The low percentage of detached endothelial cells obtained by the use of MC can evidently be explained by the fact that the molecule of this enzyme contains a Ca^{++} ion [7]. In the presence of EDTA the ion of this metal is extracted from the active center, with the formation of an apoenzyme, which leads to inactivation of MC. In contrast to MC, CHC activity was unchanged in the presence of 2.4 mM EDTA or 2.5 mM o-phenanthroline. Ca^{++}, Cd^{++}, Mn^{++}, Mg^{++}, and Hg^{++} ions in a concentration of 25 mM likewise have no appreciable effect on CHC activity. Taken together, these data explain the high efficacy of CHC for the removal of endothelial cells from the substrate.

Trials of the CHC preparation showed that it is virtually not inferior in collagenolytic activity to collagenase obtained from the firm "Worthington" and is suitable for work with cultures of endothelial cells — one of the most delicate and "capricious" test objects. This preparation can be used on a large scale, because the method of its preparation is exceedingly simple and inexpensive, and what is no less important, it does away with the need to work with a pathogenic raw material.

LITERATURE CITED

1. S. M. Danilov, E. Y. Allikmets, and A. V. Martynov, J. Cell. Biol., 99, 274A (1984).
2. A. Z. Eisen, K. O. Henderson, J. J. Jeffrey, and R. A. Bradshaw, Biochemistry, 12, 1814 (1973).
3. E. A. Jaffe, R. L. Nachman, C. C. Becker, and C. R. Minick, J. Clin. Invest., 52, 2745 (1973).
4. E. A. Jaffe, Biology of Endothelial Cells, ed. by E. A. Jaffe, Boston (1984), p. 1.
5. C. J. Kirkpatrick, I. Melzner, and T. Goller, Biochim. Biophys. Acta, 846, 120 (1985).
6. T. Maciag, Proc. Nat. Acad. Sci. USA, 76, 5674 (1976).
7. S. Seiffer and E. Harper, Methods Enzymol., 19, 613 (1970).
8. D. G. S. Thilo-Korner, D. Heinrich, and H. Temme, The Endothelial Cell — A Pluripotent Control Cell of the Vessel Wall, ed. by D. G. S. Thiol-Korner and R. I. Freshney, Besel (1982), p. 158.

A METHOD OF STUDYING RESPONSES OF SINGLE VESSELS IN SITU

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A very important parameter of the functional state of blood vessels and of changes in that state is hydraulic resistance. This can be calculated by known equations [2], given the geometric dimensions of the vessel, the viscosity of the blood, and the character of its flow. To record changes in the diameter of blood vessels various methods have been developed, based on the use of contact diameter transducers [4], miniature ultrasonic transducers fixed to the vessel wall [9], television systems [10], and x-ray or ultrasonic angiography [8]. Some of these methods are applicable only to superficial vessels or they require quite traumatic dissection, whereas others cannot guarantee sufficient accuracy of measurement of the internal diameter of blood vessels under 1 mm in caliber. An alternative method of determining resistance is based on measurement of the pressure drop on the vessel and the blood flow along it [7].

The method we have developed consists essentially as follows. Blood flowing along the vessel is passed through a standard hydraulic resistance (standard) and returned to the

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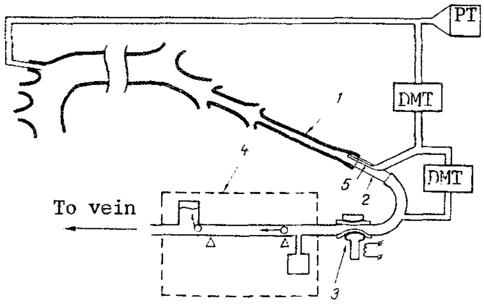

Fig. 1. Scheme of experimental method. PT) Pressure transducer, DMT) differential manometric transducer. Diameter of cannula 2 is 180 μ, that of cannula 5 is 480 μ. Explanation in text.

animal. The ratio of the pressure drop on the vessel (ΔP_1) to the pressure drop on the standard (ΔP_2) is the ratio of the resistance of the vessel (R_1) to the standard (R_2). In fact, $\Delta P_1/\Delta P_2 = QR_1/QR_2 = R_1/R_2$, where Q is the blood flow along the artery and through the standard. Moreover, since the pressure drop both on the vessel and on the standard is proportional to the viscosity of the blood, the ratio between them can be regarded as a parameter of the relationship between the geometric component of vascular resistance and the corresponding parameter of the standard.

A scheme showing the method of realization is given in Fig. 1. Blood from the artery 1 enters an inextensible plastic tube 2, passes through the electromechanical throttle 3 of the flow control unit, and discharges into the vein 4. The pressure drops along the vessel and tube are measured by differential manometric transducers. Signals from the latter are amplified and led to a voltage divider. A signal directly proportional to the ratio between the pressure drops is led to a recorder as parameter of the geometric component of resistance of the artery.

Let us dwell in more detail on individual elements of the measuring system. The blood flow control unit either stabilizes the flow or changes it in accordance with the signal from the master unit 5. The signal of the pressure drop on the standard resistance, which is an indicator of the blood flow, is led to one input of an operating amplifier. The voltage from the master system 5, which consists of a controllable voltage source or functional generator, is led to the second input. The mismatching signal is integrated and led to the electromechanical throttle 3. This compresses the polyvinyl chloride tube by the corresponding degree, and thereby regulates the resistance to the outflow of blood from the test artery into the vein. The amplification factor in the control system is chosen so as to ensure the decay time of the transition process of about 1 sec.

The differential manometric transducers are based on DMI-0.1 transducers. The chambers of the latter are filled in vacuo with polymethylsiloxane PMS-6 to increase the bulk rigidity. The intrinsic frequency of the transducer with the polyethylene catheters (length 20 cm, internal diameter 2 mm) connected to it and filled with water, was about 20 Hz. The sensitivity of the system consisting of transducer and 4ANCh-22 strain-gauge amplifier, at maximal amplification, was 2 V/0.1 Torr. The temperature drift, relative to the input, did not exceed 0.03 Torr/°C.

The voltage divider is based on one channel of a KSPP-4 potentiometer using the circuit of an electromechanical divider [1]. To measure the pressure drop on the artery, thin-walled polyethylene catheters were introduced into the branches leaving the artery, by a method described by the writers previously [5]. Examples of the use of the method are given below. Wistar rats weighing 250-300 g were anesthetized with pentobarbital (50 mg/kg, intraperitoneally, and thereafter every hour in a dose of 15 mg/kg, also intraperitoneally). After the end of dissection of the vessels hepain (1500 U/kg, and thereafter 150 U/mg hourly) was given intravenously. The scheme of the experiment is shown in Fig 2. Blood passes along the polyethylene cannula introduced into the distal end of a. saphena, into the standard resistance, flows through the electromechanical throttle of the flow control unit,

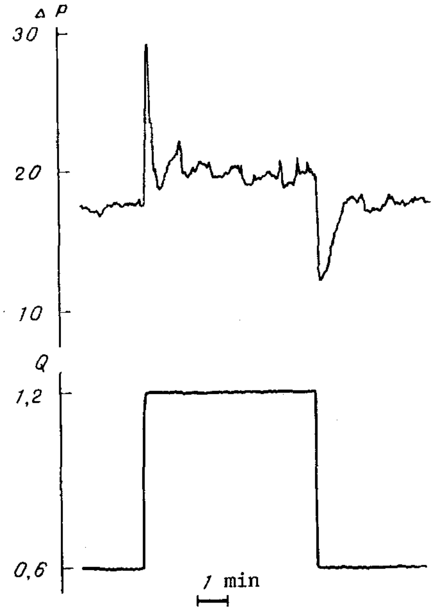

Fig. 2

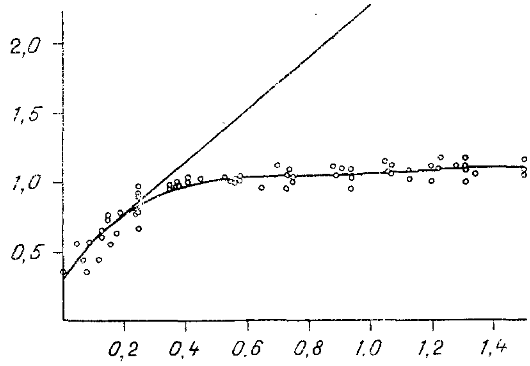

Fig. 3

Fig. 2. Changes in pressure drop (ΔP, mm Hg) between arch of aorta and distal end of a. saphena during stepwise changes in blood flow (Q, ml/min).

Fig. 3. Averaged curve showing dependence of pressure drop between arch of aorta and distal end of a. saphena during a slow (in the course of 600 sec) increase of the blood flow with a constant rate of 0.1 to 1.5 ml/min. Abscissa, blood flow in a. saphena (in ml/min); ordinate, normalized pressure drop between arch of aorta and distal end of a. saphena (in relative units). Individual curves normalized for pressure drop with blood flow of 0.5 ml/min. Straight line with slope of 36 mm Hg/ml/min corresponds to relationship between pressure drop and blood flow with a rapid (in the course of 3 sec) increase in the latter from 0.1 to 1 ml/min. The pressure drop with a blood flow of 0.5 ml/min was 19 ± 1 mm Hg ($M \pm m$, $n = 14$).

and then is directed into the femoral vein. To measure the pressure drop on the region of the vascular bed to be investigated, catheters were introduced into a. epigastrica superficialis and into a branch of a. saphena.

A. poplitea and two small branches of a. saphena were ligated. In this way the total resistance of a. saphena and of a small part of a. femoralis was recorded. However, since the diameter of the latter was about 1.5 times greater, and its length 4-5 times less than that of a. saphena, its contribution can be disregarded. The pressure in the arch of the aorta was measured by a W-102 electromanometer (RFT, East Germany), by means of a catheter passed through the subclavian artery. Values of the pressure in the aorta, the pressure drop on the artery, the resistance of the artery, and the blood flow through it were recorded on KSPP-4 two-channel potentiometers.

A trace of the response of the artery to injection (through a. epigastrica superficialis) of 10 μl of physiological saline containing $2 \cdot 10^{-5}$ g/ml of noradrenalin hydrotartrate (dose 0.2 μg) into it in the course of 2 sec, is shown in Fig. 3a. It can be concluded from this trace that constriction of the artery took place, and as a result its resistance was increased by 5.5 times.

The response of the artery to an increase in blood flow through it is shown in Fig. 3b. After an increase in the blood flow the resistance of the artery begins to fall, and quite considerably. This is evidently due to the fact that this artery, like the arteries of cats and dogs [3, 6], is sensitive to flow and can dilate in response to an increase in it.

The use of the method described above experimentally showed that it possesses the following advantages: 1) it enables responses of both large and comparatively small (to

0.3 mm) blood vessels to be studied in situ; 2) it is possible to study virtually intact vessels, for there is no need to isolate them from the surrounding tissues, or to separate the artery from more proximal regions of the vascular bed; 3) the animals' own blood flows along the vessel, thus ruling out any changes in reactivity of the vessel which are ordinarily observed during perfusion with blood substitute solution; 4) the integral value of the response along the whole length of the vessel is measured, and in many cases it is this which is interesting; 5) changes are recorded in the course of the response only of the geometric component of the hydraulic resistance of the vessel, and changes in viscosity of the blood have no influence; 6) the blood flow control unit enables physiological values of the blood flow in the vessel to be assigned when studying the action of drugs, but when the effect of hemodynamic factors on vascular tone is investigated, the necessary changes in blood flow can be provided.

LITERATURE CITED

1. R.-P. P. Zhilinskis, Ratio Meters and their Use in Radiometric Techniques [in Russian], Moscow (1975).
2. C. G. Caro, T. J. Pedley, R. C. Schroter, and W. Seed, Mechanics of the Circulation, Oxford (1978).
3. A. M. Mel'kumyants, E. S. Veselova, and V. M. Khayutin, Byull. Éksp. Biol. Med., No. 8, 7 (1981).
4. A. N. Rogoza, Byull. Éksp. Biol. Med., No. 5, 626 (1981).
5. A. N. Rogoza and V. P. Nikol'skii, Byull. Éksp. Biol. Med., No. 3, 380 (1985).
6. V. Smieshko, V. M. Khayutin, M. Gerova, et al., Fiziol. Zh. SSSR, No. 2, 291 (1979).
7. V. M. Khayutin and A. N. Rogoza, Methods of Investigation of the Circulation [in Russian], Leningrad (1976), pp. 51-63.
8. R. M. Olson and D. K. Shelton, J. Appl. Physiol., 32, 147 (1972).
9. H. Tomoike, H. Ootsubo, and K. Sakai, Am. J. Physiol., 240, H73 (1981).
10. C. A. Wiederhelm, Fed. Proc., 24, 1075 (1965).