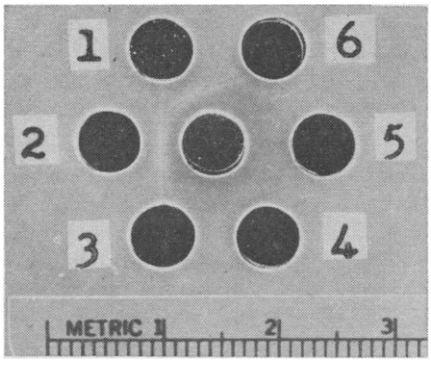

Fig. 1. A gel-precipitin plate. Note typical precipitin bands between serum (center well) and *Pasteurella pestis* (well 1) and *P. pseudotuberculosis* (well 2). Joining of bands indicates that the reacting antigens are identical or closely related. The serum did not react with *Salmonella paratyphi* A (well 3), *Shigella flexneri* (well 4), *Proteus vulgaris* (well 5), *Pseudomonas aeruginosa* (well 6), or any other of the organisms tested.

clotted blood was stored at 5°C until the serum could be tested.

The agar-gel diffusion technique of Ouchterlony (*6*) was used to detect precipitin antibody to the following human pathogens: *Pasteurella pestis, P. pseudotuberculosis, Salmonella paratyphi* A, *Shigella flexneri, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli* B, *Aeromonas hydrophila*, and *A. shigelloides*. Cultures of these organisms are maintained at Fort Detrick and their identities were verified (*7*). For preparation of the antigens, each species was cultured in Difco heart infusion broth for 48 hours at 26°C; the bacteria were then disrupted by exposure of the cultures to sonic vibrations for 10 minutes (Raytheon Sonic Oscillator). Finally the cultures were sterilized by filtration, and merthiolate was added at 0.1 mg/ml to maintain sterility. The prepared cultures and white perch serums were stored at 5°C pending the gel-precipitin tests. Agar-gel test plates were prepared with 1 percent Oxoid ion agar No. 2 in physiological saline containing merthiolate at 0.1 mg/ml; they were incubated at 23°C. When present, precipitin bands were easily visible and well defined within 24 hours (Fig. 1).

Precipitin antibodies to each of these human pathogens were detected exclusively in fish netted near heavily populated areas (Table 1). The precipitins were specific in that none of the positive serums reacted with more than one of the organisms, except that eight serums reacted with both *Pasteurella pestis* and *P. pseudotuberculosis*. There two species share most of their antigens (*8*), so that cross-reactions with specific antiserums to either organism are expected. *Pasteurella pestis* was included in the survey, as an indicator of the specificity of the antibodies detected, since it has never been detected in the Chesapeake Bay area and because it shares antigens with several other Gram-negative bacteria (*9*); therefore antibodies reacting with *P. pestis* must be considered nonspecific. The fact that serums reacting with organisms other than *P. pseudotuberculosis* failed to cross-react with *P. pestis* is a further indication of the specific nature of the antibodies involved. The possibilities that the precipitin bands may have been due to nonspecific reactions between antigens and serum lysozyme was ruled out by testing the antigens against lysozyme by the method of Leonard and Thorne (*10*); the results were negative.

Our detection in fish of antibodies to the bacteria that cause human pseudotuberculosis, paratyphoid fever, bacillary dysentery, and a variety of chronic infections is especially ominous since the fish were caught in waters most likely to be contaminated by such bacteria. It is possible that the antibodies were produced in response to infections with bacteria other than those tested, but the close antigenic relations with the human pathogens in question make it likely that the organisms responsible were potentially dangerous to man. In any case, the possibility that fish may become active vectors of human disease, as a result of their infection with pathogenic bacteria in contaminated water, deserves much more attention and study.

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11. We thank M. J. Surgalla (Fort Detrick), S. F. Snieszko (Eastern Fish Disease Laboratory, Leetown, W. Va.), and L. E. Cronin (Chesapeake Biological Laboratory) for support and assistance. Contribution No. 342 from the Chesapeake Biological Laboratory.

12 December 1967

Phenol Oxidases of a Lozenge Mutant of Drosophila

Abstract. *Monophenol oxidase (or tyrosinase-A_1) activity appears to be absent from extracts from pupae of homozygous lozenge-glossy* (lz^g/lz^g) *females and* (lz^g/δ) *males of* Drosophila melanogaster. *Diphenol oxidase (tyrosinase-A_2) activity is less in the mutant extracts than in extracts of wild-type Oregon-R pupae of the same age and sex.*

Monophenol oxidase (tyrosinase-A_1), oxidizes tyrosine to 3,4-dihydroxyphenylalanine (dopa) in one of the reactions related to tanning and hardening of the cuticle of the blowfly *Calliphora erythrocephala* (*1*). In addition, both monophenol oxidase and diphenol oxidase (tyrosinase-A_2) have been shown in *Drosophila melanogaster* to be able to oxidize dopa to dopa quinone, which spontaneously polymerizes to the pigment melanin (*2*). Our search for the biochemical aberrations in lozenge pseudoallelic mutants of *D. melanogaster* has demonstrated that the lozenge-glossy lz^g (*g*) has no tyrosinase-A_1 activity and reduced tyrosinase-A_2 activity as tested by either acrylamide-gel electrophoresis or an enzyme assay.

Lozenge-glossy is one of more than 20 known lozenge mutants characterized by less-than-normal development of eyes, female accessory sex organs, claws, and other ectodermally derived organs. The mutant phenotype results from the pleiotropic expression of a gene located at the extreme right locus of four pseudoallelic loci 27.7 map units from the left end of the X-chromosome of *D. melanogaster*. Phenotypically,

lozenge-glossy belongs to a class of lozenge mutants that can be mapped at all four pseudoallelic sites (*3*).

We compared phenol oxidase activities from late larvae, pupae, and young adults taken from an isogenic (1965) strain of wild-type Oregon R (*R*), and this same strain of flies, with the *g* gene inserted into the X-chromosome by a double crossover between the singed[3] gene (map position 21) and the vermilion gene (map position 33). Both stocks were balanced to the In(1)-FM3 X-chromosome carrying the Bar (*B*) eye gene as a marker because of the very low fertility of the *g/g* homozygous females, as well as to prevent crossing-over between X-chromosomes. The flies were grown in 0.24-liter milk bottles on standard *Drosophila* media at 27° ± 0.5°C.

The procedure used for acrylamide-gel electrophoresis was adapted from that of Mitchell and Weber (*2*). Single pupae were glass-ground in 20 μl of sample buffer [10 mg of disodium ethylenediaminetetraacetate (EDTA) per milliliter of 0.1*M* tris-glycine buffer, *p*H 8.9, with 10 percent sucrose]. The slurry was centrifuged for 5 minutes at 17,000*g* in an International hematocrit centrifuge, and the supernatant was applied to the gel slots of an E-C-470 (E-C Apparatus Corp.) vertical electrophoresis cell. The cell buffer was 0.1*M* tris-glycine, *p*H 8.9, and electrophoresis was for 90 minutes at 400 volts, 35 ma, and 17°C. After electrophoresis, the proenzymes in the gel were activated by incubation of the gel for 4 hours at 6°C in an activator solution (*4*). The *R* pupae used to prepare the activator were usually less than 48 hours beyond formation of the white pupa.

Further incubation with dopa or tyrosine, or both (0.4 mg/ml in phosphate buffer), gave dark melanin bands at the sites of enzyme activity. In Fig. 1 melanin bands appear at both A_1 and A_2 sites for electrophoresed samples of *R* male pupae sexed as white pupae and processed immediately for electrophoresis, as well as for *R* male pupae maintained under controlled conditions for 24, 48, 72, or 96 hours before being processed. Samples from *g* males, on the contrary, show no production of melanin at the A_1 region and reduced production at the A_2 region for these same hours of pupal development. The gel pattern of Fig. 2 is from individual pupae collected as white pupae and held for 65 hours, at which time increasing pigmentation permits males to be separated from females by characteristics seen through the pupal case; at the same time, non-Bar *g/g* homozygous females can be separated from *g/B* heterozygous females by examination of the eye as seen through the pupal case. The extracts from homozygous females and males of the mutant stock again show no tyrosinase-A_1 activity and reduced tyrosinase-A_2 activity, but the heterozygous female (phenotypically normal for the *g* gene) does show banding equivalent to that of the *R/R* and *R/B* females for both tyrosinases, while exhibiting darker bands than the *R/♂* sample.

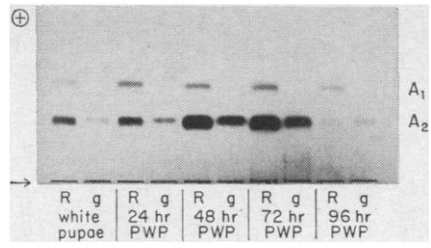

Fig. 1. Gel electrophoresis of tyrosinase-A components obtained from males of Oregon R (*R*) wild-type and of lozenge-glossy (*g*) mutant stocks of *Drosophila melanogaster*. White pupae were sexed, and the males were held under controlled conditions until they were processed for electrophoresis at the times indicated. The gel was first incubated with a 41-percent $(NH_4)_2SO_4$ fraction of wild-type pupae and then with dopa and tyrosine. The origin is marked by an arrow; A_1 and A_2 indicate distinct A components. *PWP*, Post-white-pupa.

Extracts from 24 *g/g* homozygous females and 55 *g/♂* males have shown no tyrosinase-A_1 bands and lighter tyrosinase-A_2 bands than have wild-type samples at the same age, but the extracts of 24 heterozygous (*g/B*) females processed to date have all shown tyrosinase bands similar to those from samples of *R* females. The mating of *g/B* females with *g/♂* males used to maintain the *g* stocks produces only *g/♂* males because the FM3 chromosome carries a male-lethal gene: that is, the male inheriting the FM3 chromosome dies early in development. However, four pair matings of virgin *R/g* females with *R/♂* males produced male offspring whose gel-banding patterns, when they were processed as white pupae, produced 13 *R*-type banding patterns to 15 *g*-type banding patterns; this result is indicative of the 1 : 1 ratio expected of a sex-linked trait. Samples from pigmented pupae of known phenotype, taken from these same matings, each gave the expected banding pattern.

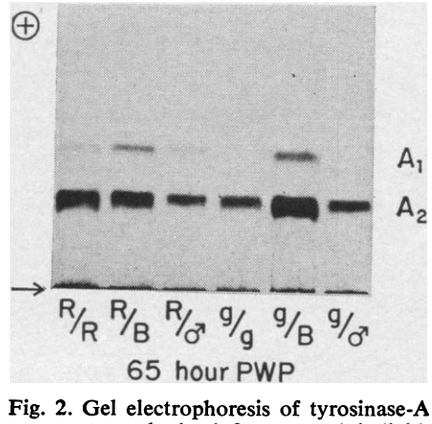

Fig. 2. Gel electrophoresis of tyrosinase-A components obtained from sexed individuals of Oregon R (*R*) wild-type and lozenge-glossy (*g*) mutant stocks of *Drosophila melanogaster*. The heterozygote females of each stock carry the In (1) FM3 chromosome marked by the Bar (*B*) gene affecting appearance of the eye. Individual samples were collected as white pupae and maintained under controlled conditions for 65 hours before selection and processing for electrophoresis. Symbols and abbreviations: as in Fig. 1.

Figure 3 shows the results of assays of enzyme activity [according to Mitchell (*5*)] with either 0.0024*M* L-tyrosine or 0.02*M* L-dopa as the substrate. Figure 3, A–D, shows the results of assays of extracts from *R/♂* males and *g/♂* males collected as white pupae and assayed immediately, with L-tyrosine as substrate, and 6, 12, and 24 hours thereafter. These results are in agreement with the acrylamide gel results in that no activity is apparent in extracts of *g/♂* male mutants as late larvae (not shown) or at the developmental ages shown. Furthermore, the monophenol oxidase of the wild-type fly is seen (Fig. 3, A–D) to decrease in activity between the white pupal stage of development and that 24 hours later. Mitchell (*5*) reported a period of unmeasurable activity for the diphenol oxidase during 4 to 8 hours of the prepupal stage of development (4 to 8 hours after the white pupal stage), and associated a decrease in measurable activity of the diphenol oxidase, at a later stage in development, with its incorporation into a structural body pigment.

Spectrophotometric assays with L-dopa used as substrate (Fig. 3F) reveal that *g/♂* male extracts have a lower rate of diphenol oxidase activity than *R/♂* male extracts obtained 24 hours after the white pupal stage. This finding, too, is in agreement with the acrylamide-gel results. The addition of 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine, a synthetic cofactor for the tyrosine hydroxylation reaction (*6*),

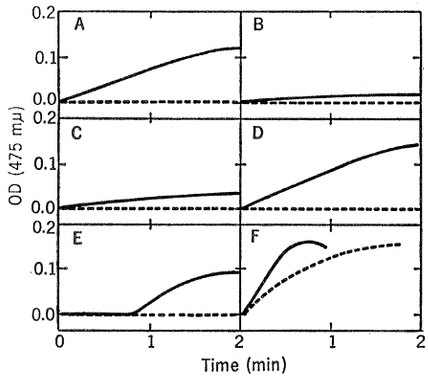

Fig. 3. Phenol oxidase activity in *Drosophila*. Oregon R (*R*/♂) wild-type males, solid lines; lozenge-glossy (*g*/♂) mutant males, dashed lines. The substrate for A–D was $2.4 \times 10^{-3}M$ L-tyrosine; for E, $2.4 \times 10^{-3}M$ L-tyrosine with $2.4 \times 10^{-4}M$ 2-amino-4-hydroxy-6,7-dimethyltetrahydropteridine cofactor; for F, $0.02M$ L-3,4-dihydroxyphenylalanine (L-dopa). Activation of homogenates was for from 90 to 110 minutes at 0°C for the monophenol oxidase assays and for 60 minutes at 0°C for the diphenol oxidase assays. (A) White pupal stage; (B) 6 hours after the white pupal stage (PWP); (C) 12 hours after PWP; (D) 24 hour PWP; (E) 24 hour PWP with cofactor; (F) 24 hour PWP–diphenol oxidase. Reactions were carried out in microcuvettes in a Cary recording spectrophotometer at 30°C; measurements were made at a wavelength of 475 mμ. PWP, Post-white-pupa.

to the *g*/♂ reaction mixture effected no increase in monophenol oxidase activity, although the added pteridine seemed to increase the rate of reaction after an initial lag in the *R*/♂ male extract (see Fig. 3D for comparison). Heat-treated extracts of *R*/♂ male pupae, when added to *g*/♂ male-pupal extracts, did not increase activity of the enzymes from the *g*/♂ males (not shown). Mixtures of *g*/♂ male and *R*/♂ male extracts had approximately half the activity of the *R*/♂ male samples; thus no inhibition of *R*/♂ male oxidase activity by *g*/♂ male extracts was indicated.

Cytological observations are that the initial failure of normal development of the accessory sex organs (*7*) and eyes (*8*) of the *g* mutant occurs within a few hours after the white pupal period in development; this period would coincide with the period of decreased oxidase activity in the wild-type fly (Fig. 3, A–D). The absence of monophenol oxidase activity, as well as the reduced diphenol oxidase activity in the *g* mutant, may result in reduction in the production of quinones necessary for either the initial development or secondary hardening of such structures as claws, female accessory sex organs, and basement membrane of the eyes. In turn, the malformed ommatidia of the eyes may alter production of pigment in the granules associated with them (*9*). An interesting difference at this point is between the light tan color of the smaller claws of the *g* mutants and the dark brown color of the claws of the *R* wild-type fly; such color is presumed to be a direct result of quinone interaction with proteins in hardening of the claws (*1*).

The mutants lozenge-spectacle (*s*), lozenge (*lz*), and lozenge-krivshenko (*k*), as representatives of mutants located at the other three pseudoallelic loci of the lozenge series, also show variations from wild-type oxidase activity, as will be reported later. Further study of these lozenge mutants and their phenol oxidases may lead to significant improvement in our understanding of developmental abnormalities and gene action in *Drosophila*.

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4. Preparation of activator [method adapted from Mitchell and Weber (*2*)]: Approximately 3 g of pupae in 60 ml of $0.1M$ potassium phosphate buffer, *p*H 6.3, was homogenized in a Virtis blender for 1 minute. The homogenate was immediately centrifuged at 0°C for 5 minutes at 18,000*g*, and the supernatant was adjusted quickly to 41 percent saturation with $(NH_4)_2SO_4$. The precipitate was dissolved in phosphate buffer (10 ml per gram of pupae) and either used immediately or stored at −8°C.
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10. Supported by PHS grant AM 08331-03. We thank H. S. Forrest for advice, and our assistants.

22 October 1967

Modulation of Elicited Behavior

Morse, Mead, and Kelleher (*1*) note the following basic relationship: "When responses have followed recurrent shock they tend to occur before the shock." The authors then question "the need for a response to precede an event in order to be reinforced by that event" (*1*, p. 217).

Using inescapable and unavoidable shock these investigators found that "the initially elicited pattern of maximal responding just after each shock was altered by the recurrent shock and by the added fixed-interval schedule to a pattern of maximal responding just before each shock." Subjects (monkeys) in this experiment had the option of being passive and receiving shock every 60 seconds or receiving shock after a 30-second period if they responded. Morse *et al.* found that most shocks were produced by responses; that is, subjects responded to receive shock after the 30-second period instead of not responding and receiving shock every 60 seconds. If the basic relationship as stated by them does obtain, it is puzzling why the frequency of responding before shock on a 30- or 60-second schedule alone is lower than when the 30- and 60-second schedules are both operative. This difference would not be expected and seems incompatible with their basic relationship. Further, an interpretation (relationship) not considered by the authors, but strongly suggested by their data, is that subjects prefer short, as opposed to long, delays before shock. Since the frequency of subject-initiated shock increased, the suggestion is that short delays are less aversive (preferred) than longer delays of shock. Data supporting this have been reported for both humans (*2*) and infra-humans (*3*).

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6 November 1967