i.e., significantly less. An increase in the accuracy of determination of the gravimetric index for z.g. can be attained by allowing for the theoretically expected error which, according to our calculations, when the ratio between the areas of maximal cross section coinciding with the largest and smallest axes was 1.1-1.25, is 3%; if the ratio is 1.26-1.49 the error is 4%; and if the ratio is 1.5-2, it is 5%. Under these circumstances the adrenals must lie in a strictly assigned position when cut into sections. If the maximal plane of section coincides with the longest axis the sign of the error will be plus; if it coincides with the shortest axis, it will be minus.

Mathematical analysis of the plasticine model of a spherical adrenal gland and the ratio between the gravimetric indices of the component elements similar in actual relative proportions to the adrenals of sexually mature female rats thus revealed that the method of quantitative determination of gravimetric indices of the adrenal cortical zones and medulla is particularly accurate for measuring the weight of z.f.r. and z.g., and less accurate for determining the weight of the medulla. This method has some disadvantages. However, in the writer's view its use will give results closer to the actual values than those obtained on the basis of any other morphological feature, such as the width or area of the zones of the adrenals.

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973).
2. G. G. Avtandilov and I. S. Kruglova, Arkh. Patol., No. 12, 76 (1975).
3. K. Kovacs, E. Zambo, and F. Laszlo, Z. Mikrosk. Anat. Forsch., 79, 413 (1968).

THE TECHNIQUE OF RESISTOGRAPHY

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UDC 612.13-087.5

To abolish the error in resistography connected with fluctuations in the animal's arterial pressure an automatic stabilizer of the input pressure (an electromagnetic valve) is suggested. To switch off the resistograph pump automatically when the blood flow into the apparatus is obstructed or stops, a monitoring device is proposed. A pressure stabilizer and monitoring device are controlled by electrical contact mercury manometers connected to the input channel of the resistograph.

KEY WORDS: resistography; design of the resistograph.

Even if all the requirements of a resistograph with external electromagnetic valves are strictly met [3], the possibility that its flow rate depends on the input pressure of the apparatus cannot be ruled out. This leads to a definite error which, according to data given by different workers [1, 3], may be 5-8%.

To reduce the error it is suggested that the external electromagnetic valves be replaced by internal valves [1], which can reduce the error in a single-channel resistograph to 1-1.5% and in a two-channel instrument to 2-3%. From the design point of view, internal valves, working compulsorily [1], are much more complicated than external electromagnetic valves, and it is particularly difficult to ensure that they operate in phase with the pump of the instrument in the extension working head. Disadvantages of internal "automatic" valves have been examined in detail by Khayutin [2, 3].

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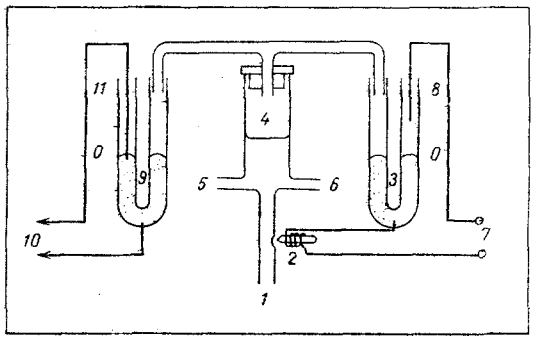

Fig. 1

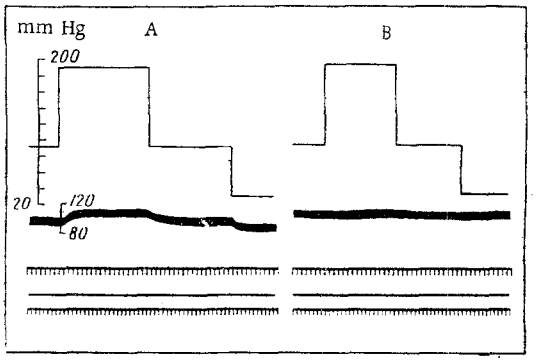

Fig. 2

Fig. 1. Electrical circuit of monitoring system of resistograph (explanation in text).

Fig. 2. Resistogram recorded at different levels of pressure in inlet of resistograph. A) Before switching on pressure stabilizer, B) after switching on stabilizer. From top to bottom: pressure at inlet of resistograph; resistogram; flow of fluid (distance between marks 1 ml); marker of stimulation; time marker (5 sec).

To abolish the error of the resistograph it is suggested that the pressure at the input of the instrument be stabilized (Fig. 1). For this purpose, an electromagnetic valve 2, controlled by an electrical contact mercury manometer 3, is fitted to the rubber tube 1 connecting the inlet of the resistograph to the artery. The manometer is connected by an air pipe to the chamber 4 which supplies blood directly to the channels of the resistograph 5, 6. The electromagnetic valve 2 is connected to a power supply 7.

The electrode 8 is set at a definite level above 0. The distance between this electrode and the column of mercury corresponds to the blood pressure at the inlet of the resistograph. When the column of mercury is not touching the electrode the valve 2 opens and blood enters the chamber. The pressure in the chamber is transmitted along the air pipe to the manometer and the column of mercury is raised toward the electrode. When contact is made between the mercury and the electrode, the electromagnetic valve is actuated and the supply of blood to the chamber stops. As the blood is used up, the pressure in the chamber and, correspondingly, the pressure in the manometer fall, so that contact is broken and the electromagnetic valve switched on. This simple device keeps the pressure in the chamber stable; fluctuations are possible only within the range of 1-3 mm Hg.

By raising or lowering the electrode 8 in the manometer the system of the stabilizer can be adjusted to any level of pressure at the inlet to the resistograph. Naturally this level must not exceed the animal's blood pressure. If a considerable fall of blood pressure is a possibility during an experiment, the stabilizer must be adjusted appropriately beforehand.

The error of a resistograph due to a change in pressure at the inlet of the instrument (A) and the correction of this error by means of the suggested stabilizer (B) are illustrated in Fig. 2.

To monitor the flow of perfusion fluid a flowmeter is connected to the outlet of the resistograph and its readings agreed with the calculated data (frequency of oscillation of the pump multiplied by the stroke volume).

In experiments using resistography, sometimes obstacles arise to the entry of blood into the inlet of the instrument. This can happen as a result of a sharp fall in the systemic arterial pressure and collapse of the artery supplying blood to the instrument. Under these circumstances the reserve blood in the chamber is used up quickly and there is the risk of aspiration of air from the chamber and embolism of the perfused vessels. The same situation may arise during thrombus formation in the cannula, displacement of the cannula relative to the artery, or accidental compression of the artery.

To prevent embolism in such cases the use of an additional electrical contact mercury manometer 9, the electrodes 10 of which are included in the circuit of the electromagnetic valves of the pump, is suggested (Fig. 1). The warning electrode 11 must touch the mercury at level 0, or 2-3 mm below 0. If the entry of blood to the inlet of the resistograph is obstructed a negative pressure is created in the chamber and this is transmitted along the tube to the manometer. The column of mercury falls below zero and breaks the circuit supplying the electromagnetic valves of the pump with current, and they are switched off. The monitoring

system suggested gives timely warning of the inadequate supply of blood to the inlet of the resistograph, and the pressure stabilizer at the inlet of the instrument enables its error to be eliminated.

LITERATURE CITED

1. B. I. Tkachenko, D. P. Dvoretskii, V. I. Ovsyannikov, et al., Regional and Systemic Vasomotor Responses [in Russian], Leningrad (1971).
2. V. M. Khayutin, Farmakol. Toksikol., No. 3, 78 (1958).
3. V. M. Khayutin, Fiziol. Zh. SSSR, No. 7, 645 (1958).

MOLECULAR WEIGHT FRACTIONATION OF PROTEINS WITHIN THE RANGE 10^{-7}-10^{-8} g BY MICROGEL CHROMATOGRAPHY

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A method of microgel chromatography of proteins on a Sephadex G-75 (superfine) column with a working volume of 110-150 μl is described. By means of the method molecular weight fractionation of proteins can be carried out within the range 10^{-7}-10^{-8} g in the course of 45-60 min.

KEY WORDS: microgel chromatography; proteins.

The use of gel chromatography on the macroscale for the molecular weight fractionation of proteins and enzymes of tissue microhomogenates (1-5 mg) is difficult for the following reasons: 1) because of dilution of the small amounts of protein on the column to concentrations which cannot be recorded by the ordinary optical instruments; 2) the considerable volume of the eluted fractions, containing a small quantity of radioactivity, makes radiometric measurements in an anhydrous scintillator difficult. These difficulties can be overcome if the procedure of protein chromatography is converted to the microscale, as was first suggested for fractionation of nucleic acids within the range 10^{-8}-10^{-9} g [1, 2]. The same workers suggested a special technique for measuring the optical density of the microeluate and this was used in the present investigation also.

The procedure of microgel chromatography of proteins is not yet fully worked out, and for that reason the investigation described below was carried out.

The chromatographic columns were made from thick-walled glass capillary tubes with an internal diameter of 1-1.5 mm and a length of 10-15 cm (internal volume of the column 100-150 μl). The bottom end of the column was drawn out over a burner. Quartz sand was placed in the bottom of the column. The column was filled with Sephadex G-75 gel (superfine). The preliminary treatment of the gel was carried out in the usual way [3]. Three volumes of working buffer (0.06 M Tris-HCl, pH 7.1) was run through the filled column. To calibrate the column the following "marker" polymers with known molecular weights were used: blue dextran (2,000,000), bovine serum albumin (68,000), egg albumin (45,000), trypsin (24,000), chymotrypsin (22,500), lysozyme (17,500), ribonuclease (13,600), cytochrome c (13,000), and 5'-AMP (480). The "marker" was applied to the surface of the gel in a concentration of 10 μg in 3 μl. After absorption of the sample into the gel by means of a special micropipet the upper part of the column was filled with buffer and connected to an automatic syringe, supplying eluting buffer at the rate of 3 μl/min. The bottom end of the column was connected by a

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