also served to control for possible variations in response rate due to changes in the chemical environment or the temperature and depth of the water.

The effects of the conditioning procedure are illustrated in Table 1, which compares the response rates for the experimental and control subjects. With 15 minutes' light termination, one would expect a conditioned subject to respond at a rate near one response per 15 minutes. Since the data were evaluated by counting the number of responses per half hour, the percentage of half-hour intervals in which the response rate was one to two responses per half hour is compared. All eight experimental subjects had higher percentages than the matched control subjects. The probability of this result occurring by chance is 0.004 by the randomization test for matched pairs. Another measure of conditioning was median latencies of response (time interval between the onset of the stimulus light and the next response) computed for successive 6-hour intervals. The experimental subject of session I was particularly striking in this regard, showing a monotonic decrease from 30 minutes for the first interval to 3 minutes for the seventh.

Only the reinforced subjects produced steady, spaced behavior. The response pattern of the control subjects generally consisted of a burst of rapid responding (as high as 62 per half hour) during the long latencies of the experimental animals and a depression of responding when the experimental subjects had short latencies. Occasional observations of the subjects indicated that after a brief period of exploratory behavior, the motion of the control subjects consisted of a repeated circling of the chamber wall. The experimental subjects at first showed similar behavior, but after 20 or 30 hours would occasionally move directly towards the photocell beam. After a period of steady responding, usually about 50 to 60 hours from the beginning of the experiment, some of the reinforced subjects became "lethargic" in their motion and showed a decrease in response rate. When removed from the chamber (but not simply when the water was changed) the subjects resumed their usual rate of movement. Best and Rubinstein (*3*) noted a similar effect for a conditioned subject in a maze: "There is, in Planaria, some process that, following the phase of rejection of the reinforced alternative, leads to a lethargic state which is not a simple fatigue or injury state."

After the response rate stabilized, the experimental subject of session 3 (Fig. 1) was exposed to a modified extinction procedure. The planarian (together with its matched control subject) was presented, independently of the response, with 15 minutes of darkness alternated with 7.5 minutes of light. The 7.5-minute period was the maximum latency of response for the last six trials of conditioning.

As discussed above, the basis for this procedure was to control for changes in general activity due to differences in illumination. Accidental reinforcement was avoided in this procedure by programming a 1.0-minute delay of light termination if a response occurred in the last minute of the light-on period. The response rate greatly decreased in extinction and increased again with reconditioning.

All of the six additional subjects had a lower percentage of response rates at one to two responses per ½ hour than the lowest reinforced subject. The two subjects with continuous light for 72 hours produced high rates of response between periods of inactivity. A similar pattern of response was produced by subjects having the light off continuously for 62 hours except that the rates were about one-tenth as high. Most similar to the experimental subjects' performance were the performances of subjects exposed to alternating 15-minute periods of darkness and light. They began with high rates which gradually decreased and became more distributed, but were not as steady as the experimental subjects. The results provide further evidence that the response rate for the experimental subjects was dependent upon the reinforcement contingency (*4*).

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### References and Notes

1. In free operant conditioning, a subject may at any time and repeatedly emit a specified response which leads to a reward or the termination of an aversive condition (for example, pressing a lever for a food reward).
2. R. Thompson and J. McConnell, *J. Comp. Physiol. Psychol.* **48**, 65 (1955).
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4. This report is based on a master's thesis submitted to the department of psychology of the University of Maryland in 1963. The research was conducted in the laboratory of psychopharmacology under the supervision of Dr. Lewis R. Gollub. Supported in part by research grant MY-1604 from the National Institutes of Health and research grant NsG-189 from the National Aeronautics and Space Administration. The advice and encouragement of Dr. Travis I. Thompson are gratefully acknowledged.

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## Enzyme Changes in Flight Muscle Correlated with Aging and Flight Ability in the Male Housefly

Abstract. *Magnesium-activated adenosine triphosphatase activity in the giant mitochondria (sarcosomes) of the flight muscle of aging male houseflies decreases concomitantly with failure in flight as reflected in the loss of wings during the second week of adult life. Preceding the loss of wings, however, there is a rapid decline in the activity of an α-glycerophosphate dehydrogenase which is located in the extramitochondrial fraction and is dependent on nicotinamide adenine dinucleotide.*

Earlier reports (*1*, *2*) have shown that the male housefly, *Musca domestica* L., not only has a much shorter life span than the female, but during the second week of adult life exhibits an abrading, and ultimately total loss, of both wings. A marked decline in activity of total body acid, sodium β-glycerophosphatase, and thoracic magnesium-activated adenosine triphosphatase (ATPase) accompanied these gross structural and functional manifestations of senescence (*1*). There is also a time-dependent reciprocal relationship between this failure in ATPase and a sixfold accumulation of total thoracic adenosine triphosphate (ATP) (*3*). By use of differential centrifugation and solubility to separate the various muscle components, particularly the giant mitochondria (sarcosomes) (*4*), it was possible to establish the biochemical mechanisms linked to alterations in flight ability of the aging male housefly and to locate within the flight muscle itself the actual sites of these mechanisms.

Houseflies (strain NAIDM) (*5*) were bred on a regularized 14-day cycle from generation to generation. The larvae were maintained on a standardized artificial laboratory medium, and the adults were reared in screened cages on cane sugar, water, and powdered whole milk. Conditions of constant lighting, temperature (26.7°C), and relative humidity (50 percent) were carefully controlled. All flies were examined, separated by sex, and counted under continuous $CO_2$ anesthesia. The sarcosomes were isolated in an ice-cold (*6*) isotonic 0.9 percent KCl solution (*7*). The Mg-activated ATPase activity was determined at 37°C for this mitochondrial fraction in

an incubation mixture of 0.4 ml of 0.05*M* tris buffer (*p*H 7.4), 0.24 ml of 0.01*M* $MgCl_2$, 0.12 ml of 0.05*M* ATP (disodium salt, Pabst), 3.08 ml of 0.9 percent KCl, and 0.16 ml of brei plus 1 drop of chloroform. One-milliliter samples, inactivated with trichloroacetic acid, were taken at 5 minutes (control) and after 15 minutes of incubation (experimental) and assayed for enzyme activity, which was measured by the number of micrograms of inorganic phosphate from ATP released each 10 minutes per fly (*8*); this was linear with respect to time for a period of more than 30 minutes.

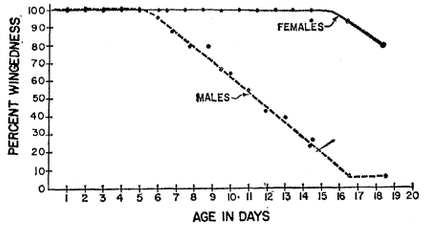


Fig. 1. Wing retention in aging male and female NAIDM houseflies.

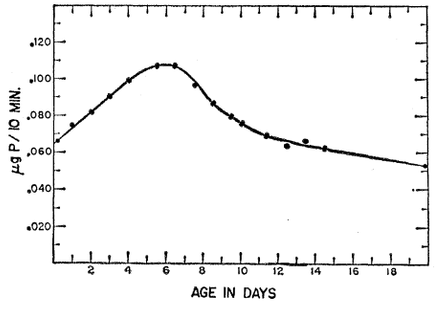


Fig. 2. Magnesium-activated adenosine triphosphatase in mitochondria from the flight muscle of aging male houseflies. Activity is expressed in micrograms of phosphorus released per fly per 10 minutes.

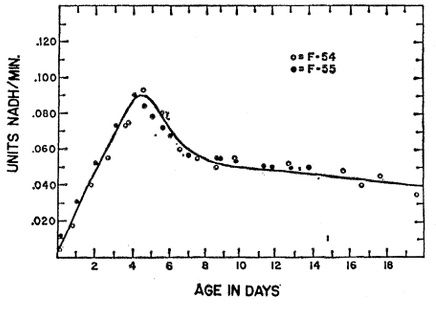


Fig. 3. Changes related to age in extramitochondrial, NAD-dependent α-glycerophosphate dehydrogenase in two generations of male houseflies. Results shown are expressed in units of NADH produced per fly per minute.

Figures 1 and 2 show the virtually identical correspondence of failure in flight ability in terms of the percentage of wing loss (in a population of 1800 male houseflies), with the precipitous decline in activity of mitochondrial Mg-activated ATPase during the second week of adult life.

Scattered reports (*9, 10*) have suggested that the terminal stages of carbohydrate metabolism, especially the reconstitution of nicotinamide adenine dinucleotide (NAD) from the reduced state (which in vertebrates is accomplished by the reduction of pyruvate to lactate by lactic dehydrogenase), may be effected in the flight muscles of insects by the corresponding enzyme α-glycerophosphate dehydrogenase. This enzyme was extracted from the extramitochondrial (that is, the combined remaining fibrillar and sarcoplasmic) fraction of the flight muscle into 25 ml of 0.1*M* tris buffer (*p*H 8.6). To an incubation mixture containing 2.4 ml of 0.1*M* tris buffer (*p*H 8.6), 0.2 ml of β-NAD (20 mg/ml tris buffer) and 0.2 ml of 0.5*M* α-glycerophosphate in tris buffer, all at room temperature, was added 0.2 ml of either the ice-cold extramitochondrial brei or a blank of tris buffer. The contents of the vial were mixed by inversion and quickly transferred to a 1-cm quartz cuvette. The activity of α-glycerophosphate dehydrogenase was quantitatively determined in a Beckman DU spectrophotometer from the increasing absorption of ultraviolet light (340 mµ) at 30-second intervals for a 5-minute period (during which the rate of reduction of NAD coupled with the oxidation of α-glycerophosphate remains constant). Figure 3 shows that the failure of the NAD-dependent α-glycerophosphate dehydrogenase system (which is found only in the extramitochondrial fraction) precedes by about 48 hours the highly increased rate of wing loss in male houseflies, which occurs between the 6th and 7th days. (Fig. 1).

Inasmuch as the studies of Zebe and McShan (*10*) and others (*11*) have shown that the highest concentrations of α-glycerophosphate dehydrogenase occur in the flight muscle of strong-flying insects, this enzyme system should serve as an important starting point for further investigation of the biochemical manifestations of senescense in flying insects. Experiments, in which the quantitative enzyme changes that follow the removal of wings from houseflies, are evaluated, should provide further relevant information.

Two of our recent papers (*12*) and related reports by others (*13*) suggest that the corresponding enzyme in vertebrate skeletal muscle, lactic acid dehydrogenase, may participate in the biochemical manifestations of senescence in vertebrate muscle. The results of these and other reports (*14*) imply that these biochemical systems may likewise be involved in the still poorly understood "muscular dystrophy-like" failure of skeletal muscle (*15*).

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4. These giant mitochondria are strategically placed in enormous numbers in a linear fashion between adjacent muscle fibrils; they represent an unusual morphological-physiological modification within the flight muscles of strong-flying insects, dipteran and hymenopteran insects particularly, by which are met the inordinately high energy demands of these rapidly contracting muscles. This is accomplished by the high concentration, within these relatively massive, discrete bodies of enzymes and coenzymes concerned with the aerobic aspects of intermediary metabolism, particularly oxidative phosphorylation.
5. A strain of highly inbred flies originally supplied by Dr. Albert S. Perry of the Savannah Technical Development Laboratories, U.S. Public Health Service.
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10. E. C. Zebe and W. H. McShan, *J. Gen. Physiol.* **40,** 779 (1957). These authors also describe an α-glycerophosphate dehydrogenase (GDH) that is not dependent on NAD. It is similar to the particulate GDH of vertebrate muscle. Subsequent work of R. W. Estabrook and B. Sacktor [*J. Biol. Chem.* **233,** 1014 (1958)], R. W. Estabrook, B. Sacktor, and B. Chance, [*Proc. 15th Intern. Congr. Zool* (London, 1958), sect. VI, paper 57], and B. Sacktor and D. G. Cochran [*Biochim. Biophys. Acta.* **26,** 200 (1957)] have established the site of this highly active GDH—a flavin enzyme linked to the cytochrome system—as the sarcosomes in the flight muscle of the housefly.
11. In fact, this enzyme is replaced by lactic

acid dehydrogenase in the leg muscles as well as in the thoracic muscle of nonflying insects like the tree weta discussed by G. B. Kitto and M. H. Briggs [*Science* **135**, 3507 (1962)].
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15. This work was begun at the New York University School of Medicine under U.S. Public Health Service research grant 7099 and continued at the University of Miami School of Medicine under grant 9680.

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## Dopamine: Its Occurrence in Molluscan Ganglia

Abstract. *Fluorometric and paper chromatographic evidence indicates that dopamine is the only catecholamine present in the ganglia of a number of lamellibranch and gastropod species.*

Dopamine has been identified in many vertebrate tissues, such as adrenal medulla (*1*), sympathetic nerve (*2*), brain (*3*), and lung (*4*), but it has never been clearly demonstrated in invertebrate animals. In assaying the ganglia of several molluscan species for catecholamines, I found substantial amounts of dopamine with little or no trace of norepinephrine or epinephrine.

The three sets of ganglia of each lamellibranch were dissected free and pooled, and, in gastropods, the entire circumesophageal ganglionic complex was removed. For each species used, the ganglia from several individuals were combined, weighed fresh, and assayed by the fluorometric method of Bertler *et al.* for epinephrine and norepinephrine (*5*); for dopamine, a modification (*3*) of the Carlsson technique (*6*) was used.

Table 1. Estimated concentrations of dopamine in molluscan ganglia. All weights are of fresh tissue. The first three species are gastropods. The others are pelecypods.

| Assays (No.) | Av. wet wt. (mg) | Dopamine (μg/g) | |
|---|---|---|---|
| | | Range | Av. |
| | *Melongena corona* | | |
| 3 | 51 | 51–82 | 63 |
| | *Lunatia heros* | | |
| 3 | 119 | 12–38 | 27 |
| | *Busycon canaliculatum* | | |
| 4 | 310 | 6–22 | 14 |
| | *Mercenaria mercenaria* | | |
| 8 | 31 | 137–405 | 261 |
| | *Modiolus modiolus* | | |
| 3 | 25 | 35–118 | 85 |
| | *Ensis directus* | | |
| 3 | 27 | 31–49 | 37 |
| | *Mya arenaria* | | |
| 1 | 11 | — | 96 |
| | *Mytilus edulis* | | |
| 1 | 41 | — | 35 |
| | *Aequipecten irradians* | | |
| 3 | 39 | 60–88 | 74 |
| | *Spisula solidissima* | | |
| 2 | 42 | 26 | 26 |

The excitation and fluorescence spectra for the ganglia of every species sampled indicated that a substance fluorometrically identical with dopamine was present, but in no case were epinephrine or norepinephrine peaks clearly evident. In addition, paper chromatography of *Mercenaria* and *Busycon* ganglia, in which the technique of Bertler and Rosengren (*3*) and such solvents as phenol and 0.1*N* HCl or butanol, acetic acid, and water (4:1:5) were used, revealed a spot corresponding to the spot produced by standard dopamine. Again, epinephrine and norepinephrine were not detected. When the three *Mercenaria* ganglia (cerebropleural, pedal, and visceral) were assayed separately, high levels of dopamine were found in each ganglion, while fluorometric assay of other *Mercenaria* tissues such as gill, mantle, heart, and intestine failed to show any appreciable dopamine content. Thus it appears that dopamine, at least in *Mercenaria,* is concentrated in the ganglia and occurs in each of the three ganglia.

The concentration of dopamine can be estimated from microammeter readings taken at the excitation and fluorescence peaks, but these values (Table 1) only indicate relative orders of magnitude. Although the recovery of standard dopamine averaged 90 percent, I found that it could vary widely between the extremes of 68 and 136 percent. Nevertheless, these data demonstrate the relatively high concentrations of dopamine which appeared in the ganglia of every bivalve and gastropod mollusk sampled.

These results are consistent with the reported absence of epinephrine and norepinephrine from *Mytilus* (*7*) and with chromatographic evidence for dopamine in *Helix aspersa* mentioned by Kerkut and Walker (*8*). Östlund's survey of catecholamines in lower animals (*9*) is inconclusive for the mollusks, possibly because he used whole animal extracts.

In the past, dopamine has been regarded as merely the precursor of norepinephrine (*10*), but recent findings have suggested an additional, physiological role for this substance; possibly it is a neurohumor in mammalian brain (*3*) and in *Helix* brain (*8*), and it may function in the regulation of the *Mercenaria* (*Venus*) heart (*11*). This demonstration that dopamine is the principal catecholamine in molluscan ganglia also suggests that dopamine has a function independent of its role as the precursor to norepinephrine.

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11 January 1963

## Ribonucleic Acid Synthesis in Protoplasts of Escherichia coli: Inhibition by Actinomycin D

Actinomycin D inhibits specifically and effectively DNA-dependant RNA synthesis in mammalian cells (*1*) and in several bacteria (*2*). *Escherichia coli* however, even in the form of spheroplasts, is resistant to the antibiotic (*3*). Since DNA-dependant RNA synthesis by *E. coli* extracts is sensitive to actinomycin (*3*), the resistance of intact *E. coli* bacteria might be attributed to impermeability of this organism to the drug. Because of the relevance of the actinomycin effect to the study of gene action, and in view of the unusual amount of information available about nucleic acid metabolism, phage infection, and the regulation of protein synthesis in *E. coli*, an actinomycin-sensitive *E. coli* system would be useful for studying these problems.

We have prepared protoplasts from