The volunteers in Group I, who were all known to have negative tests before experimental inoculation with hepatitis virus, failed to develop positive reactions during the 2nd, 3rd or 4th week after onset of disease. In Group II, 2 patients had positive tests with a titer of 1:32, which reverted to negative after standing at room temperature for 3 hours.

Summary. Heterophile antibody agglutination, Kahn, and cold agglutinin tests were performed in 2 groups of patients with viral hepatitis. Sixteen out of 508 patients (3%) developed positive heterophile antibody tests with titers of 1:56 which were reduced to 1:7 or negative by absorption on boiled guinea pig kidney. A rise and/or fall in titer of antibody was demonstrable in serial weekly determinations. Out of 388 patients who were known not to have syphilis, 6 (1.5%) developed positive Kahn tests, and 10 others (2.5%) had doubtful tests. Two out of 323 patients had cold agglutinins present in a titer of 1:32.

The relatively small number of positive heterophile antibody agglutination and Kahn tests is in contrast to reports of others[2,5] who have found a considerably higher percentage of positive tests.

16334

Factors Influencing the Growth of Integumentary Pigment in Fishes. I. The Role of Light.*†

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It is a common observation that light is associated with changes in body pigmentation such as "tanning." Numerous clinical observations[1-5] indicate that certain types of pigmentation deficiencies in man may be corrected by light treatment. In lower mammals Bissonnette and others have demonstrated that pigmentation may be influenced by illumination while experimental evidence has accumulated indicating the importance of light in producing integumentary pigmentation in lower vertebrates. Cunningham[6-8] working with flatfishes; Herbst and Ascher[9] using salamander larvae; Vilter[10] studying axolotls; Sumner and Wells;[11] Sumner and Doudoroff;[12] Odiorne;[13] Sumner;[14-16] Os-

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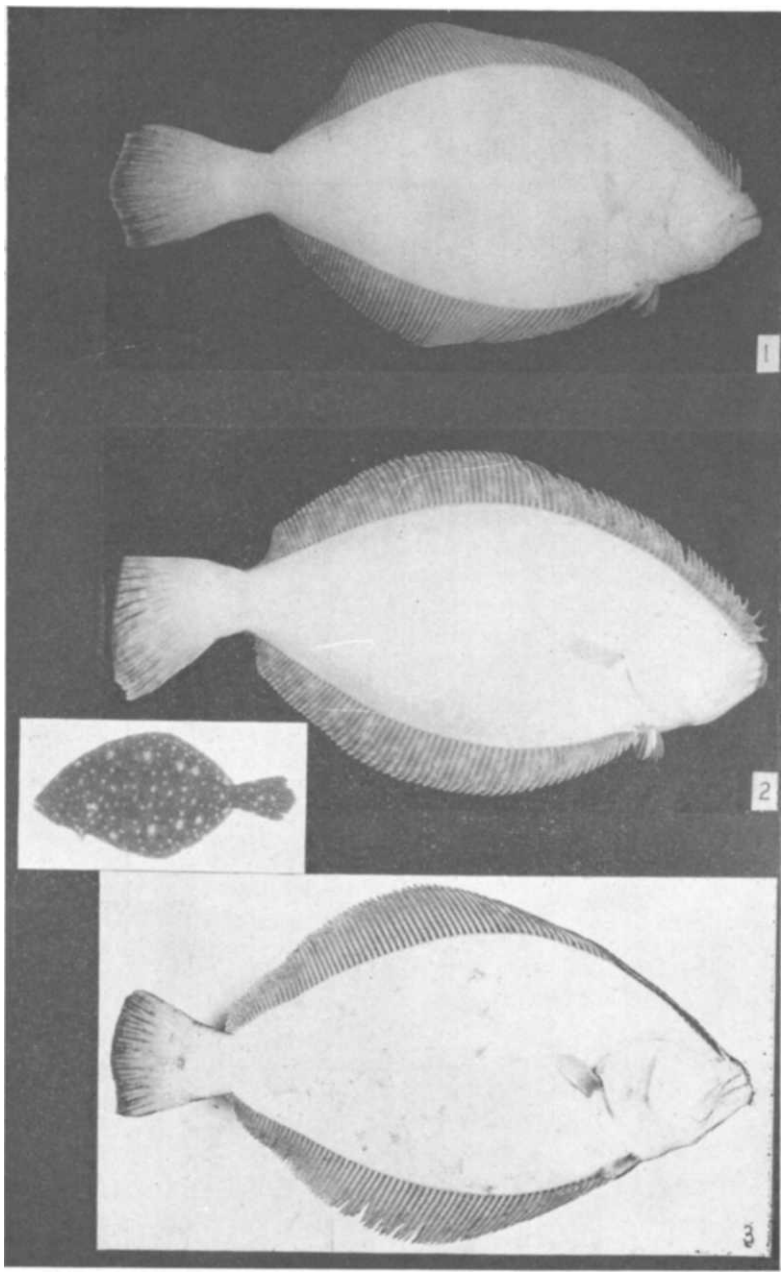

PLATE I.

All figures shown are photographs of the lower surface of living summer flounders. Each animal is selected as typical for the particular experimental situation.

FIG. 1. Normal control freshly caught. The entire ventral surface is free from melanophores.

FIG. 2. Fish black-adapted 2 days, blinded and maintained in darkness 30 days. Entire surface is white as in the control fish except for a few pigmented regenerated scales.

FIG. 3. Animal black-adapted 3 days, blinded and kept for 41 days in a black tank brightly lighted from above. About 2.0 Weston units of light. Somewhat more pigment has developed here than in either of the above figures.

born;[17-21] and Dawes[22] experimenting with various species of fishes have all reported experiments concerning integumentary pigmentation in which light was an important factor. In recent work[17-21] it was found that melanophore development in normally non-melaninated areas depended upon the maintenance of two general conditions. First, the internal environment must be one which favors darkening in the normally pigmented areas; and, second, the external environment must provide illumination (direct or by reflection) to the integument.

The experiments reported here were designed to obtain more detailed information on the role of light in the development of melanophores on the normally unpigmented surface of the summer flounder.

Materials and Methods. Summer flounders freshly caught from Woods Hole waters were used for the experiments. The water temperature for all experiments ranged between 18 and 21° C. The experimental fishes were background-adapted in black tanks illuminated artificially from above. Light intensity (reflected or transmitted) was measured by a Weston exposure meter. The following modifications were made on the ventral illumination tubs previously described:[17] (a) removable false bottoms of perforated glass were rested upon glass supports ¾ inches above the permanent glass floor to allow water to circulate between the 2 glass plates and protect the lower surface of the fishes from overheating; (b) three independent water inlets near the bottom of the tub provided rapid circulation and adequate overflows connected to variable level standpipes allowed easy adjustment of the water level; (c) the light intensity was increased by using a total of 600 Watts for each glass bottomed tub and the walls and ceiling of these special tubs were white to produce a high incidence of internal reflection.

After being black-adapted, the experimental flounders, 106 in all, were totally blinded by enucleation. Opaque masks of black cloth were held in position by suturing each corner to the base of an adjacent fin ray. This kept the mask securely in place without hampering the normal movements of the fishes. Photographic records of the living fishes were made at frequent intervals, supplemented by others of preserved animals.

Adequate unoperated control animals (Fig. 1) in a natural environment were available for purposes of comparison as the experiments progressed.

Experimental. Summer flounders previously black-adapted and totally blinded were placed in total darkness (Exp. 1); subjected to different amounts of overhead illumination where mostly *reflected* light reached the ventral surface (Exp. 2, 3 and 4); exposed to *direct* intense illumination ventrally without masking local areas (Exp. 5) or protecting parts of the ventral surface with opaque masks (Exp. 6).

Exp. 1. Summer flounders black-adapted then blinded as previously described were kept in total darkness from 2 to 6 weeks. Without exception these animals failed to develop any general melanination on the ventral surface (Fig. 2). Here and there a black scale did appear but all were cases of regenerating scales where the original had been lost during experimental manipulation. This will be considered as a constant in subsequent experiments described here. The pigmentation of regenerated scales comprises a special situation which must not confuse the objectives of these experiments.

Exp. 2, 3, 4. These 3 experiments embraced changes in intensity of light source and shade of background so that the light reflected from the surfaces was 0.1 to 0.2, 2.0, and 16.0 Weston Units respectively. Considering the findings from these experiments collectively it was observed that the most ventral melanin appeared on those fishes illuminated most intensely and long-

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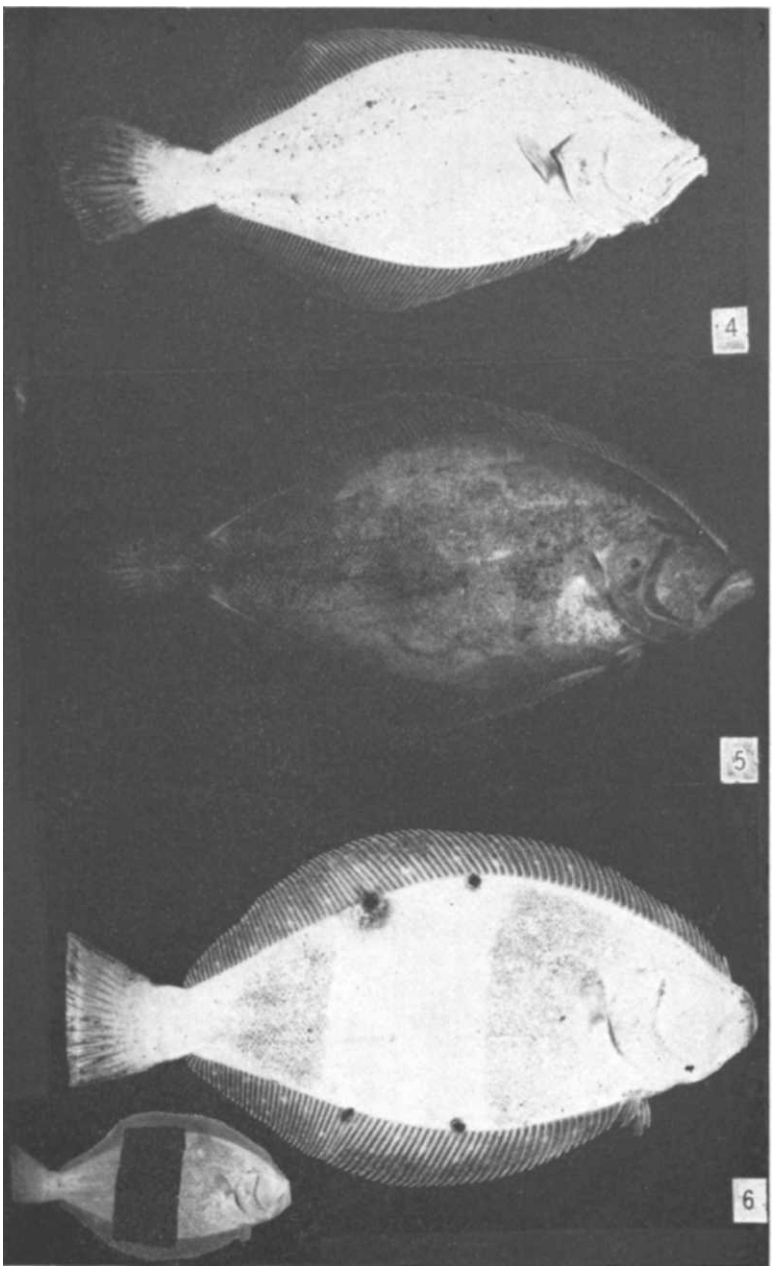

PLATE II.

All photographs are of the normally unpigmented ventral surface of living summer flounders. All inserts are further reductions of the corresponding animal.

FIG. 4. Summer flounder black-adapted 2½ days, blinded and placed in a brightly illuminated white tank (reflecting about 16 Weston units) for 28 days. A considerable increase in ventral melanophore production over that seen in the previous figures.

FIG. 5. Black-adapted 2 days, blinded and maintained in the special apparatus providing direct ventral illumination of high intensity (about 400 Weston units) for 26 days. First evidence of melanin formation was noted at about 90 hours. Note that the ventral surface has become quite completely darkened by an extensive covering of melanophores.

FIG. 6. This fish was maintained under the same conditions as described for Fig. 5. In addition an opaque mask was affixed to cover part of the ventral surface (see insert). This mask was kept in place during the entire sojourn of the animal in the ventral illumination apparatus (26 days). The mask was removed and the photograph taken at the end of that time. The area protected from the light is free from melanophores.

est. Fig. 3 and 4 illustrate typical cases.

Exp. 5. In this experiment intense light (400 Weston Units or 25X as much as was used in Exp. 4) was directed to the ventral surfaces of the fishes through glass bottomed tanks. This method provides two significant advantages. First, it is possible by using various lamps to subject the lower surface of the experimental animal to any intensity desired; and second, it is possible to measure with accuracy the amount of light falling directly upon the lower surface of the fish. Thus, illumination may be rigidly controlled.

Under such favorable conditions pigment formation proceeded rapidly. Frequently it was possible to detect some evidence of melanophore formation during the fourth day of illumination and after 4 weeks of such treatment the entire ventral surface was found to be generally darkened with an extensive growth of melanophores (Fig. 5).

Exp. 6. Success in blackening the ventral surface so completely with melanophores in a relatively short time suggested an experiment designed to determine whether the pigmentation response was due to the *direct* effect of illumination, assuming the internal environment to be optimum in all the experiments, or whether it was necessary only that light *be present* for the reaction to continue. It was reasoned, therefore, that if it were necessary for light to fall directly upon the cell (direct effect) areas covered by an opaque mask would remain unpigmented while exposed areas would develop melanophores as in Exp. 5. On the other hand if it was necessary only that a fish with optimum *internal* physiological conditions be generally illuminated one might expect that the pigmentation reaction would go on underneath the mask regardless.

Accordingly, flounders prepared as in Exp. 5 were further provided with opaque masks as described previously (Fig. 6—insert). They were illuminated ventrally (400 Weston Units) as before and simple, clean-cut results were obtained. The exposed surfaces became melaninated but the protected area remained as free from pigmentation as in the control fishes. This striking result seen in Fig. 6 seems to indicate clearly that light provides a direct stimulus to the particular cell destined to develop into a melanophore by producing melanin.

Discussion. It becomes clear from the data presented that light is not only a very significant factor in the experimental development of melanophores in a normally unpigmented area but also that there is a correlation between the amount of pigment grown, the intensity of the light and duration of the exposure. Data of a sufficiently quantitative nature to determine whether this relationship is direct over all ranges of light intensity are not available. It is probable, however, that a direct relationship between light intensity per unit time and degree of melanination would not hold in ranges of very high intensity. The physiological limit of the rate at which melanin could be formed would probably be reached before arriving at extremely high intensities of light.

The impression should not be gained from these experiments, however, that light is the most important single factor in the production of melanophores. Although it is obviously an essential factor (except perhaps in the case of regenerated scales) it must be emphasized that the condition inside the cell as evidenced by the degree of dispersion of melanin granules in the cells of normally pigmented areas must represent the darkening phase of the fish. The ineffectiveness of high intensities of light directed on the surface of a flounder while in a physiological condition not favoring darkening is strikingly shown in the case of a white-adapted fish where, instead of melanin production one witnesses an actual decrease in dark pigmentation due to melanophore degeneration.[12,13,22] The observation that in physiologically dark flounders regenerating scales develop melanophores even in the absence of illumination is further evidence that in certain instances light is not necessary for the growth of melanin in melanophores. The problem of the pigmentation of regenerating scales represents a special case which deserves more detailed investigation in the future.

Summary. 1. In addition to a favorable internal environment in the cell, light is necessary for the development of melanin in

melanophores of intact scales.

2. Light passing directly through glass bottomed tanks onto the lower surface of the flounders was the most effective in producing melanophores. This means of illumination delivered the most intense light to the ventral surface of the fish and could be most accurately measured.

3. The use of opaque masks indicates that light affects the cell directly to produce melanin.

4. The melanination of regenerating scales is apparently not dependent upon the presence of light. Investigation of this special problem is being continued.

16335

Technique for the Biomicroscopic Study of the Ovary and the Fallopian Tube.

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In order to explore the ovary microscopically *in vivo* a method is described, the first step of which is the eventration of the ovary, with or without the Fallopian tube, and its placing in a subcutaneous loggia. To do this, the animal (rabbit) is fastened on its back, with the posterior part of its body rotated 30° to 45° on the longitudinal axis. Ether or some other anesthetic is administered. Then the ovary, with or without the Fallopian tube, is reached via a discreet section of the abdominal wall, slightly under the dorso-lateral line projected by the course of the Fallopian tube, and behind the kidney. Then the great external obliquus muscle is pierced by means of a surgical knife or the tip of a scissors slightly above this first incision, and the broad ligament is gently clamped; without being touched, the ovary is thus eventrated through the small opening in the muscle, the aponeurosis of which serves as a bed. All suturing close to the ovary should be avoided and is not necessary, as the ovary remains in its new position by simply placing it perpendicularly to the incision (Fig. 1). When removing the ovary from the abdomen great care must be taken not to provoke any circulatory or nervous disturbances; this point is of the utmost importance as regards all further activity of the ovary. The wound in the skin should not coincide with the ovarian surface or with the wound in the muscle which forms the bottom of the subcutaneous loggia. A flap-like portion of skin should cover the ovary, or the Fallopian tube, or both, as the case may be. Neither the suture of the muscle nor that of the skin should be in contact with the organs in the subcutaneous loggia. Thus, the ovary, the tube, or both can be easily reached without causing trauma. The best material for study is that in which no adhesion between skin and ovary arises; this is usually prevented by the secretions of the transferred organs, as well as by the frequent opening of the loggia necessary for repeated macro and microscopic studies of the ovary and the Fallopian tube.

Visualization capsule. The first experiment performed tried to keep the ovary visible through a grafted cornea. This procedure was a failure.

The visualization capsule is made up of 4 parts: (a) an ebonite ring of varying diameter and height, threaded on the inside and having a flat perforated base which allows it to be sutured under the skin, and large enough to accommodate the ovary and the Fallopian tube; (b) a second ring which screws into the first to which is fastened an extremely fine, transparent and elastic, removable diaphragm; 2 small holes in the ring