compatibility, and the immunologic reactivity of both hybrid and inbred hosts to the grafts was about equal. One or more necrotic areas appeared on graft surfaces about 2 weeks after grafting (Fig. 1); these would enlarge and coalesce to include the entire graft surface. When rejection was complete, all that remained was a dermal collagen pad, somewhat reduced in size, overlaid with host epithelium in which hair growth usually remained absent. The partial rejection of grafts began in the same fashion except that, after varying amounts of graft destruction, which reached a maximum at 19.8 and 21.3 days in immune and nonimmune mice respectively, progressive necrosis would cease, and these areas would heal. Once healing had occurred graft size was usually reduced, and no further evidence of incompatibility was observed; this left the impression that only certain groups of cells were antigenic. The occurrence of partial rejection was about equally distributed between immune and nonimmune hosts. Small amounts of necrosis were seen in many grafts taken from leukemic donors, but these grafts have not been included in the table because of their doubtful significance.

Tumors often developed under grafts on nonimmune hosts after an average time of 46 days. Eight of these tumors that arose in hybrid hosts were subjected to transplantation studies which showed them to be of donor origin. This observation, in addition to the fact that this virus does not induce solid tumors at the site of inoculation, indicates that these tumors arose from leukemic cells present in donor skin at the time of grafting, rather than being virus induced subsequently. The response of immune hosts was probably sufficient to destroy these cells before tumors could develop.

The immunologic nature of this skin graft rejection is evidenced by the significantly larger ($P < .001$) number of complete graft rejections in immune as opposed to nonimmune hosts. It is possible that graft rejection is a consequence of the destruction of leukemic cells within the graft. There is some evidence against this possibility in that skin grafts from weanlings, injected as newborns with virus and showing no overt signs of leukemia, are similarly rejected in the apparent absence of leukemic cells. Thus there appears to be an antigen common to both the skin of leukemic mice and the induced tumor that elicits immunity in normal Balb/c mice. Thus, in animals infected with a leukemia virus, the cells of other "normal" tissues may also acquire virus-associated antigenic properties. This is supported by the reported occurrence of virus particles in the mammary glands of nonleukemic C3H(f) females injected with passage A leukemia virus (*4*). We used skin grafts because of the ease with which their survival can be followed, but whether grafts of other tissues might also show evidence of rejection is undetermined.

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Reversible Sonic Inhibition of Protein, Purine, and Pyrimidine Biosynthesis in the Living Cell

Abstract. *Yeast cells, carrying on normal biosyntheses in the treatment cup of a sonic oscillator, cease synthesis when the oscillator is operated at a critical low power level. Synthesis is resumed immediately when the oscillator is turned off. At the sonic intensities employed the cells are not extracted, uptake of ammonium ion is unaffected, and most cells remain viable. The inhibition may be the result of disruption of supramacromolecular organization in the cell.*

The disruption of cells by sound has been widely used as a technique for extracting enzymes and other components. Different structures of the cell may be broken down at different rates; certain enzymes may be extracted from the cell at different rates in sonic fields (*1*) and in the Mickle disintegrator (*2*). The attachment of respiratory enzymes in *Azotobacter* to membrane envelopes within the cell has been demonstrated in this way (*2*). Hughes and Nyborg (*3*) have demonstrated that cells can be disrupted under conditions whereby treatment with sound ("sonication") produces gas microbubbles, but the collapse of the bubbles, which produces cavitation, is suppressed. In experiments in which a vibrating needle was placed next to cell walls, violent microeddies could be seen in large cells with intact cell walls. This phenomenon provides a mechanism whereby one might be able to alter the spatial arrangement of organelles and fragile compartments without necessarily destroying the cell. If the several steps of a given biosynthetic pathway proceed efficiently in the cell only when the required enzymes are arranged or compartmentalized in a certain order, disruption of that order would be expected to inhibit the biosynthesis. On the basis of these ideas an investigation was undertaken to determine if the biosynthesis of certain compounds in the intact cell could be inhibited by sound at a frequency of 10 kc/sec.

The yeast employed was a haploid strain of *Saccharomyces cerevisae*, S1237, with biochemical deficiencies for adenine, uracil, and histidine. Aminoimidazole ribotide (AIR) accumulates in this strain in the absence of adenine as the result of a genetic block of purine synthesis (*4*). The biosynthesis of purines proceeds normally up to the reaction in which AIR is produced. When the yeast is grown in the presence of adenine, synthesis of AIR is inhibited, but when adenine is removed AIR synthesis proceeds at a high rate. The assay for AIR has been described (*4*). The block in the pyrimidine pathway leads to the accumulation of ureidosuccinic acid and dihydroorotic acid in the absence of uracil (*5*). These compounds were assayed by the Gerhart and Pardee modification (*6*) of the Koritz and Cohn carbamylamino tests; dihydroorotic acid is converted to ureidosuccinic acid in the assay. Protein was determined by use of the phenol reagent (*7*). The Raytheon 250-w 10 kc sonic disintegrator was used for sonic treatment. From a thermostatically controlled refrigerated bath, water at 30°C was circulated through the jacket of the sonic cup. The temperature of the cell suspension in the sonic cup was measured with a thermistor device during and after soni-

cation; it stayed within 0.2° of 30°C. Control suspensions were incubated in the bath. The output current used in the reversible inhibition experiments was usually 0.2 amp radio-frequency, corresponding to setting 15 on the power control (available settings ranged from 0 to 100). At this setting the cavitation hissing heard at higher levels is absent. The meter for recording output current was not sufficiently sensitive for monitoring purposes, so the voltage across the drive coil was also measured during the experiments. Calorimetric measurements of absorbed sonic energy at power setting 15 gave an estimated 1.4 cal/g min for volumes of cell suspensions ranging from 20 to 40 ml in the cup.

The yeast was grown in liquid synthetic medium (*4*) in a water bath at 30°C, the culture being shaken continuously. When the mid-log phase of growth (10^7 cells per milliliter) was reached the suspensions were kept at 4°C until used. Cell suspensions in 20 to 40 ml of synthetic medium were treated in the sonic cup. Reversible inhibition of biosynthesis was found only over a narrow range of power settings —13 to 15—and there was some evidence that the physiological state of the cell may have affected its fragility at the time of sonication, so the method of cell preparation is probably of importance. After treatment for 30 minutes at power setting 20, the cell compounds were extracted to an appreciable extent; at power setting 10 there was little or no inhibition of the biosynthetic processes tested.

To test for the inhibition of AIR synthesis the cells were transferred to a medium lacking adenine and incubated in the sonic cup, the suspension being sonicated continuously. Five-milliliter samples were withdrawn at various times during and after sonication and were immediately assayed for AIR. The results of five experiments are summarized in Fig. 1. The control cells synthesized and accumulated AIR at a high rate for 2 hours or more. In the experimental cells sonicated at power setting 15, the synthesis of AIR was almost completely inhibited. When the oscillator was turned off, AIR synthesis was resumed within 5 minutes or less and continued at a lower rate than in the control; the highest rate of synthesis observed after termination of such treatment was 70 percent of the control rate. Therefore, the cell population did not completely recover the ability to synthesize AIR. Since a fraction of the population was rendered by sonication incapable of further division, it is probable that this fraction does not regain the ability to synthesize AIR at all, while the rest of the cells recover completely.

Two types of experiments were performed to demonstrate that the low rate of AIR accumulation during sonication was not due to the amount of AIR synthesized balancing the amount leaking out of the cell. In one, adenine was added to the suspension just before it was subjected to sonic vibration. This inhibited AIR synthesis, so that if leakage occurred during sonication or if AIR itself were destroyed a net decrease in AIR per cell would be observed; no change was found. In the second type of experiment, cells were sonicated for various periods up to 2 hours; if the number of cells leaking AIR increased with duration of the sonic treatment a net decline in AIR per cell would be observed. Again the amount of AIR per cell remained constant. It was concluded that the extraction of AIR was not caused by the sonic treatment.

The effect of sonication on the biosynthesis of ureidosuccinic acid and dihydroorotic acid was tested by a method similar to that employed for AIR. The synthesis of the former was inhibited most effectively when the power setting was between 13 and 15, showing that AIR and ureidosuccinic acid biosyntheses are equally sensitive to sonication. The effect of sonication at power setting 15 on ureidosuccinic acid is shown in Fig. 2. Virtually complete inhibition of ureidosuccinic acid synthesis occurred, and there was no leakage of the acid from the cells. Soon after the sonic oscillator was turned off, the ability to synthesize ureidosuccinic acid was recovered nearly 100 percent. This complete reversibility of ureidosuccinic acid inhibition is the one characteristic yet found that distinguishes it from AIR inhibition.

It seemed possible that sonic inhibition of intracellular biosyntheses could be the indirect result of inhibition of uptake of nutrients by the cell from the medium. Thus the sonic vibration might impair the function of the cell membrane instead of (or in addition to) interfering directly with internal syntheses. We have observed that yeast cells of strain S1237 will synthesize AIR at a normal rate for an hour or so

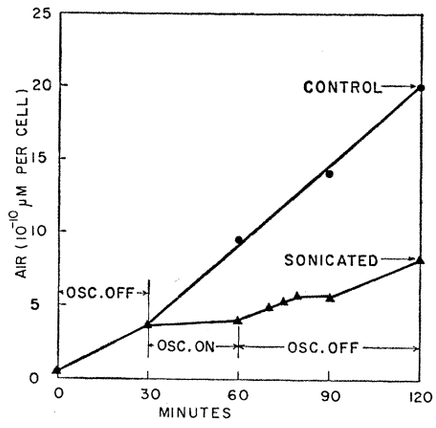

Fig. 1. Sonic inhibition of synthesis of aminoimidazole ribotide (AIR) in yeast. The sonic oscillator (OSC) was on at power setting 15 only during the 30- to 60-minute period. The control suspension was not sonicated at any time.

in a simple medium containing only phosphate buffer, glucose, and ammonium sulfate. Synthesis of AIR does not take place in the absence of ammonium ion, indicating that the cells must take up this sole source of nitrogen in order to make AIR. Hence, if sonic vibration inhibits ammonium uptake, a mechanism for sonic inhibition of AIR is provided. To test this hypothesis, cells were incubated in a medium containing the phosphate buffer and glucose, and were then provided with ammonium sulfate at a concentration of 0.01 percent. During the succeeding hour, the rate of uptake of

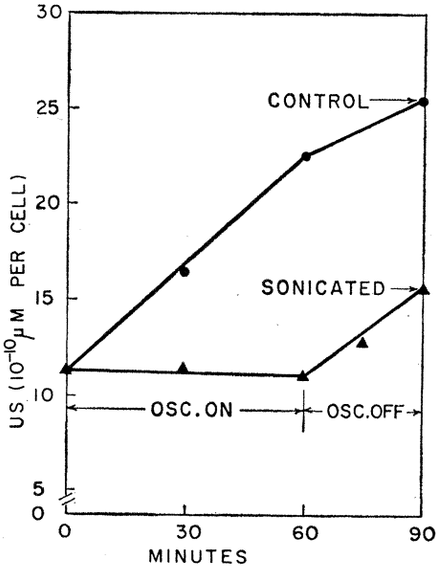

Fig. 2. Sonic inhibition of synthesis of ureidosuccinic acid (US) in yeast. The sonic oscillator was on at power setting 15 only during the 0- to 60-minute period.

Table 1. Effect of sonication on protein content of yeast. Results expressed as micrograms of protein (in cells) per milliliter of suspension.

Time (min)	Sonic power setting	Protein content	
		Sonicated	Control
0			296
	0		
30		308	308
	14		
60		288	336
	0		
105		286	348

ammonium ion was determined from analyses of the amount of ammonium remaining in the medium as a function of time. The collected ammonia was assayed by the Conway microdiffusion technique and the Nessler test. Two cell suspensions were treated identically except that one was sonicated (at power setting 15) during the uptake of ammonium ion, and the other was not. In both suspensions 24 percent of the ammonium ion was taken up per hour, indicating that sonic vibration does not interfere with the normal functioning of the cell wall and membrane.

The effect of sonication at low intensity on the net protein content of the cells was investigated; the results of a typical experiment are shown in Table 1. There was small decrease in the protein content during sonication, but thereafter protein content remained constant during 45 minutes of recovery. Apparently the protein synthesizing system was unable to recover as rapidly as the systems participating in the synthesis of AIR and ureidosuccinic acid. It is noteworthy that the latter systems recovered from sonication during the period when there was no net synthesis of protein in the population. This suggests that recovery of the ability to synthesize AIR and ureidosuccinic acid does not require the synthesis of new enzymes. The rapid recovery of these systems after sonication gave no evidence of the lag to be expected if enzymes had to be resynthesized. It is unlikely that the enzymes are damaged at the low sonic powers used in these experiments; the enzyme that forms ureidosuccinic acid from aspartate and carbamyl phosphate, aspartate transcarbamylase, can be extracted in active form from *Escherichia coli* by subjecting cultures to sonication at full power for 20 minutes (*8*).

Viability tests were made to determine whether the cells recover not only the ability to synthesize ureidosuccinic acid and AIR but also the ability to continue division through many generations. Viability was tested by plating suitable dilutions of sonicated cells on nutrient agar and scoring colonies after incubation for 2 days. Before being sonicated at power setting 15, the cells were treated briefly at power setting 10, so that biosynthesis was not inhibited but cell clumps if present were broken up. The results showed that 75 percent of the cells were viable after treatment for 30 minutes at power setting 15. Since the synthesis of both ureidosuccinic acid and AIR was completely inhibited during sonication, one can conclude that inhibition occurred in cells capable of further reproduction after sonication. Sonicated cells were also observed at magnification × 1000 in a phase microscope. No lysed cells were seen, the complement of granules was normal, and buds were not knocked off mother cells; there was some indication that the vacuoles were enlarged.

What is the mechanism of inhibition by sonic treatment? The high viability of sonicated cell populations and the rapid recovery of biosynthesis after sonication indicate that DNA and the enzyme-forming systems at least, and probably the enzymes, are not inactivated by low-power sonication. If eddies form inside the cells during sonication it would be expected that the weakest bonds, such as those involved in intermacromolecular interaction, would be most easily disrupted. A loosely linked but ordered arrangement of macromolecules in the cell has been described by Frey-Wyssling (*9*). A kinetic analysis by Pollard (*10*) indicates that the enzymes of certain pathways would have to be confined to small volumes, such as membrane channels, to account for the rates of synthesis observed in the cell. The disruption of such structures may account for the inhibition of biosyntheses during sonication.

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Adhesiveness of Spider Silk

Abstract. *Moths, by virtue of the loose scales that cover their wings and bodies, are admirably adapted to elude capture by orb-weaving spiders. Rather than sticking to the web, they may simply lose some of their scales to the viscid threads, and then fly on. Other insects, covered with detachable hairs or waxy powder, are similarly protected against entrapment. Quantitative data are presented on the adhesiveness of spider thread to insect cuticles of various kinds.*

Moths, like any insects that fly by night, profit from the relative absence of diurnal predators such as birds, yet their nocturnal excursions are fraught with other hazards. For those that fly consistently high and above the existing vegetation, the chief enemy is probably the foraging bat. Those that fly lower face danger from still another source, the spider web.

Recent work has shown that some moths are remarkably equipped for defense against bats. Special ears on the thorax enable the moths to hear the ultrasonic chirps emitted by bats in their attempts to echo-locate insect prey. Upon hearing the chirps, the moths dive downward or enter upon otherwise evasive flight, thereby eluding capture (*1*). The special way in which moths and certain other insects are adapted for escape from spider webs is the subject of this report. The study was prompted by the casual observation that moths do not necessarily get caught in a web, but may simply bounce off or fly through it.

The web of an orb-weaving spider