and Yalow(1), but a higher dosage of P^{32} was used than was recommended by either of these authors. The final cell preparation contained approximately 100 microcuries of P^{32}. A few ml of plasma containing 1.5 microcuries of iodinated (I^{131}) albumin were then added to the cell suspension and mixed thoroughly. Prior to the injection into the dog, an aliquot of the mixture was taken and diluted in alkaline saline to serve as a reference standard from which to analyze the dose per liter activity concentration for each agent. Blood samples were drawn at 10-minute intervals. Three ml amounts of heparinized whole blood were counted and the separate activity concentration for each isotope was determined as described. The P^{32} counts from blood were divided by the factor of 0.96 to correct for the loss of counts from the some-

what denser blood as compared to the alkaline saline standard. Red cells were prevented from settling in the whole blood samples by manual agitation with wire loops prior to the counting. The cells were hemolyzed with saponin in the standard solution. The results (Fig. 1) show close superimposition of the values indicating near equality of the volumes of distribution of these 2 agents.

Summary. The use of the well-type sodium iodide crystal scintillation counter to measure P^{32} and I^{131} from a single sample of whole blood is described. The individual activities are calculated from data obtained with and without a metal cup absorber.

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3. Hahn, L., and Hevesy, G., *Acta Physiol. Scandinav.*, 1940, v1, 3.

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Phosphate (P^{32}) Exchange in Brain Phospholipids *in vivo* and *in vitro*.*† (20823)

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It is now well established that adult neural tissue can synthesize phospholipids *in vitro* as well as *in vivo*(1-7), and the inference that these "structural" components of the cell are involved in normal functional metabolism has some direct support(6-8). On the other hand, the slow phosphorus turnover of brain phospholipids would preclude a very active metabolism unless the apparent low overall specific activity in fact represents high activity of a small fraction. In favor of this, it had been shown(1,2) that P^{32} is incorporated to a greater extent into brain cephalins and sphingomyelin than into lecithins; and in *in vitro* studies we observed much higher specific activities in some lipid fractions than in others. Further study shows a highly active protein moiety (derived from lipoprotein) which may represent a part of the inositol metadiphosphate complex isolated by Folch and Lees(9).

Methods. In vitro, the brains of 6 to 12 male Sprague-Dawley rats were used in each experiment. Each brain was homogenized in 8.0 cc of 0.01 M potassium phosphate buffer (pH 7.4) immediately after it was removed from the skull and kept at −7°C until the brain tissues of the different animals were combined. The entire homogenate, together with $Na_2HP^{32}O_4$, glucose (10^{-1} M), K ATP (3.1×10^{-4} M), DPN (6.1×10^{-5} M), cytochrome c (5×10^{-5} M), $MgCl_2$ (8.0×10^{-3} M), and KF (8.0×10^{-3} M) was incubated at 37°C in the Warburg water bath. After shaking for 20 minutes, one hour, or 2 hours, the tissue was separated from the incubating medium by centrifugation at −7°C for 20 minutes at an R.C.F. of 2700 times gravity. The lipids were extracted from the brain tissue at 4°C with 20 volumes of cold chloroform-methanol (2:1) per g of tissue(9), and the extract was separated from the residue by filtration at 4°C. The residue was reextracted 2 more times, the 3 filtrates were combined, and were washed several times with distilled water at 4°C by the method of Folch(9) to remove water-soluble contaminants. The chloroform-methanol extract was then dried *in vacuo*. This process released some of the protein from lipoprotein elements which were soluble in chloroform-methanol(9).

The lipids were redissolved in 20 volumes

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