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8. This is in direct contrast to the report of D. Levenbook [*J. Histochem. and Cytochem.* **1**, 242 (1953)], in which he inferred from circumstantial evidence that "the muscle adenine triphosphate must be largely in the sarcosomes" of *Phormia regina*.

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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

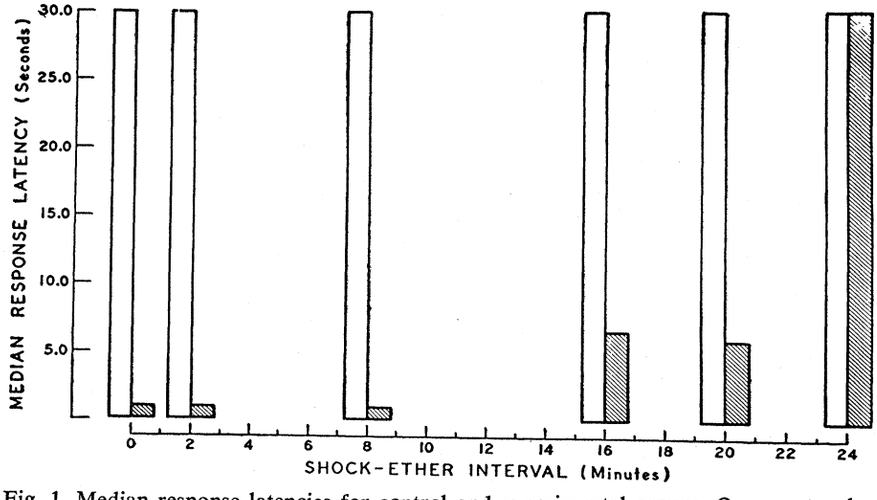

Fig. 1. Median response latencies for control and experimental groups. Open rectangles, control group; crosshatched rectangles, experimental group.

diethyl ether given to mice 1 hour after a single-trial conditioning situation will not impair the retention of a response tested 24 hours after training. This observation, supported by the results of another study employing ether anesthesia in rats (*4*), leads one to infer that consolidation is complete between 20 and 24 minutes after training in this type of situation (*5*).

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Detection of Lycopene in Pink Orange Fruit

Abstract. Lycopene is shown to be the pink coloring pigment in Sarah—a pink sport of the Shamouti (Jaffa) orange—together with other unidentified carotenoids. This is a condition similar to that found in pink and red grapefruits, while the red pigments of blood oranges are anthocyanins.

Matlack (*1*) had suggested that the red coloration in blood oranges is caused by anthocyanins, which have quite recently been identified as cyanidin-3-glucoside and delphinidin-3-glucoside, dissolved in cell sap of juice vesicles of some blood orange varieties (*2*).

On the other hand, the pink and red color of some grapefruit and shaddock varieties has been found to be caused by plastid-contained lycopene and beta-carotene (*3*), which have been quite recently investigated by Lime *et al.* (*4*), Purcell (*5*), and others. As far as we are aware (*6*), no report of the presence of lycopene in oranges has been published to date.

Sarah, a pink bud sport of Shamouti (Jaffa) orange, which was first detected in the late 1930's and described in 1944 (*7*), presents at an even superficial examination many of the characters of pink grapefruits: (i) the filtered juice is almost colorless and does not present any pink tinge; (ii) the pink color is mainly present in the inner mesocarp, carpel walls, and vesicle stalks; and (iii) large concentrations of dark-yellow to pink plastids are detected especially around main conducting bundles and their anastomoses (*8*) even when fruit is fully ripe.

Carpels of ripe Sarah fruits were blended, extracted, and chromatographed on 1:1 W/W Magnesia-Hyflo Super Cel columns, as described under method A, by Lime *et al.* (*4*).

Two main bands were obtained, an upper red and a lower yellow-orange, which were eluted with 10 percent acetone in hexane and 5 percent methanol in hexane, respectively, into two separate fractions.

Spectral curves of both fractions were determined with a Beckman DU spectrophotometer. The curve obtained from the upper band was identical with that of lycopene in hexane, with peaks at 445, 470, and 503 mμ. This is in accordance with the situation found in pink and red grapefruits (*4, 5*). The second fraction which had been found to contain only beta-carotene in grapefruits (*4*) did not yield a curve identical with that of beta-carotene in hexane, since it has peaks at 440, 470, and 500 mμ instead of two peaks at 455 and 480 mμ (*4*); it seems to contain some unidentified carotenoids.

No anthocyanins could be detected in the aqueous and methanolic extracts of Sarah carpels or mesocarp portions. It is therefore concluded that the pink coloration observed is induced by substantial amounts of lycopene and not by anthocyanins as is usual in blood oranges.

Purcell (*8*) has pointed out that a carotenoid precursor must be assumed to enter fruit through the vascular system and to diffuse into the surrounding parenchyma where it produces pink coloration in pink and red grapefruits, especially before full ripeness is attained. It seems worth adding that this must be true for most citrus fruits. Concentrations of carotenoids can be seen easily around bundles, especially at the main fruit axis in many varieties, for example, Shamouti and Valencia oranges, Dancy tangerine, Eureka lemon, and Marsh seedless grapefruit, and especially so, because of the emphasizing pink tinge, in the Sarah orange.

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A World-wide Stratospheric Aerosol Layer

Abstract. An aerosol layer has been identified by a stratospheric balloon and aircraft aerosol collection program. Measurements of horizontal extension and vertical distribution indicate that this layer is a world-wide phenomenon, displaying little variation with time and latitude. The particles in this layer range in size between 0.1 and 2 μ in radius, are water soluble, and contain sulfur as the predominant constituent. It is most likely that they are formed within the stratosphere.

A program of stratospheric aerosol collection with high-altitude balloon equipment was started about 3 years ago and was recently supplemented by collection with high-altitude aircraft (*1*). The aim was to obtain basic information on a heretofore neglected subject of some importance to radioactive fallout and stratospheric circulation studies. The instrumentation was designed to study vertical profiles of number concentration of two particle size ranges, as well as the horizontal distribution, size distribution, and chemical composition of the particles. It is evident, from the results now available, that a large, persistent aerosol layer exists in the stratosphere at an altitude of about 20 km. This layer is composed of particles between approximately 0.1 and 2 μ in radius. Particles smaller than 0.1 μ are distributed with entirely different vertical profiles, the form of which indicates that they are of tropospheric origin and are brought into the stratosphere by mixing. On the other hand, a 2-μ radius appears to be a rather sharp upper limit, and almost no particles larger than this seem to be present. The characteristic features of this aerosol layer are listed below.

1) Vertical profiles obtained in middle north latitudes show a very broad maximum in number concentration at altitudes between about 18 and 23 km. The number concentration of particles at this maximum is about 1 per cubic centimeter, which is higher by a factor of 3 than that at the tropopause, and in most cases decreases rapidly above 24 km. The time variation at this maximum over a period of more than 2 years does not exceed a factor of 3. The shape of the vertical profile indi-