nucleolo primario, che rimane piuttosto distanziato dalla membrana nucleare, si manifesta sempre rossofilo e privo di elementi nucleolonemali (Fig. 2), gli anfinucleoli, che contro la membrana nucleare vanno infine ad aderire strettamente, si dimostrano azzurrofili e con elementi nucleolonemali più o meno complessi (Fig. 2). Ulteriori esami citochimici[9] hanno permesso di individuare ancora nel nucleolo primario un complesso ribonucleoproteico con gruppi sulfidrilici, e negli anfinucleoli un complesso lipoglicoproteico con gli stessi gruppi. Entrambi i complessi sembrano regolati da una attività fosfatasica. Per i granuli nucleolonemali si è rinvenuto un complesso molto generico di glicoproteine, complesso nel quale, oltre ad essere assente l'acido ribonucleico, sembrano mancanti anche i lipidi, i gruppi sulfidrilici e l'attività fosfatasica. Tali diversità di costituzione chimica trovano il loro più convincente fondamento nei caratteri ultrastrutturali rilevati al microscopio elettronico[10,11].

I dati ricavati specialmente dagli ovociti di *Patella*, portano ad individuare nel nucleolo almeno due funzionalità principali, delle quali una legata essenzialmente alle ribonucleoproteine, e l'altra legata a sostanze non ancora bene identificate, ma comunque connesse con la presenza degli elementi nucleolonemali. Le manifestazioni relative alla seconda funzionalità si riscontrano con frequenza molto notevole tanto negli ovociti in accrescimento, che nelle cellule dei tumori, cioè dove si hanno le forme di metabolismo più esaltate. Non è improbabile che l'accostamento fra le attività nucleolari degli ovociti e quelle dei tumori possa portare in seguito ad una delucidazione sugli intimi meccanismi fisico-chimici intracellulari, che costituiscono il fondamento dell'insorgenza e del decorso delle cellule degli stessi tumori. Le nuove prospettive aperte dalla citofisicochimica, integrata dai dati forniti dalla microscopia elettronica, potranno essere di giovamento in tal senso.

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Summary

On the basis of research carried out on various animal and plant tissues, both normal and pathologic, the author reaches the conclusion that two chief functions can be attributed to the nucleolus. One of them is related to the ribonucleoproteins, the other to less well known substances which are probably connected with nucleolonemal elements. It is also suggested that the second function seems to be a characteristic of cells with high metabolic activities, such as growing oocytes and tumor cells.

Maintenance of Visual Cells in vitro[1]

The chemical reaction of the rhodopsin cycle has mainly been analysed by means of a rhodopsin solution which is extractable with detergents, particularly digitonin, from the whole retina or from the isolated outer segments of the visual cells[2,3]. Recently, ARDEN[4] measured the absorption spectrum of visual pigment in suspensions of intact rod outer segments from the frog's retina. In such a suspension the shifts of the absorption maximum toward the longer wave length compared to that of the extracted visual pigment was observed by BARER and SIDMAN[5], WALD and BROWN[6], and by SIDMAN[7]. It is of interest to study the biochemical changes of the visual pigments of a single living photoreceptor cell following stimulation. This paper is concerned with a preliminary observation on the maintenance of single visual cells in tissue culture.

Retinae excised from dark-adapted frogs, *Rana pipiens*, were cut into small pieces on a cover glass which served as the bottom wall of a Rose chamber in order to keep all fragments of outer segments of the visual cells. The explants were cultivated under dialysis membranes in Rose chambers[8]. The fluid nutrient used was composed of 50% amphibian Tyrode, 45% human ascitic fluid, and of 5% chick embryonic extract, reinforced with glucose at a final concentration of 300 mg%. These Rose chambers were kept in darkness at room temperature.

A large number of the rod outer segments with or without ellipsoids, and single visual cells equipped with both inner and outer segments and a nucleus were also studied under cellophane covering the entire culture area. Some of the isolated rod outer segments were found to lose their cylindrical structure within about 1 h after they were set up in Rose chambers. During the first 18 h the complete outer segments were present and showed refractivity and their usual smooth sheath. After the 2nd day most of the outer segments began to rupture. Such a morphological change of the rod outer segments might be caused by damage to their rod sheaths during the course of cutting the retinae. After the 7th day it was observed that most of the outer segments, which had undergone degeneration, assumed round or oval shapes, and others were transformed into elongated structures, as indicated in Figure a and b. On the other hand, even after the 20th day, the refractivity and smooth sheath of the outer segments were well maintained in isolated single photoreceptor cells as well as in that of the retinal explants which kept their complete structure even after being cultured for one month. Epithelial outgrowth from explants was observed after 14 days of cultivation. However, the rate of outgrowth was not as high as that observed in embryonic chick retinae.

The presence of rhodopsin as indicated by the red tint was observed both in the isolated photoreceptor cell as well as in the cell clusters of explanted retinal fragments. Rhodopsin regenerated readily when Rose chambers were kept in darkness. The color intensity of rhodopsin, however, decreased after explantation and was weaker than in freshly excised retinae from dark-adapted frogs. No indication of the presence of rhodopsin was observed in the elongated outer segments, while it was demonstrable in the rounded outer segments after the 14th day. Autofluorescence indicating the presence of vitamin A was noticed in the rounded outer segments in which rhodopsin was diminished. When the Rose chambers were kept in the refrigerator, both the structure and rhodopsin in the outer segments of photoreceptor cells were preserved in contrast to those kept at room temperature.

It might be possible to study the mechanism of the rhodopsin cycle in a single photoreceptor cell and to in-

[1] Aided by a grant from the National Society for the Prevention of Blindness.

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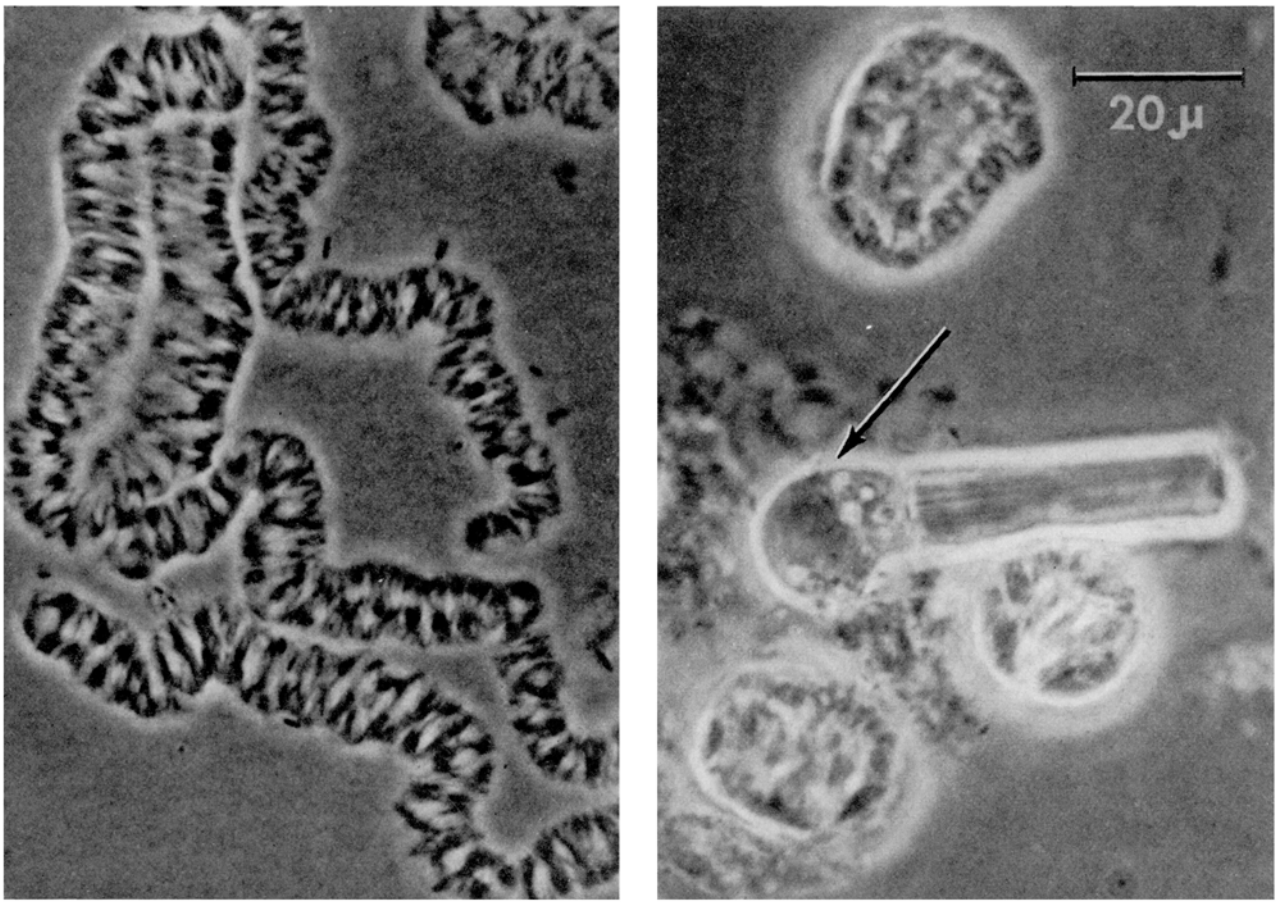

a b

a and b. Photomicrographs showing the rod outer segments from a frog's retina.

a) Elongated rod outer segments indicating the band-like structure which resulted from the loose separation of discs in the rod outer segments. b) Round-shaped rod outer segments and a single rod cell in which a smooth sheath of the outer segment and a nucleus (indicated by an arrow) are seen. The length of the inner segments of the single rod cell appeared to be relatively short. Photomicrographs were taken from a 16-day culture.

vestigate whether there are action potentials in relation to light stimuli. Further microspectrophotometric measurements on visual pigments in the single photoreceptor cell are being carried out.

Acknowledgement. The author acknowledges his sincere gratitude to Professor C. M. POMERAT for valuable suggestions and encouragement.

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Résumé

Des fragments de rétine et des cellules visuelles isolées de la grenouille *Rana pipiens* ont été cultivés dans la chambre de Rose. Bien qu'une dégénérescence morphologique des segments extérieurs des cellules visuelles ait été observée pendant la culture, le pouvoir de réfraction normal et la présence du pigment visuel ont été constatés dans ces segments, les segments intérieurs avec leurs noyaux étant conservés. Mais, l'intensité du pigment visuel a été plus faible que dans la rétine fraîche. Ces résultats montrent que la cellule individuelle du photorécepteur se prête à l'étude physiologique *in vitro*.

Genetic Nature of Self-Incompatibility in Annual Chrysanthemum

Studies on the genetics of self-incompatibility in various plants have proved to be of considerable interest for several aspects of gene action. An important discovery in recent years has been that in some members of the family compositae, the imcompatibility alleles show a sporophytic determination of their behaviour in one parent and gametophytic in the other[1,2]. Additional evidence suggesting that the incompatibility genes in this family do not conform to an easily predictable system with regard to their dominance relationships, has been presented by CROWE[3] on the basis of her observations on *Cosmos*. In view of these findings, it is of interest to undertake further investigations on the breeding systems of other members of this group. In the present brief report, evidence relating to the genetic nature of self-incompatibility in *Chrysanthemum carinatum* ($2n = 18$) is presented.

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