multiplied by approximately 1.6 to permit a direct comparison with uridine labeling in interphase cells.

A possible significant error in this method of estimation would arise from a slow equilibration of the methyl donor pools with the exogenously added radioactive methylmethionine. While previous work has indicated that equilibration is rapid in interphase cells (*4*), the equilibration with exogenous radioactive methionine might be slower in mitotic cells. This would lead to an underestimation of the correction factor. Thus, if the methyl donor pool equilibrates more slowly in metaphase cells than in interphase cells the true rates of synthesis in the metaphase cells of 5*S* and 4*S* RNA would be even higher than obtained here.

An estimation of the relative rate of synthesis of 5*S* RNA in metaphase-arrested cells requires still another correction. In interphase cells, newly synthesized 5*S* RNA is first found free in the cytoplasmic fraction (*5*). After a considerable lag, the 5*S* RNA is incorporated into nucleoli and subsequently appears in mature ribosomes. Thus, in interphase cells radioactivity in cytoplasmic 5*S* RNA does not represent the total 5*S* RNA. No such process occurs in the mitotic cells since nucleoli are not found during metaphase. In Table 2 the amount of 5*S* RNA in interphase cells is corrected for the amount that has entered the nuclear fraction. The final estimate is that 5*S* RNA is synthesized in metaphase-arrested cells at approximately 74 percent the rate of interphase cells. This estimate is taken to indicate merely that a significant synthesis of 5*S* RNA occurs in metaphase-arrested cells.

A direct comparison of the methyl label in 4*S* RNA indicates that this species is apparently synthesized in metaphase-arrested cells at a rate approximately 34 percent of that found in interphase cells. Further evidence that the labeling of these RNA species is not due to contaminating interphase cells is obtained from the ratio of methyl to uridine in the small amount of 18*S* RNA of the metaphase preparation. The ratio of methyl to uridine of this RNA species is exactly the same as that found in the interphase culture, and this indicates that 18*S* RNA is derived from the 8 percent contaminating interphase cells. This is further supported by the fact that the amount of label in 18*S* RNA for the metaphase culture is 8 percent of the amount in the interphase culture. Thus, the pyrimidine pools from the contaminating interphase cells appear to be unaltered. If these contaminating cells continue to synthesize 5*S* and 4*S* RNA, their contribution to these species should constitute approximately 8 percent of the amounts of 5*S* and 4*S* RNA labeling in the interphase culture. This would result in small corrections downward for the relative rates of synthesis shown in Table 2.

The labeling of 5*S* and 4*S* RNA in metaphase-arrested cells proceeds at a linear rate for at least 90 minutes (Fig. 2). Thus, the incorporation seen here is not a transient phenomenon occurring only at the beginning of metaphase. The formation of cytoplasmic 4*S* RNA appears to be normal; the first product is a molecule of slightly higher molecular weight which is subsequently transformed into the mature form of 4*S* RNA (*6*).

Identical results have been obtained with Chinese hamster cells. The phenomenon, therefore, appears to be characteristic of cells of both human and rodent origin. The results suggest that some regions of the condensed chromosomes are transcribed. However, the possibility exists that some of the RNA labeled during metaphase may arise from nonchromosome-associated templates (*7*).

ESTER A. ZYLBER
SHELDON PENMAN
Department of Biology, Massachusetts Institute of Technology, Cambridge 02139

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Periodical Cicada: Mechanism of Sound Production

Abstract. *The species* Magicicada septendecim *and* Magicicada cassini *of the 17-year cicada produce sound by sequentially buckling a series of stiff ribs embedded in a flexible tymbal. Each such collapse excites a damped oscillation in a resonant cavity. By this means the cavity (an abdominal air sac) is excited 10 to 12 times per muscle contraction, which permits a normal muscle to perform a task requiring very rapid repetitive activity.*

The periodical cicadas (*Magicicada*) are among the noisiest of insects (*1*, *2*). A single male cicada can produce a sustained call lasting several seconds, and repeat this call many times per day without apparent fatigue. The sound is produced by a rapidly repeated series of damped oscillations produced in the abdominal air sac by activity of the tymbal organs (Fig. 1A). In *M. cassini*, direct tap excitation of an air sac produces a brief oscillation which is damped out in about 2 msec (Fig. 2A). To maintain sustained sound, the air sac must be excited several hundred times per second. Pringle (*3*) has described the sound-producing mechanism in several species of Ceylonese cicadas; in these a "clicker" mechanism is popped in and out at rates up to 480 per second by a specialized muscle with highly unusual excitation-contraction properties.

In June 1970, I took advantage of the local emergence of brood X of the 17-year cicada to examine the mechanism of sound production in these species (*4*). Preliminary work was done on *M. septendecim*, but the bulk of the experiments were done on *M. cassini*, as specimens of this species were much more tolerant of surgical manipulation in the laboratory. In both species the tymbal organ of the male consists of 12 stiff ribs connected by a flexible sheet (Fig. 1, C and D). The tymbal muscle is connected close to the posterior end of this sheet by a flexible, tendon-like apodeme (Fig. 1B). Recordings of the mechanical behavior of the tymbal were obtained by slipping a fine, stainless steel wire hook through

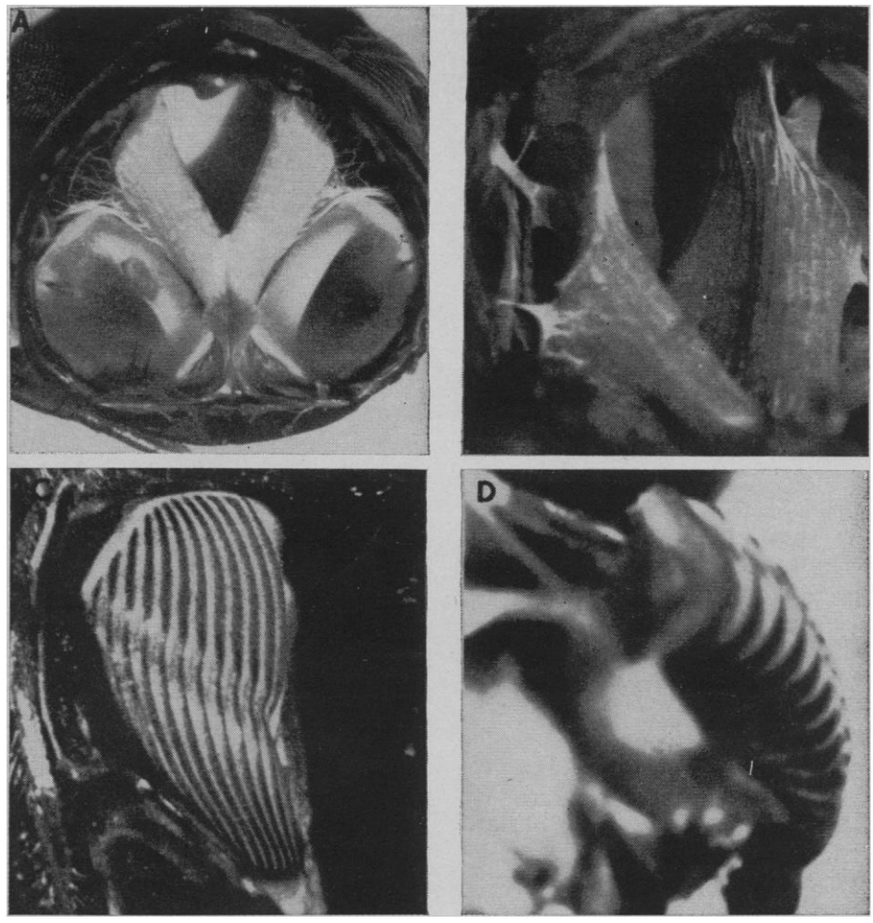

Fig. 1. (A) Section through 8-mm diameter *M. cassini* just caudal to the thoracic-abdominal junction. View from rear. Tymbals visible at top corners; tymbal muscles are V in center; below are the pair of ducts, each covered with a thin flexible membrane, connecting the tymbal cavity with the air-filled abdomen. (B) View of tymbal muscles from front, showing attachments (apodemes) linking them with the tymbals. (C) Lateral exterior view of tymbal, showing ribs. (D) Freshly dissected tymbal, obliquely illuminated, showing functionally separate ribs.

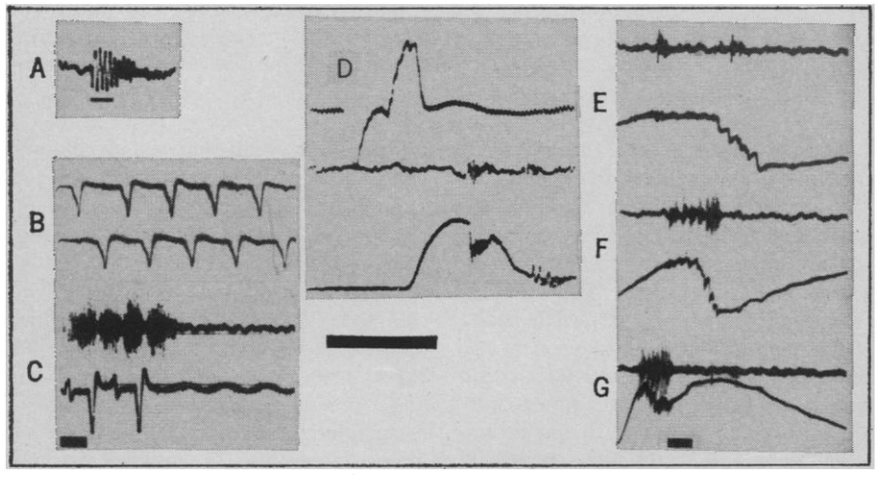

Fig. 2. (A) Minimum excitation of cavity; damped oscillation of about 5 khz, lasting less than 2 msec. Time line, 1 msec. (B) Electromyograms from the two tymbal muscles during a burst of sound; muscles contract alternately. (C) Sound output and EMG at termination of a sound burst. The EMG from both tymbal muscles are recorded on the lower channel, at different amplitudes. Time line for B and C, 10 msec. (D) The EMG, sound, and contractile force during a weak contraction. Tension on apodeme drops abruptly as tymbal buckling begins; at end of twitch ribs snap back into place producing brief increases in tension. Time line, 10 msec. Peak tension was about 1.8 g. (E–G) Sound and tension records during manual "twitch." See text. Time line, 10 msec.

a slit in the tymbal membrane and hooking it around the apodeme; the other end of the wire was attached to a Pitran (*5*) force transducer by a small dab of Caulk dental cement. The frequency response of the transducer-hook combination was in excess of 30 khz (*6*).

Single muscle twitches were excited by electrical stimulation of the motor nerve dorsal to its entrance into the tymbal muscle (*7*). Sustained bursts of sound similar to the natural call could be elicited by brief electrical stimulation in the region of the thoracic ganglion. Some records were also taken during spontaneous sound emission (*8*).

Electromyographic (EMG) records (Fig. 2B) showed that the two tymbal muscles were excited alternately during the call. These alternate excitations produced a continuous burst of sound (Fig. 2C). Changes in EMG, sound, and tension during a single contraction are shown in Fig. 2D. Tension rises in the tymbal until buckling begins, and the ribs then collapse in orderly succession, from posterior to anterior, producing a burst of sound. As the tymbal muscle relaxes, the ribs snap back to their original positions in reverse order, producing a second weaker burst of sound (*9*).

To confirm this mechanism, chirps were elicited manually by pulling on the apodeme in the direction of tymbal muscle contraction (*10*). Changes of force and sound output associated with slow, moderate, and rapid movement are shown in Fig. 2, E–G. The sequential stepwise reduction of tension accompanying rib buckling is shown clearly in the upper tracing, where the rate of movement is too slow to produce much sound. The lower tracing (Fig. 2G) shows a typical chirp elicited in this manner.

The fine structure and properties of the tymbal muscle were studied in some detail (*11*). During sound production each tymbal muscle contracts at about 50 twitches per second in *M. cassini*. The cicada can maintain this rate for several seconds—300 to 500 twitches—before showing any signs of fatigue. The tymbal muscles are well able to handle this rate of operation, for a twitch contraction reaches maximum tension in less than 7 msec and tetanic fusion of contraction does not occur until the rate of stimulation exceeds 150 to 200 per second.

In summary, the periodical cicadas possess a highly efficient mechanism for exciting a small resonant cavity at

a very high rate (up to 1000 per second) with a minimum expenditure of muscular energy. The high efficiency of this mechanism permits the male cicadas to emit a large volume of sound for a long period of time (1 to 2 weeks) despite a total lack of food intake during that time. The behavioral significance of this sonic activity has recently been discussed by Simmons *et al.* (*2*); the main functions are the attraction of mates, the separation of the different species within a brood, and the repulsion of avian predators.

KENNETH H. REID
Department of Physiology and Biophysics, University of Louisville School of Medicine, Health Sciences Center, Louisville, Kentucky 40202

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2. J. A. Simmons, E. G. Wever, J. M. Pylka [*Science* **171**, 212 (1971)] measured sound levels on the ground beneath cicada-filled trees as 800 dyne/cm² and estimated the level within the swarm as 1000 dyne/cm² or more.
3. J. W. S. Pringle, *J. Physiol. (London)* **124**, 269 (1954). In the genera studied by Pringle the tymbals were not ribbed, but were smooth domelike structures, "clicked" in and out at a rate high enough to produce a sustained sound.
4. A preliminary presentation of these data was made at the American Physiological Society meetings in Bloomington, Indiana, on 1 September 1970.
5. The Pitran force transducer (Stow Laboratories, Hudson, Maine) is a pressure-sensitive transistor employing a piezoelectric sensing element. Sensitivity of the models used (PT-2, PT-5) is of the order of 1 volt/g force, and the mechanical self-resonance frequency is in excess of 100 khz.
6. The modified transducers, with attached wire hooks, could easily follow ultrasonic vibrations at 30 khz, and had a frequency response essentially flat over the range of interest.
7. Nerve stimuli were delivered through PE-10 tubing filled with mammalian Ringer solution. Electromyographic recordings from the tymbal muscle were also made with these electrodes. Stimulation of the thoracic ganglion was done with a pair of fine needle electrodes. Stimulus durations were 0.1 to 0.5 msec.
8. Neurophysiological studies were begun, but no useful results were obtained before the brood died off. S. Hagiwara and A. Watanabe [*J. Cell. Comp. Physiol.* **47**, 415 (1956)] have described a similar pattern of tymbal muscle activity in the Japanese cicada *Grapsopsaltaria nigrofuscata* and have recorded intracellularly from the tymbal motoneurons. With extracellular recording, they detected 200 per second activity in interneurons during the call, and proposed that the motoneurons respond alternately to a pacemaker neuron discharging at 200 per second, with a mutual inhibitory interaction ensuring alternation.
9. A. D. Blest, T. S. Collett, J. D. Pye [*Proc. Roy. Soc. Sec. B* **158**, 196 (1963)] have described a very similar mechanism in the arctiid moth *Melese laodamia* Druce. In this species the tymbal cavities are much smaller than in the cicada, and the sound produced is ultrasonic, in the range 30 to 90 khz. Each tymbal has 15 to 20 ribs, whose buckling excites the resonators at rates between 500 and 1200 per second. Sound bursts (contraction-relaxation cycles) are produced at an average rate of 2.4 per second; their function is believed to be to "jam" the echolocation mechanisms of the bats which prey on these moths.
10. The force transducer was mounted on a rod held in a fairly stiff ball joint (Grass Instrument Co. cortical electrode holder). By flicking the other end of the rod with a finger, a rapid smooth "twitch" of the appropriate amplitude could be made, with reasonably good control of the speed of motion.
11. S. M. Walker and K. H. Reid, in preparation.
12. I thank Dr. S. M. Walker for introducing me to this insect and suggesting this study, and Dr. E. Roseman for the loan of equipment.

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Residues of Total Mercury and Methylmercuric Salts in Lake Trout as a Function of Age

Abstract. *An analysis of the concentrations of total mercury and methylmercuric salts in lake trout of precisely known ages from 1 to 12 years has been carried out. The concentrations of both total mercury and methylmercury increased with the age of the fish. The proportion of methylmercury to total mercury also increased with age.*

There have been numerous reports of relatively high concentrations of mercury in fish (*1*). Although many analyses of fish for mercury have been carried out, it is usually difficult to relate concentrations to time of exposure since judging age by scale examination is very difficult, particularly in older fish. In a study of northern pike (*Esox lucius*) Johnels and Westermark (*2*) found the total mercury concentration proportional to the age of the fish but admitted the unreliability of judging their ages by scale examination. In the work reported here residues of mercury have been determined in lake trout of known age.

We were fortunate to have available lake trout (*Salvelinus namaycush*) from Cayuga Lake in Ithaca, New York, of known age since they are tagged and stocked there annually as fingerlings. It was not known what concentrations of mercury might be expected in the fish. Mercury reaching the lake could, however, result from its use in laboratory research, in dental and medical services, in agriculture, in coal burnt in power plants, and from other sources. In October 1970, fish were netted in order to obtain samples of as many different ages as possible. Without evisceration, each was mechanically chopped, ground, and thoroughly mixed. A 1-g

Table 1. Corrected* concentrations of total mercury and mercury as methylmercury in Cayuga Lake trout.

Fish code	Age (years)	Mercury (total) (ppm)	Methylmercury (calculated in terms of mercury) (ppm)	Percent of total mercury as methylmercury
95	1	0.24	0.074	30.8
99	1	.28	.098	35.0
101	1	.19	.066	34.7
59	2	.25	.108	43.2
78	2	.26	.096	36.9
89	2	.31	.121	39.0
80	3	.38	.208	54.7
82	3	.45	.271	60.2
112	3	.28	.157	56.1
104	4	.44	.375	85.2
105	4	.41	.288	70.2
151	4	.44	.346	78.6
155	5	.43	.349	81.2
10	6	.46	.412	89.6
11	6	.55	.479	87.1
13	6	.50	.445	89.0
2	7	.40	.283	70.8
4	7	.46	.403	87.6
5	7	.44	.349	79.3
1	8	.60	.534	89.0
6	8	.59	.519	88.0
8	8	.47	.479	101.9
19	9	.53	.433	81.7
3	11	.58	.407	70.2
15	12	.62	.415	66.9
16	12	.66	.503	76.2
22	12	.44	.389	88.4

* Corrected for percent recovery (see text).