SYNTHESIS OF HYALURONIC ACID BY A SOLUBLE ENZYME SYSTEM FROM MAMMALIAN TISSUE*

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In a previous communication (Schiller, Slover and Dorfman, 1961a) it was demonstrated that cell-free preparations of skin from rat fetuses were capable of catalyzing the synthesis of hyaluronic acid from uridine diphospho-N-acetylglucosamine (UDP-GNAc) and uridine diphosphoglucuronic acid (UDP-GUA). The hyaluronic acid synthesizing activity was reported to be associated with a fraction which was sedimentable at 20,000 x g. Attempts to solubilize "hyaluronic acid synthetase" by a variety of orthodox procedures resulted, in most instances, in complete loss of activity.

Recently, Semenza and Auricchio (1962) reported the preparation of soluble intestinal disaccharidases following treatment of particles that sedimented between 3,000 and 105,000 x g with papain. Further studies revealed that the intestinal dextranase of rats could be solubilized by trypsin (Dahlqvist, 1963) whereas papain was required for solubilization of human intestinal disaccharidases (Auricchio et al, 1963).

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The present communication is concerned with the effect of papain on the activity of hyaluronic acid synthetase in homogenates of embryonic rat skin. A brief account of the effect of papain on solubilization of the enzyme(s) will also be presented.

Skin from rat embryos, obtained by Caesarean section as described (Schiller et al, 1961a), was homogenized in 2 volumes of 0.1 M potassium phosphate-0.01 M $MgCl_2$-0.004 M cysteine buffer, pH 7.5, and 0.5 ml samples were removed for incubation at 38° with uridine nucleotides, as indicated in Table I. The remainder of the homogenate was first preincubated for 30 min. at 37° with no additives or with either papain and cysteine, or papain, cysteine and EDTA. Following the 30 min. preincubation period the samples were chilled in crushed ice and a 0.5 ml aliquot was used for incubation with nucleotides, as above. The reaction was terminated by immersing the tubes in boiling water for 1 min. Zero time controls were likewise heated at 100° for 1 min. before incubation. Three mg. of carrier hyaluronic acid were added to the reaction mixtures and the hyaluronic acid was isolated by a method described previously (Schiller, Slover and Dorfman, 1961b). The isolated hyaluronic acid was precipitated with glacial acetic acid, to reduce spurious counts, and identified as described (Schiller et al, 1961a). The samples were then dissolved in water, diluted to 5 ml and analyzed for uronic acid (Dische, 1947). Aliquots were removed for counting in the Packard Tri-Carb liquid scintillation spectrometer.

UDP-GNAc-H^3 with tritium in the acetyl position was prepared as described by Markovitz et al (1959) from uridine triphosphate and N-acetyl-glucosamine-1-phosphate-H^3. The latter compound had

TABLE I

Effect of Papain on Activity of Hyaluronic Acid Synthetase in Homogenates of Embryonic Rat Skin

Preincubation*	Incubation**	Specific Activity
	hrs.	cpm/mg UA
-	0	20
-	2.5	174
No Additives	2.5	155
papain, cysteine	2.5	348
papain, cysteine, EDTA	2.5	544

* Preincubation was carried out at 37° for 30 min. Where indicated, 2 mg crystalline papain, 8 micromoles cysteine and 1 micromole EDTA (potassium salt) were added per ml of homogenate, prepared as described.

**The incubation mixture contained 0.22 micromoles UDP-GNAc-H^3 with 9.2×10^3 cpm, 0.2 micromoles UDP-GNAc, 0.05 micromoles DPN, 0.2 micromoles ATP, and 0.5 ml of homogenate equivalent to 15 mg protein, in a total volume of 0.56 ml.

been prepared chemically from glucosamine-1-phosphate and tritiated acetic anhydride.*

Table I summarizes the results of a typical experiment and demonstrates a 3-fold increase in hyaluronic acid synthetase activity following preincubation of cell-free homogenates of embryonic rat skin with papain. Preincubation with papain for periods longer than 30 min. resulted in unchanged or somewhat decreased enzyme activity. Erratic

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results were obtained with increased concentrations of papain. Since enzyme activity was found to be depressed in the presence of Na ions, 0.1 M potassium phosphate buffer was used in all instances. The addition of EDTA to the preincubation mixture enhanced the hyaluronic acid synthetase activity: this compound, however, was without effect in the absence of prior preincubation. No inhibition of synthetase activity could be demonstrated by puromycin. The increase in enzyme activity of tissue homogenates following preincubation with proteolytic enzymes is a consistent and reproducible finding although not unique to this system. In rat liver homogenates, incubation with papain increased fructose-1, 6-diphosphatase activity (Freedland and Waisman, 1960); with trypsin, glucose-6-phosphatase activity was increased (Heise and Görlich, 1963).

Unlike its effect on the whole homogenate, preincubation of 600 x g supernatant fractions did not result in increased synthetase activity; in fact, some degree of inactivation was obtained.

Approximately 36 to 40% of the hyaluronic acid synthetase activity of cell-free homogenates (non-preincubated) can be found in the 105,000 x g supernatant (Table II) following preincubation with papain. In contrast, no solubilization of enzyme activity was achieved by sonication, detergents, snake venoms or deoxycholate, and in many instances, the use of these agents and techniques resulted in complete inactivation of the tissue preparation.

It is not possible from these studies to localize the effect of papain to a particular cellular organelle. The fact that no increase in enzyme activity was obtained after preincubation of 600 x g supernatant fractions with papain suggests the possibility that surfaces of unbroken cells were

TABLE II

Synthesis of Hyaluronic Acid from UDP-GNAc-H^3 and UDP-GUA

Tissue Preparation	Preincubation*	Incubation**	Specific Activity
		hrs.	cpm/mg UA
1. Homogenate	—	0	23
2. Homogenate	—	2.5	553
3. Homogenate	No additives	2.5	586
105,000 x g supernatant of 3		2.5	15
4. Homogenate	papain, cysteine	2.5	1027
105,000 x g supernatant of 4		2.5	172
5. Homogenate	papain, cysteine, EDTA	2.5	1043
105,000 x g supernatant of 5		2.5	210

*Preincubation was carried out at 37° for 30 min. Where indicated, 2 mg crystalline papain, 8 micromoles cysteine and 1 micromole EDTA (K salt) were added per ml of homogenate (29 mg protein), prepared as described in the text.

**The incubation system contained 0.17 micromoles UDP-GNAc-H^3 with 1 x 10^6 cpm, 0.2 micromoles UDP-GUA, 0.5 mg albumin, 0.05 micromoles DPN, 0.2 micromoles ATP, and 0.5 ml of enzyme in a total volume of 0.65 ml.

disrupted or that enzyme sites, enveloped by non-specific particular material, were unmasked by proteolysis.

Full experimental details on the soluble hyaluronic acid synthetase will be presented elsewhere.

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