against this yellow background, as may be seen in Fig. 1.

The blue color developed with this method is not permanent but oxidizes to a red color in 5 to 10 min when exposed to air. Acidification of the sample also changes the blue color to red. The red color persists for a month or more. Treated tissue may be placed in lactic acid after staining if it is to be kept for any length of time. The lactic acid not only changes the blue color to a more stable red, but at the same time makes the leaf more transparent.

The results with the whole-leaf staining procedure can be observed macroscopically or with the aid of a low power microscope, so that the usual histological techniques of embedding, sectioning, and staining were not employed. However, the method presumably could be adapted for more precise histological studies.

It should be noted that the procedure is not applicable to all plant species but is restricted to those which have a polyphenol system similar to that present in deciduous fruit trees. The following virus-infected plants were found to give good staining reactions: peaches, cherries, apricots, and plums (*Prunus*); apples (*Malus*); pears (*Pyrus*); strawberry (*Fragaria*); rose (*Rosa*); and high bush cranberry (*Viburnum*). Negative results were obtained with all the viruliferous herbaceous plants—other than the semiherbaceous strawberry—thus far tested.

Since this method is based upon the accumulation of certain polyphenols in virus-infected tissue, care should be taken in the sampling procedure and the interpretation of results, because conditions other than virus infection may induce accumulation of the same or similar phenolic compounds. Nectaries on the leaf normally stain very deeply. Any type of phloem blockage (girdling) may produce phenol accumulation. Similarly, mechanical injuries to the leaf often induce the accumulation of the phenolic compounds, as a wound reaction, immediately adjacent to the injury. Virus diseases, however, produce characteristic distribution patterns of the phenolic compounds and, therefore, can usually be distinguished from other more localized conditions.

The type of staining pattern produced in leaf tissue invaded by different viruses depends upon the kind of virus and the stage of development of the disease. In the case of western X-disease of peaches, a yellows-type virus disease, staining is confined to the phloem in the veins of the leaf during the early stages of the disease. As the disease progresses, localized areas of parenchyma tissue adjacent to the veins become involved, and finally most of the leaf stains deeply throughout. The ringspot diseases of cherries, either in latent or visible stages, produce striking staining patterns (Fig. 1). In the strictly latent stage the staining is confined to the phloem in the leaf veins. In those forms showing symptoms, even though so faint as to be nearly indiscernible, characteristic distribution patterns in the leaf parenchyma are produced that correspond to the symptom patterns.

Stem sections have not been as useful as those of leaves, because the nature of the tissue precludes the production of specific staining patterns. The staining is usually confined to the phloem, but xylem and phloem rays, pericyclic parenchyma, and sometimes cortical and pith parenchyma also will stain, depending upon the severity of the disease.

Genetic variegations may produce chlorophyll distribution patterns in a leaf that resemble virus disease symptoms. None of the genetic variegations tested has reacted to the stain test. Similarly, certain insecticides and other chemicals will induce viruslike symptoms in the leaf, and these also failed to give a stain reaction. The accumulation of polyphenols in virus-affected tissue is not completely understood, but apparently it is not related to the reactions involved in chlorophyll destruction. It seems likely that one or both of the following mechanisms is involved: (1) partial or complete blockage of the phloem, resulting in the dehydration of accumulated sugars to polyphenols; (2) mutual precipitation of virus protein and polyphenol, and by mass action a subsequent accumulation of polyphenol-virus aggregate. In the latter event, the staining reaction may be a test for the localization of inactivated virus particles.

References

1. KELLY, S. *Science*, 1949, **109**, 573.
2. LINDNER, R. C. *Science*, 1948, **107**, 17.

Biological Experiments on *Drosophila melanogaster* with Supersonic Vibrations

Hedi Fritz-Niggli and Albert Böni

Institute for Physical Therapy, University of Zurich, Zurich, Switzerland

Recently, supersonic waves have begun to be widely used by physicians to treat such diseases as rheumatism, ulcus cruris, etc. It is, therefore, necessary to make an exact examination of the biological effects of these vibrations. It is first of all important to know whether supersonic waves have any mutagenic influence on the genes.

Many biological experiments with supersonic vibrations (hereafter to be called S.V.) have been made, but mostly with the purpose of determining the killing effect against protozoa, bacteria, or small experimental animals such as mice. Hersh *et al.* (*2*), who made genetic experiments with *Drosophila melanogaster*, treated them in the air, not being aware that air cannot conduct sonic waves of high frequency. We have made some further genetic experiments by investigating the effects of S.V. on mutations and development in *Drosophila melanogaster*.

We used a wild stock of *Drosophila*, treating all stages of development, i.e., egg, larval, and pupal. The insects were placed in an isotonic NaCl solution in a tube of artificial resin, Trolitul, with a bottom of special thickness to let the waves through. This receiver was put on the vibrator head of a piece of equipment used by physicians (an ultravibrator) which has an intensity of 0.7–4 w/cm^2 (800,000 vibrations per sec). To exclude a heat effect, we cooled the receiver with water, thus allowing a treatment of over 25 min without a significant increase in the temperature of the NaCl solution.

TABLE 1

DIFFERENCE IN SUSCEPTIBILITY TO S.V. (INTENSITY 1.75 W/CM²) IN DIFFERENT STAGES OF DEVELOPMENT

Age in hr when treated	Stage	Time of exposure	Percentage of survivors after treatment	Percentage of imagoes	Modified organs
2	egg	5 sec	12.1	12.1	none
15	"	60 "	75.6	47.1	legs, wings, abd.*
22	larval I	10 "	57.3	51.2	eyes, hyp.*
42	" I	30 "	57.7	37.2	abd.
70	larval III	30 "	13.2	7.3	abd., wings
70	" III	60 "	8.3	4.2	none
100	larval III	15 "	83.3	60.7	hyp.
100	" III	30 "	82.4	28.9	hyp.
100	" III	60 "	63.9	11.8	hyp.
108	prepupal	60 "	53.8	25.6	hyp.
110	"	30 "	64.0	41.7	hyp., legs
113	"	60 "	81.8	44.2	hyp., legs, eyes
115	"	30 "	100.0	85.7	hyp., legs, eyes, wings
122	pupal	4 min	63.6	45.5	" " " "
126	"	5 "	100.0	96.2	" " " "
130	"	10 "	52.2	41.5	" " " "
130	"	20 "	33.3	25.0	" " " "
160	"	10 "	55.0	65.0	" " " "
180	"	10 "	100.0	100.0	wings
200	"	10 "	100.0	100.0	"

* Abd. = abdomen; hyp. = hypodermis.

We observed a big difference in susceptibility in the various stages of development (see Table 1). The eggs, larvae, and early pupae (prepupae) are extremely susceptible to S.V. Usually the insects survive after treatment, but some time afterwards a few of them die, while others die at a later stage of development. The metamorphosis, which begins with the formation of a puparium, seems to be a very sensitive one. After a pupation of about 7 hr, resistance begins to increase slowly, and 6 hr after metamorphosis is finished, resistance increases rapidly.

In 22-hr pupae exposed to S.V. for 20 min at an intensity of 1.75 w/cm², mortality was 75%. A treatment lasting 1 min, however, killed the same number of 1-hr pupae. This means that resistance increases quickly to approximately twentyfold what it is at the beginning of puparium formation, and to nearly a thousandfold as compared with the resistance of the egg when laid. In the other stages the same effect has been observed, i.e., resistance increases with age, and this more strikingly in the egg stage than in the larval. This result parallels the effects on *Drosophila* eggs with x-rays (*4*). It seems probable that morphological changes in prepupal or early egg stages of a growing insect are more susceptible to S.V. than are simple proliferations or cytological differentiations of tissues. A possible explanation of the great resistance of old pupae might be the existence of air, between pupa and puparium, that does not conduct S.V.,

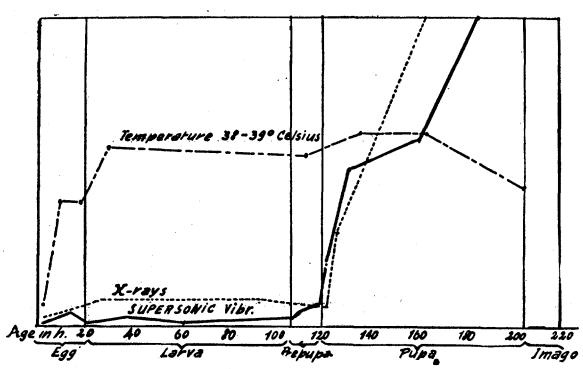

FIG. 1. Differences in susceptibility to different agents in various stages of development. Ordinates: for broken line (temperature 38°–39° C during the 2–3-hr period of the test)—% of survivors (Henke [*1*]); for dotted line (x-rays, dose to kill 50%)—0–2,400 r (Mavor [*3*]); for solid line (supersonic vibrations, 1.75 w/cm² for time needed to kill 50%)—0–20 min.

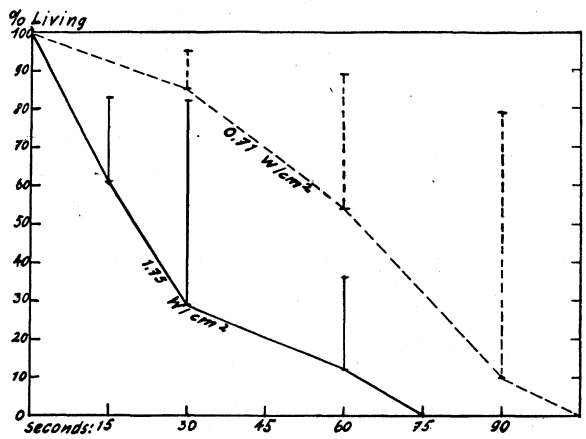

FIG. 2. Variation in survival rate of larvae (100 hr old) with time; treatment with 0.71 and 1.75 w/cm². Curve represents number of emerged imagoes. The percentage of survivors some time after treatment is plotted for each value.

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.

References

1. HENKE, K., v. FUNK, E., and MA, SUNG-YÜN. *Z. indukt. Abstamm. Vereb.*, Lehre, 1941, **79**, 267.
2. HERSH, A. H., KARRER, E., and LOOMIS, A. L. *Amer. Nat.*, 1930, **64**, 552.
3. MAYOR, J. W. *J. exp. Zool.*, 1927, **47**, 63.
4. PACKARD, CH. *Radiology*, 1935, **25**, 223.

A Simplified Method of Lyophilizing Microorganisms[1]

R. W. Barratt and E. L. Tatum[2]

Department of Biological Sciences, Stanford University, Stanford, California

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

[1] This work was done in part under grants-in-aid from the American Cancer Society upon recommendation of the Committee on Growth, National Research Council, and from the Jane Coffin Childs Fund for Medical Research.

[2] The authors acknowledge the technical assistance of Dorothy Van Hacht and Carol Fuller.

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