with that of the tetrazolium pattern of *Zea mays* embryo slices. Attempts were made to inhibit the sulfhydryl reaction with the tetrazolium reduction inhibitors. It was found that several of the tetrazolium reduction inhibitors also blocked the sulfhydryl groups. Embryo slices treated with thioglycollic acid gave no sulfhydryl pattern, iodoacetic acid resulted in a weak reaction, and 2,4-dinitrophenol in a medium reaction.

It appears highly probable that dehydrogenase enzyme systems are responsible for the oxidation of various substrates and the concomitant reduction of tetrazolium to formazan. The reducing agent is heat-labile, but remains undamaged by freezing. It has also been observed by the author that homogenized tissues give a much weaker reaction. The classic experiments of Thunberg demonstrated that dehydrogenases are responsible for reduction of reversible redox dyes. However, because of the lack of specificity for the reaction, as shown by inhibition studies, it is probable that no one reductase system is responsible for the characteristic reduction in plant tissues. It seems more likely that a general redox potential level, maintained by the operation of several physiologically active systems, brings about the reduction of tetrazolium.

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The Relationship of Acoustical Energy to the Lethal Effects of Ultrasonic Vibrations on *E. coli*[1]

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In a previous paper (*1*) it was shown that the rate of destruction of *E. coli* by ultrasonic vibrations at 400 kc was influenced significantly by the environmental temperature. Many other factors undoubtedly influence the germicidal properties of these vibrations, one of which should be the energy input to the sample under treatment. Accordingly, it was desirable to investigate this factor and to determine its significance.

Using the apparatus previously described (*1*), a series of tests was conducted wherein the energy input to a sample containing an aqueous suspension of *E. coli* was varied by controlling the variable transformer in the electronic driving circuit.

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TABLE 1

PERCENTAGE OF *E. coli* SURVIVING ULTRASONIC VIBRATIONS AFTER VARIOUS EXPOSURE TIMES AND AT VARIOUS ENERGY INTENSITIES AT 15.5° C

Exposure duration (min)	Energy intensity, acoustical w/cm²								
	4.8	5.8	7.2	9.1	11.5	14.4	18.6	24.0	31.3
3	97.5	100.1	88.6	83.3	88.6	74.7	71.5	73.6	76.9
6	85.4	85.0	75.7	73.3	71.8	56.4	52.8	58.1	59.4
15	65.1	66.5	54.5	50.5	40.2	22.4	15.9	17.9	26.4
25	53.2	46.1	35.3	36.3	21.5	13.2	10.5	7.1	9.9
40	39.1	34.1	20.9	15.9	9.24	5.04	3.41	1.77	2.69
60	21.0	18.0	9.87	4.11	0.10	0.42	0.08	0.34	0.62

In order to determine the amount of acoustical energy that reached the sample, a Siemens power meter was suspended in the oil bath at the same position with regard to the crystal that the sample normally occupied. Varying amounts of acoustical energy were beamed to the meter by changing the setting of the variable transformer in the driving circuit. The readings of the power meter were correlated with readings obtained simultaneously from a voltmeter inserted in the electronic circuit across the crystal. In this manner the voltmeter was calibrated to read in terms of the acoustical energy applied to the sample. Since the power meter used did not cover the entire range of energy intensities available from the generator, it was necessary to extrapolate the calibration curve for high intensity energy inputs.

Altogether, sixty-seven 1-hr runs were made on *E. coli* suspended in buffered water. The suspension was prepared by introducing 1 ml of a 24-hr broth culture of *E. coli* into 100 ml of sterile buffered water. The environmental temperature for all the observations was maintained at 15.5° C. The initial concentration of viable cells in all cases was approximately 80,000/ml. Statistical analyses of the results obtained were made and are presented herewith (Table 1 and Fig. 1).

It is apparent from Fig. 1 that the killing curve is essentially logarithmic at all energy intensities. In some cases, toward the end of the run, the curves tend to level out somewhat, but generally speaking the straight-line relationship applies. In those cases where the rate of killing showed a slight curvature, the initial killing rate, obtained during the first 30 min, was chosen as the characteristic rate for that particular energy intensity. It may also be concluded from Fig. 1 that, although an increase in energy intensity (within limits) results in an increase in the killing rate, an intensity is finally reached which yields the maximum killing rate; and that further increases in energy intensity result merely in reduced lethal effects. This is clearly shown in Table 2 and Fig. 2.

Fig. 2 was obtained by plotting the killing rate constant, as determined by the slope of the killing curve,

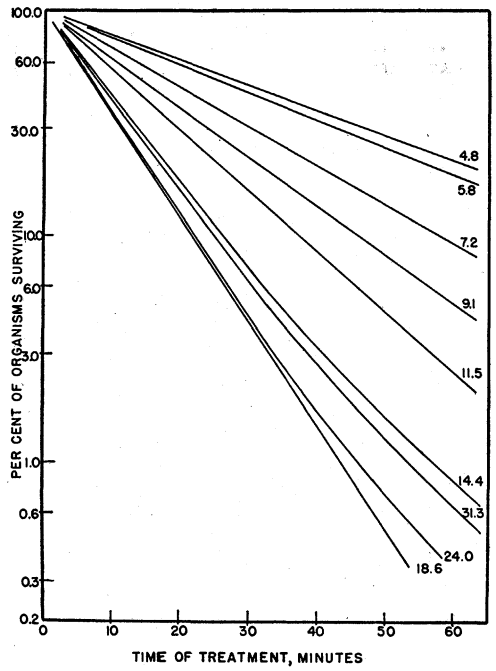

FIG. 1. Relation of energy intensity to the survival by *E. coli* of ultrasonic vibrations. Intensities are in acoustical w/cm².

against the energy necessary to obtain that rate. The curve shown in Fig. 2 is similar to that obtained by Weissler (*2*) in his investigations on the relationship of acoustical energy intensity to the liberation of iodine from potassium iodide solutions. The ordinate in Fig. 2 is the slope-dependent form of the rate constant M, from the equation of the killing curve

$$Y = 10^{(2-\frac{X}{M})},$$

where Y is the percentage of *E. coli* surviving ultrasonic treatment at any time X. In all cases the slope used to determine the factor M was the initial slope of the killing curve.

Fig. 2 indicates that the killing effect of ultrasonic vibrations is decreased after a certain peak, or optimum, energy intensity is reached, despite further apparent increases in the energy intensity. This is probably due to the fact that at higher energy intensities the sound wave is attenuated because of the presence of excessive cavitation bubbles in its path. The cavitation bubbles, which are filled with the gases dissolved in the liquid medium, are formed in large numbers during the low pressure phase of the higher intensity sound waves. Thus they effectively prevent the uninterrupted passage of the sound wave. In consequence, only a small portion of the sample receives a continuous application of sound energy, and the net effect is to decrease the killing rate.

This attenuation of the sound wave may also be observed qualitatively by considering the typical radiation fountain formed in the water at the top of the sample container as the output of the generating unit is increased. The radiation fountain increases in size until it is approximately an inch above the normal water surface. As the energy output increases, there is a sudden cutoff of the fountain, the water surface is only gently agitated, and a buzzing sound is heard.

TABLE 2

KILLING RATE AS CHARACTERIZED BY CONSTANT, M, IN EQUATION $Y = 10^{(2-\frac{X}{M})}$*

Energy intensity w/cm²	Rate constant, M
4.8	92.3
5.8	82.9
7.2	58.3
9.1	46.4
11.5	37.7
14.4	26.3
18.6	21.8
24.0	22.8
31.3	25.3

* Y = percentage of *E. coli* surviving at given time X; M = initial slope of killing curve.

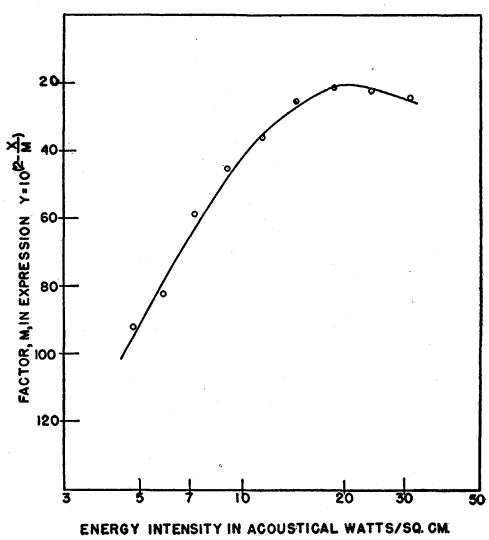

FIG. 2. Effect of acoustical energy on the ultrasonic killing rate with *E. coli*.

The sharp buzzing that emanates from within the sample container is probably due to the violent collapse of cavitation bubbles.

Under these circumstances it is reasonable to assume that the energy intensity in the sample is not that indicated by the voltmeter. If the excessive cavitation had been inhibited by superimposing an adequate additional pressure on the surface of the sample, it is apparent that the energy intensity necessary to induce an amount of cavitation sufficient to attenuate the sound wave would have been higher than that shown in Fig. 2, where cavitation was not inhibited. The important fact is that, because cavitation attenuates the sound wave at high energy intensities, there is a definite upper limit to the effective energy in-

tensity that may be introduced into a sample with a flat crystal, unless cavitation is inhibited by excess pressure at the surface or by degasifying the sample. However, since many of the effects of ultrasonic vibrations are directly attributable to cavitation, its inhibition or prevention would defeat the purpose of applying acoustical energy to a sample.

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Biosynthesis of Radioactive Asparagine from $C^{14}O_2$[1]

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As a first step toward the elucidation of the metabolism of asparagine and other nitrogen compounds, the following method of preparing radioactive asparagine has been developed. Since leguminous seedlings synthesize exceptionally large amounts of asparagine, it was decided to try blue lupin, *Lupinus angustifolius,* as experimental material. Preliminary work (*1*) has indicated that in this plant the peak in asparagine content is reached on the twelfth day after germination. This suggested the use of seedlings prior to this date.

It was reported by several workers (*2*) that plants supplied with glucose synthesize more asparagine than those which are not. Assuming that the asparagine carbon chain is produced directly from sugar, this observation suggests two different methods for the synthesis of radioactive asparagine. In both cases plants should be placed under the conditions favoring accumulation of asparagine, and either infiltrated with radioactive glucose or permitted to carry on photosynthesis in the presence of $C^{14}O_2$. The second method was adopted, and preliminary tests have indicated that in 8-day-old lupin seedlings the photosynthetic mechanism has already been developed.

In our earlier experiments about 50 g of seeds were soaked for 4 hr in distilled water and sown in vermiculite in small plastic dishes. Seedlings were grown at 25° C on a 15-hr day in a constant temperature and light chamber. When they were 8–9 days old, the plastic dish with the seedlings was placed in an 8-l desiccator, and this was filled with air containing 5% CO_2 and about 0.25 mC of $C_{14}O_2$. After 24 hr of continuous illumination with fluorescent bulbs at a light intensity of 400 ft-c, the desiccator was aerated to remove the residual CO_2. From 97–99% of the CO_2 present initially was absorbed by the seedlings. The plants were then frozen at −40° C, thawed out, and minced in a Waring Blendor. The brei was suspended in about 600 ml of distilled water, brought to 90° C, and held there for 5 min. After cooling to room temperature it was acidified with glacial acetic acid to pH 4 and left standing at 4° C for 12 hr. At the end of this time the precipitate formed was filtered off, the filtrate was decolorized with charcoal and concentrated to about 5 ml. Radioactive asparagine crystallized out on cooling without the addition of carrier. As is seen from Table 1, the activity of the asparagine obtained in these first four experiments was quite low.

TABLE 1

ACTIVITY OF THE ASPARAGINE ISOLATED

Experiment No.	Material	Duration of experiment (days)	Isolated asparagine (g)	Activity in asparagine (μc/mM)	Mc given	Percentage mc recovered
1	Blue lupin seedlings*	1	——	0.688	0.25	—
2	Blue lupin seedlings*	1	——	0.238	.25	—
3	Blue lupin seedlings*	1	——	0.279	.25	—
4	Blue lupin seedlings*	1	——	0.301	.25	—
5	Tobacco leaves†	11	——	3.72	.25	—
6	Tobacco leaves†	6	0.176	4.74	.25	2.5
7	White lupin seedlings*	3	2.724	0.73	.5	3.6
8	Blue lupin seedlings*	6	0.620	2.00	0.25	3.0
9	Blue lupin seedlings*	6	1.789	1.47	1.00	2.0

* Asparagine crystallized out without carrier.
† Asparagine crystallized out only after the addition of about 0.5 g of the carrier.

It was observed earlier (*3*) that when tobacco leaves were permitted to carry on photosynthesis for 24 hr under approximately the same conditions, they produced glucose with the activity of about 338 μc/mM, or about 1,000 times stronger than the asparagine. Assuming that the sugars in lupin seedlings had a comparable activity, the low activity of the asparagine obtained might be due to two causes. Either the immediate precursor of the asparagine carbon chain is not a sugar, but is, for example, a protein, or the amounts of radioactive asparagine produced were greatly diluted by ordinary asparagine present in the seedlings. On the basis of either explanation, it appeared desirable to extend the time of contact with $C^{14}O_2$.

Fifteen young tobacco leaves were detached and placed on 0.1% NH_4Cl in an 8-l desiccator in an atmosphere of 5% CO_2 with 0.25 mc of $C^{14}O_2$. The desiccator was placed between two 200-w incandescent bulbs, with light being filtered through about 8 cm of

[1] This work was aided by a grant from the National Research Council of Canada.

[2] Holder of a Research Council of Ontario scholarship.