above can be dried in 8 hours. Following this drying period the specimen is removed to a vacuum chamber for embedding in paraffin. The quality of the results, in terms of histological detail, appears equivalent to that of results obtained by the conventional vacuum freeze-drying approach. The efficiency of this method compares favorably with the best reported vacuum freeze-drying and is substantially superior to that of devices which do not have the cold trap in line of sight from the specimen.

The mechanical simplicity achieved through elimination of vacuum pumps and use of a vacuum-tight system is considerable. An additional advantage of this approach, however, appears to be in situations where a number of specimens are to be dried simultaneously, where the many specimens and their supports constitute physical obstacles which diminish the effectiveness of the vacuum system by preventing straight-line passage of vapor to the trap. Above all, these experiments (*5*) confirm the supposition that passage of vapor through the dried specimen shell is primarily a matter of vapor pressure gradient rather than of total pressure in the system.

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## Photodynamic Inactivation of Monkey Kidney Cell Monolayers

*Abstract.* Monkey kidney cell monolayers exposed to white light in the presence of neutral red (1:40,000) undergo degeneration within 24 hours after exposure.

This communication concerns the effect of white light on monkey kidney cell monolayer cultures in the presence of the vital stain neutral red. In the course of attempts to photoreactivate ultraviolet-light-inactivated monkey kidney cell monolayers grown in 100-mm petri dishes, it was observed that unirradiated monolayers, when exposed to white light in the presence of the vital stain neutral red (1:40,000), degenerate within 24 hours after exposure. Similar plates exposed to the same light for the same time period, but in the absence of the neutral red, showed no ill effects from the exposure. Neutral red applied to the cells after exposure to the white light did not produce cell degeneration and was picked up by the cytoplasm as in unexposed cells. If the light was first passed through a solution of neutral red (1:40,000), the particular wavelengths of light responsible for the damage were filtered out, and damage to the cells was prevented. However, when methylene blue (1:10,000) or trypan blue (1:10,000) were used as filters, damage occurred as it did with unfiltered white light. The results are summarized in Table 1.

Table 1. Tissue degeneration with various dyes applied to the culture.

| Time of exposure (min) | Tissue degeneration* | | | |
|---|---|---|---|---|
| | No dye | Neutral red (1:40,000) | Trypan blue (1:10,000) | Methylene blue (1:10,000) |
| 0 | 0 | 0 | 0 | 0 |
| 2.5 | 0 | 0 | † | † |
| 5.0 | 0 | 0 | † | † |
| 10 | 0 | 1 | 0 | 0 |
| 20 | 0 | 3 | † | † |
| 30 | 0 | 4 | 0 | 0 |
| 60 | 0 | 4 | 0 | 0 |
| 90 | 0 | 4 | † | † |
| *Light filtered through trypan blue* | | | | |
| 60 | | 4 | | |
| *Light filtered through methylene blue* | | | | |
| 60 | | 4 | | |
| *Light filtered through neutral red* | | | | |
| 60 | | 0 | | |

* Degeneration: 100 percent, 4; 50 to 75 percent, 3; 0 to 25 percent, 1; none, 0.
† Not determined.

The source of the white light was a bank of three 20-watt fluorescent bulbs at a distance of 15 cm. The light was filtered through a 3-cm solution of 1-percent copper chloride, and the temperature of the air above the solution did not rise above 38° to 39°C.

It is suggested that perhaps one cause for the occasional degeneration of monkey kidney monolayers or for the loss in plaque count (*1*) seen after the addition of the nutrient-agar–neutral-red mixture used in the Dulbecco Plaque Technique is inadvertent exposure to white light. It is evident from these findings that in the presence of neutral red, the exposure of the tissue to white light should be kept to a minimm.

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## Effects of Differential Infantile Handling upon Weight Gain and Mortality in the Rat and Mouse

*Abstract.* Animals were handled during ages 1 to 10, 11 to 20, or 1 to 20 days, or were nonhandled controls. Animals handled for 20 days weighed the most in adulthood, while the controls weighed the least. Animals handled on days 1 to 10 survived food and water deprivation the longest of any group. Mice handled for 20 days died earlier than controls, while the reverse was true for the rat.

The general procedure followed in studying the effects of infantile experience upon adult behavior has been to stimulate the organism from birth until weaning and then test for effects of the stimulation later in life. Since the rat and mouse undergo tremendous developmental changes during the pre-weaning period, it is likely that the same stimulation affects the organisms differently at different developmental stages. This is suggested by Scott's critical period hypothesis (*1*) and by the work of Levine and Lewis (*2*). Therefore, if animals are stimulated only during certain parts of the pre-weaning period, the stimulation may interact with maturational processes which are at different stages of development to differentially modify adult behavior.

Complete litters of rats descended from the Harvard Wistar strain, and C57BL/10Sc mice were randomly assigned to one of the following infantile experience groups: handled on days 1 to 10, handled on days 11 to 20, handled on days 1 to 20, and nonhandled controls (*3*). At least two litters were used per experimental treatment. Handling consisted of removing the pups from the home cage, placing them in a container (a 1-gal can filled with sawdust for the rat, and a wooden mouse box for the mouse) where they remained for 3 minutes, and then returning them to the home cage. This procedure was followed once daily on the appropriate days. All animals were weaned at 21 days and reared thereafter with like-sexed members of their own litter in small groups. Food and water were always available. At 69 days the rats were weighed and placed on total food and water deprivation in individual cages. The same was done with the mice at 54 days. All animals had received 10 days of testing of avoidance learning just prior to this. Hours until death occurred were recorded.

The group means for weight and mortality for both species are presented in Table 1. The data were analyzed in a $2 \times 2$ factorial design: presence or absence of handling on days 1 to 10 was one factor, while presence or absence of handling on days 11 to 20 was the

Table 1. Mean weight (in grams) and survival time (in hours) for the rat and mouse as a function of infantile handling.

| Days on which handled | Weight | Survival time | No. of animals |
|---|---|---|---|
| | *Rat* | | |
| None | 166.79 | 173.50 | 16 |
| 1 to 10 | 191.33 | 229.58 | 12 |
| 11 to 20 | 176.77 | 223.92 | 13 |
| 1 to 20 | 194.12 | 199.60 | 15 |
| | *Mouse* | | |
| None | 17.14 | 90.47 | 17 |
| 1 to 10 | 18.81 | 95.00 | 10 |
| 11 to 20 | 19.22 | 90.31 | 16 |
| 1 to 20 | 19.87 | 72.83 | 12 |

second factor. This analysis permits the evaluation of the interaction between the two factors.

*Weight.* For both species a monotonic relationship is found between amount of handling in infancy and weight in later life. The animals handled for 20 days were the heaviest, while the controls were the lightest. Tests of significance showed that rats handled during the first 10 days of life (groups handled on days 1 to 10 and 1 to 20) weighed significantly more than the two groups not handled at this time ($P < .05$). The same finding was obtained with the mouse ($P < .01$). In addition, the presence of handling during the second 10 days of life also led to a significant increase in body weight for the mouse ($P < .01$). In neither species was the interaction significant ($F < 1.0$ in both analyses). We conclude, therefore, that for both species the effects of handling during the first and second 10 days of life affect later body weight in an additive manner, with the presence of handling during the first 10 days bringing about a significant weight gain.

*Mortality.* For both species a *non*-monotonic relationship is found between amount of handling in infancy and number of hours of survival in later life. For both the rat and mouse the interaction between presence or absence of handling during the first and second 10 days is significant beyond the .01 level. Inspection of Table 1 indicates that the reason for the significant interaction is that the animals which were handled on days 1 to 10 lived the longest, while those handled on days 1 to 20 died quite early. A test for trend, using the control group, the group handled on days 1 to 10, and the group handled on days 1 to 20, showed a significant quadratic (curvilinear) function beyond the .01 level for both species. We conclude, therefore, that handling during days 1 to 10 will lead to longer survival under total food and water deprivation but that lack of handling or prolonged handling will reduce survival time.

As one of us has reported elsewhere (*4*), the mouse data may be biased because of significantly differential "spontaneous" deaths which occurred between ages 28 and 35 days. That this is not a major bias is shown by a recent study by Levine and Cohen (*5*), who handled DBA/2 mice for the first 24 days of life and then injected leukemia virus into the experimental animals and nonhandled controls at about 50 days. They found that their handled mice died significantly earlier than the controls, which is consistent with our findings that the mice handled on days 1 to 20 died earlier than control mice. Our data for rats are also consistent with the findings of Levine and Otis (*6*), who reported a significantly lower proportion of deaths for rats handled for the first 20 days of life than for controls when both groups were placed on 120 hours of food and water deprivation.

Thus we see that when mice and rats are given 20 or more days of infantile handling the rats will survive longer than their controls while the reverse is true for the mice. It appears, therefore, that the same physical stimulation has a more severe effect upon the mouse. Though these two species differ in many respects, one of the outstanding differences between the C57BL/10Sc mouse and the albino rat is that the mouse is a more rapidly developing organism. This suggests the hypothesis that the more rapid an organism's development, the greater the effect of infantile experience. In addition to incorporating our data as well as the findings of Levine and Otis and Levine and Cohen, this hypothesis also accounts for the findings of King and Eleftheriou (*7*), who studied the effects of infantile stimulation upon two subspecies of the deer mouse, *Peromyscus maniculatus*. They found that *P. maniculatus bairdii,* a rapidly maturing organism, was deleteriously affected by the infantile stimulation, while *P. maniculatus gracilis,* a slowly maturing organism, was facilitated by the identical stimulation, as measured by behavioral tests of activity and learning, and by adrenal weights. Since a more rapidly maturing organism may have a shorter infantile period, the use of developmental indices such as eye opening may be a more appropriate basis for the administration of stimulation than the use of absolute age.

The mechanisms underlying these phenomena are as yet unknown. However, it is apparent that body weight is not related to survival time in any simple manner. It is also apparent that there is more similarity between the weight data for the rat and mouse than between the survival results. Denenberg has suggested (*4*) that the effect of infantile handling is to stress the organism, and this stress acts to reduce the animal's responsiveness to later stressing agents. The greater the magnitude or duration of the infantile stress, the greater is the reduction in responsiveness. The initial effect of reducing responsiveness to stress is to bring about a facilitation in performance (for example, through reducing emotionality), while prolongation of the infantile experience results in an organism which exhibits impaired performance. Within each species the present results are consistent with this hypothesis, but it is apparent that the age during which the organism is stimulated is another critical parameter. When making comparison across species, developmental rates, and probably other parameters, must be considered.

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## Procedure for Studying Olfactory Discrimination in Pigeons

*Abstract.* A discrimination, based on olfactory stimuli, was established in two pigeons by an operant conditioning procedure. Results from control sessions demonstrate that the discrimination can be attributed only to the presence or absence of olfactory stimuli.

Experiments designed to determine whether birds possess a sense of smell have had equivocal results. Some workers have reported that birds are able to make precise olfactory discriminations, while others have found that birds fail completely in discriminating the presence of olfactory stimuli (*1*). Procedural difficulties and artifacts have marred the many experimental procedures that have been used by these workers. An experi-