

tions. Further details of procedures and figures will be published in the near future.

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The Use of Ion-exchange Resin in the Purification of Hyaluronidase

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It has been shown that high molecular weight polyanions produce a complete reversible inhibition of hyaluronidase even in small amounts¹⁻⁴. This led to the idea that a cation exchanger of a weakly acid type could be used for the isolation and purification of hyaluronidase.

Decapsulated ground bull testes were extracted with equal volumes of 0.1 M acetic acid. Extracts fractionated with $(\text{NH}_4)_2\text{SO}_4$. Fraction obtained between 30 and 70 % saturation was retained. An aqueous solution of this fraction was dialysed salt free. In order to denature proteins of high molecular weight the solution was vigorously shaken at pH 6 at room temperature for 15 minutes with 1/2 parts of chloroform without loss of activity. After centrifugation the upper aqueous layer was separated and dialysed against distilled water.

The hyaluronidase activity was determined as described by Diczfalusy *et al.*³ The crude fraction had an activity of 700 VRU per mg N. The resin used was Amberlite XE-64 as the ammonium salt.

In a typical experiment 200 ml of crude hyaluronidase with a potency of 3 500 VRU per ml was run through a 1 cm × 11 cm Amberlite XE-64 column (2 g) at room temperature. Rate of flow: 1 ml per min. A gold-coloured impurity passed into the effluent. About 95 % of the activity was adsorbed on the resin. The resin was rinsed with 200 ml of water, 150 ml 0.1 M ammonium acetate, and 25 ml 0.1 M ammonia, respectively. The hyaluronidase was then eluted with minimal volumes of a 0.1 M KCl-HCl-buffer, pH 1.5.

On a nitrogen basis the eluate had an activity about 100 times that of the original material. The purified hyaluronidase fractions, thus obtained, are homogeneous in electrophoretic analyses. The protein impurity not adsorbed

has neither activating nor inhibitory effects on purified hyaluronidase.

The method described showed that bacterial hyaluronidase from different origin cannot be adsorbed. These phenomena make it possible to separate testes hyaluronidase from bacterial hyaluronidase.

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On the Action of the Intestinal Flora on Conjugated Bile Acids

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Recently Bergström and Norman¹ have investigated the labelled products in bile and feces after injection of cholesterol-4-¹⁴C in the rat. This compound is transformed and excreted into the bile to more than 90 % as taurocholic acid. However, only a minor part of the labelled product in feces consists of taurocholic acid. Most of the conjugates had been split and the cholic acid further modified. This is in accord with a number of older observations that the bile acids present in bile cannot be found in feces.

To obtain some information regarding the role played by the microorganisms in the biological splitting of the conjugated bile acids and the further modifications of the bile acid molecules, antibiotics were given to rats and the fecal bile acids were analyzed.

100 mg of terramycin and 250 mg of "sulfatyl"^{*} was given by stomach-tube twice a day to rats. If the decrease in the total aerobic count was satisfactory, the rats were given 3—3.5 mg of cholic acid-24-¹⁴C on the third day after administration of antibiotics. The feces were collected daily for five days. No increase in the amount of viable bacteria was found during this period. The labelled bile acid products were extracted and fractionated by reversed partition chromatography as described by Bergström and Norman¹.

Intraperitoneally administered cholic acid-24-¹⁴C is excreted in the bile almost totally

^{*} Pharmacia.