that red oats, *Avena byzantina* C. Koch., are more heat resistant than common or "white" oats, *A. sativa*. It appears, however, that no data on heat resistance in oat plants have ever been published, except for a brief preliminary note by me (*3*). The experiments were continued and the additional data obtained confirm the preliminary results and provide additional information. The more recent data were obtained while a specially constructed heat chamber was being used.

The relative winter hardiness of the varieties listed in Table 1 has been determined (*4*), and the varietal type and morphological classification as indicated by Stanton (*5*) is shown. Bond and Victoria are resistant to many races of both crown rust and smut, Appler to many smut races as well as to some minor diseases; Bond and Fulghum are rather quick growing, large culmed oats; Winter Turf, Appler, and Victoria are late-developing, large-culmed varieties, whereas others listed in Table 1 are early, comparatively small-culmed oats.

Plants of different species, varieties, and ages were exposed to temperatures of 48.5° to 51°C for 45 minutes. Such exposures had previously proved effective in differentiating differences in heat resistance. Results from experiments indicated that some varieties of both *A. byzantina* and *A. sativa* are heat resistant, but others are not. There appeared to be little or no relationship between heat resistance and resistance to any major disease of oats. Quick-growing oats with thick culms were less heat resistant than slower growing oats with more slender culms. Many of the latter group were winter oats, whereas many but not all of the former were spring varieties. On the average, early maturing oats, whether spring or winter, appeared to be more heat resistant than late to very late varieties. There was, however, definite correlation between heat resistance and winter resistance in oats (Table 1). These data were compiled from extensive field seedings to determine winter hardiness and from heat experiments conducted on greenhouse-grown oats.

Black Mesdag, a heat-susceptible spring oat, was crossed with Lee, a heat-resistant winter variety. The F_3 behavior indicated that heat susceptibility was dominant. The segregation ratio of approximately 37 (susceptible)/27 (resistant) indicated that three factors were involved, but transgressive segregation for increased heat resistance above that of the Lee parent also was observed. Transgressive segregation resulting in increased winter resistance in winter × spring oat crosses likewise has been observed. Since heat and winter resistance appear to be correlated, transgressive segregation for increased heat resistance might be expected in certain crosses.

Oat plants apparently are more resistant to heat in the early boot stage than earlier or later in their development. Maximum resistance to heat was reached in plants some 40 to 45 days old in spring varieties and in plants 45 to 50 days in the slower-developing winter oats. Heat resistance in both groups dropped precipitously thereafter.

Heat resistance in oats was greater in plants after exposure on bright days than it was in those treated on dark days. In winter oats, an apparent conditioning response resulted from exposure to warm temperatures prior to heat treatment. This was not apparent in spring oats.

Table 1. Relative winter hardiness and heat resistance of 12 varieties of oats (Fulghum check, 100 percent).*

C.I. No.	Type and variety	*Avena* species	Winter survival (%)	Heat survival (%)
		True winter		
3168	Fulwin	*A. byzantina*	137.6	123.4
3218	Bicknell	*A. sativa*	130.1	124.7
2505	Hairy Culberson	*A. sativa*	129.8	122.2
2499	Pentagon	*A. byzantina*	128.2	103.1*
947	Tech (V.P.I. No. 1)	*A. sativa*	125.1	105.5*
3296	Winter Turf	*A. sativa*	120.1	111.2
2042	Lee	*A. sativa*	116.8	100.0*
3217	Culred	*A. byzantina*	108.5	120.0
		Intermediate winter		
1815	Appler	*A. byzantina*	100.3	99.8
708	Fulghum (check)†	*A. byzantina*	100.0	100.0
		Spring		
2733	Bond	*A. byzantina*	63.8	68.2
2401	Victoria	*A. byzantina*	46.8	43.0

* Additional data, not fully comparable with those shown, reveal that this variety is somewhat more heat resistant than indicated here.
† The average heat survival for Fulghum was 56.3 percent of 451 plants tested, and it survived 56.1 percent in 560 field-grown nurseries in which differential winter killing was recorded.

It appears probable that those physiological factors conditioning cold resistance in the oat plant cell also condition heat resistance. This suggests the possibility of testing for winter resistance in oats by the cheaper, more easily conducted heat test. Exploratory tests have indicated that heat resistance and winter resistance may also be associated in wheat and in barley.

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26 February 1957

Singing Female Canaries

Pet-bird dealers have reported that some of their imported canaries sing as typical males for a time but later refuse to sing. Some have been identified as females. One dealer stated that he would no longer handle imported birds because of complaints from customers.

Since it is known that female chickens and turkeys that are treated with male sex hormone will give voice in a manner similar to males (*1*), treatment of the imported canaries with hormones was suspected. To determine whether female canaries would "sing" after receiving male sex hormone, birds were treated with this hormone.

Nine female canaries, young but fully grown, were selected for the test. Five were injected with 0.1 ml each of microcrystalline suspension of male sex hormone, testosterone phenylacetate (Perandren, Ciba). Each bird was given a single injection per test period. Each injection of 0.1 ml of the preparation contained 5 mg of active material. Four birds were not treated.

Nine days after the injection, two of the treated canaries made a series of chirps that were more closely connected than previous chirps had been. Twelve days after the injection, all treated birds were definitely "singing," although the song lasted for only a few seconds at a time. In the following days the length of sustained song became greater, and the song was indistinguishable from that

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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