METHOD OF RECORDING THE MINUTE VOLUME OF THE HEART IN RABBITS BY MEANS OF P^{32}

(UDC 612.171.1-088.9:546.18.02.32)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 11,
pp. 118-121, November, 1965
Original article submitted January 22, 1965

Among the methods of determining the minute volume of the heart those which are based on a measurement of the degree and rate of dilution of foreign substances—dyes and isotopes—in the blood are acknowledged to be some of the most accurate [3, 5, 8].

The use of methods employing β - and γ-radiators, especially when working with small laboratory animals, has its difficulties. Thus, the use of β-radiators which requires numerous and frequent sampling of the arterial blood is limited because the blood samples must be taken at intervals that are shorter, the smaller the animal. For the rabbit this interval should not exceed 0.5 sec, which is difficult to accomplish. Furthermore, frequent venesection changes the minute volume and this introduces an error into the determination [7]. The use of γ-radiators requires careful collimation of the counter for cutting off the flux of quanta issuing from the place of injection of the isotope and from other regions of the vascular system, which is practically unachievable when working with small and medium-sized laboratory animals.

These considerations forced us to use β-radiators having a spectral hardness sufficient for recording through an undamaged vessel. The phosphorus isotope P^{32} completely satisfies these demands.

Our method requires that we obtain the curve of the change in the concentration of the intravenously injected isotope at a certain point on the arterial canal. The character of mixing of the isotope with the blood determines the rapid increase and slow drop off of activity at the given point. As a rule a secondary increase of activity occurs before the first cycle of passage of the isotope past a given point is completed. Hamilton et al., [4] proved in model experiments that the drop off of activity after its first maximum is governed by the exponential law. This permits us to find the continuation of the curve of the first drop off up to the intersection with the time axis by extrapolation of the curve on semi-logarithmic paper. The base of the obtained figure is the time of the first cycle of passage of the isotope past a given point of the arterial canal or some other time during which the heart is free from all the isotope injected into the vein. The volume of the solution ejected by the heart during this time is easily calculated if we know the original quantity of solute and its end concentration. Since the quantity (activity) of the injected isotope is known, it remains to find its concentration in an unknown volume of blood ejected by the heart during the time found. This concentration (the average concentration of the isotope in the blood flowing past a given point of the vessel during the first cycle of passage of the isotope) is equal to the quotient from dividing the area of the obtained figure by its base. For a further calculation of the minute volume MacIntyre's formula is used [6], to which we will return later.

To ensure long retention of P^{32} in the vascular stream, we used the method of labelling erythrocytes with radiophosphorus. This made it possible, simultaneously with determining the change of concentration of the isotope in the vessel, to obtain data on the volume of the circulating blood which were needed for the final calculation of the minute volume. Control measurements showed that the radioactivity of the blood is kept at a constant level for about an hour. The drop in activity after an hour usually did not exceed 5%. According to our data the optimal period for a uniform distribution of the labeled erythrocytes in the blood is 10 min after the injection of the isotope

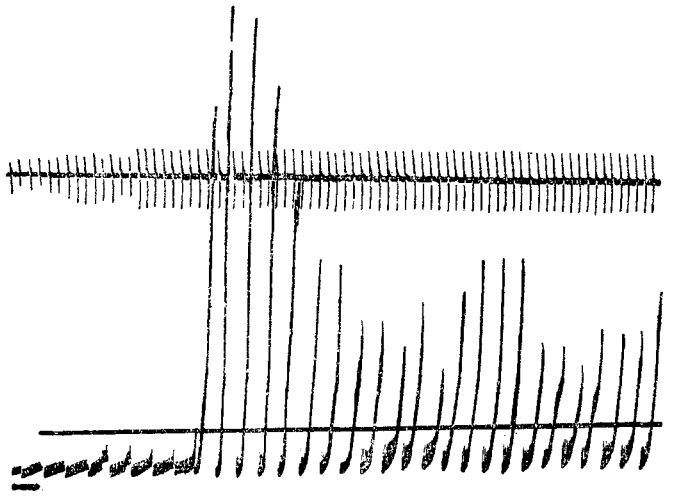

Fig. 1. Recording of the change of radioactivity of the blood over the femoral artery (with the use of an integrator and simultaneous recording of the EGK). The interruption of the base line is the instant of injecting the suspension of labelled erythrocytes into the jugular vein.

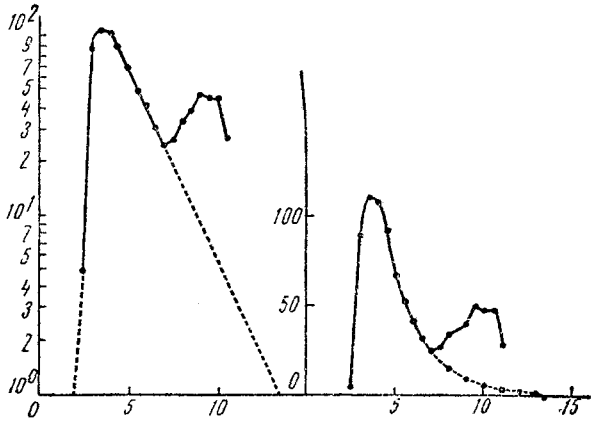

Fig. 2. Principle of extrapolating the data shown in Fig. 1. Left) Semilogarithmic scale; Right) ordinary scale. On the x-axis is the time in sec; on the y-axis is the height of the deflection of the trace of the galvanometer in mm. Explanation in text.

into the vascular stream. In this case the error in calculating the volume of circulating blood does not exceed the error of the method.

METHOD

In rabbits under urethan narcosis (1 g/kg) we exposed the jugular vein and the femoral artery. Above the latter we placed a BFL-25 end-window counter fastened on a flexible hose and special hinged device which enabled us to place it in the necessary position. The counter was arranged so that at the maximal approach of the mica window to the vessel surface, the vessel was not compressed by the metal rims surrounding the window. In our experiments the usual distance of the counter window from the vessel surface was 1 mm. Into the jugular vein we injected a suspension of labelled erythrocytes at a rate of about 5-7 μCi per 1 kg of rabbit weight. The total volume of the injected suspension should not exceed 0.1-0.12 ml, otherwise the time of the first cycle of passage of the isotope can be too long which makes its accurate determination by extrapolation impossible. The injection was made by means of a tuberculin syringe to which we attached contacts for automatic recording of the instant of injection on an oscillograph. The recording was made on photographic paper at a constant rate of 1 cm/sec for 20 sec after injection of the labelled erythrocytes. The recorder was again switched on after 10 min, and the radioactivity of the blood was recorded over the femoral artery for 10 sec. After this we took 0.1 ml of blood from the marginal vein of the rabbit's ear for preparing the target. During the entire experiment the electrocardiogram was recorded.

To avoid recording each impulse, which would greatly hamper subsequent analysis of the recordings, we worked out a scheme,and with the help of engineer K. N. Kulikov,constructed an integrator which sums the individual impulses during a specific time. The fixed time constant in our experiments was 0.5 sec. Such a small time

Cardiac Output of the Rabbits (in ml per 1 kg of weight)

Author and year of investigation	Minute volume	Method of investigation
Legaard, 1926 [2]	121.0	Fick's principle
Fasold and Hartle, 1928 [2]	93.8	
Meyer et al., 1949 [2]	122.0	
	175.0	Dye dilution method
Korner and Smith, 1954 [2]	218.0	
Edwards et al., 1959 [2]	175.0	
	213.0	
	218.0	
Cumming and Nutt, 1962 [1]	97.0	Fick's principle
Malvaux et al., 1960 [7]	325.9	Radiometric (I^{131})
M. Éster and V. I. Kandror, 1964	266.7	" (P^{32})

constantly necessitates the use of the aforementioned comparatively high radioactivity (5-7 μCi). Otherwise the error arising as a consequence of nonuniformity of radioactive decay can greatly lower the accuracy of the determination.

The operating principle of the integrator reduces to the following. The electrical impulses from the B-2 counter system are accumulated at the capacitor, the charge of which is transmitted to a galvanometer through an amplifier. The amplitude of the trace of the galvanometer increases as the charge increases. At precisely every 0.5 sec the plates of the capacitor close and the trace of the galvanometer returns to the zero line. The entire recording when using the integrator fits on a paper 20-30 cm long, which greatly facilitates its decipherment (Fig. 1).

To decipher the recording, the amplitudes of the deflections of the trace (in millimeters) were transferred as points to semi-logarithmic paper, and then by extrapolation we found the entire curve of the change of isotope concentration during the first cycle of circulation (Fig. 2). Using a planimeter we determined the area bounded by this curve and by a segment of the x-axis corresponding to the time of the first cycle of isotope circulation. The quotient from dividing the area of the obtained figure by its base (in millimeters) gives the value corresponding to the average concentration of the isotope during the first cycle of circulation. The minute volume (MO) was calculated by the formula:

$$MO = \frac{C_g \cdot V \cdot 60}{c \cdot T},$$

where C_d is the activity of the blood after complete mixing of the isotope (10 min after injecting the labelled erythrocytes); V is the precalculated volume of the circulating blood; c is the average isotope concentration during the first cycle; T is the time of the first cycle of isotope circulation (in sec).

It is important to provide complete linearity of the dependence of the trace amplitude on the radioactivity value (i.e., ultimately, on the value of the capacitor charge), since only in this case can we avoid the need to express C_d and c in absolute figures of isotope concentration (which requires additional cumbersome calculations) and substitute into the formula the corresponding values in millimeters. To preclude the error arising as a consequence of nonuniformity in radioactive decay, we calculated the values of all trace amplitudes for 10 sec of the recording, after complete mixing of the isotopes with the blood. The arithmetic mean value was substituted into the formula.

By means of this method we determined the minute volume of the heart of 20 healthy male Chinchilla rabbits weighing from 2300 to 3620 g. The value of the minute volume in our determinations varied from 694 to 800 ml, averaging 763±9.2 ml. The standard deviation was 41.3, the coefficient of variation was 5.4%. On converting the minute volume to 1 kg of animal weight we obtained the following values: the limits of variation were 221-300 ml, the arithmetic mean and mean error was 266.7±5.8, the standard deviation was 26, and the coefficient of variation was 9.8%. For the degree of confidence (P = 0.05) the confidence interval was 255.1-278.3 ml/kg. It is characteristic that the amount of blood ejected by the heart per 1 kg of animal weight proved to be progressively lower as the rabbits' weight increased. Thus, for animals weighing up to 3 kg, this value averaged 282.7±3.3 ml/kg, whereas for animals weighing more than 3 kg the average was 237±3.3 ml/kg. The significant difference was quite high ($P < 0.001$).

The table shows the values of the minute volume of the heart obtained by different methods by various authors. Without going into an analysis of the causes for such an appreciable divergence of data obtained by different authors, we note that Fick's principle gives markedly underestimated results in comparison with the dye dilution and radiometric methods, whereas our data differ little from the results of the determination carried out by other authors by means of analogous methods.

The value of the proposed method is increased in connection with the possibility of simultaneous recording of a number of other hemodynamic values. The parallel determination of the volume of the circulating blood was already mentioned. The incubation time can be appreciably reduced by using a microelectromotor set in the stopper of the test tube for incubating the erythrocytes in a solution of P^{32} and rotating a stirring rod during the entire incubation period. Furthermore, the placement of contacts on the syringe, which permits one to objectively record the instant of injecting the suspension of labeled erythrocytes, makes possible the determination of the linear rate of circulation at a given section of the vascular stream. Another favorable aspect of the proposed method, which does not require incising and ligating the arteries, is the possibility of many repeated determinations of the cardiac output in the same animal, which is extremely important for dynamic investigations. Our experiments showed that a week after the first determination the residual radioactivity of the blood is not an obstacle to a redetermination.

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