Recording the rate of propagation of the pulse wave consists of the following. The pulse wave P proceeding along the artery A acts successively on the pulse recorders  $Pc_1$  and  $Pc_2$ , changing their electrical resistance. The passage of the pulse wave along the artery A is recorded, as tracings  $K_1$  and  $K_2$  on the photosensitive paper, with the aid of the corresponding vibrators  $B_1$  and  $B_2$ . At the same time, the time marker Tm records time intervals (0.1 seconds) on the same paper.

The rate of propagation of the pulse wave is derived from the tracings by the following calculation:

$$v_p = \frac{L}{t}$$
,

where  $v_p$  is the velocity of propagation of the wave, in milliseconds, L is the distance between the two counters, along the artery, in meters,  $\underline{t}$  is the distance, expressed as seconds, between the beginning of an upward wave in tracing  $K_1$  and of the corresponding wave in  $K_2$ , read from the number of time intervals  $t_0 = 0.1$  seconds.

Before beginning the recording, the vibrators should be so adjusted that the spot of reflected light thrown on the paper by each of them is on the same vertical line, perpendicular to its length. If this is not done, the ordinates of the pulse tracings will be shifted, relatively to each other, which would lead to considerable error in calculating the absolute value of the speed of propagation of the pulse wave.

Care should also be taken that the pulse waves recorded are of about the same amplitude, as otherwise it may be difficult to establish accurately the beginning of the upward part of the pulse waves. The amplitudes are regulated by adjusting the variable resistances in the control panels.

Greater accuracy is achieved by making 3-4 readings of  $\underline{t}$ , at different points of tracings  $K_1$  and  $K_2$ , and using the arithmetic mean for the calculation.

Pulse wave tracings may be taken from human subjects, using the radial, iliac, carotid, or temporal arteries, as well as the digital arteries of the hands and feet; the heart beat can also be recorded. It is thus possible to determine the rate of propagation of the pulse wave separately for different sections of the arterial system, or, if an oscillograph with a large number of vibrators is available, simultaneously for the whole system.

For animals, we usually determine the rate of propagation of the pulse wave along the section: origin of the aorta (from recordings of the heart beat or from the electrocardiogram) — anterior calcaneal artery.

## LITERATURE CITED

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## METHOD FOR DETERMINING THE CIRCULATING BLOOD VOLUME, FOR EXPERIMENTAL PURPOSES

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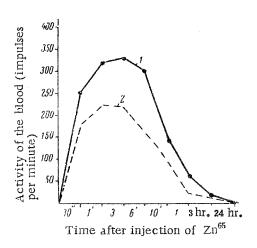
The circulating blood volume is one of the basic indicators of the condition of the hemodynamic function of the cardiovascular system. This volume is usually derived from measurements of the dilution of dyes introduced into the blood stream, usually Congo red or Evans blue. This technique cannot, however, be applied to the evaluation of circulating blood volume regularly over a prolonged period. The measurements cannot be repeated at shorter intervals than 6-7 days, since the dyes are retained in the blood for a long time.

There are also a number of methods for determination of blood volume based on gas analysis, but they are very time-consuming, and unsuitable for the execution of mass experiments.

A method for the determination of circulating blood volume was published by Wasserman, Joe, and Rashkof in 1951, depending on the use of radioactive phosphorus (P<sup>32</sup>). Blood is taken from an animal, centrifuged, and the erythrocytes are suspended in a solution containing P<sup>32</sup>. After a certain time the erythrocytes are separated and washed free of the solution, the specific activity of the packed cells is measured, and the cells are reintroduced into the blood stream, after suspension of an aliquot in physiological saline. Blood samples are then taken, and the circulating blood volume is evaluated from the degree of dilution of the labeled erythrocytes. This method is laborious and time-consuming. Morover, the lebeled erythrocytes persist in the blood stream for a long time, which interferes with repetition of the procedure.

It appeared to us that the procedure would be simplified if a solution of some radioactive isotope were to be introduced into the blood stream, instead of labeled cells. Our experiments showed that a radioactive isotope of zinc, Zn<sup>85</sup>, is applicable to the determination of circulating blood volume. Radioactive isotopes of other elements, such as phosphorus, iron and sodium are very rapidly eliminated from the blood stream after injection of small indicator quantities. Thus the amount of radiophosphorus found in blood samples 1-2 minutes after its introduction is so small that the circulating blood volume based on calculations of its apparent dilution is impossibly large. Radioactive zinc, introduced in indicator quantities, is retained in the blood stream for a much longer time. This permits the derivation of circulating blood volume from the dilution of injected Zn<sup>85</sup>.

A number of experiments were performed in order to ascertain the optimum times of sampling after injection of radioactive zinc. The results found for activity of samples of blood taken at different times after injection of  $Zn^{65}$  into an ear vein of a rabbit are shown in the Figure.



Activity of blood samples taken at various times after injection of Zn<sup>65</sup>. 1) Iterations in activity of 1 cc of blood after injection of 1 cc of Zn<sup>65</sup> solution of an activity of 49,400 impulses per minute; 2) the same, after injection two days after the previous experiments of 1 cc of Zn<sup>65</sup> solution of activity 34,509 impulses per minute, into the same rabbit.

As appears from the Figure, the highest Zn<sup>65</sup> contents are found in blood samples taken 1-3 minutes after injection. The activity of the blood falls considerably after 6 minutes, and it follows that radioactive zinc is being eliminated from the blood stream, and is practically undetectable after 24 hours. Blood samples should therefore be taken between 1 and 3 minutes after injection of the Zn<sup>65</sup> solution. We usually took a blood sample 2 minutes after the injection. This gives enough time for the thorough and uniform mixing of the injected solution with the blood, as is shown by the close agreement between the values obtained for circulating blood volume using our method, and using other generally approved methods.

For determining the circulating blood volume of rabbits we usually took an indicator solution containing 30-40  $\mu$  curies of Zn<sup>65</sup> per cc , while the solution for dogs contained 80-100  $\mu$  curies of Zn<sup>65</sup> per cc.

The procedure for determining the circulating blood volume of rabbits consists of the following. One cc of  $Zn^{65}$  solution is injected into an ear vein.

The activity of 1 cc of solution is determined before every injection. For this purpose 0.01 cc of solution is spread by means of a micropipette on a

slide, and its activity is determined, in impulses per minute. The background count is subtracted from that found for 0.01 cc of solution, and the result is multiplied by 100 to give the value for 1 cc.

Two minutes after injecting the solution 0.5-1 cc of blood is withdrawn from a vein of the other ear. In repeating the experiments it is necessary to inject solution and withdraw blood from the same ear as before. An even smear of 0.1 cc of blood, measured from a micropipette, is made on a slide, covering an accurately delimited area (we usually took an area of  $1 \times 4$  cm), marked on the slide with a glass pencil.

Two such smears are made, the activity of each of them is measured, and the mean value is taken for calculating the activity of 1 cc of blood. The activity found for 1 cc of Zn<sup>65</sup> solution is divided by the calculated activity for 1 cc of blood. The quotient gives the volume of circulating blood:

$$M = \frac{A}{a}$$
,

where M is the circulating blood volume in cc, A is the activity of the injected solution of  $Zn^{65}$ , in impulses per minute, and a is the activity of 1 cc of blood, in impulses per minute.

The following illustrates the calculation of the circulating blood volume of a rabbit, using the data presented in the Figure. The calculation is based on the activity of the blood sample taken 3 minutes after injection of  $Zn^{65}$  solution.

The data are: 0.01 cc of  $Zn^{65}$  solution gave 552 impulses per minute, and the background count was 58 impulses per minute, giving an activity for 1 cc of  $Zn^{65}$  solution (552-58)  $\cdot$  100 = 49,400 impulses per minute; 0.1 cc of blood gave 93 impulses per minute, at a background count of 60 impulses per minute, giving an activity for 1 cc of blood of (93-60)  $\cdot$  10 = 330 impulses per minute.

The circulating blood volume is:

$$M = \frac{A}{a} = \frac{49,400}{330} = 147.7 \text{ cc} \sim 150 \text{ cc}.$$

In repeating experiments on the same animal a blood sample was, for greater accuracy, taken before injection of radioactive zinc, and a smear was made of 0.1 cc of the blood; the activity of the smear served as the background count (it was usually identical with that of the counter itself). In most cases the activity of the pre-injection blood was zero.

The circulating blood volume of dogs is determined in the same way as for rabbits, except that injection of solution and taking of blood samples were performed on veins of the hind legs.

Determinations performed on large groups of animals gave values for circulating blood volume of 145-220 cc for rabbits, i.e., 5-6% of body weight; for dogs, the corresponding figures were 733-910 cc and 5-5.9%.

Our method determining circulating blood volume is simple, takes very little time, and provides the possibility of determining this important indicator of the state of the hemodynamic function of the cardiovascular system at daily intervals, and hence of studying the dynamics of changes in circulating blood volume.

## PHOTOGRAPHIC METHOD OF STUDY OF THE VASCULAR PERMEABILITY OF THE HEMATO-OPHTHALMICBARRIER AND THE SKIN

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The permeability of the vessels of the anterior portion of the eye has been studied either by registering entry of dyes into the anterior chamber, or by withdrawing aqueous humor and determining its optical density [1]. Amsler and Huber [2] have proposed a visual method, depending on determination of the amount of fluorescein entering the anterior chamber; the eye of the patient was examined with the aid of a split lamp. By varying the resistance in the lamp circuit the intensity of the light could be adjusted until the green fluorescence of the fluorescein was no longer discernable. The strength of current at this moment was proportional to the reciprocal of the fluorescein concentration in the aqueous humor. Kurt Lange and Linn Boyd [3] have devized a photoelectric method, for the study of the permeability of skin. Fluoresceinwas injected intravenously, the skin was