

sisted unchanged for about 24 hours. No convulsions were observed.

In Table I there are tabulated for each dose, the time of death of each animal, and the time at which 50% of the animals remained alive. This last figure was obtained by plotting against time in minutes a curve representing the percentage of animals remaining alive at each minute; and then locating on the time axis the point where the smoothed curve crossed the 50% line. For doses of 90 mg. per kg. or greater, this value is determined solely by the animals presenting the first type clinical course; below 70 mg. per kg. solely by those presenting the second type.

The data indicate that the minimum dose killing any animal was 70 mg. per kg.; the maximum dose survived by any animal was 50 mg. per kg. The minimum lethal dose thus lies between 30 and 50 mg. per kg. Experimenters will be more interested in the doses producing the poisoning. The second type of clinical course (prolonged prostration, death without immediate rigor) was obtained in doses ranging from 70 to 110 mg. per kg., the minimum producing it in 50% of cases being 70 mg. per kg. The first type (convulsions with immediate rigor) was obtained with doses of 70 mg. and greater, the minimum producing death in this manner in 50% of cases being 90 mg. per kg.; and the minimum for 100% being 120 mg. per kg.

While this study has not included any analysis of the mechanisms involved, the impression is given that the earliest action on the intact animal is on the central nervous system, including particularly a stimulation of the respiratory center. Provided that the dose be high enough, and that the central nervous system action has produced vigorous convulsions, the rigor producing action on muscles, seen by Lundsgaard in frogs, is evident.

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Ultrasonic Radiation and Yeast Cells.

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A system capable of producing a series of high frequency waves in fluid may be constructed by placing a piezo-electric crystal of

carefully prepared quartz in a di-electric liquid such as xylol. A pair of electrodes is then placed upon opposite sides of the quartz crystal and should be connected with a high frequency oscillator. Such an arrangement of apparatus allows one to set up compression waves even of radio frequency in the liquid. A system similar to this has been described by Wood and Loomis.¹ Radiation by the resultant ultrasonic vibration has been shown by Wood and Loomis¹ to be destructive to certain varieties of organisms including algae, but it was their opinion that bacteria are not injured by such treatment. Radiation by ultrasonic vibration may be attended by cavitation of included gases and it is considered probable by Johnson² and by Schmitt and Uhlemeyer³ that this mechanical expulsion of the gases is the cause of disruption of living cells. Recent observations of Wu and Liu⁴ indicate that these rays coagulate protein and thus it becomes probable that protein changes contribute to the cause of death.

To test the effects of ultrasonic radiation upon yeasts, the following series of experiments was performed: A wine yeast was incubated in grape juice for 24 to 48 hours at 37°C. This organism, listed in our cultures as a *Saccharomyces elliposoideus*, was chosen because of its relatively large size and active growth.

Treatment was carried out by placing the culture to a depth of approximately one inch within a glass tube 1½ inches in diameter. The bottom of this tube, however, was of heavy cellophane cemented to the side walls. The culture within this tube was now lowered into the di-electric of xylol in which had been placed the piezo crystal of quartz. Thus the yeast culture was separated from the xylol only by a cellophane layer in order that the minimum amount of energy absorption might take place during transmission through the intervening wall. When current was applied a standing wave system appeared attended by formation of a pyramid of fluid in violent agitation and by much fog of finely divided fluid. Temperature tended to rise but a circulatory cooling system held the thermometer reading below 39°C. At least 25 tests of the effect of ultrasonic vibration upon this and other similar yeasts have been made with results indicated in Table I. Figures are to be translated not as

¹ Wood, R. W., and Loomis, A. L., *London, Edinburgh and Dublin Philos. Mag. and J. of Sci.*, 1927, **4**, 417.

² Johnson, C. H., *J. Physiol.*, 1929, **67**, 356.

³ Schmitt, F. O., and Uhlemeyer, B., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 626.

⁴ Wu, H., and Liu, S., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 782.

counts per cc. but rather as indicators of the relative numbers of viable forms present. They were obtained by plating upon Sabouraud's agar.

TABLE I.
The Effectiveness of Ultrasonic Vibration in Killing Actively Growing Yeasts.

Before Treatment	100,000	28,000	54,000	50,000	21,000	22,800	15,000	125,000	36,500
1 min.	17,000	39,000	2,100	1,800	12,600	22,800	12,500	31,500	4,200
3 "	0	4,800	0	450	9,100	9,100	10,500	3,000	8
5 "	0	110	0	20	3,500	7,700	11,200	360	5
10 "	0	21	0	8	2,200	1,750	5,800	13	5
15 "	0	0	0	0	1,600	lost	3,500	1	5

Microscopic examination of treated culture material revealed much cellular debris and relatively few formed yeast cells. Moreover, transfer from a treated culture to a fresh tube of medium in many instances was followed by no subsequent growth, indicating, therefore, complete sterilization. In other instances, however, these subcultures did show delayed growth.

It appears, therefore, that ultrasonic radiation is an effective agent in killing yeasts. Moreover, the death of the yeast cell may be attended by its disruption.

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Prevention of Heat-Coagulation, Mercuric-Precipitation of Proteins, and of Precipitation of Alkaloids by Colloidal Dyes.

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Congo-red and some other colloidal dyes possess the unique action of preventing death in animals injected with surely fatal doses of potent bacterial toxins and drugs.¹ This protective action appears to be mediated through the physical properties of these colloidal dyes. Presumably correlated with this physical protection *in vivo* are some striking protective effects of the colloidal dyes on powerful reactions *in vitro*, such as the heat-coagulation and mercuric precipitation of proteins and the alkaloidal precipitation by Mayer's reagent. As far as I know, these marked protective effects *in vitro* have not

¹ Hanzlik and Butt, *J. Pharm. Exp. Therap.*, 1928, **33**, 260.