QUANTITATIVE DETERMINATION OF SODIUM EXCRETORY FACTOR IN BIOLOGICAL FLUIDS

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Quantitative determination of sodium-excretory factor was carried out on isolated everted segments of rat jejunum. After addition of the test blood plasma to the incubation medium changes in absorption of fluid by the intestinal epithelium were recorded. The quantity of sodium-excretory factor capable of completely blocking the transport activity of the intestinal epithelium was taken as one intestinal unit of sodium excretory factor.
KEY WORDS: *sodium excretory factor; transport in the small intestine.*

With an increase in the volume of extracellular fluid in animals and man an increase in the excretion of sodium by the urine takes place as a result of the entry of the so-called sodium excretory factor (SEF) into the blood stream in response to expansion of the extracellular space [1]; this factor, acting on the proximal portion of the nephron, blocks reabsorption [2, 3].

Several methods are currently used to determine SEF. Its activity is tested most frequently by the method of Cort et al. [4] on hyperhydrated rats anesthetized with ethanol, this method being a slightly modified method of Heller [7], which was suggested for determination of antidiuretic hormones. According to this method, the blood plasma to be tested is injected intravenously and samples of urine are collected every 10 min; this method requires catheterization of the urinary bladder or a preliminary operation for the formation of a microbladder. Because of the duration and complexity of these experiments, the determination is carried out on a small number of animals; this makes the analysis of the results difficult and, in addition, the method is insufficiently sensitive. The SEF concentration in this method is expressed as a percentage of the change in sodium excretion compared with the control period.

To determine SEF activity, Cort et al. [4] introduced a different method of testing. The blood plasma for testing was injected into the renal artery of anesthetized cats and changes in the sodium excretion with the urine were recorded. If the sodium excretion was increased, it was considered that the blood plasma tested contained a higher concentration of SEF.

A method of determination of SEF based on the ability of blood plasma to change the short-circuited current in model experiments on frog skin after the addition of blood plasma to the nutrient medium has also been described [4]. This method likewise has its disadvantages, for it does not allow quantitative interpretation and, in addition, it uses the frog, a cold-blooded animal, in which sensitivity to SEF may differ.

All the methods mentioned above, moreover, are complicated, cumbersome, and laborious and they require special skills. They do not allow quantitative determination of the SEF concentration in blood plasma, they are carried out on a small number of biological objects, and mathematical analysis of the results is accordingly difficult.

The method of determination of SEF suggested by the writers is based on changes in the absorption of fluid by an isolated segment of rat small intestine. Considering that sodium transport in the intestine is linked with water absorption by an equimolar relationship [5, 6], the transport function of the intestinal epithelium can be judged from changes in the weight of the segment of intestine. Testing of SEF was carried out on rats. The animals were deprived of food for 18-22 h before the beginning of the experiment, but water was not

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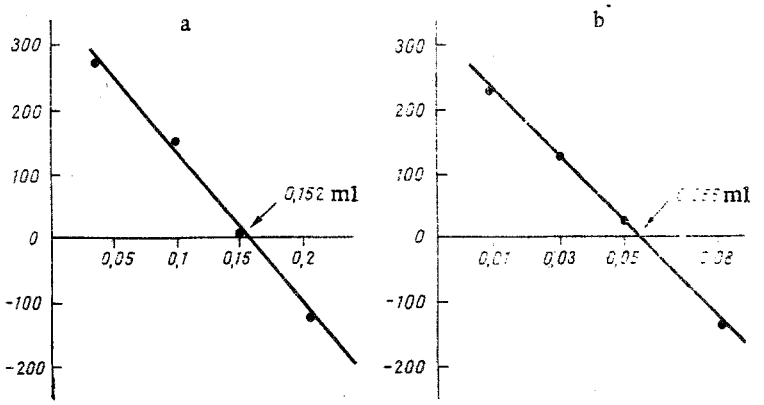

Fig. 1. Calculation of SEF content in rat blood plasma: a) graphic determination of quantity of plasma containing one intestinal unit SEF in intact rats; b) the same, in rats after an increase in volume of extracellular fluid. Abscissa, volume of blood plasma tested (in ml); ordinate, change in weight of intestine (gain or loss, in mg/g dry weight/30 min).

restricted. After decapitation a segment of jejunum 4-5 cm long was isolated (from one animal several segments of jejunum can be taken). The segment of jejunum was everted and ligated at both ends. The resulting everted sac was placed for incubation in Krebs-bicarbonate solution of the following composition (in mM): NaCl 135, KCl 4.5, $CaCl_2$ 2.5, $MgSO_4$ 1.18, $NaHCO_3$ 5.0, NaH_2PO_4 1.84 Na_2HPO_4 0.46, glucose 12.2, pH 7.4, at a temperature of 37°C and with constant aeration of the medium.

To determine SEF in the material for testing three or four such segments of jejunum were prepared. The test material (blood plasma) was added in the proportion of 0.01-0.2 ml to 10 ml incubation medium, depending on the quantity of SEF presumed to be present, to each segment of jejunum 1 h after the beginning of incubation in nutrient medium. The segment was reweighed 30 min later. After the experiment the everted sac was dried to constant weight. The changes in weight of the preparation (gain or loss) were calculated per gram dry weight of jejunal tissue. The results were plotted on a graph. Changes in weight (gain or loss) in mg/g after addition of the test material were plotted along the ordinate and the quantity of material tested along the abscissa (Fig. 1).

A graph of the change in weight of the intestinal sac on addition of rat blood plasma to the nutrient solution is given in Fig. 1. The intestinal unit (int. u.) of action of SEF was taken to be that quantity of SEF which completely blocked the absorption of fluid by the jejunal segment. This quantity corresponded to intersection of the graph with the abscissa (Fig. 1). The quantity of SEF in one ml of test material (int. u./ml) was calculated by the equation:

$$K = 1/M,$$

where K is the quantity of SEF (in int. u./ml); M the value obtained from the graph.

The quantity of SEF in blood plasma of intact rats, determined by the above method, was 6.0 ± 0.42 int. u./ml (Fig. 1a). After an increase in the volume of extracellular fluid by intravenous injection of isotonic saline into the rats in a dose of 3% of body weight the quantity of SEF was 15.6 ± 1.12 int. u./ml (Fig. 1b). i.e., it was increased. The quantity of SEF in the blood plasma of intact rabbits determined by the above method was 8.6 ± 0.99 int. u./ml, whereas in rabbits after an increase in the volume of extracellular fluid the quantity of SEF determined by the same method was 22.4 ± 1.86 int. u./ml.

With an increase in the volume of extracellular fluid in the animals the concentration of SEF in the blood plasma, determined by the above method, thus increased statistically signficantly ($P < 0.001$).

The suggested method is superior to those already known in its simplicity of performance and in its informativeness, for the quantity of SEF is expressed in intestinal units/ml. The results can accordingly be subjected to statistical analysis. Furthermore, unlike in other known methods, in the one described above, the effect of general anesthesia and operative trauma on the animal is ruled out. A particularly important feature is that small quantities of test material (not more than 0.5 ml) are required for determination of SEF. With all these advantages, the above method can be widely used for clinical-physiological investigations.

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A DOUBLE LABELING METHOD IN ELECTRON-MICROSCOPIC AUTORADIOGRAPHY

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A method of investigation of synthesis of two different substances (RNA and protein) in the same cell is described. Two specific precursors, uridine and proline, each labeled with tritium, were injected into animals. Differentiation of RNA and protein labeling was based on the fact that the biosynthesis of these macromolecules is located in different parts of the cell. Comparison of the results with those of two control experiments in which only one precursor was used showed that the suggested method is suitable for a simultaneous study of RNA and protein synthesis in wound fibroblasts.

KEY WORDS: *autoradiography; wound; RNA metabolism; protein metabolism.*

The double labeling method is used in light-microscopic autoradiography to investigate the metabolism of two different substances in the same preparation or metabolism of the same substance, usually thymidine, but injected on two occasions separated by a definite time interval, so that the fate of the labeled substance can be studied after being present in the body for different periods. Double labeling has widened the scope of autoradiography, for the method can be used to study problems beyond the reach of biochemistry and of ordinary autoradiography. To identify cells labeled at the first and second injections of thymidine, the labeled amino acid is given in sharply different doses, and the density of the tracks is then studied [4, 7]. When two precursors are injected they are labeled with different isotopes, usually ^{3}H and ^{14}C. Radioactivity of ^{3}H and ^{14}C is identified in sections coated with a double [2, 3] or a thick single [6] layer of emulsion. These methods of differentiating the two labeled substances are unsuitable for electron-microscopic autoradiography and, for that reason, no method of double labeling suitable for use with it has hitherto been described.

The object of this investigation was to determine the possibility of studying DNA and protein synthesis in granulation tissue cells by injecting two precursors. The basis for this investigation was the following arguments. RNA synthesis takes place in the cell nucleus; the cytoplasm, except the mitochondria, does not produce RNA, at least in quantities sufficient to be detected by methods of investigation used. Protein, however, is synthesized

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