of a sperm ATPase, perhaps one not associated with motility.

Summary. Low concentrations of DNP did not stimulate respiration or affect motility of sea-urchin sperm suspended in sea water; high concentrations of DNP inhibited sperm respiration and motility, the inhibition of motility being reversible. Dinitrophenol stimulated the respiratory rate of sperm in the presence of Versene and caused respiration to be partially dissociated from motility. Versene, as compared with sea water, prolonged the motile life and respiration of sperm treated with high concentrations of DNP. Dinitrophenol and dilution have similar effects on sea-urchin sperm, raising the possibility that both act through similar mechanisms.

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Effect of Age on Polysaccharide Composition of Cartilage. (21931)

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Human cartilage has been subjected to chemical analysis by Hass(1) who studied the effect of aging on its composition. His methods for studies of polysaccharide components consisted of the determination of reducing substances and of sulfate in washed cartilage after hydrolysis with 4.2 N HCl. All of the sulfate and one-half of the reducing substances were assumed to be derived from chondroitin-sulfuric acid under these circumstances. As methods for hexosamine, hexose, and uronic acids are now available and have been used in this laboratory for studies of tissue, it is possible to make more specific studies of the polysaccharide moieties of cartilage. The following study was accordingly initiated.

Methods. Costal cartilage samples were obtained at autopsy from patients who had died from a number of different pathological conditions. The cartilage was freed from extraneous tissue, cut in fine pieces with a surgical knife and dried in a vacuum oven at 60°C. The dried cartilage was ground to pass through a 60 mesh screen.

Uronic acid was determined by a method which was modified from the carbazole method of Dische(2). Ten mg samples of cartilage were weighed into glass stoppered test tubes (16 x 150 mm). Ten ml of 85.7% by volume of sulfuric acid (concentrated H_2SO_4; Sp. Gr. 1.84, diluted 6:1 with distilled water) were added and the tubes placed in a boiling water bath for 15 minutes. After cooling 2 ml aliquots were pipetted into each of 3 glass stoppered test tubes. Twelve ml of 85.7% sul-

TABLE I. Uronic Acid and Hexosamine Contents* of Human Costal Cartilage.

Age group	No.	Uronic acid	Chondroitin sulfate†	Hexosamine	
				Total	Excess‡
Fetal	5	7.8 (7.4–8.0)	19.1	8.2 (7.9–8.5)	1.0 (.6–1.1)
Newborn	2	7.5 (7.3–7.7)	18.4	8.7 (8.5–8.9)	1.3 (1.2–1.4)
4 mo	1	7.0	17.2	7.1	1.6
6–11 yr	2	3.6 (3.6–3.7)	9.0	6.1 (6.0–6.2)	2.7 (2.6–2.8)
25–40 "	5	3.3 (2.9–3.9)	8.0	7.3 (6.9–7.7)	4.3 (3.4–4.6)
41–55 "	5	2.4 (2.0–3.2)	5.8	6.8 (5.4–7.7)	4.6 (3.9–5.1)
56–70 "	10	1.6 (1.3–1.9)	4.0	6.1 (4.9–6.6)	4.6 (3.6–5.0)
71–88 "	4	1.3 (1.1–1.6)	3.2	5.4 (4.7–6.0)	4.3 (3.6–4.6)

* All results are expressed as % of dry material.
† Calculated from uronic acid data using factor 2.46.
‡ Hexosamine in excess of that calculated to be in the chondroitin sulfate.

furic acid were added to each tube. The tubes were cooled 10 minutes in an ice bath. 0.4 ml of carbazole reagent (0.1 g/100 ml absolute ethanol) was added to 2 of the tubes; 0.4 ml of absolute ethanol was added to the third tube which served as an experimental blank. The tubes were mixed by inversion and placed in a 37°C incubator for 1 hour and then in a 30°C water bath for 1 hour. Samples were then read in a Coleman Spectrophotometer at 530 mu against the respective blank. A standard solution of glucuronic lactone* equivalent to 100 μg of glucuronic acid in 2 ml of water was cooled 10 minutes in an ice bath and then diluted with 12 ml of concentrated H_2SO_4. It was hydrolysed for 20 minutes in the boiling water bath. After cooling for 10 minutes in the ice bath, the carbazole reagent was added and the tubes were carried through the procedure as described above. This standard was read against a blank containing glucuronic lactone, sulfuric acid and absolute alcohol, subjected to the same procedure. Hexosamine was determined by a modified Elson-Morgan method as previously described by Shetlar, *et al.*(3).

Results. Results of analytical determinations are summarized in Table I. It can readily be seen that uronic acid is quite high in young cartilage, but decreases drastically in cartilage of children and mature individuals and decreases even more in old age. Hexosamine content also decreases with age, but the decrease is much less striking than it is for uronic acid. If one assumes that all the uronic acid of cartilage is contained in chondroitin sulfuric acid, the latter component may be calculated from the uronic acid content. The excess hexosamine may logically be assumed to be contained in neutral polysaccharides with hexosamine and hexose as carbohydrate constituents. These calculated data indicate that although the acid polysaccharide of cartilage decreases with age, neutral polysaccharide increases in cartilage until maturity. After maturity no appreciable change of neutral polysaccharide occurs.

Discussion. The chondroitin sulfate values reported in Table I are in general somewhat lower than those reported by Hass(1). These differences may be due to the different methods used to arrive at the values found for chondroitin sulfate. Hass assumed that one-half the reducing substance and all of the sulfate of cartilage was derived from the chondroitin sulfate. Obviously these are only approximations. It is noteworthy that results obtained by isolation as reported by Hass are much lower. cartilage from 35, 46, 50, and 66-year-old individuals having 11.4, 8.5, 6.9, and 5.8% of chondroitin sulfate, respectively. These results compare more favorably with the data of Table I. The other possibility is that the uronic acid determinations made as described above are too low. In the presence of some proteins the carbazole method does give results which are too low(2). However, optical densities of glucuronic acid lactone solutions carried through the analytical procedure described above were not affected by the presence of gelatin in concentrations equivalent in nitrogen content to that found in cartilage. Further studies of the carbazole method are in progress.

* General Biochemicals, Chagrin Falls, O.

In any case the data of Table I indicate chondroitin sulfate is higher in young cartilage and decreases with age. Hass reported an increase between ages 2 and 21 followed by a gradual decline. The data obtained in this study confirmed the gradual decline after 21 years, but were insufficient between the ages of 2 and 21 to confirm or fail to confirm the observation of an elevation between these ages. Hass' studies did not include cartilage samples from fetal or of newborns which have a uronic acid content much higher than that of children. When such data are considered, it appears that a high acid polysaccharide level is characteristic of young growing cartilage and may be correlated with active proliferation. A more rapid fixation of sulfate in young tissue has been reported(4,5). This fixation in part is probably a reflection of a higher chondroitin sulfate content.

Conclusions. A study was made of the polysaccharide constituents of cartilage from individuals of various ages. Fetal cartilage was found to be very high in uronic acid. The uronic acid content was strikingly lower by the sixth year and then declined slowly with age. Hexosamine in excess of uronic acid increased with age until maturity was reached after which there was no appreciable change.

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Complement-Fixing Antigens in Concentrates of Streptococcal Culture Supernates.* (21932)

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Recent research on the etiology of rheumatic fever has been directed at the only known association of this disease with any microorganism—association with hemolytic streptococcus. This relationship, which has been reviewed in terms of epidemiologic(1), serologic(2,3) and bacteriologic(4) data, suggested to several investigators that some immunologic relationship might exist between a substance derived from the hemolytic streptococcus and the tissues of the rheumatic host. Within the area of metabolic products of the streptococcal cell there has been considerable study of those antigens which have some biologic or biochemical activity, and to which antibodies can be measured in neutralization tests. Such streptococcal antigens include the hemolysins, hyaluronidase, desoxyribonuclease and kinase (plasminogen activator). Other possible antigens, to which antibodies would be measurable by aggregation phenomena, have not been studied except for 2 studies of hemagglutination reactions with streptococcal supernate concentrates(5,6). The present report concerns an investigation of this potential group of streptococcal antigens.

Methods and materials. Medium. Streptococci were cultivated in either of 2 media, a dialysate medium and, for the most part, in the synthetic medium described by Bernheimer and Pappenheimer(7). The dialysate

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