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.

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[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.
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## 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.

In actual use, the combined parts of the template are put on top of a thin-layer plate. The base line of the diagram on part B is aligned with the starting line of the chromatogram, and part B is then moved to either side so as to have the Rf-line corresponding to a value of 1.0 to intercept the solvent front exactly perpendicular to a particular spot or series of spots whose Rf-values are to be measured. This alignment is facilitated by the vertical lines which are drawn onto part A, 2 cm apart from each other. The actual Rf-values can now be read off directly, without need of further calculation, by taking the Rf-equivalent of the line crossing the centre of a spot. Intermediate values could be estimated with ease and accuracy; according to available facilities, however, additional thin lines for the second decimals may be interposed. The Rf-lines on the template cover travel distances of solvents of up to 18 cm; this range will suffice for all practical purposes.

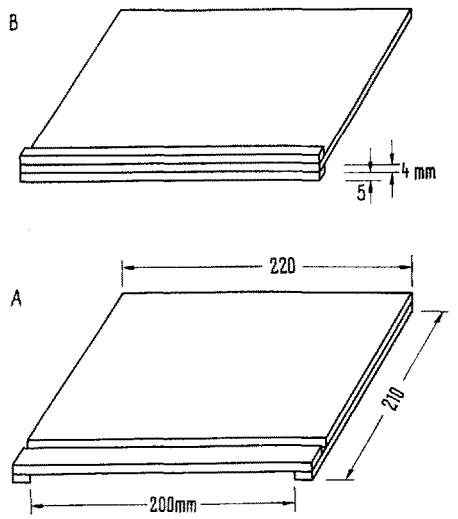


Fig. 1. Parts A and B of the Rf-measuring template.

The principle of this template is similar to that of other devices recommended for use in paper chromatography[1,2]. The overall design of the template is adapted to the particular requirements of thin-layer plates, as the coating is well protected by the base plate and adjustments to variations of origins of the spots and solvent fronts on individual plates can be made very easily and rapidly. Among a number of possible ways of documenting thin-layer data, the measurement of Rf-values is an economical and most commonly used method. However, an appreciable time factor may be involved when a large number of chromatograms is to be dealt with. The template described proved to be a valuable and time-saving aid in this respect.

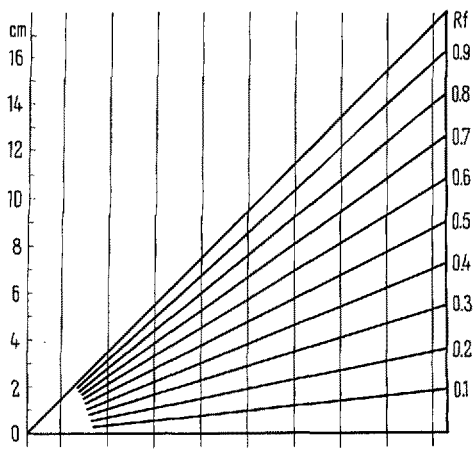


Fig. 2.

*Zusammenfassung.* Eine Schablone zur direkten Messung von Rf-Werten auf Dünnschichtplatten wird beschrieben, deren Ausführung den Besonderheiten der Dünnschichtchromatographie angepasst ist. Mit Hilfe dieser Schablone konnte die Auswertung von Chromatogrammen vereinfacht und wesentlich beschleunigt werden.

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*October 25, 1966.*

[1] D. M. P. PHILLIPS, Nature *162*, 29 (1948).
[2] L. B. ROCKLAND and M. S. DUNN, Science *111*, 332 (1950).

## Stereotaxic Localization of Amygdaloid Nuclei in Rats from Weaning to Adulthood[1]

The function of the limbic system is of considerable interest to neurophysiologists, and one approach to the study of this system is through electrolytic destruction of selected areas. Although the excellent atlas of DE GROOT[2] provides the coordinates for the localization of forebrain structures in adult rats (200–300 g body weight), there is no consideration of the coordinates for localizing such structures in younger and lighter animals. The present study was performed to determine workable coordinates for the localization of amygdaloid nuclei in rats from 54–236 g body weight and 24–80 days of age, respectively.

Female Holtzman rats were received at the age of 22 days and accommodated in single cages; Lab Chow and tap water were available ad libitum. At the ages of 24, 34, 45 and 80 days, respectively, 5 rats each were anesthetized with ether and placed in a stereotaxic instrument

[1] This investigation was supported by U.S.P.H.S. Grant No. HE 06975 of the National Heart Institute.
[2] J. DE GROOT, Verh. K. ned. Akad. Wet. *52*, 4 (1959).