except that the paramecia were taken from a culture containing a single strain of bacteria, *Achromobacter pinnatum,* and were washed according to the method of Parpart. This bacterium is extremely easy to remove, so it was possible to sterilize large numbers of the ciliates. The following results were obtained. The sterile 0.5 per cent. liver extract killed *P. caudatum* in about one hour. After the yeast suspension and kidney were added, a marked reduction in toxicity was evident. If only a few paramecia were inoculated, survival continued for about three hours. If a large number, *i.e.,* 100, was inoculated, a few lived six to eight hours. If a similar number was added with a few bacteria, some of the paramecia survived and started to increase.

These results were checked, using other ciliates, some of which the author had previously obtained in pure culture. The small and resistant *Glaucoma piriformis,* when taken from a pure culture and added to the liver extract, largely subsides in six hours, but a few swim about for twelve hours. *Colpidium campylum* is slightly less resistant. *Colpidium colpoda,* on the other hand, behaves like *Paramecium,* for it is killed even in the complete medium in a few hours. Since these relatively resistant ciliates are killed by the medium, it is improbable that it is well suited to *Paramecium.*

The most difficult bacteria to remove when washing ciliates are certain slow-growing, adherent forms of very deceiving nature. Peters found that what he first interpreted as "patches of cilia" in his cultures on ammonium glycerophosphate were bacteria and that the growth of *Colpidium* was due to these bacteria. Hetherington[2] repeated these experiments and obtained the same results.

It is instructive to note the characteristics of these bacteria: (1) They are killed at 37° C.; (2) they grow very slowly—colonies on nutrient agar plates are easily overlooked on the third day of incubation at 25° C. and are still very small on the fourth; (3) they do not cloud liquid media even after considerable periods; (4) they are remarkably adherent, usually appearing affixed to the slide or cover-slip in somewhat uniform rows.

These facts suggest that Glaser and Coria probably did not have sterile paramecia.

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A HOLDER FOR SMALL ANIMALS

THE following animal holder is being offered as a result of our effort to secure a holder that is not only practical but also simple and inexpensive. Because of its ease of adjustment it is suitable for all small animals, including cats and small dogs. Notwithstanding its simplicity, it permits the arrangement of the animal in the position most satisfactory for any procedure. In general, its design follows that of the holder for chickens developed by Seifried, Cain and Wulf.[1]

The board consists essentially of a metal plate (A) 28 inches long and 5½ inches wide mounted on six-inch legs (B) (Fig. 2) to form a table. The upper

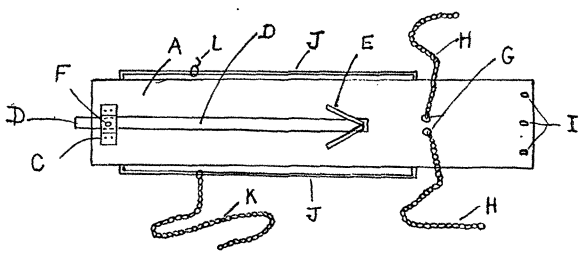

FIG. 1.

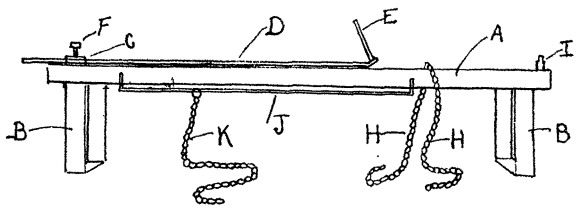

FIG. 2.

surface of the plate is slightly depressed longitudinally down the center to fit the spine of an animal in a dorsal position. At one end and on top of the plate is a bracket (C) carrying a sliding bar (D), to which is attached a forked head holder (E). After adjustment to the length of the animal, the bar, together with the head holder, is locked into position by the set screw (F). The forked head holder is set at an angle of approximately 30 degrees from the vertical as an added safeguard against the animal lifting its head. Coming through two small holes (G) in the plate seven inches from the opposite end from that carrying the bracket are two lengths of light chain (H), permanently fastened to the under side of the plate, for crossing diagonally over the hind legs and then being attached to the pins (I) at the end of the board. On each side of the board are welded two rods (J), extending slightly down and out from it and running from the position of the bracket down about three quarters of the length. Attached to one of these rods is another length of chain (K) and to the other a hook (L). Both the chain and hook are so attached to the rods that they may slide along them. The purpose of this chain is

[1] Oskar Seifried, C. B. Cain and Harro Wulf, SCIENCE, 75: 1942, March 18, 1932.

[2] *Arch. Protistenk.,* Bd. 80: 255, 1933.

to pass over the upper part of the body and when tightly fastened to the hook it restrains any motion of this part of the body.

When the animal is properly stretched out before securing the three chains in position the animal is completely restrained and very little, if any, movement is permitted except by the fore paws.

The advantages of this holder appear to us to lie in its compactness, simplicity and utility with a minimum number of adjustments or attachments.

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SPECIAL ARTICLES

THE VIRUCIDAL ACTION OF HIGH FREQUENCY SOUND RADIATION[1]

THE effect of high frequency sound radiation on biological material was first studied by Wood and Loomis.[2] Since the publication of their investigations in 1927 considerable work dealing with this phenomenon has followed. Much of the literature on this subject has recently been reviewed by Chambers and Gaines.[3] In our experiments we have determined the effect of such high frequency sound on tobacco mosaic virus.

The apparatus used by us was similar to that described by Harvey, Harvey and Loomis.[4] In brief, the sound radiation originated in the vibration of a one-inch-square quartz crystal immersed in a water-cooled, circulating oil bath and excited by means of a 75-watt vacuum tube oscillator in connection with a step-up voltage arrangement. The natural frequency of the crystal was 450,000 cycles per second.

Leaves from tobacco plants affected with the virus of typical tobacco mosaic were crushed, frozen overnight and thawed, and the juice was pressed from the tissue and centrifuged. Three cubic centimeters of juice were then pipetted into a small test-tube, the end of which had been blown into a thin-walled bulb about one inch in diameter. The bulb was immersed in the oil bath directly above the quartz crystal. Separate samples from the same lot of juice were irradiated for 30, 60 and 120 minutes. The temperature of the liquid within the bulb immediately after each experiment was found to have risen from 24° C. to approximately 35° C.

It was shown by a test that this rise in temperature during the course of the experiment was not a factor in the inactivation of the virus. The total number of local lesions formed on 20 half leaves of *Nicotiana glutinosa* inoculated as described below was 1,052 for juice held at 35° C. and 1,058 for unheated juice.

After each experiment the juice treated with sound radiation and the untreated controls were diluted with distilled water, as indicated in Table 1, and were used to inoculate opposite halves of leaves of *Nicotiana glutinosa,* according to the method devised by Holmes[5] and Samuel and Bald.[6] The number of local lesions produced on the leaf halves gives an indication of the relative concentration of active virus present in the suspensions.

TABLE 1

THE INACTIVATION OF TOBACCO MOSAIC VIRUS BY HIGH FREQUENCY SOUND RADIATION

Number of experiment	Duration of experiment, minutes	Number of local lesions per 20 half leaves of *Nicotiana glutinosa* inoculated with juice diluted 1: 500, except as noted	
		Untreated juice	Juice treated with sound radiation
1	30	980	50
	60	1,446	9
	120	872	0
2	30	1,301	584*
	60	1,218	52*
	120	1,116	0*

* Diluted 1: 50.

The results obtained indicate that the tobacco mosaic virus is inactivated by high frequency sound radiation. A progressive inactivation of virus with time of exposure was found to take place. After exposing the juice for two hours the presence of active virus could not be detected by inoculating

[1] A joint contribution from the Division of Plant Pathology and the Fruit Products Laboratory, University of California.

[2] R. W. Wood and A. L. Loomis. "The Physical and Biological Effects of High Frequency Sound Waves of Great Intensity," *Phil. Mag.,* 4: 417–436, 1927.

[3] 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.

[4] E. N. Harvey, E. B. Harvey and A. L. Loomis. "Further Observations on the Effect of High Frequency Sound Waves on Living Matter," *Biol. Bull.,* 55: 459, 1928.

[5] F. O. Holmes, "Local Lesion in Tobacco Mosaic," *Bot. Gaz.,* 87: 39–55, 1929.

[6] Geoffrey Samuel and J. G. Bald. "On the Use of the Primary Lesions in Quantitative Work with Two Plant Viruses," *The Annals of Applied Biology,* 20: 70–99, 1933.