some equiperdum) somewhat longer than control animals(6). Ascorbic acid deficiencies in monkeys markedly prolonged the course of an otherwise rapidly fatal infection with *Plasmodium knowlesi*(7). Healthy well nourished fowl were found to be more susceptible to virus chicken sarcoma than thin and ill ones(16). In experimental Murine typhus infection of guinea pig, the starved animals showed considerably less fever and scrotal reaction(8). Undernourished rabbits were less reactive to vaccine virus(17), and vitamin deficient mice failed to show characteristic signs of infection with Western equine encephalitis(18). In children, the original observation of Heine that poliomyelitis seems to attack the better fed children with relatively higher incidence has been confirmed(18).

In the above cited cases it would seem that lack of nutrition has rendered the host a relatively unsuitable culture medium for the parasite. Perhaps the increased phagocytic ability of the starved animals, as demonstrated by the rats in the experiment here reported, is a factor in rendering the host more resistant to invasion by the parasite.

Conclusions. Leucocytes obtained from rats well fed for 4 weeks prior to a 36-hour period of starvation were compared with leucocytes from continuously well fed rats. The starved group showed twice the percentage of phagocytosing W.B.C. which had engulfed 3 times the number of bacteria.

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A Rapid Method for Clinical Total Blood Volume Determination Using Radioactive Iodinated Human Serum Albumin (RIHSA)* (18832)

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This paper reports a rapid method of directly measuring blood volumes using radioactive iodinated human serum albumin as a tracer agent. Human serum albumin is iodinated with I_{131} (RIHSA) in an essentially neutral buffer. Each cc of RIHSA† contains 5 mg of human serum albumin and 2 mg of salt(4). Metabolic studies of RIHSA have been carefully done and reported elsewhere(1,3). By the end of 24 hours after administration, approximately 55-65% is still in the circulation and since it is gradually metabolized, free I_{131} is liberated, and may be taken up by the thyroid. This can be successfully prevented by "blocking" the thyroid with stable iodine in the form of Lugol's solution, 10 drops, 3 times daily for a week, starting at least 24 hours previous to the administration of RIHSA(1). RIHSA was selected as a tracer agent for blood volume determination because it fulfills better than any other substance known at present, the basic criteria as laid down by Keith and Rowntree(2).

Fig. 1 shows the design of the 3-tube Geiger counter constructed for the blood volume determinations. The 3 tubes were held in place on a steel disc by a lucite plate. A thin wall brass tube was soldered in the center to hold

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† Obtained from Abbott Laboratories.

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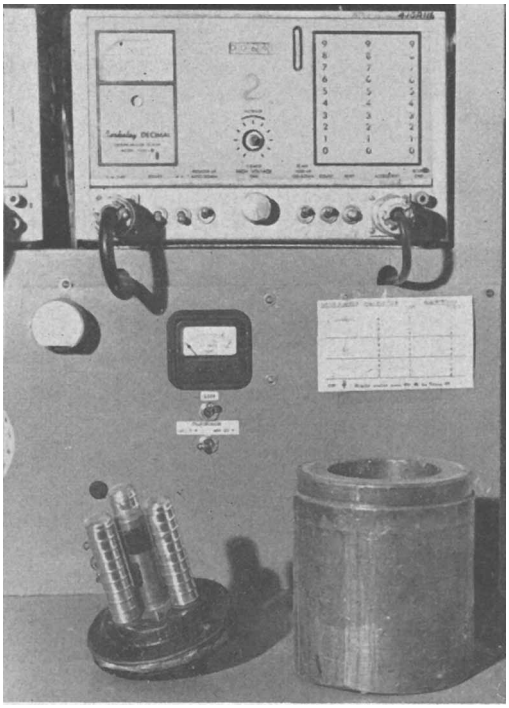

FIG. 1.

the cuvettes. This counter was mounted in the lead cylinder to reduce the background count. A five foot coaxial cable connects the counter to a Berkeley Scaler, Model 1000B, which is controlled by a predetermined time-interval clock. Coleman 6-304A cuvettes which accurately fit the central brass tube of the triple thyrode G-M counter were calibrated to contain 10 ml. Heparin solution was used as an anticoagulant; other anticoagulants have been investigated and found, as will be pointed out later, extremely unsuccessful.

The selection of a counting system was dictated by the requirements of rapid measurement of liquid samples with an accuracy of 1-2%, and a counting efficiency which would make feasible blood volume determinations with the tracer dose containing less than 60 μc of I_{131}. Construction of the counter made possible the direct comparison of this method with dye methods, since the same blood specimen and cuvettes were used for both technics. Thus the counting procedure was analogous to the colorimetric technics. The use of 3 Geiger tubes surrounding the central brass tube (which holds the cuvette) eliminates errors due to positioning the sample. The system was designed to count 10 ml of either blood or a standard "active solution" in a selected cuvette. The size of cuvette and position of the brass tube are such that the first 5 cc and the second 5 cc of solution gave equal contributions to the count. This eliminates, almost completely, errors introduced by sedimentation of the blood if this be allowed to occur. Measurements indicated this error is less than 1%.

Comparison of solutions of I_{131} in water and in blood indicated that the error of absorption, introduced by the change in the medium is less than 1%. In this study aqueous comparison solutions were used instead of making up these solutions with blood as the diluting medium. The sensitivity of the counter was 2820 counts per minute for 1 μc of iodine (point source) in the center of the tube or 2540 counts per minute per μc of I_{131} in 10 ml of solution (National Bureau of Standards distribution of 1-15-51). Efficiency of the counter was 0.11% for iodine in 10 ml of solution. (Not corrected for failure to achieve 4 π geometry). Substitution of bismuth cathode Geiger tubes‡ of the same size will increase this by a factor approximating 5. Comparison with a multiple anode cup counter§ indicated this counter would increase the sensitivity by a factor just exceeding 20, and would allow a considerable reduction in the required dose.

Procedure. From every batch of RIHSA delivered, a sterile tracer solution is prepared in such a dilution that 10 cc of it contains 60 μc of I_{131}. Of the tracer solution 1 cc is further diluted to 100 cc of which 10 cc is used as the standard. This standard therefore contains 0.6 μc which will give about 7500 counts per 5 minutes in the triple thyrode counter. Using calibrated syringes, each patient is given 10 cc of the tracer solution intravenously containing from 30-60 μc

‡ Constructed for us by Radiation Counter Laboratories.

§ Type H18-20TR (cup 20) supplied through courtesy of Dr. Herzog of Texas Company, Bellair, Texas.

I_{131} depending on the decay-factor; 10 minutes after administration, blood is withdrawn from the antecubital vein of the opposite side with a heparinized syringe and 10 cc immediately delivered into a selected cuvette. The cuvette is inverted several times to assure homogeneous suspension of the blood corpuscles. It is then put into the triple thyrode counter and counted for 5 minutes. The total blood volume can be calculated by the following formula:

$$\text{TBV} = \frac{\text{Counts/5 min of the standard} \times \text{No. of cc given of tracer solution}}{\text{Dilution of standard} \times \text{counts/5 min of the specimen.}}$$

In order to study the validity of the technic under investigation, T-1824 dye was used simultaneously in patients to study blood volumes. We have also studied plasma volumes with RIHSA technic in a group of patients. This was done by centrifuging about 30 cc of heparinized blood that had been withdrawn through the same venipuncture made for obtaining the 10-minute blood specimen. Centrifugation at 3000 RPM was carried out for 30 minutes and the clear supernatant plasma pipetted into the calibrated cuvette up to the 10 cc mark. The plasma activity is counted and plasma volume calculated in the same manner previously described. From the same blood sample, 2 Wintrobe hematocrit tubes were filled and centrifuged at the same speed together with the plasma specimens. From the blood volume and plasma volume figures obtained, "hematocrit" values can be calculated. These were compared with direct Wintrobe tube hematocrit values in the same patients giving further indices of accuracy.

Results and discussion. A total of 40 cases has been studied up to the time of this report. In all the cases, no effort was made to consider patient's body weight, and height. Since this group was made up of patients with various physiological and pathological conditions, any attempt to express their blood volumes, as determined by the RIHSA technic in terms of body weight will undoubtedly give false indices and therefore be misleading. For purposes of discussion they are separated into 2 groups, the first group consists of 8 cases in whom simultaneous blood volume studies with RIHSA and T-1824 dye were made. The results obtained and the percentage of difference between the two methods are tabulated in Table I.

TABLE I.

Cases	Blood vol I_{131} P technic	Blood vol Evans blue technic	Diff.
1	4120	4920	—16
2	4100	4080	± 0
3	4950	5320	— 7
4	4840	5900	—18
5	4840	5380	—10
6	4915	6250	—20
7	4970	5390	— 8
8	5210	5200	± 0

In case 6, a difference of 20% was observed, this was the highest percentage of difference in this series. In cases 2 and 8, however, virtually no difference existed between the results obtained. It is interesting to notice that almost all figures obtained with the Evans blue technic were higher than those using RIHSA. This finding agrees with those of other workers in the same field(3). It has been suggested that probably Evans blue diffuses out from the vascular system much more quickly than RIHSA. Assuming that all the figures in our series were obtained with the minimum technical error, it seems difficult to evaluate the validity of the technic by merely looking at the percentage of difference listed in Table I. In cases 2 and 8, the results obtained by two technics were almost identical. This is in disagreement with the theory that Evans blue diffuses out from the vascular bed more quickly and should result in a larger volume.

It was then decided to measure plasma volumes which, when calculated against blood volumes of the same patients, would give "hematocrit" values. These values were compared directly with Wintrobe tube readings. Comparison between these two should, for all practical purposes, give more accurate indices. We are justified in carrying out the comparison between the calculated and the actual hematocrit values based upon the following assumptions: (1) That the tracer solution is homogeneously mixed with the circulating blood in a practically closed system by the end of 10 minutes after its injection. This

TABLE II. Statistical Analysis of 30 Cases.

Range in B.V.	1642 to 6390 cc
" " P.V.	952 " 3625 cc
" " calc. HT	23.4 " 66.7
" " actual HT	25.5 " 67
" " % of diff.	−8 " +8

Mean diff. (actual-calc. HT) = +0.63%
Std deviation = ±3.95%

Cases	B.V.	Plasma vol	Hemato-crit calc.	Actual HT	% of diff.
5	5820	3100	47	69	−32*
6	4660	3500	25	43	−42*

* See text.

TABLE III.

	Total c/5 min	Absolute c/5 min	%
10 cc plasma + heparin	1577	1009	
10 cc plasma + Na-oxalate	1332	764	
Diff.	245	245	25

was found correct by counting serial blood specimens at 2-minute intervals up to 30 minutes. They were found to contain almost the same amount of radioactivity; (2) That the tracer material is present in plasma only, having no binding or tagging properties on the corpuscles. That is, a given volume of whole blood, actually is composed of 2 compartments, namely a radioactive plasma and a radioactivity-free corpuscular compartment. This was found correct and as will be shown later on, the calculated "hematocrit" and the actual "hematocrit" do actually agree in all cases.

Table II presents the second group of cases in whom the blood volume study was controlled by plasma volume and hematocrit studies. Note cases 5 and 6 in which an extreme difference between the calculated and actual hematocrit was observed. They were the two instances in which sodium oxalate was added as an anticoagulant agent in addition to merely heparinizing the syringes which were used to withdraw blood specimens. The diminishing of calculated hematocrit value was attributed to a false increase in plasma volumes resulted from technical loss of radioactivity from the plasma. Investigation was then started to study this problem and the following case will illustrate the difference in counts of the plasma specimen of the same patient using heparin and sodium oxalate, respectively (Table III).

It was found that approximately 25% of the radioactivity was missing from the oxalated plasma. Since these 2 specimens were treated under the same conditions except for the different anticoagulants used, it was felt that it must be due to Na-oxalate which probably acts on the radioactive compound in such a way that when the blood is centrifuged, the radioactive compound is spun down together with the blood cells. This was subsequently found to be true as indicated by the finding that 10 cc of saline suspension of the packed blood corpuscles from the specimen treated with Na-oxalate contained more radioactivity than that of the specimen treated with heparin. Na-citrate has also been tried and it has a similar effect on the relative distribution of the radioactive material in the centrifuged specimens. This observation is extremely important. It points out the tremendous error that could occur if blood volume determination is to be calculated from hematocrit and plasma volumes with the latter being determined by RIHSA using either Na-oxalate or Na-citrate as anticoagulant. In the technic described by Storaasli *et al.*(3), crystal heparin was used; no mention was made as to whether any other anticoagulants had been tried. However, in our technic for the direct determination of whole blood volume, it makes no difference as to the type of anticoagulant that is employed.

The advantages of direct blood volume determination using RIHSA are worth summarizing: (1) It is a very simple and rapid method; (2) only one blood specimen is required; (3) calculation is minimal, and (4) repeated blood volume determination is possible by giving further doses of RIHSA after determining the residual activity left in the blood stream.

Summary. (1) A clinical laboratory procedure for directly measuring total circulating blood volume using RIHSA as the tracer agent has been described. (2) A series of 40 cases in whom total circulating blood volume

was determined by this technic. (3) The advantages of this technic over the dye method have been discussed.

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Effect of Thyroidectomy on ACTH Content of Rat Adenohypophysis.* (18833)

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The site of formation of ACTH within the adenohypophysis has not yet been conclusively established. Finerty and co-workers (1,2), who observed a transient rise in the number of acidophils after hemiadrenalectomy in the rat, regard this class of cells as the source of ACTH. On the other hand, one of the rat's 2 types of pituitary basophils, the beta cell, has been suggested as the possible producer of the hormone(3). In these studies conclusions were reached by comparing pituitary cell counts with the state of the adrenal gland. It should be recognized, however, that the count of a given pituitary cell type, if due allowance is made for the size and density of granulation of the individual cells, reflects the storage of its secretory material, whereas the condition of the target organ is an index of the level of its governing pituitary principle in the circulation. As storage and discharge of pituitary trophins do not always run parallel, the comparison between the histological structure and the stored amounts of hormones in the pituitary appears to be a more promising approach to the problems of its histophysiology. Since thyroidectomy is known to lead to an extremely conspicuous drop in the number of both the acidophils and the beta cells, while the delta cells become progressively hyperplastic and vacuolated(3), it was of interest to determine how these profound histological changes affect the ACTH stores of the pituitary.

Method. A shipment of 22 young adult male Sprague-Dawley rats (180-200 g) was divided into 2 groups. Nine animals were thyroidectomized, 13 served as controls. The rats received Purina Laboratory Chow and tap water *ad lib.* Thirty days later the members of both groups were sacrificed by decapitation. At this time the thyroidectomized rats weighed 228-256 g (mean 248 g), the intact animals 288-337 g (mean 322 g). The pituitaries of 3 thyroidectomized and 4 control rats were saved for histological examination. The hypophyses of the remaining animals were prepared for a comparative assay of their ACTH content by the ascorbic acid depletion method of Sayers *et al.*(4). In view of the unstableness of ACTH in alkaline solutions(5), lyophilization and subsequent extraction of the glands with saline made alkaline to 0.01 N with sodium hydroxide(6) was abandoned in favor of a technic devised by Sayers(7). The anterior lobes were pooled in a solution of 0.85% sodium chloride made to 10th molar with hydrochloric acid (one pituitary per ml fluid), homogenized and extracted at room temperature. The extracts were transferred to a boiling water bath for 60 minutes,

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