

percent being attributable to the beta rays from lead and bismuth. As a basis for comparison, this dose is about one-fifth that which may be calculated for  $Ra^{226}$  and its daughters when the total body content of a standard man is 120 pc of  $Ra^{226}$ .

It may be pertinent to note that the individuals whose cadavers provided the ashed material were in the upper age brackets. Since a major contributing factor to the level of body lead is the level of lead in the environment, and since this has risen during the past several decades, a group of younger subjects who are actively forming bone mineral might yield a higher result.

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6. No direct experimental proof can be cited, but no loss from ashing has been shown for radium [J. B. Hursh and A. A. Gates, *Nucleonics* **7**, 46 (1950)], and it is known that lead is incorporated in the bone mineral in a combination like that for radium.

8 June 1960

### Previous Breeding Experience and Hormone-Induced Incubation Behavior in the Ring Dove

**Abstract.** Injected progesterone induces incubation behavior much faster, and in a higher percentage of cases, in doves with previous breeding experience than in those without such experience. The nature of the animal's previous experience is thus one of the variables influencing behavioral responses to exogenous hormones.

When a male and female ring dove (*Streptopelia risoria*), each of which has had previous breeding experience but not with the other, are introduced into a breeding cage containing a nest and eggs, they do not sit on the eggs until after 3 to 6 days, during which time they engage in nest-building activity (1). If, however, the birds are injected daily with progesterone during the 7-day period before they are placed in the cage together, they sit on the eggs almost immediately (2). The purpose of the experiment reported here was to

Table 1. Latency of response to eggs by pairs of progesterone-treated ring doves with and without previous breeding experience. Time was measured from the introduction of the birds into the cage to the first occurrence of the response. Range is given in parentheses.

| Response               | Latency (median and range) |                         |
|------------------------|----------------------------|-------------------------|
|                        | Experienced doves          | Inexperienced doves     |
| Standing near nest     | <1 min (<1 min- 1 min)     | 34.5 min ( 3 min->2 hr) |
| Standing on nest       | 1 min (<1 min- 3 min)      | 60.5 min (<1 min->2 hr) |
| Settling on eggs       | 6.5 min ( 3 min-26 min)    | >2 hr ( 56 min->2 hr)   |
| Established incubation | 21.5 min ( 6 min- 3 hr )   | 24.5 hr ( 82 min-nil* ) |

\* Represents three pairs which never established incubation.

study the effect of progesterone upon incubation behavior in birds that had had no previous breeding experience, and to compare birds that had and had not had breeding experience with respect to their responses to eggs after progesterone injection.

Male and female ring doves were separated from their parents at the age of 21 days and placed in stock cages in groups of six to ten birds. At 4 months of age, the sex of each bird was determined by exploratory laparotomy (3), and the birds were placed individually in small cages in which they were visually isolated from other birds. When the birds were 5 to 6 months old (that is, sexually mature), ten breeding pairs were constituted by selecting males and females from the isolation cages and placing them in breeding cages. These birds were permitted to carry out a complete breeding cycle, which consisted of building the nest, laying and incubating the eggs, and rearing the young to the age of 21 days. The parents were then returned to the individual isolation cages. After spending 3 to 5 weeks in the isolation cages they were considered available for use in our experiment, constituting the "experienced" group.

The "inexperienced" group consisted of birds which remained in the small isolation cages throughout the period in which the birds of the "experienced" group were acquiring their breeding experience. These birds were matched with the birds of the experienced group with respect to date of hatching and age at time of testing (for details of housing and maintenance, see 1 and 3).

All birds were given seven daily injections of 100  $\mu$ g of progesterone (4) in sesame oil, injected into the pectoral muscles on alternate sides on alternate days. At approximately 10:00 A.M. on the day following the last injection, a pair of birds was introduced into a breeding cage containing a nest with two eggs. The arrangement of the cage was such that the nest and eggs were always in full view of any bird in the cage. The birds were watched continuously for 2 hours, during which time a verbal report of their behavior was recorded on a dictating machine, and subsequently they were visited

briefly at hourly intervals, for up to 10 days when necessary (5).

Table 1 shows the results of the experiment. "Standing near nest" means that the bird was in the quadrant of the cage which contained the nest for longer than 15 seconds. "Settling on eggs" means that the bird sat on the eggs like an incubating bird, raising the ventral abdominal feathers so that the area of naked skin on the underside of the body (6) came into contact with the eggs. "Established incubation" means that the bird sat for 30 minutes or more. [Observations on these and other birds (7) indicate that, once doves have established incubation by this criterion, they continue to incubate for at least the normal incubation period of 14 days.]

Regardless of which response is considered, there is obviously a striking difference between the two groups; more of the experienced than of the inexperienced birds incubated, and the experienced birds did so sooner than the inexperienced ones. It may be noted that the latency scores for the two groups did not overlap at all with respect to two of the measures. The overlap in the ranges for the other two measures was based on only one pair in each group. In the case of the last measure (established incubation), the overlap is due entirely to the fact that one of the experienced birds, which first settled on the eggs 6 minutes after being introduced into the cage, spent the time between 6 minutes and 3 hours alternately settling on the eggs and repairing the nest. With respect to each of the four measures, a median test, by Fisher's exact probability method (8), shows that the differences between experienced and inexperienced birds are significant at the .005 level. Qualitative differences were immediately apparent to the observer: the experienced birds usually went directly to the nest, in contrast to the inexperienced ones.

Although the occurrence of incubation behavior in response to exogenous progesterone is here shown to be greatly facilitated by previous breeding experience, the present data do not indicate what this "experience" consists of, or how it affects subsequent behavior. It will be interesting to discover exactly

what aspects of the previous breeding experience are required in order that a bird should act like an "experienced" bird in this situation, what contribution is made by the purely hormonal aspects of the earlier events, what the exact details of the differences in behavior between experienced and inexperienced birds are, and so forth (9).

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4. The progesterone ("Lutocylin") was kindly supplied by CIBA Pharmaceutical Products, Inc.
5. The lights in all experimental and rearing rooms were clock-controlled, with a daily light period of 14 hours (6:00 A.M. to 8:00 P.M.). Temperature was constant at  $74 \pm 2^\circ\text{F}$ , except for a few brief and irregularly distributed periods of malfunctioning of the temperature-control apparatus.
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### Thioctic Acid Contained in Visual Cell

**Abstract.** The D- $\alpha$ -thioctic acid content of the eye of the frog (*Rana nigromaculata*) was determined quantitatively. Outer segments of rods, whole retinas, and choroids contained, respectively 2.0, 8.6, and 13.0 m $\mu$ g of D- $\alpha$ -thioctic acid per milligram (dry weight). DL- $\alpha$ -Thioctic acid had no accelerating effect on the regeneration of rhodopsin in vivo.

Several years ago, Strauss (1) proposed a hypothetical role of 6-8-dithioctic acid in vision. He assumed that rhodopsin should contain one molecule of 6-8-dithioctic acid per molecule of retinene and that the -S-S-bond of 6-8-dithioctic acid might be split by illumination. Since that time, however, there has been no report about this subject. In the experiment reported here the D- $\alpha$ -thioctic acid content of the dark-adapted frog eye (retina, choroid, and outer segments of rods) was measured, and the effects of DL- $\alpha$ -thioctic acid on the regeneration of rhodopsin in vivo were observed by means of the intraocular injection method (2).

To measure the D- $\alpha$ -thioctic acid content of the frog eye (*Rana nigromaculata*), 75 dark-adapted frogs were used.

They were kept in a dark room overnight, and all operations were carried out under dim red light. The outer segments of rods were isolated, by Kimura's sugar flotation method (3) at  $0^\circ\text{C}$ , from 60 frogs. Retinas and choroids were separated from the rear half of eyeballs of 15 frogs at room temperature (about  $15^\circ\text{C}$ ). To determine D- $\alpha$ -thioctic acid, tissues were homogenized in a Potter-Elevehjem homogenizer and were hydrolyzed with 6N H $_2$ SO $_4$  for 1 hour at  $120^\circ\text{C}$ . For the microbiological assay of D- $\alpha$ -thioctic acid, *Streptococcus faecalis* (10 CL) was used.

Outer segments of rods, whole retinas, and choroids contained, respectively, 2.0, 8.6, and 13.0 m $\mu$ g of D- $\alpha$ -thioctic acid per milligram (dry weight). According to Hubbard (4), the rhodopsin content of outer segments of rods in the frog is about 35 percent of the dry weight, and frog rhodopsin has a molecular weight of about 40,000 and contains only one retinene per molecule. If Strauss's hypothesis were correct, the thioctic acid content of the outer segments of rods might be about 1600 m $\mu$ g per milligram (dry weight). However, only 2.0 m $\mu$ g of D- $\alpha$ -thioctic acid per milligram (dry weight) was contained in the outer segments of rods.

Table 1 gives data for the effect of DL- $\alpha$ -thioctic acid on the regeneration of rhodopsin in vivo. In each experiment, ten frogs (*Rana nigromaculata*) were light-adapted for 2 hours under white light (2000 lux). A DL- $\alpha$ -thioctic acid solution (0.02 ml neutralized with NaOH, containing 400  $\mu$ g of DL- $\alpha$ -thioctic acid) was injected into the vitreous humor of the left eye (2). The right eye was used as the control. After the injection, the frogs were placed in the dark at about  $20^\circ\text{C}$  for 1 hour. Then the outer segments of rods were isolated under dim red light at  $0^\circ\text{C}$ . For the quantitative determination of rhodopsin contained in separated outer segments of rods, Kimura's method was used (5). The DL- $\alpha$ -thioctic acid had no accelerating effect on the regeneration of rhodopsin in vivo, as shown in Table 1.

From these results it cannot be concluded that D- $\alpha$ -thioctic acid is directly related to the construction of rhodop-

Table 1. The effect of DL- $\alpha$ -thioctic acid on the regeneration of rhodopsin in vivo. All data are expressed as follows:  $R = [\Delta E/\text{Dry wt. (mg) of separated outer segments}] \times 100$ . Here  $\Delta E$  is the difference in optical density at 500 m $\mu$  before and after illumination.

| Experiment No. | With thioctic acid | Control |
|----------------|--------------------|---------|
| 1              | 5.6                | 6.2     |
| 2              | 6.0                | 7.0     |
| 3              | 4.5                | 5.1     |
| 4              | 7.0                | 7.5     |
| 5              | 7.0                | 7.8     |

sin. However, the observation that D- $\alpha$ -thioctic acid is found abundantly in the choroid as well as in the liver is particularly interesting, suggesting a possible relationship to the function of visual cells. Further studies are being carried out in our laboratory on the role of D- $\alpha$ -thioctic acid in vision.

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### Brightness Scaling of White and Colored Stimuli

**Abstract.** Brightness scales for chromatic and achromatic test stimuli have been obtained by the methods of ratio production and magnitude estimation for the dark-adapted observer and conditions of brief foveal stimulation. All scales adhere to the form of a power law for brightness, and scales obtained under the differing psychophysical procedures agree.

There is now considerable evidence from several laboratories both here and abroad relating to the form of the brightness scale for the dark-adapted observer (1). The universal finding is that brightness is related to luminance by a power law with an exponent of about 0.3. That the results of studies obtained under widely different conditions should happen to agree might be attributed in part to biases associated with one or another scaling procedure, or to the interaction of the scaling method with a fortuitous choice of stimulus conditions for measurement. Earlier studies employed differing stimulus areas (Stevens,  $3.3^\circ$ ; Hanes,  $4.5^\circ$ ; Hopkinson,  $2^\circ$ ), differing durations of test stimulus (2 to 3 seconds, or prolonged viewing), and differing means of controlling the effects of simultaneous contrast. These studies all employed stimuli large enough to stimulate extrafoveal areas and long enough to permit some local adaptation to the test flash. Hanes employed the method of ratio production, Stevens and Hopkinson the method of magnitude estimation.

The purpose of the investigation reported here was to compare brightness scales obtained by differing scaling