mucosa of a frog (in the hibernation phase) in both "nutrient" chambers after complete exhaustion of spontaneous secretion. Clearly methionine caused almost identical stimulation of H^+ secretion in both halves of the mucosa. Against this background, antimycin A, an inhibitor of mitochondrial respiration, was added to one of the nutrient chambers. Under these circumstances, the stimulation of H^+ secretion by methionine in the half of the mucosa whose serous side was in contact with the added inhibitor, was inhibited completely after 5-6 min, whereas the other half, without addition of antimycin A, continued to secrete acid for 30 min. It will be clear from this example that the stimulation of acid secretion in both halves of the mucous membrane coincided with sufficiently high accuracy, and the stimulation of secretion itself was evidently connected with the function of the mitochondrial respiratory chain.

The use of a two-channel system for recording H^+ secretion in symmetrical halves of the same piece of gastric mucosa thus has definite advantages over the single-channel scheme. To begin with these advantages are expressed as minimal scatter of the data (the very substantial individual variations in the rate of H^+ secretion are leveled out) and data on differences in the physiological state of the two parts of the same piece of gastric mucosa can be obtained quickly and simultaneously. Another advantage of this method is that it can be used for the spectrophotometric measurement of the redox level of the cytochromes in the gastric mucosa by the use of a dual-beam system with simultaneous recording of the H^+ secretion.

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RAPID METHOD OF STANDARDIZING CHOLAGOGUES IN MICE

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A rapid method of selection and quantitative estimation of the specific activity of cholagogues in mice is described: The gall bladder is removed from the animals, weighed initially together with bile and then without it, so that the difference is the quantity of bile collected. The accuracy of the method was tested in experiments in which insulin, dehydrocholic acid, and 2-mercaptobenzthiazole (Mebetizole) were administered. The method as described can be used for pharmacological screening.

KEY WORDS: cholagogues.

Cholagogue activity is usually determined either in chronic experiments on animals with fistulas [7, 8] or in acute experiments on anesthetized rats [1, 5, 6]. However, the high cost of the animals, the complexity of the operations, the long duration of the postoperative period, and also the low sensitivity of anesthetized rats to the substances tested (which distort the results of the corresponding determinations) all combine to restrict the usefulness of these methods and to make essential the development of more simple methods in which cheaper biological material can be used. The writer suggests a rapid method of standardization of cholagogues on mice which he has developed.

After enteral or parenteral administration of the cholagogues to mice, at the height of their action (30, 60, or 90 min after administration) the unanesthetized animals animals are killed by bleeding from the carotid arteries. Laparotomy is performed, the liver exposed, and a No. 75 silk ligature is tied around the cystic duct,

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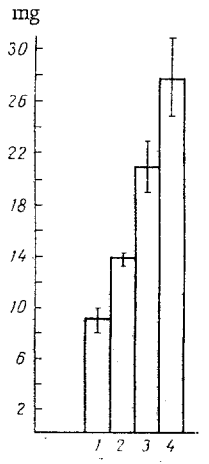

Fig. 1

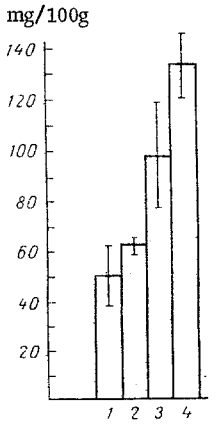

Fig. 2

Fig. 1. Effect of various substances on choleretic function of the albino mouse liver. Ordinate, quantity of bile in gall bladder (in mg). 1) Control, 2) administration of insulin, 3) of dehydrocholic acid; 4) of Mebetizole. Values of M ± m given.

Fig. 2. Effect of various substances on choleretic function of albino mouse liver (expressed per 100 g body weight). Ordinate, quantity of bile (in mg/100 g). Remainder of legend as in Fig. 1.

which is detached from the bile ducts and removed from the peritoneal cavity. If a large volume of bile has been obtained, the full gall bladder is removed together with the bile ducts. The isolated gall bladder is weighed on torsion scales, after which the contents are removed, the bladder walls are washed with distilled water and dried on filter paper, and the organ is again weighed. The difference in weight of the full and empty gall bladder indicates the quantity of bile secreted during a measured time, i.e., the cholagogue activity of the substance tested can be estimated. The concentration of cholates, bilirubin, and cholesterol in the bile can be determined.

Determination of the choleretic activity of a single substance on 12 to 14 mice by this method takes on the average 2-2.5 h.

The method was tested on 44 albino mice. As standard cholagogues, dehydrocholic acid (in a dose of 50 mg/kg by mouth), insulin (1 unit/10 g body weight subcutaneously), and Mebetizole (2-mercaptobenzthiazole) (50 mg/kg by mouth), which is 1.5-2.5 times stronger than dehydrocholic acid in its choleretic activity [2-4], were used. Control mice received physiological saline. The mice were killed 90 min after administration of the substances and the quantity of bile in the gall bladder was determined by the method described above. The quantity of bile in the control animals varied within wide limits and its mean value was 9 ± 0.3 mg. Subcutaneous injection of insulin greatly increased the quantity of bile in the gall bladder. Dehydrocholic acid and, in particular, Mebetizole were more effective still; under the influence of the latter the quantity of bile in the gall bladder was increased by 211.1% compared with the control (Fig. 1). The same pattern was observed when the results were expressed per 100 g body weight (Fig. 2).

The suggested method of standardization of cholagogues on mice is thus simple, rapid, and cheap and it can accordingly be used for screening therapeutic substances.

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DETERMINATION OF GLUCOKINASE AND HEXOKINASE ACTIVITY IN LIVER EXTRACTS PREVIOUSLY PURIFIED ON MOLSELECT G-50 DEXTRAN GEL

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Hexokinase and glucokinase activity in the supernatant of a rabbit liver homogenate obtained at 18,000g was determined by a spectrophotometric method. Preliminary purification to remove low-molecular-weight components by gel filtration on Molselect G-50 dextran was shown to prevent reduction of NADP unconnected with the hexokinase reaction.

KEY WORDS: hexokinase; glucokinase; rabbit liver; gel filtration; method of determination of enzyme activity.

The spectrophotometric method of determination of glucokinase (GK) and hexokinase (HK) activity of the liver, based on recording the increase in optical density at 340 nm due to reduction of NADP in the presence of an excess of glucose-6-phosphate dehydrogenase, is widely used at the present time [4, 5, 8-13]. The method requires preliminary purification of the liver extract by dialysis from low-molecular-weight components [4]. However, prolonged analysis can lead to considerable inactivation of the enzymes [12]. The gel-filtration method has considerable advantages over dialysis.

This paper describes an investigation of the effectiveness of preliminary purification of liver extract by gel filtration on Molselect G-50 dextran during determination of liver hexokinase activity.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits weighing 2.5-3 kg. To determine GK and HK activity the supernatant obtained by centrifugation of the liver homogenate at 18,000g and at 0-2° C for 1 h was used. The supernatant was purified on Molselect G-50 dextran gel (Reanal, Hungary) [13]. Fractions containing protein and free from low-molecular-weight compounds were used to determine the liver hexokinase activity. Activity of GK and HK was determined by a spectrophotometric method [10]. Protein was determined by Lowry's method [7].

EXPERIMENTAL RESULTS

Typical curves characterizing the rate of reduction of NADP are given in Figs. 1-3. On addition of different amounts of protein of the unpurified liver supernatant to the incubation medium, the rate of reduction of NADP was not directly proportional to the quantity of protein added (Fig. 1). The reaction of NADP reduction on the addition of unpurified supernatant took place fairly intensively if the incubation medium did not contain ATP and glucose, which are necessary for the hexokinase reaction (Fig. 2). Under those conditions, when the spectrophotometric method was used to determine GK and HK activity, considerable deviations from the true value took place. It was shown that during determination of the GK and HK activity of the purified liver supernatant the rate of NADP reduction was directly proportional to the quantity of added protein of the eluate (Fig. 1). No reduction of NADP took place on the addition of purified liver supernatant to incubation medium without ATP or glucose (Fig. 3).

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