sumption is verified by the results in Fig. 1, which yield a linear relationship between the relative concentration of the salivary amylase and the reciprocal of the time required to attain given optical density.

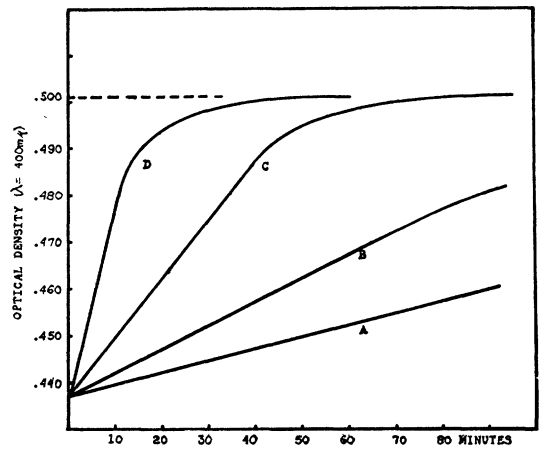

FIG. 1. Effect of salivary amylase on the optical density of 0.05% (Lintner's) starch solution containing 3×10^{-5} *M* Congo Red. The relative concentrations of enzyme are 1, 2, 5, and 15 for curves *A*, *B*, *C*, and *D*, respectively. Broken line is the optical density for the dye in the absence of starch. Solution contained 0.01 *M* NaCl and phosphate buffer at pH = 7.0; temperature, 24° C.

Unlike iodine, which will immediately arrest enzymatic action, the above results indicate that the assay of an α-amylase may be carried out in the presence of the dye. The extent of inhibition by Congo Red appears to be small and is considered in further detail elsewhere (*4*). It may be said here that with the exception of native serum albumin, most proteins bind anionic dyes rather poorly (*5*). In the enzymatic hydrolysis of the starch the concentration of dye is kept at 10^{-5} *M*; thus adsorption of the dye by the protein α-amylase is probably extremely small. The substrate, on the other hand, binds the dye, possibly preventing the accessibility of the substrate to the enzyme. But when one considers that the molecular ratio of dye to glucose units is about 1:100, the inhibiting effect of the dye should not be appreciable. However, for those enzymes where the pH optimum is quite removed from the pH required to give the greatest spectral change, the precision may be improved by following the dextrinogenic activity of the amylase by the addition of the dye to successive samples of the hydrolysate, in a manner similar to that used in the iodine method.

It is interesting to compare the behavior of an adsorption indicator in the hydrolysis of starch with that in the hydrolysis of the protein substrate, native bovine albumin (*1*). Unpublished results from this laboratory indicate that few of the approximately 600 peptide bonds have to be broken before the protein molecule completely loses its binding capacity for the indicator ion. The indicator ion used here lacks such specificity for the starch substrate. One may speculate hopefully that additional work may lead to a series of dyestuffs that may be used to probe the starch substrate and cast additional light upon the mechanism of the starch-amylase reaction.

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Systemic Stress as an Inhibitor of Experimental Tumors in Swiss Mice

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The role of systemic stress has been investigated in a wide variety of human ills; yet its relationship to neoplastic disease has remained obscure. Since the known effects of systemic stress are largely catabolic (*1*), it might be expected, because of this, that the growth of tumors might be hindered rather than abetted. Indeed, when one considers the many agents that have been effective experimentally against neoplasms, it seems quite likely that, in addition to the special effects of each, many or all of these agents share in a general action as a systemic stressor. We have accordingly tested the hypothesis that organisms under stress will show less tendency to develop experimentally produced tumors than will their normal controls. This hypothesis has been tested in the Swiss albino mouse, employing forced swimming as a stressor and the ascites tumor and the methylcholanthrene-induced sarcoma as experimental neoplasms.

Fifty young adult male Swiss albino mice were inoculated intraperitoneally with 0.15 ml of a fluid ascites tumor in a 1:4 dilution with normal saline. At the time of inoculation 25 of these mice (the experimental group) had been subjected to forced swimming in glass jars (approx 18 cm^2), containing water at room temperature, with 6–8 mice in each jar. Over a period of 17 days this group had swum a total of 48¾ hr in daily sessions increasing in duration from 1½ to 4¼ hr. Following inoculation, the forced swimming was continued for 34 additional hr over a period of 14 days, in daily sessions the duration of which decreased from 4¼ to ¼ hr. Discontinuance of swimming was necessitated by the growth of tumors in the experimental group, a circumstance that increased the danger of death by drowning.

One member of the control group died with a large fluid tumor on the seventh day following inoculation, and 2 days later one of the experimental group died. By the fourteenth day, when forced swimming was

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discontinued, 10 of the control group had died with large fluid tumors as compared with 3 members of the experimental group. Following discontinuance of swimming, the experimental group developed tumors and died at a greatly accelerated rate (Fig. 1, *Stress*

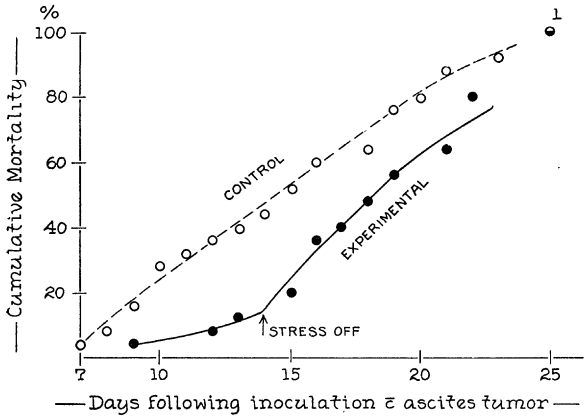

FIG. 1. Cumulative mortality curves for the experimental and control groups following intraperitoneal inoculation with a fluid ascites tumor. (1, Survivors sacrificed.)

Off). The median survival time of the experimental group (18 days) was 20% longer than that of the control group (15 days).

Because of the short survival time of mice inoculated with the ascites tumor, a different neoplasm with a much longer latency was employed to study the effects of varied amounts of stress. Seventy-six young adult male Swiss albino mice were injected subcutaneously in the lower back, at the base of the tail, with 1.0 mg methylcholanthrene in 0.25 ml mineral oil. At the time of injection 28 of these mice had endured 5–12 consecutive days of forced swimming. On each day each mouse was forced to swim until he appeared to be on the verge of drowning; this required an average of 8 hr, with a range of ½–15 hr. On the day following injection with methylcholanthrene, swimming was resumed for 16 mice; the remaining 12 were subjected to no further stress. At the same time 7 mice were added to the group of swimmers, and swimming was continued for this group of 23 mice for a total of 24 days following the injection of methylcholanthrene. In accordance with the findings of Buu-Hoï and Ratsimamaga (*2*), who studied the effects of carcinogens as stressors, the mice appeared to tire more quickly following the injection.

The total experimental group consisted of 35 mice, each of which had swum to what appeared to be exhaustion on each of 5–36 days. Twelve mice had swum only prior to injection, 7 only following injection, and 16 had swum throughout the pre- and post-injection periods. A control group of 41 mice did no swimming, but was injected with 1.0 mg methylcholanthrene at the same time as the experimental group. While their opposite numbers were swimming, the appropriate members of the control group were deprived of food, but not water. As each experimental mouse was housed individually and swam in an individual container, observations on his condition could be made throughout the course of the experiment. This latter factor is of importance, because there is no simple correlation between what Selye (*1*) calls the "stressor," or stress situation, and the amount of stress experienced by the organism or the effected modifications in its physiology.

In this experiment two types of tumors developed. One of these was a papilloma, or epithelial tumor; the other was the typical subcutaneous sarcoma obtained with methylcholanthrene.

By 100 days following injection, 9 members of the control group and 1 of the 7 mice who had swum only following injection were dead, with papillomas that had become invasive and scirrhous, involving the entire back of the 10 animals. By 132 days, 23 (72%) of the surviving control group and 17 (50%) of the surviving experimental group had developed typical sarcomas, 7 of the controls and 5 of the experimental group having died with these tumors. By 165 days, all but 3 of the controls and 1 of the experimental group were dead with sarcomas. Fig. 2, depicting the cumulative mortality for both groups, demonstrates how the early protection afforded the experimental group was lost once true sarcomas began to develop.

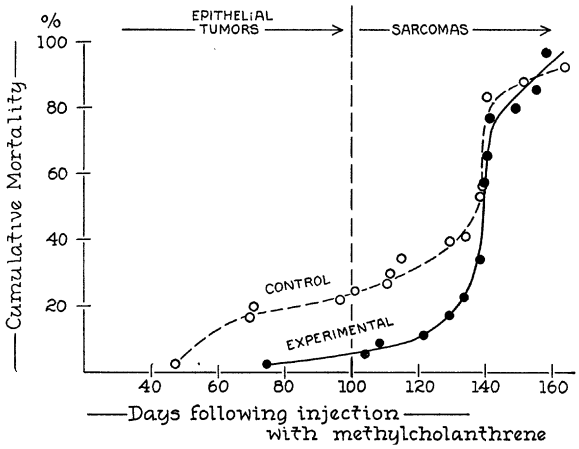

FIG. 2. Cumulative mortality curves including deaths from both epithelial tumors and sarcomas for the experimental and control groups following subcutaneous injection of 1.0 mg methylcholanthrene.

The modal survival time for the entire population developing sarcomas was 139 days, with the majority surviving between 138 and 142 days. It is of interest that the 7 members of the experimental group surviving longer than 142 days were among those mice who had sustained the least stress either because they had done a minimal amount of forced swimming or because they had withstood the ordeal better than had the other experimental animals. Conversely, the first 2 experimental mice to succumb had endured the greatest amount of forced swimming (35 and 36 days, respectively), both appearing exhausted and one having developed an infected paw accompanied by a leu-

cocytosis. If these findings prove verifiable, it may be that there is an optimal amount of stress affording maximal protection against tumor development.

In evaluating the results of this investigation it must be recognized that both the ascites tumor and the methylcholanthrene-induced sarcoma are extremely malignant and rapidly prove fatal. Moreover, a large dose of methylcholanthrene (1.0 mg) was employed. These factors would tend to minimize any inhibitory effects in the experimental procedure.

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A "Purkinje Shift" in Insect Vision

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The well-known Purkinje shift in vertebrates is due to the shift from cone to rod vision as the light intensity becomes subliminal for the cones. This phenomenon is manifested by a shift of the over-all spectral sensitivity of the eye toward the shorter wavelengths because of the different action spectra of the cones and rods. There has been no previous report, however, of a similar shift occurring in any invertebrate.

Hanström (*1*), having found both long- and short-axoned retinula cells in several Arthropoda, postulated that these are analogous to the cones and rods, respectively, of the vertebrate retina, and that the long-axoned retinula cells probably mediate color vision. This is, then, the anatomical basis for the application of the Duplicity Theory to the compound eye (*1*). Power (*2*) later demonstrated long and short visual axons in the optic lobes of the brain of *Drosophila* that were similar to those previously described by Hanström. More recently, Fingerman (*3*) has obtained highly suggestive evidence of true color vision in wild-type red-eyed *Drosophila melanogaster.* In addition, there was indication that the spectral response curve of Drosophila changes shape as the intensity of the monochromatic light stimulus is diminished. The present investigation was therefore undertaken to determine the manner in which the spectral response curve of Drosophila is altered as the intensity of light is decreased.

Wild-type red-eyed *D. melanogaster,*[1] not less than one week old, were used in the present investigation. The method employed here to determine the response of the flies to the colored light has been described in detail by Fingerman (*3*). The experiments were performed in a darkroom. Briefly, the test chamber consisted of a Y-tube the arm and stem of which had been

[1] The authors are indebted to G. H. Mickey for the Drosophila used in this investigation.

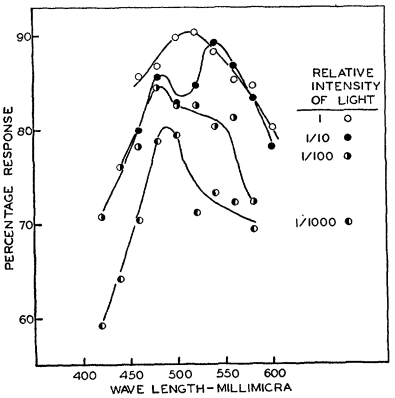

FIG. 1. The percentage response of wild-type red-eyed *Drosophila melanogaster* at each of four intensities of monochromatic light.

covered with black tape to within 1 cm of their junction after cover glasses had been affixed onto the end of each arm. The Y-tube was then placed in a box the top and bottom of which had been removed, in such a fashion that the arms and stem of the tube protruded and the junction could be observed. As a result, the light beam could stimulate the flies at the junction only through one of the arms. Thirty flies were placed in the chamber and shaken down to the stem by two or three brisk taps. The apparatus was next placed on an observation box in such a position that a dim ruby-red light shining through a window on top of the box illuminated the junction, allowing only observation of the silhouettes of the flies for counting purposes. Simultaneously, one arm of the Y-tube was directed toward the stimulating monochromatic light source and the other arm was covered with an opaque vial. As a result, the flies were faced with monochromatic light in one arm and darkness in the other. The monochromatic light was obtained by the use of a constant deviation quartz-prism monochromator[2] in conjunction with an 8.5-v concentrated filament lamp. The distance from the end of the Y-tube arm to the slit of the monochromator through which monochromatic light was emitted was 63 cm. The number of flies entering each arm was then recorded. After about 25 flies were counted, they were again shaken down to the stem and the counting was repeated. The percentage response of the flies is expressed as the percentage of all flies counted that entered the arm directed toward the oriented beam of light. In the earlier experiments approximately 100 flies were counted for each determination. Later it was observed that equally replicative results were obtained when only 50 flies were counted for each determination, and this was done in approximately 75% of the experiments. Each point depicted in Fig. 1 represents the average of eight such determinations. The earlier data of Fingerman (*3*) are included in the averages, since all the experiments were performed

[2] The authors are grateful to R. J. Cashman, of the Physics Department, Northwestern University, through whose generosity this instrument was made available.