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].

1. Jensen, C. E. *Acta Chem. Scand.* **7** (1953) 603.
2. Lundquist, F. *Acta Physiol. Scand.* **17** (1949) 44.
3. Dorfman, A. *J. Biol. Chem.* **172** (1948) 377.
4. Andersen, S. O. and Graae, J. *Acta Chem. Scand.* **9** (1955) 1431.

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	—	—