the presence of the pancreas the action of insulin is limited by the secretion of HGF. 5. It is suggested that HGF and insulin might be 2 mutually regulated pancreatic hormones and that their balanced secretion might be an important factor in the maintenance of a normal blood sugar concentration.

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Measurements of Radioisotopes in Blood Applied to Determinations of the True Hematocrit.* (19716)

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A convenient and accurate technic for sample preparation for use in measurement of concentrations of soft beta-ray emitting radioisotopes in blood is required in clinical and experimental investigations. Solid samples, especially at or near "infinite thickness," prepared by the isolation of a precipitate of the radioactive ion give reproducible results(1,2). However, their preparation requires considerable time and the counting rate of the sample is lowered by absorption of beta-rays by the sample. Measurement of radioisotopes in liquid samples(3,4) exposed to a thin-window counter has been recommended. Such liquid samples, like thick solid samples, reduce the counting rate by self-absorption, thus necessitating the administration of large amounts of labeled isotopes. Liquid samples suffer the additional disadvantage of possible evaporation, unless covered during counting, and are easily spilled.

Rapid and simple methods for sample preparation, particularly applicable to blood containing beta-ray emitters of low energy, have been developed and tested. These procedures

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have been found to give reproducible results and have few of the disadvantages of other methods. The procedures have a definite application, when I^{131} labeled albumin is employed, to the measurements of blood and plasma volumes and in the determination of the true hematocrit. The methods have also been successfully applied to the measurement of Ca^{45} and Na^{22} (a positron emitter) in plasma and blood. It is probable that they could also be employed for the measurement of other non-volatile electron emitters with maximum energies down to 0.15 mev.

The accuracy of relative corpuscle-plasma volumes obtained from the hematocrit has been repeatedly questioned on the score of the amount of plasma trapped in the corpuscle columns. The consensus(5,6) of the most consistent observations published prior to 1952 gave estimates of trapped plasma as 3-5% of the packed cell column. Maizels(7), however, found that only 2.2% of the packed cell column is plasma, while McLain and Ruhe(8) reported 4-17.9% of the cell column of defibrinated ox blood to be plasma. Leeson and Reeve(5) employed determinations of the dilution of I^{131} labeled serum proteins in blood by a complicated procedure and also made measurements of radioactivity in the packed cell columns and in the supernatant plasma. These workers reached the conclusion that about 5% of the cell column of heparinized human blood in the hematocrit tube is plasma. Jackson and Nutt(6) measured the concentration of T-1824 dye, added to whole blood, in the cell column and in the plasma and found that not over 2.2% of the cell column is plasma when the centrifugation is carried out at 4000 times gravity.

Methods. A 5-inch diameter circle was drawn on heavy paper attached to a plane surface of a transite board and the board was fixed in a level position as determined with a mechanic's bubble level. Whole blood was hemolyzed by the addition of an equal volume of water. The hemolyzed blood solution, or undiluted plasma, was treated with 0.08 ml of glycerol per milliliter and with 3-6 flakes of a solid surface tension depressant† and then shaken for several minutes to assure homogeneity of the solution. Glycerol was employed as a plasticizer to prevent brittleness, cracking, and flaking of the sample after drying. Half milliliter samples of the solutions were pipetted onto the center of 1-inch diameter copper planchets arranged on the perimeter of the circle mentioned above. A thin straight wire, such as hypodermic needle obturator, was used to distribute the sample evenly over the surface of the planchet. An infra-red radiant heating bulb was suspended approximately 4 inches above the center of the circle, and the samples were dried for one hour. The dried samples were firm and tough and adhered smoothly to the planchets. The radioactivity measurements were made with thin mica window Geiger-Mueller counters and care was taken to maintain a fixed geometric relationship between the sample and the counter window. All radioactivity counts were made within a statistical deviation of less than 1%.

Radiocalcium concentrations in whole blood and plasma were also determined by counting "infinitely thick" samples of calcium oxalate collected in previously described counting dishes(1). In these experiments protein-free filtrates of blood and plasma were prepared from trichloracetic acid and inert carrier calcium ion was added to the filtrates to give the required amount of calcium oxalate precipitate.

Hematocrit determinations were made with Wintrobe tubes centrifuged at 2000 r.p.m. (18.5 cm radius) until a constant packed cell column was obtained (1-1.5 hours). Duplicate measurements were made.

Results and discussion. The reproducibility of the results obtained by the methods of sample preparation described above was examined by counting several samples prepared from a single volume of plasma or blood containing I^{131} labeled albumin, Ca^{45}, or Na^{22}. The mean results, as counts per minute, with standard deviations of the means follow: 10 samples I^{131} albumin in plasma, 1034 ± 20.4; 10 samples I^{131} albumin in whole blood, $25{,}688 \pm 230$; 6 samples Ca^{45} in plasma, 3083

† Nacconol, a laboratory cleansing aid, manufactured by National Aniline Division, Allied Chemical and Dye Corp., Merchandise Mart, Chicago, was used.

± 31.6; 10 samples Ca^{45} in whole blood, 1983 ± 66.5; 6 samples of Na^{22} in plasma, 4936 ± 81.5; 10 samples of Na^{22} in whole blood, 1646 ± 20.1.

It was desired to compare the radioactivity measurements of whole blood and its plasma, each containing I^{131} labeled albumin, in order that the relative volumes of plasma and corpuscles in whole blood might be calculated. The greater solids content of whole blood results in the weight and thickness of the dried residues on the planchets from whole blood (diluted 1:1) being greater than those of the plasma residues. It was, therefore, necessary to examine the magnitude of radiation losses due to self-absorption in the whole blood residues. Five dilutions of whole blood containing I^{131} labeled albumin were made with water in such manner that half milliliter volumes contained the equivalent of 0.05-0.23‡ ml of whole blood when further diluted with 0.08 ml of glycerol per ml. The weights of the residues of these solutions, prepared as described above on tared planchets, varied from 56 to 94 mg of which 43 mg was due to glycerol. A plot of the counts per minute of the planchets against volume of blood represented in the samples, or against weights of the residues, gave a straight line. It is thus evident that self-absorption by the samples of I^{131} radiation is not a factor under the conditions of sample preparation described in this paper and that measurements of I^{131} activity in plasma and whole blood, by these technics, can be related directly.

The beta radiation of Ca^{45} exhibits considerable self-absorption in samples(9). The use of these technics with Ca^{45} would, therefore, require standard planchets prepared from plasma or whole blood, depending upon which fluid is to be studied. It would also be preferable to prepare the Ca^{45} standards concurrently with the experimental samples in order to control possible variations in degree of drying among the samples.

The plasma fraction of blood was calculated as the ratio of the radioactivity measurement of whole blood to that of an equal volume of plasma from the same blood sample. The apparently valid assumption is made that I^{131} from iodinated albumin added to blood remains in the plasma(5). The measurements were carried out with heparinized human blood treated with I^{131} labeled human albumin and also with the same blood after increasing its plasma volume. Plasma was collected from the original blood after addition of I^{131} albumin and added to aliquots of the original blood to give 2 blood samples with increased plasma fractions. The results compared with those obtained by the hematocrit are given in Table I.

The direct and derived results in Table I show that the hematocrit determination is liable to a considerable error due to plasma trapping in the cell column. This error decreases with increasing plasma volume (decreasing cell volume as, for example, in cytopenic or microcytic anemias). This observation may account for some of the variations as to plasma trapping in the hematocrit tube reported in the literature. On the basis of this observation it is suggested that ordinary hematocrit determinations applied to polycythemic blood considerably overestimate the true cell volume. Undoubtedly, the increasing centrifugal force(6) toward the closed end of the hematocrit tube is an important factor in reducing plasma trapping in short cell columns.

Although the results obtained with Sample C (Table I) by the 2 methods are virtually identical, it seems unlikely that the cell columns in the hematocrit tubes were devoid of plasma. It is possible that experimental errors, particularly in the hematocrit determination which became of increasing importance as the cell volume decreases, account for the agreement of these results.

The plasma volumes of blood samples and the intercellular plasma of the cell column of the hematocrit were also studied by measurements of Ca^{45} concentrations in whole blood and plasma. In this work, carried out before the sample preparation technics described in this paper were developed, Ca^{45} was measured in infinitely thick preparations of calcium oxalate as mentioned in the section on

‡ 0.23 ml is the volume of blood represented in the residue on the planchets prepared from whole blood by the routine technique.

TABLE I. Plasma Volumes of Blood Calculated from Hematocrit Determinations and from Concentrations of I^{131} Labeled Albumin in Blood and Plasma.

Material	Plasma vol. as % of blood vol. from Hematocrit	Plasma vol. as % of blood vol. from I^{131} albumin	Plasma vol. deficit by hematocrit (% of actual vol.) †	Trapped plasma as % of vol. of packed cell column
Original blood *(A)*	54.08	58.65 (6P,3B)*	7.8	9.9
A diluted with plasma *(B)*	69.31	70.72 (6P,4B)*	2	4.6
A further diluted with plasma *(C)*	74	73.97 (6P,8B)*	0	0

* These numbers denote the number of separate samples of plasma (P) and whole blood (B) counted.

† The actual volume taken to be that obtained from the radioactivity measurements (third column).

Methods. The plasma content of whole blood was taken to be the ratio of the Ca^{45} count of 1 ml of whole blood to that of an equal volume of plasma of the same blood.§

Small volumes of high specific activity Ca^{45} solutions were mixed with 18 heparinized whole blood samples (17 dog, 1 human). Plasma samples were promptly removed. The plasma content of each sample of whole blood was measured by the hematocrit and calculated from the Ca^{45} concentrations of duplicate samples of whole blood and plasma. The mean results of the 18 samples, with standard deviations, as percentage of plasma in blood follow: 55.66 ± 4.80 (by hematocrit) and 61.60 ± 4.12 (by Ca^{45}). In no case was the plasma fraction read from the hematocrit greater than that obtained by calculation from the Ca^{45} concentrations. The mean plasma volume deficit by hematocrit was 2.94 ± 1.59% of the whole blood volume. These results allow the calculation that, on an average, 7.1% of the packed cell columns in the hematocrit tubes was plasma. One sample of dog blood was treated with I^{131} labeled albumin and with Ca^{45}. The plasma fraction was estimated by each of the methods with the following results: 53.25% (hematocrit), 56.45% (I^{131} albumin), and 57.38% (Ca^{45}).

§ The assumption is made, as in the case with I^{131} labeled albumin, that Ca^{45} added to blood remains in the plasma. This assumption is justified by the very low, probably zero, calcium content of red cells (10). We shall show, however, in a subsequent report that detectable quantities of Ca^{45} are removed from plasma by red cells after 24-48 hours.

Summary. Simplified methods for sample preparation for use in estimation of I^{131}, Ca^{45}, and Na^{22} in blood and plasma are described. These methods will permit the administration of minimum amounts of I^{131} labeled plasma proteins in studies of blood and plasma volumes. The degree of plasma trapping in the centrifuged hematocrit was determined from measurements of I^{131} labeled albumin and Ca^{45} concentrations in equal volumes of whole blood and plasma. With normal human and dog blood 7-9% of the packed cell column in the hematocrit tube is plasma. The amount of plasma trapped decreases with decreasing cell column height. The practical implications of the last observation are mentioned.

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