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

Bertil Högberg

Research Department, AB Leo, Hälsingborg, Sweden

It has been shown that high molecular weight polyanions produce a complete reversible inhibition of hyaluronidase even in small amounts [1-4]. 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 $(NH_4)_2SO_4$. 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 vigourously 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 eluated with minimal volumes of a 0.1 *M* KCl-HCl-buffer, pH 1.5.

On a nitrogen basis the eluate had en 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

Arne Norman

Department of Physiological Chemistry, University of Lund, Lund, Sweden

Recently Bergström and Norman [1] have investigated the labelled products in bile and feces after injection of cholesterol-4-^{14}C 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 "sulftalyl"* 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-^{14}C 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 fractioned by reversed partition chromatography as described by Bergström and Norman [1].

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

* Pharmacia.

conjugated with taurine [2,3]. Only 1—2 % of the labelled cholic acid is present as glycin conjugate and none was found free. Chromatographic separation of the labelled bile acid products excreted in feces of the rats treated with antibiotics [1] showed that the major part is excreted as taurocholic acid (70—80 %), 5—10 % being presumably excreted as glycocholic acid. About 10 % of the labelled compound was, however, found at the place where free cholic acid occurred. None of the bile acid metabolites found in normal rat feces were observed after administration of antibiotics. This indicates that most of the peptide bonds of the conjugated bile acids are split by the intestinal microorganisms and these also cause further modifications of the bile acid molecules.

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Quantitative Determination of Bile Acids on Paper Chromatograms

Sten Eriksson and Jan Sjövall

Department of Physiological Chemistry, University of Lund, Lund, Sweden

Bile acids like other steroids give characteristic ultraviolet spectra when dissolved in strong sulfuric acid [1]. Kier [2] has developed a method for the simultaneous determination of cholic acid and desoxycholic acid using 65 % sulfuric acid. This method has been improved by Mosbach *et al.*[3]

We have studied the absorbtion spectra of free and conjugated bile acids in 96 % and 65 % sulfuric acid in order to develop a method for the determination of individual bile acids after separation by paper chromatography. It was found that tauro- and glycodesoxycholic acid did not behave like free desoxycholic acid when heated in 65 % sulfuric acid. To get a main absorbtion maximum at 390 mμ the conjugated desoxycholic acid has to be heated 60 minutes at 60° whereas free desoxycholic acid gets a maximum at 385 mμ after 15 minutes at 60°.

Cholic, taurocholic and glycocholic acid had absorbtion maxima at 320 mμ after heating for 15 minutes at 60° in 65 % sulfuric acid. On further heating a second maximum appeared at 389 mμ but after about 150 minutes at 60° the maxima decreased.

In 96 % sulfuric acid cholic acid has its main absorbtion maximum at 389 mμ whereas conjugated and free desoxycholic acid and chenodesoxycholic acid have their maxima at 310 mμ.

We have used these methods for the quantitative determination of bile acids after paper chromatography. The acids were run on 1.5 cm wide strips of Whatman 3 MM filter paper. The strips had to be washed with ethanol and 10 % acetic acid before use. After chromatography according to Sjövall [4] the spots were located by spraying with phosphomolybdic acid in ethanol. The spots on the other strips including one blank were cut out and eluted with ethanol into small test tubes. The ethanol was evaporated at 100° C and the acids were determined by using the reactions in 65 % sulfuric acid. Nine determinations of mixtures of cholic acid and desoxycholic acid (0.02 mg of each acid) gave a standard deviation of about ± 6 % for each of the acids.

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A Paper-chromatographic Method for the Estimation of Peptidase Activity

Torsten Ekstrand

Centrallaboratoriet, Astra, Södertälje, Sweden

The activity of proteolytic enzymes is usually determined by titrimetric methods. The well known Linderstrøm-Lang and Holter method, for instance, is based upon the acidimetric titration, in acetone, of the amino groups liberated from the peptides after the enzymatic cleavage. By this method it is possible to determine the reaction velocity of an enzymatic cleavage of, for instance, D,L-leucylglycylglycine (LGG), but nothing can be said about the order of splitting of the peptide linkages. As we were interested to know if, by certain peptidase preparations, leucine or glycine was initially liberated, we have used a semi-quantitative paper chromatographic method.