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Hydrolysis of Hyaluronic Acid of Human Joint Fluid *in vivo*.

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There is present in joint fluid a polysaccharide which Meyer[1] had named hyaluronic acid. This polysaccharide is believed to be the substance which is responsible for the increased viscosity of synovial fluid. One may obtain a rough estimate of the amount of polysaccharide present in the joint fluid from the relative viscosity of the fluid. A specific enzyme which hydrolyzes this polysaccharide has been recovered from bacterial filtrates and from various animal organs.[2] The enzyme has been shown to have many properties similar to the spreading factor of Duran-Reynals.[3, 4] The enzyme has been found to be active *in vitro* and so an attempt was made to determine its activity *in vivo*. In the experiments to be reported here the enzyme was injected directly into the joint space.

Methods. Four patients were taken by chance as they were seen in the clinic or hospital. The enzyme was concentrated by Dr. Karl Meyer, who kindly furnished us with the material. It was derived from bulls' testes. An aqueous solution of the enzyme was made up in small volume. The solution was then passed through a Seitz filter. Fluid was removed from the knee, and the enzyme was immediately injected into the joint space. At varying times thereafter, fluid was again removed from the knee. The joint fluid was cooled, the clot separated by slow centrifugation and the viscosity determined on the supernatant fluid, using an Ostwald viscosimeter under standard conditions. Viscosity values are expressed as multiples of the viscosity of water. The total protein content of the synovial fluid was determined by a specific gravity-gradient method.[5]

Results. As shown in Table I, in all instances in which a potent

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3 Chain, E., and Duthie, E. S., *Nature*, 1939, **144**, 977.

4 Hobby, G. L., Dawson, M. H., Meyer, K., and Chaffee, E., *J. Exp. Med.*, 1941, **73**, 109.

5 Lowry, O. H., and Hastings, A. B., *J. Biol. Chem.*, 1942, **143**, 257.

TABLE I.

Cause of Hydrarthrosis	Amt of testicular extract injected in water	Time during which enzyme allowed to act, min	Viscosity times water		Protein g %	
			Before enzyme	After enzyme	Before enzyme	After enzyme
Rheumatoid Arthritis	(1) 2.6 mg in 2 cc	16	7.9	4.4	5.0	4.9
	(2) 6.5 mg in 5 cc	130	6.3	2.5	5.0	4.8
Rheumatoid Arthritis	(1) 6.3 mg in 5 cc	170	45.7	32.0*	3.9	3.8
	(2) 4.9 mg in 3 cc	115	119.6	24.2	4.1	4.1
Possible Traumatic Arthritis	(1) 6.3 mg in 5 cc	155	6.6	5.1*	4.9	4.4
	(2) 3.8 mg in 3 cc	90	6.0	1.6	5.1	4.7
Rheumatoid Arthritis	3.6 mg in 3 cc	105	18.0	4.6	5.6	5.4

*Enzyme was also inactive *in vitro*.

enzyme was injected, the viscosity of the joint fluid was markedly reduced. In 2 instances in which an enzyme was injected which had become inactive as measured by *in vitro* hydrolysis no significant reduction in viscosity occurred. Since the change in joint fluid protein was of small magnitude, we believe the changes in viscosity were not the result of dilution caused by adding an aqueous solution.

Another experiment was done to show that the change in viscosity of the joint fluid is brought about by the enzyme itself. This experiment is outlined in Table II. Fluid was withdrawn from the right knee at approximately hourly intervals. After the first hour, 2.5 cc of sterile water was injected into the joint. An hour later there was a small reduction in viscosity of the same order of magnitude as the fall in total protein. At this point a similar amount of water in which 3.4 mg of testicular enzyme had been dissolved was injected into the joint. There was again a slight fall in protein content, but with the addition of the enzyme, the fall in viscosity was marked.

There was no significant change in the underlying disease leading to the hydrarthrosis. There were no marked changes in erythrocyte

TABLE II.

Time	Substance injected into joint space	Joint fluid viscosity times water	Protein g %
8:30		24.3	3.9
9:20		22.8	3.9
9:22	2.5 cc of water		
10:30		19.3	3.7
10:32	3.4 mg of testicular extract in 2.5 cc of water		
11:35		2.1	3.6

sedimentation rate, agglutination with the Group A hemolytic streptococcus, or in the general course of the disease. After a week in most instances, the viscosity of the joint fluid had returned approximately to its original level. There was no change in the tendency of the fluid to reaccumulate in the joint.

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Electrophoretic Analysis of Human Semen.*

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The seminal proteins are of interest in that they are largely composed of proteoses (60%). By analytical methods globulins account for 21-40% of the total seminal proteins.[1] The present study was undertaken to investigate this nondialyzable portion.

Method. The electrophoretic patterns of normal and pathological semen were studied with the Longsworth[2] modification of the Tiselius[3] apparatus. Four pooled normal semen specimens and one specimen from a patient, whose semen contained a persistent thick coagulum, were studied. The semen was centrifuged in a high speed centrifuge at 12,000 revolutions per minute and was then diluted with 1 to 3 volumes of buffer depending on the turbidity of the solution. The solution was then dialyzed for 3 to 4 days at 0° against several liters of the same buffer consisting of 0.025 M veronal, 0.025 N hydrochloric acid and 0.025 N sodium chloride at pH 7.8. The protein solution was then centrifuged at 0° before being introduced into the electrophoretic cell.

The semen was introduced into the 2 lower compartments of the Tiselius cell. The 2 upper compartments of this cell were then disconnected, and buffer was added to the two upper cells. After equilibrium had been established in a constant temperature bath at 0° to 2° the 2 upper cells containing the buffer were made continuous with the 2 lower cells containing the protein and a direct current of 6 milliamperes was applied to the protein solution.

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3 Tiselius, A., *Tr. Faraday Soc.*, 1937, **33**, 524.