TABLE 2

THE EFFECT OF SUPERSONIC WAVES ON MUTATION RATE

| Stage when treated | Treatment | Number of tested X-chromosomes | Number of lethals |
|---|---|---|---|
| Egg | 60 sec 1.75 w/cm² | 93 | .. |
| Larval | 0.71 and 1.75 w/cm², 10–60 sec | 79 | .. |
| Pupal | 0.71 and 1.75 w/cm², 3–18 min | 161 | 1 |
| Pupal | 0.3 w/cm², 2–3 min | 51 | .. |
| Pupal | 0.71 w/cm², 25 min | 89 | .. |
| Total | | 473 | 1 |
| Eggs, larvae, pupae in controls | | 112 | 0 |

but experiments indicate that this possibility is remote.

In comparison with the resistance of *Drosophila* to x-rays and heat (Fig. 1), we found a very striking identity of effect by x-rays and by S.V. (*3*); while heat effects (*2*) do not resemble effects of S.V. To demonstrate the relation between survival rate and intensity, and/or time, we have to treat insects of identical age. A treatment with 0.71 and 1.75 w/cm² for 15–105 sec (Fig. 2) shows the importance of time; the relation is, however, not proportional. A comparable similarity to effects of x-radiation has been observed in modifications after S.V. treatment. Besides other variations (shortening of limbs, wings, etc.), a great number of treated late larvae and prepupae (in some cases even 20-hr pupae) died as late pupae, just before emergence, with a varying deficiency of chitin of the dorsal and ventral abdomen. The hypodermis was absent, while a normal pupal cuticle was present with brown spots, including the hypodermal defect. The same fact was observed by Bucher (unpublished data), when treating prepupae with x-rays, and it seems that x-rays and sonic waves of high frequency destroy the hypodermal histoblasts in the same manner. Other interesting abnormalities, like nonattachment of the testis to the vas efferens, will be described and discussed in a further paper.

During these experiments, we tried to discover whether S.V. could produce mutations. The imagoes derived from treated eggs, larvae, and pupae were examined for possible modifications, and were then used in crossing experiments in order to detect any recessive or dominant mutations. The CIB-method was applied for the X-chromosome, and, parallel to the experiment, we bred a control series, subject to the same procedure. In Table 2, we summarize some of the results of our experiments.

These first experiments seem to show that supersonic vibrations with an intensity of 0.3, 0.71, or 1.75 w/cm² do not produce many mutations. For a final answer, it would be necessary to know the effect of S.V. of small intensity or the effect upon dissected gonads, problems which we are studying further in our institute.

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# A Simplified Method of Lyophilizing Microorganisms[1]


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The lyophilization of microorganisms is almost essential for their preservation without change of characteristics. It was shown to be effective for bacteria (*1–7*), for yeast (*8, 9*), and for fungi (*4*) and has been used satisfactorily for a number of years in our laboratories as a means of maintaining original and derived strains of many microorganisms. A simplified method developed in our laboratories for lyophilizing organisms, and in use for over a year, is herein described.

The method requires a minimum of special equipment and materials. A 0.1-ml sample (Fig. 1, A) of the spores, bacterial cells, or yeast cells in sterile skim milk or serum is introduced with a capillary pipette into a 12-in. length of 8-mm pyrex tubing sealed at one end and previously plugged loosely with nonabsorbent cotton and sterilized. This cotton plug (B) is pushed down the tube to a level $3\frac{1}{2}$ in. above the sample, and 2–3 in. of the desiccant, powdered phosphoric anhydride (C), is added. A second and tight cotton plug (D) pushed down the tube wipes the loose $P_2O_5$ from the walls and holds the desiccant in place during evacuation. The $P_2O_5$ is introduced into the lyophil tubes from a dispenser such as that diagramed in Fig. 2. This device consists of a funnel turned on a lathe out of 2-in. brass stock; the lower end (Fig. 2, E) fits inside the lyophil tube and the upper end (F) is sealed with deKhotinsky cement to the open end of an inside section of a 45/50 standard taper pyrex interchangeable joint (G). The outside section of the joint (H) is fitted with a rubber stopper (I), carrying through the center a brass rod (J), which is long enough to be used as a plunger for ejecting the dry powdered $P_2O_5$. This apparatus is mounted in a ring stand. Enough $P_2O_5$ can be held in the dispenser when $\frac{3}{4}$ full for approximately 35 samples, a convenient unit of work.

After preparation of all the tubes for a given run the samples are frozen rapidly by immersing either in a solvent-dry ice mixture or in powdered dry ice alone. Each tube is individually evacuated by attaching with pressure tubing to a high vacuum electric pump. In order to keep the sample frozen during evacuation, an insulated shell vial containing powdered dry ice is held over the sample end of the tube. When evacuation is complete (usually in about 1 min or as indicated by a manometer), the tube, still under vacuum and in a horizontal position, is sealed with a flame[3] above the $P_2O_5$ (as indicated by


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[3] Hand gas-air torch, type 3A, National Welding Equipment Company, San Francisco, California, with tip size N-1.

an arrow in Fig. 1). Each tube is then placed in a rack with the sample immersed to a depth of 1 cm in a freezing mixture. A brine-ice bath or a glycol-water mixture, held at −5° C to −10° C in an open-topped refrigerator, can be used conveniently. The sample is completely dry in 2–4 hr. The lyophil tubes are removed from the freezing mixture and sealed off leaving about 2 in. of the tube above the sample (arrow in Fig. 1).

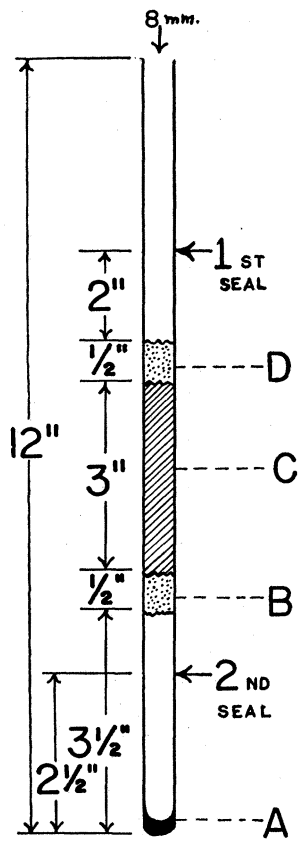


FIG. 1. Schematic drawing of a lyophil tube. A, Sample. B, First cotton plug. C, Phosphoric anhydride. D, Second cotton plug.

Several precautions should be observed: (1) For satisfactory desiccation the sample should not exceed 0.2 ml. (2) The glass surfaces to be sealed must be free of a film of $P_2O_5$. This is assured by the second cotton plug which wipes the surface. (3) Contact between $P_2O_5$ and moist air should be minimized, since it makes the compound sticky and extremely difficult to manipulate. (4) The $P_2O_5$ dispenser should be washed and thoroughly dried between each run.

In addition to the simplicity and low initial cost of the equipment, this method has the following advantages over the customary manifold method: (1) Each tube is evacuated independently, so that maintenance of a high vacuum in a large complicated glass system is obviated.

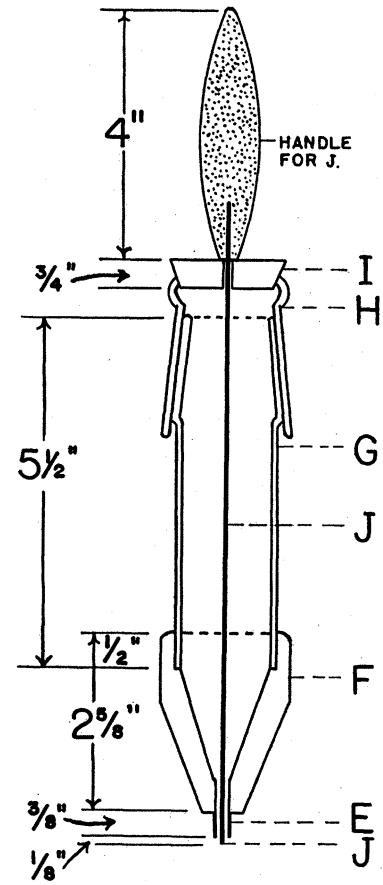


FIG. 2. Schematic drawing of the phosphoric anhydride dispenser. E, Brass tip which fits into lyophil tube. F, Brass shoulder of funnel, inside diam 1½ in. G, Inside part 45/50 ST joint. H, Outside part 45/50 ST joint. I, Rubber stopper. J, Brass rod (⅛ in.) which extends about ¼ in. beyond tip of dispenser when in lowest position.

(2) Any desired number of samples from a few to several dozen may be done at one time. (3) The lyophil tubes can be sealed while rotating in a horizontal position, and a structurally strong seal is therefore more easily made by an inexperienced worker. (4) The short distance between sample and desiccant speeds desiccation.

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