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^{65} 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

illuminated with a blue light, and the intensity of fluorescence was determined with the aid of a photoelectric cell. It has not yet been possible, however, to apply this method to the detection of the entry of the dye into the anterior chamber, because of the extreme difficulty of excluding the brilliant fluorescence of the eyelids and sclera from the instrument.

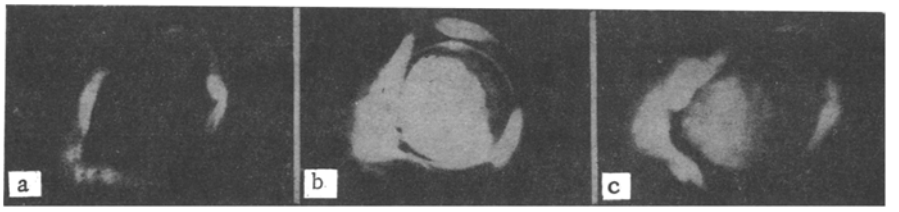

Fig. 1. Emergence of fluorescein into the anterior chamber of the eye. a) before injuring the Gasserian ganglion; b) 3 hours later; c) 48 hours after.

The above-described methods of registering emergence of dye into the anterior chamber of the eye are either insufficiently objective, or else they do not permit of a dynamic study of variations in the permeability of the hemato-ophthalmic barrier. For this reason we attempted to investigate the permeability of the blood vessels of the anterior portion of the eye by photographic registration of the intensity of fluroescence of fluorescein penetrating into the anterior chamber after intravenous injection into an animal (at a dosage level of 20 mg per kg body weight). The dye solution was made up as a 5% solution in saturated sodium bicarbonate.

A number of workers have used ultraviolet light from a PRK-4 lamp, with a Wood's filter, for exciting luminescence of fluorescein. It has been found, however, that this excites luminescence of the sclera and the crystalline lens of the eye, and this interferes with the quantitative evaluation of the intensity of fluorescence of the dye entering the anterior chamber. We therefore used a point-source flashlight 275 watt bulb, with a double thickness of SS-5 filter, which has maximum transmission for light of wave length 4250 A. A stabilized beam of light was directed on a rabbit's eye, at an angle of 45°. A Zenith camera was fixed rigidly at an angle of 90° to the beam of light, with the objective situated at a distance of more than 7 cm from the film, which made it possible to photograph the rabbit's eye at a short distance from it. Photographs of the emergence of fluorescein into the anterior chamber, as shown by the fluorescence excited by the blue light, were taken on ordinary Isopanchromatic film at fixed intervals of time after injection of the dye. Blue light reflected from the surface of the eye was absorbed by a yellow ZhS-18 filter.

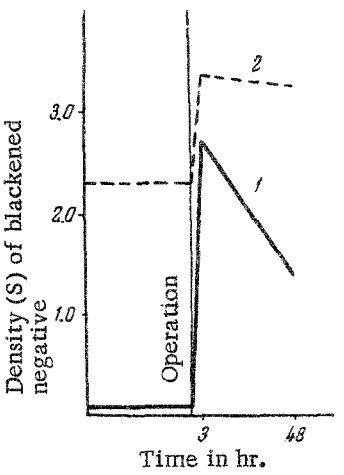

Fig. 2. Emergence of fluorescein into the anterior chamber of the eye. Derived from photodensity measurements of photographs. 1) Permeability of vessels of the anterior portion of the eye; 2) permeability of the skin.

In order to obtain comparable results, the photographs were taken on films of equal sensitivity, with a constant exposure time, measured by the strokes of an electric metronome. The films were developed under standard conditions with the developer solutions at the same temperature.

The density of the negative was measured with the aid of a MF-4 microphotometer, i.e., we measured the density S of the blackened parts of the film representing the image of the anterior chamber of the eye. These measurements were made at a number of points within the area concerned, as the intensity of fluorescence in the anterior chamber is not uniformly distributed. The mean values of S were then calculated, and served as a measure of the permeability of the vessels of the anterior portion of the eye to fluorescein. The values of S for standard solutions of fluorescein were obtained in an analogous manner, giving a factor for conversion of S values to percentage contents of fluorescein in the aqueous humor.

The proposed procedure for registering emergence of fluorescein into the anterior chamber allows us to follow

objectively the effects of various factors exerted on an intact animal on the permeability of the hemato-ophthalmic barrier. The same photographs can also serve for the study of the permeability of skin vessels; the values of S are determined for the hairless or almost hairless skin of the eyelids or around the eyes.

Figure 1 illustrates the course of change in permeability, as shown by photographs of fluorescence of a rabbit's eye, taken (a) a day before the Gasserian ganglion had been injured, (b) 3 hours later, and (c) 48 hours later. The photographs were taken 15 minutes after intravenous injection of fluorescein. Figure 2 presents the results of photometric treatment of the negatives. The degree of blackening of the relevant part of the negative (S) is expressed as the logarithm of the ratio of the intensity of light passing through the background parts of the negative to that passing through the exposed part.

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INTERPRETATION OF HUMAN PLETHYSMOGRAPHIC DATA

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The shortcomings of the techniques employed in the plethysmographic examination of human subjects emerge clearly from a study of the subject.

In particular, insufficient attention has been paid to the nature, magnitude, and frequency of distortions of the plethysmograms, due to movements of the subject. V. V. Yakovlev has shown, in experiments on dogs, that effects interpreted as plethysmographic reactions were in reality due to tonic contractions of the muscles of the extremity.

O. Bruns [12] has reported similar occurrences. It appeared that the subject (plethysmography of the arm) is usually unaware of contractions of his muscles such as can give a spurious vascular effect. From a comparison of plethysmographic tracings with those obtained as a result of muscle contractions registered in response to various stimuli (flexing and straightening the foot, pain, heat, cold), O. Bruns came to the conclusion that the use of plethysmography is justified only for the study of temperature effects, and even then only after special training of the subjects, who were enabled, by means of a separate device, to become conscious of "unconscious" movements of the arms, and to control them.

It should be noted that O. Bruns' observations were made chiefly with the aid of rubber bulbs in contact with the arm, and connected with the registering system (he makes a passing mention of the use of a galvanometer for this purpose, by one of his co-workers). It follows that some of the weaker movements, which might have had a distorting effect on the plethysmograms, were not recorded.

The object of the present research was to ascertain the effect of weak muscular contractions on a plethysmogram, obtained by one of the present-day techniques.

EXPERIMENTAL METHODS

Plethysmographic records were taken from the terminal phalanx of a finger of the left hand of 22 subjects, by the method of pneumatic finger plethysmography.

A glass cap is placed on the phalanx, and is connected by a rubber tube with a special micromanometer (1), fitted with a small mirror for photoregistration of the smallest pressure changes in the system (the micromanometer was mounted in the place of the vibrator of the oscillograph.