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In a previous paper[1] it was shown that *Limuli* "freshly collected" are predominantly positive to photic stimulation; that they show positive circus movements when a single eye is stimulated; and that these reactions are easily accounted for by the tropism hypothesis. It was also stated that considerable irregularity, and sometimes reversal, in the phototropic reactions may occur, especially in "hungry" animals, and in those stimulated severely by other means than light. More recently Northrop and Loeb[2] reported the results of their experiments on the phototropism of *Limulus*, in which only negative reactions were discussed. This apparent discrepancy regarding the sign of the phototropic reaction of *Limulus* called for explanation, and the experiments[3] reported here were done. It is stated[4] that only ten out of forty-eight specimens could be used, and it is suggested that lack of response to photic stimulation might have been caused by unfavorable laboratory conditions. In the experiments at least 1 week had elapsed between the time of collecting the animals and the time of making the tests. These facts lead to the belief that changes in the phototropism of *Limulus* are closely linked with, and probably dependent upon, the laboratory age of the specimens. Accordingly tests were made daily upon recently collected individuals, and upon the same ones at each 24 hour interval thereafter over a period of 1 week. The first tests upon each lot of animals were made within 6 hours after the speci-

[1] Cole, W. H., *J. Gen. Physiol.*, 1922–23, v, 417.

[2] Northrop, J. H., and Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 581.

[3] The tests were made in 1923 at the Marine Biological Laboratory, Woods Hole, Massachusetts and the writer wishes to express his thanks to the Director for his accommodations.

[4] Northrop and Loeb,[2] p. 583.

mens were collected from their natural habitat. All of them were small, varying from 20 to 60 mm. in width, the majority being about 30 mm. wide. Between the daily tests all the animals were kept in large aquaria with running sea water, the temperature and pH of which varied but slightly from that of the water in which the *Limuli* were found.

In various positions they were subjected to the directive stimulation of a beam of light, 30 mm. in diameter, coming from a 25 watt Mazda

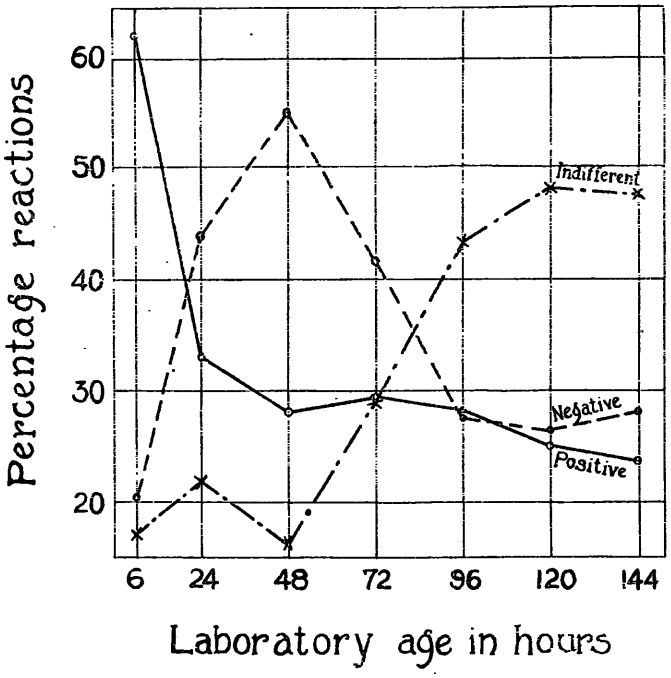

FIG. 1. Graphs showing the percentages of positive, negative, and indifferent reactions of *Limuli* at successive 24 hour intervals of laboratory confinement.

lamp at a distance of 14 cm. from the center of the shallow, glass crystallizing dish. Each animal was tested separately three successive times. If there was any doubt as to the sign of the response, the animal was set aside and tested again later three more times. If doubt still existed, the animal was classified as indifferent. The number of individuals in each lot showing positive reactions, and the number showing negative reactions were recorded. The remainder

were recorded as indifferent. The percentages for each kind of reaction for each lot were calculated. Fresh animals exhibited an accuracy and a precision of orientation which were never seen in animals of a laboratory age of 24 hours or more.

Fig. 1 shows the graphs in which the percentages of positive, negative, and indifferent reactions are plotted against laboratory age. It is seen that during the first 24 hours positive reactions markedly decrease, from about 62 to about 33 per cent. During the next 24 hours there is a slight decrease to 28 per cent, a figure which changes but little during the rest of the week. It is during the first 24 hours, therefore, that laboratory confinement profoundly affects the animal's positive reactions to light. Negative reactions also show variation, but in a different way. They increase from 20 to 55 per cent during the first 48 hours, and then show a steady decrease to about 28 per cent during the second 48 hours. Thereafter the percentage remains practically constant. In the case of indifferent reactions there is fluctuation during the first 48 hours, followed by a steady increase to approximately 50 per cent at the end of 120 hours. At the close of a week's laboratory confinement, therefore, it may be predicted that about 50 per cent of a *Limulus* population will be indifferent to light; about 30 per cent will be negative and about 20 per cent positive, although when first brought into the laboratory they showed 60 per cent positive, 20 per cent negative, and 20 per cent indifferent reactions. From the graphs in Fig. 1 it may be determined how long *Limulus* should be kept in the laboratory to exhibit the maximum number of reactions of the kind desired.

The determination of the factors of the laboratory environment which might be responsible for the changes in reactions was not attempted, the purpose having been merely to demonstrate that the changes do occur, and to clear up the discrepancy referred to above.

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