of a male bird. The first attempts at singing were similar to those of young males, but the injected female birds developed rapidly in this respect. Vigorous singing continued for nearly a month; after this period there was a gradual decrease.

At approximately 5 weeks after the injection, all treated birds had stopped singing. They made only individual chirps, typical of the untreated females. No female bird then sang for 10 weeks. After this period the previously injected birds were injected again, with the same dosage as in the first trial. One bird sang 4 days after the injection, a second sang after 6 days, and all were singing within at least 10 days. Since the birds were not under continuous observation, complete information on first singing is not available. The untreated females are not known to have sung.

The hormone that is used is a relatively new preparation that is said, by the maker, to be absorbed for therapeutic effect for about 30 days. The birds sang for nearly 1 month.

These observations do not prove that female canaries treated with male sex hormone are being sold for singers. They indicate that treated females may sing and in a manner indistinguishable from males (*2*).

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Synthesis of Hyaluronic Acid by Human Synovial Tissue Slices

Hyaluronic acid is an important constituent of the ground substance of connective tissue and of synovial fluid. In the joint, it appears to be a product of the synovial membrane (*1*) and accounts for the lubricative and mechanical protective properties of normal synovium (*2*). The interpretation of data on the synthesis of hyaluronic acid by synovial tissue culture is difficult because of the possibility of metabolic alteration in culture growth. The purpose of this report is to present data on the production of hyaluronic acid from glucose-C^{14} by human synovial tissue; tissue-slice technique was used.

Specimens were obtained at operation, and 100 to 200 mg of hand-sliced tissue was incubated at 37°C in air for 1 to 3 hours in Krebs-Ringer's phosphate buffer, *p*H 7.4 (*3*), which contained 40 to 100 mg percent of uniformly labeled glucose-C^{14} (specific activity 0.5 μc/mg). Of 54 samples incubated, 45 showed significant glucose incorporation, and the remaining samples were used as controls. Isotope incorporation from radioactive glucose was approximately 50 percent greater with substrate concentration at 100 mg percent than at 40 mg percent and was linear with time for the first 3 hours.

Following incubation, the tissue was crushed and extracted with 30 to 40 ml of 10 percent sodium acetate at *p*H 9. The *p*H of the extract was then adjusted to 4, with glacial acetic acid, and 1.5 vol of ethanol was added to precipitate hyaluronic acid. After thorough washing of the precipitate with ethanol to remove extraneous radioactivity, the crude hyaluronic acid was extracted with sodium acetate and was subjected to the following studies, which are summarized in Table 1.

Reprecipitation of a pooled sample of crude C^{14}-labeled mucopolysaccharide from sodium acetate at *p*H 8, by means of ethanol saturated with potassium acetate, yielded 95-percent recovery of radioactivity. Dialysis of six samples against distilled water for 12 to 24 hours resulted in a recovery of 96 percent. One crude sample, subjected to trypsin digestion followed by dialysis, showed no loss in radioactivity, and shaking with a 10/1 mixture of chloroform and *n*-butanol removed only 9 percent. All the radioactivity of the deproteinized crude product was precipitable with hemoglobin in acid solution, by the method of Pierce (*4*). After digestion with hyaluronidase, 76 percent of the precipitable material was lost, while identical digestion of known hyaluronic acid led to 86-percent loss of precipitable material. The radioactive product was compared with authentic hyaluronic acid and chondroitin-sulfuric acid by paper electrophoresis at *p*H 5 in 0.1*M* acetate buffer, and its mobility was identical with that of hyaluronic acid. Finally, analysis of an acid hydrolyzate of the dialyzed, reprecipitated, and deproteinized product for hexosamine and uronic acid, by the methods of Boas (*5*) and Fishman (*6*), revealed values of 42.3 percent and 50.6 percent, respectively, which were in excellent agreement with theory. Following hydrolysis, as much as 77 percent of the recoverable radioactivity, or 67 percent of the total radioactivity, could be accounted for in the hexosamine fraction (Table 2).

Table 1. Identification of hyaluronic acid.

Procedure	Recovery of hyaluronic acid		
	Before	After	
	(count/min)	(count/min)	(%)
Reprecipitation	10,700	10,200	95
Dialysis	12,954	12,464	96
Trypsin digestion	1,630	1,662	100
Organic solvent treatment	4,230	3,860	91
Hemoglobin precipitation	109	0	0
Hyaluronidase digestion	132	32.2	24

Table 2. Radioactivity of hexosamine in biosynthetic hyaluronic acid.

Item	Total radioactivity recovered after hydrolysis (%)	Recovered radioactivity as hexosamine (%)	Specific activity (count/min mg)
1	88	43	10,800
2	53	73	6,600
3	93	72	7,850
4	51	74	40,060
5	28	77	32,200

The foregoing data indicate that hyaluronic acid may be synthesized from glucose by synovial tissue *in vitro*.

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