eyes from direct and scattered radiation. It is a sheet of unexposed, processed X-ray film, which absorbs practically all the 2,537 A. ultraviolet striking it. The aluminum cover of the box has openings for dissecting instruments, cover glasses, and slides. The dissecting instruments are kept sterile by exposure to radiation whenever they are not in use. A few minutes before a set of preparations is to be made, cover glasses and depression slides are inverted over the larger openings.

The interior of the box is shown in Fig. 2. The ultraviolet source is an 8-watt, commercial, low-pressure, mercury vapor lamp, known by the trade name "germicidal lamp" or "sterilamp," with lamp holders, ballast, and starter. The box is lined with aluminum, which is a particularly good reflector of ultraviolet and insures irradiation of all sides of the instruments and of the mouths of cork-stoppered flasks. We have found it necessary to insert a piece of cellophane under the cork stoppers to prevent mold growth on their lower surfaces. Further, since many fused quartz flasks do not have perfectly round mouths and therefore seal with difficulty, the projecting edges of the cellophane help guard against the entrance of contaminants.

Every precaution should be taken to prevent exposure of the eyes of the worker or others in the room to direct or reflected radiation from the lamp. Failure to observe this may result in severe temporary pain commencing several hours after exposure. Unexposed, processed X-ray film, pieces of window glass, and most opaque materials of appreciable thickness are effective absorbers of the harmful radiation.

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The Production of Phenocopies in *Drosophila* Using Visible Light and a Photodynamic Dye

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Phenocopies, changes in development produced experimentally which mimic the phenotypes of mutant genes, have been produced by high temperatures (*5, 8*), low temperatures (*6*), X-rays (*3, 9, 11*), chemical agents (*7*), and ultraviolet rays (*2, 4, 10*). The present study was undertaken to see whether phenocopies could be produced by visible light and a photodynamic dye and to explore the possibility of using cyanin as a photodynamic dye. The dyes used previously by other experimenters have the disadvantage of being difficult to remove from the organism treated after their use so that the photodynamic action may continue. Cyanin (quinoline blue) is an indicator dye that is colorless at pH 7 and blue at pH 8, which permits its photodynamic action to be controlled by adjustment of the pH.

Drosophila larvae of known ages were obtained by allowing large numbers of flies to lay eggs for a two-hour period on corn meal-molasses-agar food in half-pint bottles. The cultures were kept at 25.5° C. before and after irradiation. For irradiation, the larvae were placed in small Petri dishes, covered with 0.04 per cent cyanin solution adjusted to pH 8, and exposed to the light from a 100-watt projection lamp at a distance of 10 inches. After irradiation the cyanin solution was made colorless by the addition of a few drops of 0.1N HCl, and the larvae removed, washed twice in Ringer's solution, and placed in shell vials in the incubator to complete development. The cyanin solution, made by grinding 0.1 gram of crystalline cyanin (Grübler) in a mortar with 20 cc. of 0.01N NaOH and diluting to 250 cc. with distilled water, is not very stable and gradually loses its blue color.

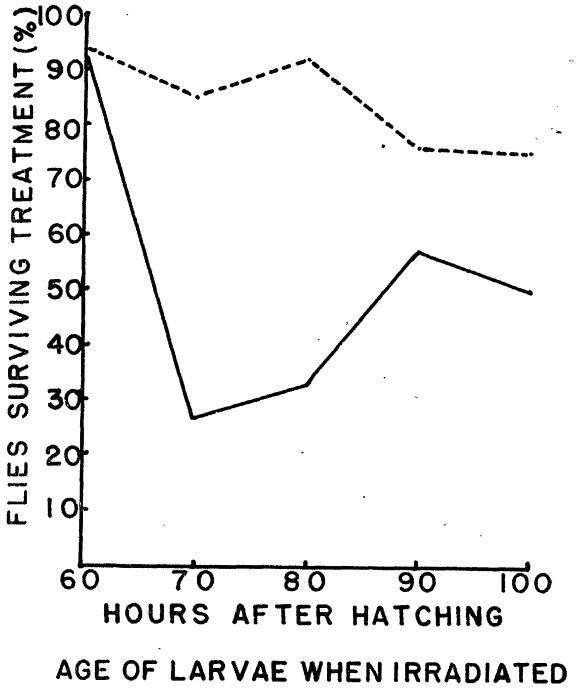

FIG. 1. Percentage of flies hatching from larvae exposed to light in cyanin solution. Dotted line = 30-minute exposure, solid line = 60-minute exposure.

Larvae 60–100 hours old (after hatching from the egg) were exposed for 30 or 60 minutes to the beam of light. The absolute intensity of the light is not known, but the constancy of its intensity throughout the experiment was checked by a photocell and milliammeter. As a result of the light a certain percentage of the larvae were killed in each experiment (Fig. 1); the larvae usually died within 12 hours of the irradiation, although some died after pupation. Other larvae, placed in the cyanin solution and subjected to the same changes in pH and washings as the irradiated flies, but not irradiated, showed no mortality. A second effect of the light was the production of a certain number of phenocopies: abnormal abdomen, dark eye color, bent or missing bristles, and folded wings (Fig. 2). The various types of phenocopies occurred at random throughout the period tested; no indication was found that an irradiation at a particular stage tended to produce a particular kind

of phenocopy. In all, 558 flies of two stocks, an isogenic wild type and the mutant aristapedia-Bridges, were irradiated. No difference in the response of the two stocks was observed. The results indicate the existence of two periods of high sensitivity to the treatment, one 70 hours after hatching and one 100 hours after hatching, just before pupation. Larvae

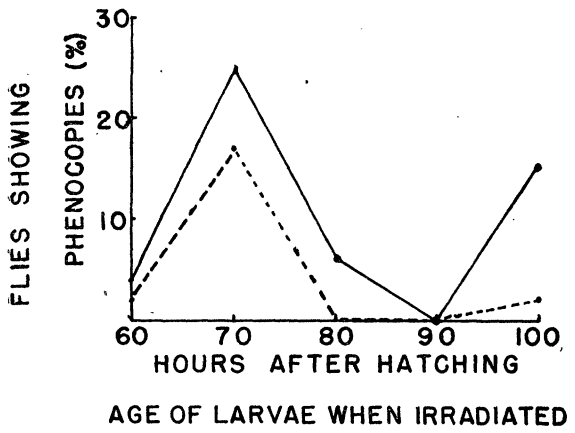

FIG. 2. Percentage of flies showing phenocopies after exposure of the larvae to light in cyanin solution. Dotted line = 30-minute exposure, solid line = 60-minute exposure.

show a gradual increase in sensitivity to ultraviolet radiation with increasing age from 50 to 100 hours after hatching (*10*) but only one period of maximum sensitivity, just before pupation.

The phenocopies produced in these experiments may be interpreted as due to the killing of individual cells or groups of cells in the larvae at the time of the exposure, which results in the failure of some part to form. More extensive damage results in the death of the larva. The larvae which pupated and then died were dissected open. These animals showed patches of black, necrotic tissue on the abdomen which resembled burns. No phenocopies resembling the pervasive changes in morphogenesis found as a result of X-radiation (*9, 11*) were produced by these treatments. Photodynamic dyes are believed (*1*) to be activated by a quantum of light and to transfer this activation to a molecule of some substrate substance in the cell which then reacts with oxygen. Since the oxidation of this substrate molecule is incompatible with life, the cell dies.

These experiments demonstrate the effectiveness of cyanin as a photodynamic dye in the production of phenocopies and suggest its use in other biological systems.

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A Qualitative Test for Penicillin

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As the list of antibiotic substances lengthens, the problem of determining whether or not a new one has been obtained becomes increasingly difficult. In a survey of new sources of antibiotic substances, the problem is further complicated by the need to identify the active substance in highly impure preparations. The fact that penicillin is widely distributed, very active, and produced in satisfactory quantities by fungi already in cultivation makes it highly desirable to determine before the solution is studied further that its activity is not due to penicillin. An enzyme from a culture of *Staphylococcus aureus*, which did not affect the activity of aspergillic acid, gliotoxin, and gramicidin but did inactivate penicillin, was used to indicate that one of the antibiotic agents formed by *Aspergillus flavus* was a penicillin (*2*).

A specific qualitative test for penicillin is available if it can be shown that penicillinase inactivates only penicillin and does not affect other antibacterial substances. Penicillinase in the form of Clarase Lot No. 1351 and Schenley Penicillinase are used in chemical (*6*) methods for the assay of penicillin concentrates and in sterility tests of penicillin preparations (*1, 4, 5*).

It was found in this laboratory that penicillinase (Clarase Lot No. 1351 or Schenley Penicillinase A) did not affect the antibacterial activity of the following substances: aspergillic acid, citrinin, gliotoxin, patulin, penicillic acid, spinulosin, streptomycin, streptothricin, the active substances formed by eight members of the higher Basidiomycetes, and a crystalline compound isolated from a green plant. The activity of one of the basidiomycete culture solutions was not affected by the Schenley Penicillinase A but was decreased by the Clarase, presumably as the result of some of the many other enzymes in it. Hence, only the Penicillinase A could be used to identify penicillin. It is possible that antibacterial agents other than penicillin will be found that are inactivated by penicillinase; but until they have been shown to occur, the failure to obtain inactivation by penicillinase may safely be taken to indicate the absence of penicillin.

The activities of the antibacterial substances were measured in serial dilution tests (*3*) set up with and without the inactivating enzyme in the broth. The test bacterium was the Heatley strain of *Staph. aureus*. Clarase at a concentration of 1 mg./ml. of broth inactivated 50 μg. of penicillin G in this test—that is, reduced the effective concentration of penicillin to less than 0.06 μg./ml. One Schenley unit of penicillinase/ml. of broth inactivated 200 μg. of penicillin G. The Clarase and Schenley Penicillinase A were used at concentrations sufficient to inactivate penicillin solutions with activities from 10 to 100 times as great as that of the antibacterial substances which were not affected by the enzyme.

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