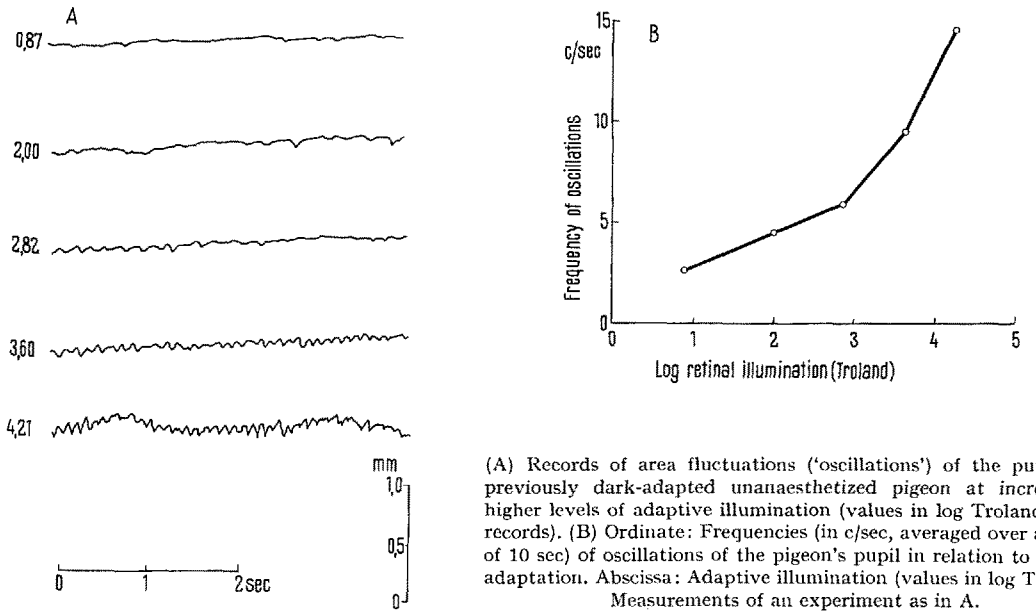

(A) Records of area fluctuations ('oscillations') of the pupil of a previously dark-adapted unanaesthetized pigeon at increasingly higher levels of adaptive illumination (values in log Troland beside records). (B) Ordinate: Frequencies (in c/sec, averaged over a period of 10 sec) of oscillations of the pigeon's pupil in relation to state of adaptation. Abscissa: Adaptive illumination (values in log Troland). Measurements of an experiment as in A.

pupillary diameter, reaching a change of up to 16% in retinal illumination (cf. Figure A, lowermost record). Changes like these may play a role in the visual process in the pigeon's eye with its high resolving power in time[10] and space[11], such as preventing the disappearance of a stabilized image.

Zusammenfassung. Die oszillatorischen Änderungen der Pupillenweite wacher Tauben wurden infrarot-reflektometrisch gemessen. Gleichzeitig mit einer Amplitudenzunahme steigt die Frequenz der Pupillenoszillationen von 1/sec bei einer adaptiven Beleuchtung unter 10 Troland bis auf 15/sec bei 1500 Troland.

E. Alexandridis

Abteilung für Experimentelle Ophthalmologie (II. Physiol. Abteilung) des W. G. Kerckhoff-Instituts der Max-Planck-Gesellschaft, Bad Nauheim (Germany), September 13, 1966.

[10] E. Dodt and A. Wirth, Acta physiol. scand. *30*, 80 (1953).
[11] G. L. Walls, The Cranbrook Institute of Science, Bloomfield Hills, Michigan (1942), 785 p.

STUDIORUM PROGRESSUS

Light Sensitivity of Melanophores in Neural Crest Explants

Changes in the state of contraction (concentration of pigment) or expansion (dispersion of pigment) of amphibian melanophores resulting from alterations in illumination have been studied frequently. In some cases, as in the tail melanophores of *Xenopus* larvae, the action of light is direct[1,2], while in others it presumably operates through indirect hormonal mechanisms. One body of information, recently discussed by Burgers et al.[3], reveals that color variation may depend upon the release of intermedin from the hypophysis. Another group of experiments[4–6] indicates that the blanching of amphibian larvae which are placed in darkness may result from the action of a pineal hormone. In order to ascertain whether these indirect hormonal effects are influenced by responses attributable to direct action of light on melanophores, the following experiments on isolated melanophores were performed.

Pieces of trunk neural fold from open neural plate stages of 3 anurans, *Xenopus laevis*, *Rana esculenta* and *R. pipiens*, and 2 urodeles, *Ambystoma mexicanum* (axolotl) and *Pleurodeles waltlii*, were excised and wrapped in sheets of ventral-lateral epidermis (with accompanying mesoderm) from the same embryo. *Xenopus* explants were made and cultured in Niu-Twitty solution, while for all the others, the initial operation was performed in full strength Holtfreter's solution followed by subsequent culture in 50% Holtfreter's solution with addition of an

[1] J. T. Bagnara, Proc. Soc. exp. Biol. Med. *94*, 572 (1957).
[2] B. van der Lek, J. de Heer, A. C. J. Burgers, and G. J. van Oordt, Acta physiol. pharmac. néerl. *7*, 169 (1958).
[3] A. C. J. Burgers, K. Imai, and G. J. van Oordt, Gen. comp. Endocr. *3*, 53 (1963).
[4] J. T. Bagnara, Science *132*, 1481 (1960).
[5] J. T. Bagnara, Gen. comp. Endocr. *3*, 86 (1963).
[6] J. T. Bagnara, Prog. Brain Res. *10*, 490 (1965).

antibiotic, Elcosine (CIBA), at a concentration of 5 ml/l. All experiments and culture were performed at room temperature (20–22 °C). A total of 67 well-formed explants were obtained for tests of the effects of light and darkness. Tests were performed by placing explants in a light-tight box for various lengths of time and the relative state of melanophore expansion or contraction was obtained from observations made with a dissecting microscope. Tests were repeated on the same explants several times during the period of culture which extended to 3 weeks in some cases.

Melanophore differentiation occurred in about 5 days on explants of all species except *Xenopus*, where it required only 3 days. In axolotl explants practically every melanophore was contracted, while in *P. waltlii*, *R. pipiens*, and *R. esculenta* the majority of melanophores were expanded. *Xenopus* explants were more variable; however, the majority had expanded melanophores. The action of light on the state of melanophore expansion or contraction is summarized in Table. Melanophores of the 2 urodele species, *A. mexicanum* and *P. waltlii*, were unaffected by darkness and always retained their original state. Melanophores on explants of all 3 anurans, however, were stimulated to contract markedly by darkness. After 15 min, only slight melanophore contraction was observed; however, after 30 min in darkness, a more prominent response was visible. Explants in darkness for 45 min displayed fully contracted melanophores. A typical response to darkness is illustrated in Figures 1 and 2, which demonstrate the degree of melanophore contraction induced in a *Xenopus* explant maintained in darkness for 45 min.

It seems that for all 3 responsive species, a darkness stimulation of more than $^1/_2$ h is necessary to evoke com-

State of melanophores on neural crest explants in darkness and in light

Explant species	State of melanophores in light	State of melanophores in darkness for 15 min	30 min	45 min	Time necessary for fully expanded melanophores to re-expand[a]
A. mexicanum (axolotl)	contracted: only a few expanded	no change	no change	no change	–
R. waltlii	almost all expanded	no change	no change	no change	–
R. pipiens	almost all expanded	little or no change	partial contraction	full contraction	6–12 min
R. esculenta	almost all expanded	little or no change	partial contraction	full contraction	8–10 min
Xenopus laevis	full expansion on most explants	slight contraction	partial contraction	full contraction	10–15 min

[a] Melanophores contracted from 1–3 h exposures to darkness took no longer to re-expand than those in darkness for only 45 min.

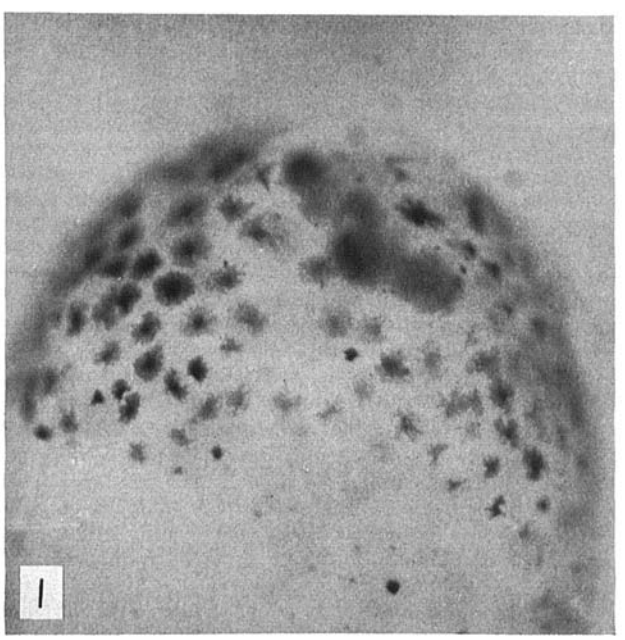

Fig. 1. Expanded melanophores on neural crest explant of *Xenopus* which was kept under normal room illumination. × 150.

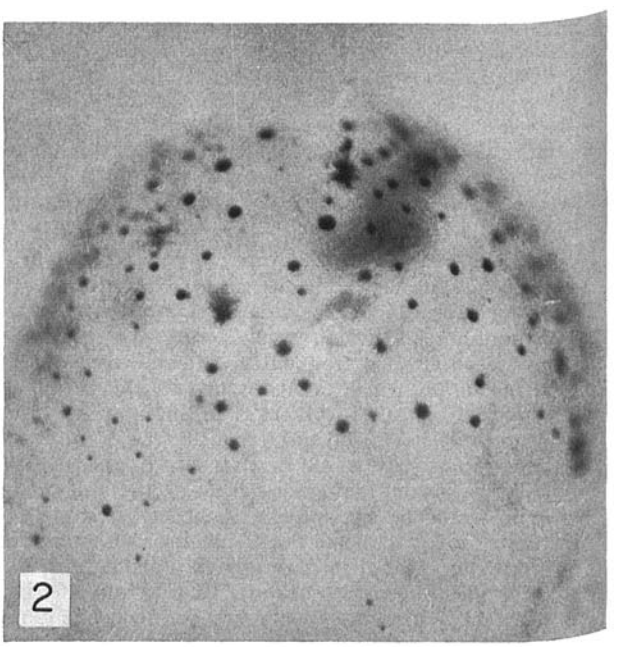

Fig. 2. Contracted melanophores on same explant which was kept in darkness for 45 min. × 150.

plete contraction. In obvious contrast to this, is the relatively short period of time required for re-expansion to occur after the explants are returned to normal room illumination. Re-expansion occurred in as early as 6 min in explants of *R. pipiens*, while in those of *R. esculenta* and *Xenopus* it took slightly longer. This relatively short period for recovery appeared to be fairly constant regardless of the length of duration of the inducing dark period.

Altogether, these data indicate that melanophores have an intrinsic sensitivity to light which enables them to respond according to their state of illumination. Temporal factors involved in expansion or contraction may provide some notion as to the mechanism of the response. It seems likely that the relatively slow melanophore contraction of explants in the dark is due to the gradual build-up of a photolabile melanophore contracting agent which evokes this response when a sufficient level is reached. When illumination is restored, the substance is quickly destroyed or modified, resulting in relatively rapid re-expansion of melanophores. Such a proposed mechanism is similar to that which has been advanced to explain the tail-darkening reaction in *Xenopus*[1,2]. It is beyond the scope of the present data to attempt to analyze the mechanism of this response; however, it should be emphasized that melanophores in neural crest explants of these 3 anuran species are light sensitive and that they can respond to this photic stimulus by concentrating their melanin. Although *P. waltlii* and axolotl did not exhibit a similar response, OBIKA[7] has since shown similar light sensitivity by melanophores in 2 explants of the urodele, *Triturus pyrrhogaster*. With respect to the physiology of melanophore control in the living amphibian, the implications of this intrinsic sensitivity of melanophores to light may be far-reaching. It seems important that one should be aware of the possible involvement of this response in pigmentary experiments which might involve changes in illumination. Of specific concern in this context is the possible super-imposition of this mechanism on the body-blanching reaction of amphibian larvae. Obviously the intrinsic mechanism is not the primary one in bringing about the blanching reaction, for the direct response to light occurs very slowly and disappears rapidly, while temporal events for the body-blanching reaction occur in the opposite manner, requiring just a few minutes for onset and about an hour for disappearance[4]. Moreover, salamander larvae display a perfectly good blanching reaction, while these same salamander melanophores in explants do not exhibit an intrinsic sensitivity to light. However, in anuran larvae at least, it seems altogether reasonable to consider that while melanophore contraction induced by the direct effect of light may not operate as the primary stimulus of the body-blanching reaction, it may function to supplement melanophore contraction later on. This is a factor which should be taken into consideration in the evaluation of a blanching reaction induced by long exposures to darkness or in the interpretation of melanophore responses in general, where a period of exposure to darkness is involved. Just as one must take into account this in vitro mechanism in interpreting in vivo results, it must be remembered that melanophores in explants are a special situation. There is no evidence to prove that melanophores in the skin of larvae are also light sensitive and, indeed, that they can contract as a result of this sensitivity. It should be pointed out, however, that generally, melanophores in culture situations respond to many of the same stimuli as do melanophores in vivo[8]. These observations, together with the results of KULEMANN[9], that *Xenopus* melanophores in hanging drop cultures are light sensitive, argue the case that melanophores have an intrinsic mechanism of response to light. The biological significance of this response is an enigma, as is the mechanism involved[10].

Résumé. Les mélanophores de la crête neurale de divers amphibiens isolés in vitro se sont montrés sensibles à la lumière. A la lumière normale, ils s'étendent, mais se contractent rapidement dans l'obscurité. Cette sensibilité intrinsèque pourrait renforcer la réaction qui produit la décoloration du corps chez les larves, mais n'en est pas la cause principale.

J. T. BAGNARA and M. OBIKA[11]

Department of Zoology, University of Arizona, Tucson (Arizona 85721, USA), September 5, 1966.

[7] M. OBIKA, unpublished results.
[8] R. R. NOVALES, Ann. N.Y. Acad. Sci. *100*, 1035 (1963).
[9] H. KULEMANN, Zool. Jb. *69*, 169 (1960).
[10] The first author is grateful for the privilege of carrying out the experiments on *Rana esculenta* as a Fulbright Research Scholar in the Laboratory of Prof. L. GALLIEN, Laboratoire d'Embryologie, Faculté des Sciences, Université de Paris. Thanks are expressed to Dr. CHRISTINA M. RICHARDS for her help on the *R. pipiens* experiments and to NSF for support of part of this work.
[11] Present address: Biological Laboratory, Keio University, Yokohama-Hiyoshi (Japan).

PRO EXPERIMENTIS

A Template for the Rapid Measurement of Rf-Values in Thin-Layer Chromatography

In the course of an extensive chromatographic screening of urinary extracts, the need arose for the rapid measurement of Rf-values on thin-layer chromatograms. A template was therefore designed and constructed allowing the direct measurement of Rf-values in a quick and very convenient way. This note will describe the features of this device.

The template is made of translucent plastic material and consists of 2 parts as shown in Figure 1. The base plate A serves to cover up the thin-layer plate to be examined and to support part B. Part B is inserted into a groove on part A by means of a prominent rim on one of its sides, and it can thus be slid from one side to the other. To minimize friction and to prevent scratching of the clear surfaces, 2 narrow pieces of tape may be fixed onto part A giving part B a 2-point support. A simple measuring grid, as shown in Figure 2, was drawn onto part B using a special lacquer for plastic surfaces. The grid represents a sliding scale model with each of the lines corresponding to a particular Rf-value over the whole range of possible solvent fronts on thinlayer chromatograms.