Kinetics of Hyaluronidase

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The enzymatic splitting of hyaluronic acid is studied by means of viscometry. The reaction is followed to more than 90 % reduction in the specific viscosity.

The substrate was potassium hyaluronate prepared from umbilical cords according to Jensen [1]. The enzyme was a commercial hyaluronidase "Invasin Lundbeck". The investigations were carried out in a modified Ostwald viscosimeter. All experiments were carried out at 20° C and pH = 7.0 in a McIlvaine phosphate-citrate-sodium chloride buffer solution.

Taking η/η_0 as a reaction parameter and calling $\eta/\eta_0 = y$ we find empirically that the results in the investigated interval fit the chronometric integral

$$E \cdot t = A(1/y—1) + B(1/y^2—1),$$

where t is time, A and B are constants and E is the enzyme concentration which varied by a factor of 4 in different runs.

The concentration of potassium hyaluronate was in all experiments 0.4 %. The above mentioned expression is different from other expressions found in the literature to describe the degradation of hyaluronic acid. Most of these involve a first order decay of the specific viscosity [2] or a Michaelis expression [3].

The interpretation of these results is not obvious. If we accept the Staudinger equation $\eta_{spc} = KMc$, (where K is a constant, M the average number of units in the high molecular substance and c the concentration in weight %) and if we furthermore assume that the enzyme splits a constant number of bonds per time unit, we can derive the expression

$$E \cdot t = K(1/y—1)$$

which accounts for the dominating term in our expression.

More details on these experiments are published at the time of the meeting [4].

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Biliary Excretion of Cholesterol and Bile Acids in Bile Fistula Rats

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The main metabolic end products of cholesterol in the rat have been shown to be taurocholic and taurochenodeoxycholic acids [1]. Several authors [2,3] have studied the daily excretion of cholic acid and cholesterol in bile fistula rats but due to the lack of a suitable analytical method no detailed analysis of the excretion pattern has so far been undertaken. In the present study bile has been collected from rats with a bile fistula. The amount of bile and its content of taurocholic and taurochenodeoxycholic acid has been determined at various intervals during the 14 days following the operation. The bile acid content was determined by the methods developed by Sjövall [4].

In Table 1 some typical values obtained are shown. The output of bile acids during the first 6 hours after the operation is considerably higher than the output during the subsequent 6 hours periods. A minimum generally occurs between 12 and 18 hours after the operation. The amount of bile acids excreted during the first 6 hours largely represents the amount of bile salts circulating in the intact animal [5]. Following the minimum excretion between 12 and 18 hours there is a rapid increase in the

Table 1. Excretion of Na-taurocholate (TC) and Na-taurochenodeoxycholate (TCD) in bile fistula rats.

Hours after operation	Rat No. 1		Rat No. 2	
	TC mg	TCD mg	TC mg	TCD mg
0— 6	10.4	3.44	13.4	4.9
6— 12	3.86	0.94	3.4	1.5
12— 18	1.97	0.78	1.7	1.0
18— 24	5.25	1.85	2.7	0.95
24— 48	49.1	10.1	33.6	4.0
48— 72	52.2	15.9	39.2	12.0
72— 96	37.6	13.8	31.2	28.0
96—120	35.3	16.5	36.4	12.0
120—144	47.3	19.1	35.8	12.0
144—168	45.2	14.9	26.0	10.8
168—192	39.4	19.2	—	—

amount excreted and a constant level is reached after the second day with a total excretion of bile salts ranging between 50 and 80 mg/day. These amounts have to be compared with the daily synthesis of bile acids in an intact animal. From the turnover time of labelled bile acids [6] and the determinations of the amount of bile acids in the enterohepatic circulation by isotope dilution [5] a daily synthesis of 2—3 mg of bile acids in a 200 g rat has been calculated. In a bile fistula rat there is thus a 20—30 fold increase of the normal bile acid production. This synthesis of bile acids in bile fistula rats is thus the result of a broken enterohepatic circulation. Further studies are in progress to elucidate factors regulating this process and its relation to the metabolism of cholesterol.

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Urea Solution as a Solvent for the Viscosimetric Determination of Lichenase Activity

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Viscosimetric methods are generally more sensitive than other methods for the determination of the activity of enzymes which are capable of breaking down polymer-homologous substances. It follows as a matter of course that a viscosimetric determination of enzymic activity can only be carried out with such substrates as are completely soluble and give solutions with a high viscosity.

Lichenin is a polysaccharide which is easily soluble only in warm alkaline water solution, and much of it is precipitated if the temperature and the pH are lowered to such values as are suitable for the investigation of the activity of lichenase. A new method for obtaining solutions of lichenin is suggested here.

It has turned out that lichenin is soluble in water solutions of urea.

The viscosimetric determination of lichenase can be carried out as follows. Lichenin is dissolved in a warm urea solution, containing 0.3 to 0.5 g urea for each gram of water. A lichenin concentration of a few per cent may have a viscosity of about 3 or 4 times that of the urea solution, which is convenient.

One ml of an enzyme solution and 1 ml of a buffer solution are transferred to an Erlenmeyer flask. Two ml of the lichenin solution are added, and the contents are thoroughly mixed. Two ml of the reaction mixtures are transferred to a dry Ostwald viscosimeter, and a timer is started. The flow time is measured several times.

The inverse value of the specific viscosity is computed for each flow time by dividing the flow time for the solvent by the flow time of the reaction mixture diminished by the flow time of the solvent.

The inverse values of the specific viscosity are plotted in a diagram *versus* the times, for which they are valid, and a straight line is fitted to the points. The inclination of the line, multiplied by the square of the concentration of lichenin in the reaction mixture, is a measure of the enzyme activity.

By this viscosimetrical method it was shown that sperm of *Paracentrotus lividus* contain lichenase in small quantities.

The method suggested here — dissolving lichenin in a urea solution — has the advantage, that the true solution of even a high molecular fraction of the naturally occurring substrate, and not its hydroxy ethyl or carboxy methyl ether — as is the case with cellulose and chitin — can be used for the assay of enzymes capable of splitting β-1,4 glucoside linkages.

Sialic Acid and Neuraminic Acid

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The structure of sialic acid and of neuraminic acid is discussed principally on the basis of group analyses and periodate oxidations and with particular regard to the ideas recently put forward by Gottschalk [1]. The structures proposed by this author are in the main in good agreement with our findings.

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