Time-consuming dialyses, which often result in a considerable degree of protein denaturation, are thus avoided. At present this method is being tested with other anti-hapten systems (*8*).

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Age Changes in Adenine Nucleotides in Flight Muscle of Male House Fly

Abstract. Under standard conditions of temperature, humidity, and (artificial) diet, loss in flight ability is paralleled by a decline of up to 66⅔ percent in activity of enzymes dephosphorylating organophosphorous compounds in the thoracic flight muscle. Concomitantly, the content of adenosine triphosphate in the flight muscle increases five times, while the content of adenosine monophosphate correspondingly diminishes.

In an earlier paper, Rockstein reported (*1*) that the male house fly, *Musca domestica* L., reared and maintained under standardized conditions of temperature, humidity, and artificial diet, consistently demonstrated failure in flight function in the form of abrading and (ultimately) loss of its wings beginning with the 10th and usually by the 14th day of adult life. Concomitant biochemical changes were also observed in the form of marked decline in the activity of sodium β-glycerophosphatase activity from five activity units at 1 day to 1.8 units by the 11th day. Follow-up determinations of activity in the thoracic flight muscle of magnesium activated adenosine triphosphatase, by the method of Sacktor (*2*), have shown a remarkably similar decline in the activity of this enzyme, both in total thoracic homogenate extracts as well as in the flight muscle mitochondria per se, especially after the 9th to 10th day.

In order to explore further the biochemical manifestations of the senescence in flying ability of the male house fly, we undertook to determine what changes might be taking place in the substrate of this important enzyme system, adenosine triphosphate and its breakdown products, in the aging flight muscle of the male fly. Flies of known age of the NAIDM (*3*) strain were anesthetized lightly with CO_2, sexed, and frozen solid by rapid plunging into liquid air. For each age, 50 thoraces were isolated and ground in cold 0.6*N* perchloric acid in an Elvehjem-Potter (Teflon and glass) homogenizer. This and all other subsequent procedures preparatory to chromatographic separation were carried out at approximately 0°C. The total homogenate was transferred quantitatively to a centrifuge tube and centrifuged at 2000*g*. The supernatant liquid was set aside and stored cold, and the centrifugate was resuspended in cold 0.2*N* perchloric acid and recentrifuged. The combined supernatant liquids were then neutralized with 5*N* sodium hydroxide and transferred to a Dowex 1-X 10 (200 to 400 mesh) ion-exchange resin column (1 by 6 cm), previously prepared in the formate form, according to the Siekevitz-Potter method (*4*). By the modified Carter and Cohn gradient elution technique (*5*), we separated the three adenine nucleotides, with adenosine monophosphate and adenosine diphosphate being eluted by the distilled water–4*N* formic acid system and adenosine triphosphate by a similar formic acid–0.6*N* ammonium formate in 4*N* formic acid system (*6*). Estimation of each nucleotide was made by absorbance at 260 mμ, in the Beckman DU spectrophotometer. Standard solutions of each of these nucleotides were run through the column once weekly to check the consistency of this fractionation system. By this method, adenosine monophosphate was eluted completely in the 6th to 15th (5 ml) collecting fractions, adenosine diphosphate between the 30th to 50th tubes, and adenosine triphosphate in tubes 75 to 90.

Table 1 shows that the content of adenosine triphosphate in the thorax of the male house fly rises significantly after the 1st week and reaches a maximum value by the 9th day, that is, at a time when the enzyme system involved in its dephosphorylation approaches a minimum. Concomitantly and reciprocally, the content of adenosine monophosphate (the breakdown product of adenosine triphosphate) reaches a minimum level. These biochemical manifestations of senescence of flight ability all reflect morphological and gross functional alterations with age in flying ability at the virtually identical point in the life history of the male fly. The recognized role of phosphorus metabolism in general and the dismutation of "high-energy" bonds in the energizing of a number of important biological processes, including muscle contraction (*7*), make these correlative positive observations of a quantitative nature highly gratifying in an area where more and more concrete evidence of a fundamental biological phenomenon (like senescence) is being sought after. In order to pinpoint the actual sites of such changes within the flight muscle itself, current investigation is being made of biochemical changes and concomitant changes in the cytochemical-cytological features of the giant mitochondria (sarcosomes), the fibrillar, and the soluble fractions of the flight muscle of the aging house fly. Preliminary findings point strongly to the primary role of a declining intramitochondrial enzyme and an extramitochondrial site of the changing adenine nucleotide content (*8, 9*).

Table 1. Adenine nucleotides in the flight muscle of aging male house flies, *Musca domestica* L. Amounts are expressed in absorbance units per total extract from 50 thoraces, at each age level. AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

Age	AMP	ADP	ATP
0–2 hr	3.9	1.2	1.0
6–8 hr	4.4	1.1	0.7
22–24 hr	5.7	0.8	1.6
44–48 hr	5.3	0.9	0.8
3–4 day	4.9	0.4	0.6
4–5 day	6.6	0.5	1.9
6–7 day	4.6	1.6	2.7
8–9 day	1.2	2.0	6.7
12–13 day	1.8	2.2	5.2
14–15 day	1.7	1.8	5.2
15–16 day	1.1	1.3	6.2

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Ether-Induced Retrograde Amnesia for One-Trial Conditioning in Mice

Abstract. Mice were tested in a situation permitting them to step readily from a small, restrictive platform to a larger one. Conditioned avoidance was established by a single training trial when animals received a shock by stepping from one platform to another. At various intervals after the single punishing shock, subjects were anesthetized with diethyl ether. Interference with retention, tested 1 day later, was shown for intervals up to 24 minutes, at which time ether no longer appeared to influence the subsequent response.

Several recent investigations (*1*) have suggested that anesthetic agents have an interfering effect upon the retention of recently acquired memories. The rationale underlying such studies has been that certain agents disrupt the consolidation of the memory trace. One such agent which also causes a depression of neural activity is diethyl ether. The purpose of the present study was to investigate the effect of anesthesia introduced at various intervals after a single conditioning trial on retention.

One hundred and twenty male Swiss-Webster mice, approximately 28 days old, were randomly assigned to six experimental and six control groups of ten subjects each. All animals were conditioned to inhibit a stepping response from a small restrictive platform to a larger platform below in order to avoid a shock. The small platform, measuring 1¾ by ⅞ in., was lowered to the larger platform, measuring 6 by 6 by ¾ in., by means of a ½-in. metal rod connected to a pinion gear arrangement; each animal was lowered from a height of 4 in. to within ⅜ in. of the lower platform. Animals received a painful electric shock of approximately 3 ma from a 270-volt d-c source when they stepped to the lower platform. Subjects in each of the six experimental groups were anesthetized in a beaker with diethyl ether at intervals of 0, 2, 8, 16, 20, or 24 minutes after the conditioning trial and were allowed to recover in their home cages. Anesthetic induction took approximately 40 seconds. Subjects in the six control groups were confined to an empty beaker for 10 seconds after delays equal to those of the experimental subjects; then they were returned to their home cages without etherization.

A testing trial for the retention of the conditioned avoidance response was performed 24 hours after the training trial. Conditions were identical with those of the training trial except that the platforms were not electrified. Response latencies were recorded for all subjects, and those animals not moving from the upper platform within 30 seconds were removed by hand.

To test for any possible effects of ether anesthesia on the unconditioned response, two groups of ten subjects each were allowed to respond on the platform apparatus in the absence of shock. One group was anesthetized with diethyl ether immediately after responding, whereas the other group was treated in the same way as the controls described above. These animals were tested 24 hours later.

Figure 1 shows the experimental and control response latencies as a function of the training-anesthesia interval. A conditioned response was defined as any response with a latency longer than 10 seconds. A chi-square analysis for the frequency of conditioned responses yielded a value of 22.70 between the experimental and control groups; this value is significant beyond the .001 level of confidence. The response latencies for the unshocked control groups were not significantly different, with none of these exceeding 2.1 seconds.

The data clearly support the hypothesis that ether anesthesia has an interfering effect on the retention of a conditioned avoidance response in the mouse. The fact that all of the untreated control subjects increased their response latencies from a median of 1.41 seconds on training to 30+ seconds on testing suggests that conditioning has certainly taken place.

The ether anesthesia was effective in interfering with retention in 100 percent of the subjects in the 0-, 2-, and 8-minute groups, 60 percent of the subjects anesthetized 16 minutes after training, 50 percent of the 20-minute group, and only 20 percent of the group treated 24 minutes after training. At this time the median response latency was again 30 seconds.

The concept of the consolidation process has been proposed (*2*) to account for the changes in neural activity which follow a behavioral event. The hypothesis states that during this period the memory trace from the original event perseveres and hence may be affected by the introduction of various chemical and other agents and by given environmental events.

The results of the present experiment suggest that anesthesia with diethyl ether will completely impair the retention of the conditioned avoidance response if it is administered within 8 minutes after training, will impair the retention less if administered from 16 to 20 minutes after training, and will not significantly affect retention if administered 24 minutes after training. A previous study (*3*) has shown that

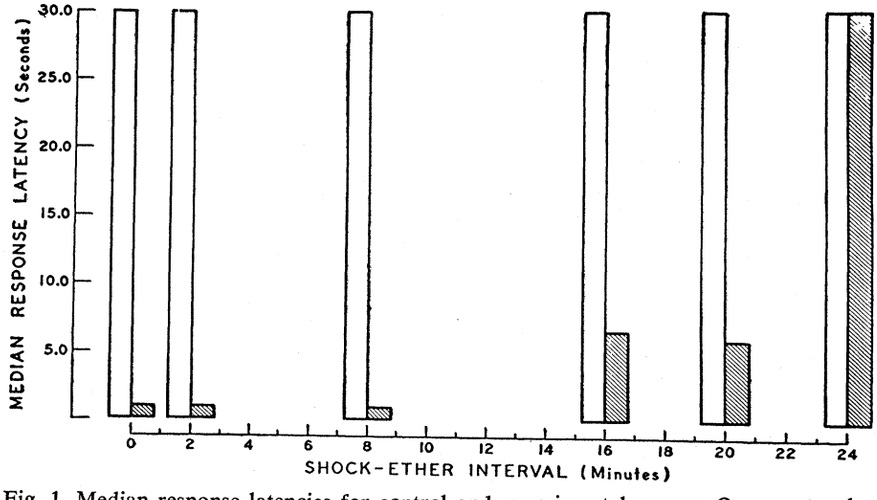

Fig. 1. Median response latencies for control and experimental groups. Open rectangles, control group; crosshatched rectangles, experimental group.