Consequently, the method of visual evaluation with instantaneous statistical control can be used in histochemical reactions when the investigator can construct a subjective scale of gradations of the feature concerned. In reactions in which proportionality does not exist between optical density and the quantity of indicator, this method is suitable only for use in comparing standard preparations.

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AN ACCURATE AND RAPID METHOD OF ASSESSING ACTIVITY OF CHOLERETICS IN RATS

M. D. Litvinchuk and Z. I. Novosilets

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KEY WORDS: choleretics.

There is no simple method for the rapid and accurate detection and screening of choleretics, for practical purposes, under experimental conditions. Choleretic activity of drugs under acute experimental conditions is usually studied in rats by Heidenhain's method [5]* or in one of its modifications [1-4]. These methods consist essentially of the introduction of a cannula into the main bile duct of anesthetized rats, through which the bile is collected. A disadvantage of these methods of testing choleretics is the presence of a foreign body in the bile duct, namely the cannula, which injures the tissue or causes constant irritation of the biliary tract, and this is often reflected in the experimental results.

The method now suggested eliminates these defects and, together with its simplicity in use and its accessibility, it always gives stable results. It is essentially as follows. The experimental rat is anesthetized with ether and urethane, fixed on an operating table, and laparotomy is performed through an epigastric incision 1.5-2 cm long. The duodenum and the point where the bile duct enters it are then located. Above this point the duodenum is ligated. Another ligature is tied below the point of entry of the bile duct into the duodenum, into the lumen of which one end of a rubber cannula-tube 1 mm in diameter and 4-4.5 m long is first introduced. A closed sac with a volume of 0.5 cm^3 is thus formed from the duodenum. Bile escapes from the bile duct into this sac and then into the cannula-tube (Fig. 1). The cannula-tube (a transparent rubber capillary tube) is first calibrated by means of a tuberculin or insulin syringe, and then wound spirally on a stand on which a scale is mounted, with divisions corresponding to calibration of the capillary tube in millimeters. In the case illustrated, the value of each scale division was 0.025 ml of the volume of the capillary tube (Fig. 1). As a result of pressure in the bile duct and in the isolated duodenal pouch, and also of the properties of the capillary tube, bile constantly fills the special reservoir (capillary tube) as it enters the duodenum. Bile collecting in the tube is measured hourly according to the scale readings for the necessary period of time. At the end of the experiment the cannula tube, filled with secretion, is cut from the duodenum, and its contents, either as a whole or in hourly portions, are subjected to biochemical analysis.

To prevent the evacuatory function of the gastrointestinal tract, an anastomosis is formed by means of a rubber tube between the proximal and distal parts of the duodenum (Fig. 1).

The duration of the operation to form the isolated sac, to introduce and fix the cannula tube, and to form the anastomosis was 5-7 min per rat. In the course of one working day the choleretic activity of one preparation can be studied in 14 rats (including seven controls) by the suggested method and the qualitative and quantitative characteristics of the preparation can be obtained.

*There is no reference 5 in the Russian original – Consultants Bureau.

Department of Pharmacology, L'vov Medical Institute.

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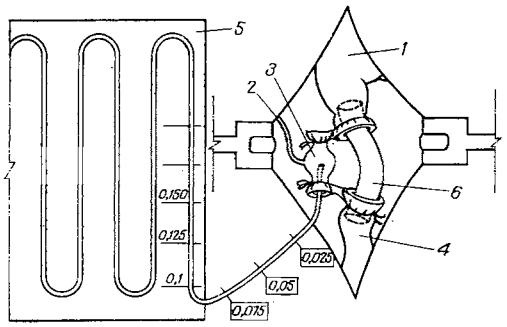

Fig. 1

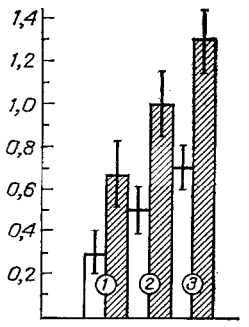

Fig. 2

Fig. 1. Scheme of operation to form an isolated sac and arrangement of cannula-tube in rats. 1) Stomach; 2) bile duct; 3) isolated sac with tube; 4) distal portion of duodenum; 5) cannula-tube mounted on scale; 6) anastomosis between stomach and small intestine.

Fig. 2. Effect of insulin and chologon on bile-secreting function of the liver in rats. Unshaded columns represent quantity of bile (in ml); shaded columns quantity of bile (in ml/100 g body weight). 1) Bile secretion under normal conditions; 2) under influence of insulin; 3) after oral administration of chologon.

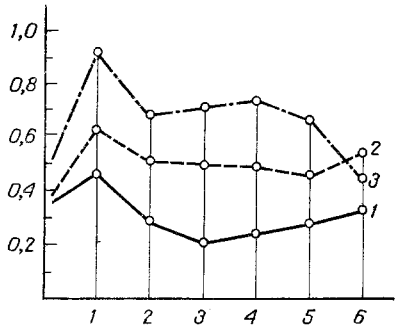

Fig. 3. Effect of insulin and chologon on choleresis in rats. 1) Choleresis under normal conditions, 2) under the influence of insulin, 3) after oral administration of chologon. Abscissa, time of exposure (in h); ordinate, volume of bile (in ml).

To study the suitability of this method for the standardization of chologon, the effect of test preparations of insulin and chologon on choleresis was studied in 27 intact albino rats weighing 250-300 g, divided beforehand into three groups with nine animals in each group.

The results indicate that hourly portions of bile in rats of the control group during a 6-h period of observation fluctuated within narrow limits and their average value was 0.3 ml (Fig. 2, 1). In the next experiments this quantity of bile was used as the standard (100%). After injection of insulin (4 units/100 g body weight subcutaneously) into rats, hourly portions of bile exceeded the control in volume on average by 66.7% (Fig. 2, 2). Intragastric administration of chologon to the animals in a dose of 0.1 g/100 g body weight caused a definite increase in choleresis on average by 132.3% (Fig. 2, 3). Similar parameters of choleretic activity of insulin and chologon were obtained when the quantity of bile secreted was calculated per 100 g body weight (Fig. 2, shaded columns).

Data on the choleretic activity of these test preparations over a period of time are shown graphically in Fig. 3.

The experimental results thus show that the suggested method of testing choleretics on rats is sufficiently sensitive and does not cause trauma to the hepatobiliary system. It can be used as a convenient and accessible method of pharmacological screening and also to study the choleretic activity of drugs.

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SIMPLIFIED METHOD OF ISOLATING β_2-MICROGLOBULIN FROM URINE

R. A. Bobreneva

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KEY WORDS: β_2-microglobulin; gel-chromatography; ion-exchange chromatography.

β_2-Microglobulin (B_2-M), a protein first described by Edelman et al. [4], is a small molecule (mol. wt. 11,800) homologous in its primary structure with the Cγ3 domain of the immunoglobulin molecule.

Interest in this protein has increased in recent years, for it has been shown that it is a component of glycoproteins coded by the principal histocompatibility locus as a subunit. It has recently become evident that the role of cell surface glycoproteins in immunity is extremely important [3, 11]. However, the function of β_2-M is not yet known. It has recently been shown [2] that β_2-M activates T lymphocytes and increases the number of Fc receptors on their surface.

Methods of determination of β_2-M in human urine and serum are of great importance for the diagnosis of kidney diseases [1, 5] and of tumors [5, 7].

The technique of isolation of β_2-M was fundamentally researched by Beggard and Bearn [1]. As its successive stages it includes ultrafiltration, zonal electrophoresis, gel chromatography, and ion-exchange chromatography. The use of this technique calls for different kinds of apparatus and also, at the testing stage (Ouchterlony's method and immunoelectrophoresis), it requires anti-β_2-M antiserum. The use of SDS-electrophoresis [7], however, enables this test protein and test antiserum to be dispensed with. For the analysis of β_2-M in human blood and urine an expensive Phadebas β_2-microtest kit, containing nanogram quantities of human β_2-M, has been suggested by the firm of Pharmacia. For diagnostic and, in particular, for research work, larger quantities of human and animal β_2-M are necessary.

The development of a simplified technique for the isolation of β_2-M in amounts required for investigations of β_2-M in certain pathological processes, and also to study the role of this protein in the composition of the HLA-glycoproteins of the cell surface, is a matter of some urgency. This paper describes a study of the possibility of obtaining sufficiently purified β_2-M by methods of column chromatography. The sequence of stages of isolation of purified (92.3 ± 2.85%) β_2-M, with the characteristics of each stage, is described below.

All operations were performed at room temperature with the addition of sodium azide (final concentration 0.04%). The source of the β_2-M was urine from patients with Fanconi's syndrome (2.2-3 mg β_2-M/liter) and patients with transplanted kidneys (5-38 mg/liter).

The quantity of β_2-M in the urine and at all stages of its isolation was determined by the Phadebas β_2-microtest in accordance with instructions provided by the firm of Pharmacia. The protein concentration was determined by Lowry's method and from the optical density at 260 nm and 280 nm.

The samples of urine were desalted on columns (5 × 20 cm) with Molselect G-25 (from Reanal). Urine was applied to the column in a volume of 250 ml and the rate of elution was 60 ml/h. The eluant was a 0.001 M

Laboratory of Cytochemistry and Molecular Biology of Immunogenesis, Institute of Human Morphology, Academy of Medical Sciences of the USSR. Institute of Pediatrics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 6, pp. 752-754, June, 1980. Original article submitted June 2, 1979.

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