microscope are then placed in the holes seen in the surface view $\frac{3}{4}$ inch from each side and $\frac{5}{8}$ inch from the lower edge. These are used to hold the large 2 inch by 3 inch slide or heavy cover glass on which the culture is placed. The cover glass is essential if the work to be done requires the higher powers of magnification. The culture is suspended in a hanging drop in the center of the square opening shown in the surface view and in the projections below and to the left. The instrument to be used is held in one hand and enters the moist chamber through the opening seen in the projection to the right.

The wet filter paper which is placed around the chamber maintains a humidity sufficiently high to prevent any considerable amount of evaporation from the culture over quite a long period of time. It is possible, therefore, to keep eggs in the chamber under constant observation until they reach the stage desired for operation and afterward to follow the immediate effects of the manipulation before transferring them to other containers without subjecting them to a hypertonic medium. This was done regularly in the experiments mentioned above. It is wise, however, if the culture is to remain in the cell for a relatively long time, to close the open end temporarily with a small door of cardboard or some similar material to reduce the area of exposure through which evaporation may take place.

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A CONSTANT-RATE DROPPING DEVICE FOR LIQUIDS[1]

IN SCIENCE, Vol. 79, No. 2059, p. 545, Dr. J. H. Wales, of Stanford University, describes a "Device for Constant Flow of Liquids," which is similar to one I devised a number of years ago, and which is described on page 76 and illustrated in Fig. 3 of the British Medical Research Council Report of 1923 entitled "The Wasserman and Sigma Reaction Compared."

Another device for the same purpose is illustrated herewith, which has the advantage that the distance between the dropping tube and the receiving vessel is held constant. In operation, fluid from the reservoir "R" is allowed to flow into the apparatus, the

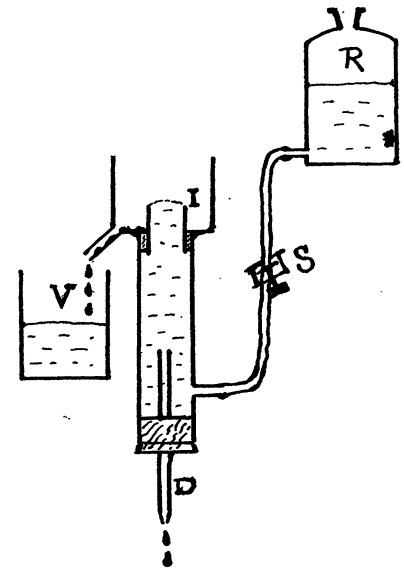

FIG. 1.

rate being adjusted by the screw-clamp "S" until a slight excess runs over the edge of the inner tube "I" continuously. This excess collects in vessel "V" and may be returned to the reservoir. The number of drops delivered per minute depends on the size of the orifice of the dropping tube "D" and the distance between this orifice and the top of the inner tube "I." The dropping rate may be adjusted, within limits, by sliding the tube "D" up or down through its cork.

The outer shell of the apparatus was made from an old student-lamp chimney; the edge of the inner tube "I" was ground flat on a rough stone so that the excess fluid would flow smoothly over it; the inner tube was held in place by a piece of thick rubber tubing, filling the space between it and the outer shell.

H. F. PIERCE

SPECIAL ARTICLES

THE ACTION OF HIGH FREQUENCY SOUND WAVES ON TOBACCO MOSAIC VIRUS[1]

RECENTLY Takahashi and Christensen[2] reported that tobacco mosaic virus is inactivated by high frequency sound waves.[3] They found that the inactivation of virus progressed with exposure, until

[1] From the Wilmer Institute of the Johns Hopkins University and Hospital.

[1] Thanks are due Professor E. Newton Harvey for the use of his laboratory where all the radiation experiments were performed, and Mr. Charles Butt for the use of his high frequency oscillator and for much helpful technical assistance.

[2] William N. Takahashi and Ralph J. Christensen, "The Virucidal Action of High Frequency Sound Radiation," SCIENCE, 79: 415, 1934.

[3] For a general survey and literature on supersonic waves see: E. Newton Harvey, "Biological Aspects of Ultrasonic Waves, a General Survey," *Biol. Bull.*, 59: 306–325, 1930; Leslie A. Chambers and Newton Gaines, "Some Effects of Intense Audible Sound on Living Organisms and Cells," *Jour. Cell. and Comp. Physiol.*, 1: 451–471, 1932.

after about 2 hours no active virus could be demonstrated by inoculating leaves of *Nicotiana glutinosa* L. The virus which they used was in the centrifuged juice from infected tobacco leaves which had been crushed, frozen overnight, then thawed and pressed. This procedure gives a virus preparation of which the solid content is made up of more than 99 per cent. extraneous matter.

It seemed possible that the inactivation of virus by supersonic radiation might be associated, in some way, with the presence of this large amount of extraneous material or with the expulsion of dissolved gas (cavitation) from the fluid. Accordingly, colorless, water-clear virus solutions prepared by the method described by Vinson and Petre,[4] which contain less than 1 per cent. of the total solids present in the infectious juice itself, were subjected to high frequency sound waves. Experiments were also made using purified virus diluted with 9 parts of juice from healthy tobacco plants prepared in a manner similar to that used by Takahashi and Christensen for infectious juice. Since cavitation can be prevented by removing dissolved gas,[5] other samples of purified virus were sealed in tubes under a high vacuum and irradiated. The tubes were placed in a water bath at 40° C. and evacuated for 10 minutes before sealing. Virus in infectious juice prepared by the method of Takahashi and Christensen was also irradiated, both at atmospheric pressure and under a high vacuum.

A 500-watt apparatus similar to that described by Harvey[6] was used in the experiments to be reported. The virus samples, usually 3 cc, were subjected to supersonic radiation at about ½ intensity for nine 10-minute periods with a 5-minute interval for cooling between periods. While it is difficult to compare the sound intensity in different laboratories, the height of the fountain of oil directly above the quartz crystal may be used as a rough measure. This fountain was about 2.5 to 3 centimeters high at the intensity used in the present experiments. It is believed that the apparatus delivered as much, and very probably a greater, sound intensity than that produced by the 75-watt apparatus used by Takahashi and Christensen. Containers for the virus were tubes of Pyrex glass 20 cm long, having a diameter of 12 mm and a bulb about 20 mm in diameter on one end. These tubes were suspended in the oil directly over the quartz crystal by means of a string attached to a wooden support, so that the bulbs were 1 or 2 mm above the electrode. Tubes used for untreated infectious juice in one experiment were used for purified virus in the next experiment in order to eliminate differences due to the tubes. The temperature of the oil bath was maintained at 23° C. by means of a glass coil of circulating cold water. The temperature of the samples of purified virus did not go above 29° C. at any time, while the temperature of the samples of infectious juice and of purified virus diluted with juice from healthy tobacco plants was about 41° C. at the end of the 10-minute periods. This temperature is insufficient to cause a noticeable inactivation of virus over the period of time required for the ex-

[4] C. G. Vinson and A. W. Petre, "Mosaic Disease of Tobacco. II. Activity of the Virus Precipitated by Lead Acetate," *Contrib. Boyce Thompson Institute*, 3: 131–145, 1931.

[5] See reference note 3.

[6] *Ibid.*

TABLE I

THE EFFECT OF HIGH FREQUENCY SOUND RADIATION ON VARIOUS PREPARATIONS OF TOBACCO MOSAIC VIRUS

Tested on →		*Nicotiana glutinosa*			*Phaseolus-vulgaris*			Average of all tests on all plants
Dilution →		1	10	100	1	10	100	
Infectious juice[a] (Atmospheric pressure)	Average[b]	8.8	3.8	1.2	8.8	5.1	1.9	4.9
	Actual[c]	6.0	1.8	0.2	7.0	5.4	0.3	
Purified virus plus non-infectious juice (Atmospheric pressure)	Average	11.7	9.5	1.7	12.0	8.6	7.6	8.5
	Actual	4.6	3.2	0.2	7.2	3.2	0.7	
Infectious juice (High vacuum)	Average	98.0	82.9	86.0	97.0	85.5	73.5	87.2
	Actual	57.5	27.5	16.3	89.0	65.9	34.6	
Purified virus (Atmospheric pressure)	Average	73.5	46.5	55.0	76.5	58.3	53.6	58.8
	Actual	17.8	11.6	5.4	79.0	59.0	28.4	
Purified virus (High vacuum)	Average	89.4	92.0	109.0	102.0	88.5	98.0	96.5
	Actual	46.6	36.5	20.2	164.3	83.0	30.2	

[a] Virus preparation similar to that described by Takahashi and Christensen.

[b] Numbers represent the quotient obtained when the average number of lesions per half-leaf obtained on 10 or more half-leaves of *N. glutinosa* or 16 or more half-leaves of *P. vulgaris* with the designated virus preparation is divided by the average number of lesions per half-leaf obtained on the other halves of the same leaves with the corresponding control preparation, multiplied by 100.

[c] Numbers represent the average of the actual number of lesions per half-leaf obtained on 10 or more half-leaves of *N. glutinosa* or 16 or more half-leaves of *P. vulgaris* on inoculation with the designated virus preparation.

periment. The fact that the temperature of virus preparations containing much extraneous matter rose about 12° C. higher than the temperature of purified virus preparations indicates that the former absorb much more energy.

Immediately after each experiment the irradiated preparations and the corresponding control preparations were tested on half-leaves of *Nicotiana glutinosa* and *Phaseolus vulgaris* L. Dilutions of infectious juice were made with distilled water and dilutions of purified virus were made with 0.1 M phosphate at pH 7. The average of the actual number of lesions per half-leaf obtained on the two species of test plants at three different dilutions of the various preparations of irradiated virus is given in Table I. Another number which represents the average of the lesions obtained with an irradiated virus sample, expressed as a percentage of the average number of lesions obtained with the corresponding control, is also given for each preparation at each dilution. This number indicates the amount of virus present.

It may be seen from Table I that virus prepared as described by Takahashi and Christensen is almost completely inactivated at atmospheric pressure. This is in complete accord with their results. Purified virus diluted with 9 parts of untreated juice from healthy tobacco plants is also almost completely inactivated. However, if infectious juice is sealed under a high vacuum in order to prevent cavitation and then irradiated there is but slight inactivation. Purified virus at atmospheric pressure is inactivated only to such an extent that it gives about 60 per cent. as many lesions as the untreated control, while there is practically no inactivation when purified virus is irradiated under a high vacuum. No reactivation of virus which had been inactivated by supersonic radiation at atmospheric pressure was found. Materials toxic to virus or to test plant are not produced during the irradiation since the addition of such inactivated virus preparation to fresh virus has no effect on the infectivity of the active virus. In general, the results are similar to those of previous workers[7] who have found that cells or particles affected by supersonic waves at atmospheric pressure were unaffected under a high vacuum.

The results indicate that inactivation of virus by supersonic radiation is associated with cavitation of dissolved gas and with the presence of extraneous matter found in untreated juice, since high frequency sound waves of great intensity have practically no effect on purified virus under a high vacuum.

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[7] *Ibid.*

GROWTH OF YEAST BELOW ZERO

THOUGH the growth of "false" yeast, as Torula species, below 0° C. has been recorded,[1,2] probably most bacteriologists incline to the belief that the minimum temperature requirements of "true" yeasts, or forms which sporulate and reproduce by budding, are above the freezing point of water. That this is not true of all representatives of the genus Saccharomyces has been proved in this laboratory in connection with the storage of cider at low temperatures. On two occasions about half the number of 500 cc portions of fresh cider in sealed containers held at −2.2° C. (28° F.) developed pressures of 15 or more pounds per square inch after 2 months storage. Such samples contained many millions of yeast cells per cc and were unmistakably alcoholic.

Of several strains of yeasts isolated from the fermented cider, one in particular has shown ability to increase at −2.2° C. Beer wort, pH 4.8, has been used as the medium, and the rise in number of cells has been followed in the haemocytometer from small samples of the well-agitated culture aseptically withdrawn at about 5-day intervals. Counts at 20, 40, 60 and 70 days are given in Table 1.

TABLE 1

GROWTH OF COLD-TOLERANT YEAST IN WORT AT −2.2° C.

Cells per cu cm				
At inoculation	After 20 days	After 40 days	After 60 days	After 70 days
1,800,000	3,840,000	17,600,000	48,400,000	49,000,000

The viable cell count on wort agar on the 66th day was 32,500,000 per cu cm.

Reproduction was most active from about the 20th to the 50th day, and as shown in the table largely ceased after the 60th day. In a portion of the culture transferred from −2.2° C. to 21° C. on the 66th day, however, reproduction was resumed, the haemocytometer count rising to 83,200,000 per cu cm in 42 hours.

The yeast in question ferments dextrose, levulose and sucrose, but not maltose or lactose. Ascospores are present in 2- to 4-week old thin wort agar plate cultures. Despite its ability to grow at −2.2° C. the yeast is not "psychrophilic" or cold-loving, as the 60-day count at the temperature mentioned is equaled in 23 hours at an incubation temperature of 21° C.

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[1] R. B. Haines, Report of the Food Investigation Board (Gt. Brit.) for the year 1931. 46–51.

[2] J. A. Berry, "How Freezing Affects Microbial Growth," *Food Industries*, 4: 6, 205, 1932.