rungen an regenerativ gebildeten Keimen zu unterscheiden.

Sicher beruht auch die Verschiebung des Verhältnisses zwischen Syncotylen und Pleiocotylen zugunsten der Syncotylen bei den mit schwächer konzentrierten Lösungen oder zu einem späteren Zeitpunkt behandelten Embryonen, wenigstens zum Teil, auf dem Wegfall der nur durch intensivere Einwirkungen zustandekommenden, hochgradig pleiocotylen Regeneratembryonen.

B. HACCIUS

Aus dem Botanischen Institut der Universität Mainz, den 3. August 1954.

Summary

Experiments on influencing cotyledon formation in embryos of *Eranthis hiemalis* are reported. By treating the seeds with 100–2000 ppm solutions of 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, α-naphthylacetic acid and even isopropyl N-phenyl carbamate one can obtain significant increases in twinning and in cotyledon anomalies (syncotyly and pleiocotyly of all degrees). Embryos can be successfully treated until approximately 2 weeks after initial opening of the follicles. X-radiations achieve the same effect, with doses of 2000–4000 *r* corresponding to the treatments with 1000–2000 ppm 2,4-D-solutions. By using weaker concentrations and with sudden temperature changes one can likewise raise the percentage of syn-, tri- and tetracotyls, but pleiocotyls of the higher degrees (5 and more cotyledons) appear only when more intensive methods of treatment are employed. These forms which exceed the normal range of variability arise as bud-like embryo regenerates on the as yet undifferentiated original embryo.

Bordure superficielle de pseudopodes au niveau des cellules mésothéliales du revêtement peritonéal chez les mammifères

L'étude au microscope optique du revêtement peritonéal des Mammifères montre que, souvent, la limite extérieure des cellules mésothéliales est marquée par une ligne particulièrement épaisse, quelquefois même par un mince plateau.

Au cours de recherches expérimentales sur l'absorption de la silice injectée dans le péritoine chez le Rat, nous avons pu étudier au microscope électronique les caractères de ce revêtement mésothélial.

Il supporte de fins pseudopodes grêles, larges d'environ 400 à 450 Å, longs de 0,6 à 0,8 μ. Ils sont peu serrés, avec des espacements allant de 0,1 à 0,3 μ. Le contenu de ces pseudopodes apparaît homogène, non limité par une membrane perceptible.

Les pseudopodes ne sont pas disposés en couche régulière, mais par touffes, avec un certain fusionnement à leur base. Ils se distinguent par là des éléments constituant les bordures en brosse du rein.

Ils ressemblent par contre aux fins pseudopodes décrits à la surface de certaines cellules: cellules endothéliales[1], cellules alvéolaires du poumon[2], cellules épithéliales de la trachée entre les cils vibratiles[3].

[1] W. BERNHARDT, Communication au Congrès international d'Hématologie, Paris, 1954 (sous presse).

[2] A. POLICARD, A. COLLET et L. GILTAIRE-RALYTE, C. r. Acad. Sci. *239*, 573 (1954).

[3] G. BLOOM et H. ENGSTRÖM, Ann. Otol. Rhin. Laryng. *62*, 15 (1953).

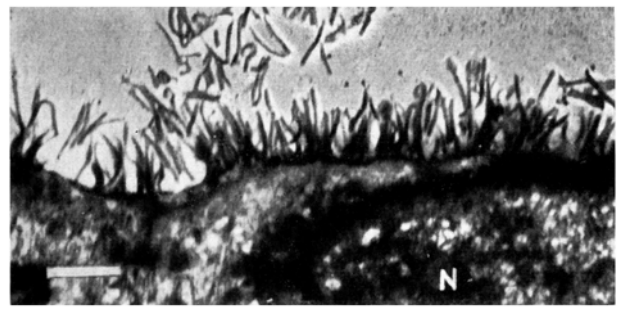

Fig. 1. Disposition des pseudopodes à la surface de la cellule mésothéliale. *N* partie supérieure du noyau. Grossissement: 12000.

Il semble plausible de rattacher ces formations aux processus d'absorption très développés au niveau du revêtement péritonéal.

A. POLICARD, A. COLLET et L. GILTAIRE-RALYTE

Centre d'études et recherches des charbonnages de France, Paris et Verneuil, Oise, le 6 décembre 1954.

Summary

The surface of the mesothelial peritoneal cells (Rat) show with electron microscope very thin pseudopods, 0·6–0·8 μ by 0·04–0·045 μ, separated by 0·1–0·3 μ spacings, and different from vibratile cilia, but similar to the submicroscopic pseudopods of alveolar or endothelial cells.

Mitotic Frequency in Normal and Regenerating Tail Tips of *Rana pipiens* Embryos[1]

The tail tip of the Amphibian embryo or larva has proved to be a most convenient material for chromosome studies, and has been used extensively for this purpose first by FANKHAUSER[2], later by COSTELLO and HENLEY[3], and others. Usually these studies are done on whole mounts of tail tips, which of necessity are amputated, at the earliest, during later embryonic stages when the fin becomes thin and transparent. Recently, however, it has been shown that squash techniques also give good results when applied to tail tips (TING[4]). This means that it is possible to get satisfactory preparations of tail tips at any stage of development, and poses the problem of selecting the stage (*s*) which will give the largest number of mitotic figures for study. Primarily in order to solve this practical problem we have measured mitotic frequencies in smears of tail tips taken from normal frog embryos at stages ranging from SHUMWAY[5] stage 18 (4 mm embryo) to stage 25 + (young tadpole). A comparable series of measurements was done on tail tips which were regenerating following amputation at stage 18 or stage 19.

Materials and Methods.–Fertilized eggs were produced in the laboratory according to the usual technique

[1] Aided by an Institutional Grant from the American Cancer Society.

[2] G. FANKHAUSER, Proc. Amer. Phil. Soc. *79*, 715 (1938); Quart. Rev. Biol. *20*, 20 (1945).

[3] D. P. COSTELLO and C. HENLEY, Proc. Amer. Phil. Soc. *93*, 428 (1949). – C. HENLEY and D. P. COSTELLO, J. Morph. *89*, 91 (1951).

[4] H. P. TING, Stain Techn. *25*, 127 (1950).

[5] W. SHUMWAY, Anat. Rec. *78*, 139 (1940).

(RUGH[1]) and were kept throughout development in spring water at 18–20°C. At stages 18 and 19 some of the embryos were set aside for tail amputation and regeneration. They were anaesthetized by exposure to M.S. 222 (ca. 0·037%) for 2–3 min; i.e. just long enough to produce immobilization[2]. Approximately three-fourths of the tail was cut off, after which the embryos developed normally and within 2 to 3 days regenerated normal tails.

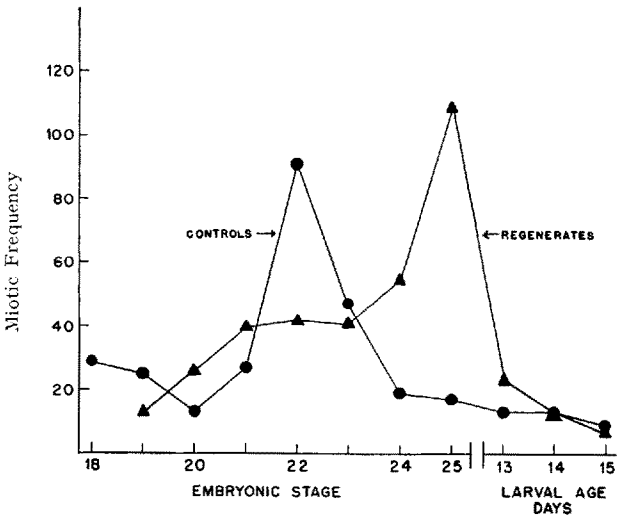

Fig. 1.–Mitotic frequency represents the mean number of mitoses per 1,000 cells.

Tail tip squashes were prepared at daily intervals starting at stage 18 for the normal (nonregenerating) series, and at stage 19 or 20 for the regenerates. The tips, approximately 3 mm in length, were first fixed for 15 min in Carnoy's fluid and were then transferred to acetic-orcein (LACOUR[3]) for 2 h. (Our stain was prepared by refluxing a 2% solution of orcein in 60% acetic acid for 1 h. The solution was filtered after cooling to room temperature.) Following the staining period the tips were squashed, stored in the alcohol vapor bath overnight, and finally mounted in diaphane. The mounting was done without removing the coverslip, simply by applying diaphane to 3 edges of the coverslip. As the alcohol evaporated the diaphane was drawn under the coverslip, giving a permanent mount.

In order to determine mitotic frequencies the squashes were observed at a magnification of about 660 diameters, the field under observation being delimited by means of an ocular grid. Within this field we counted (a) the number of mitotic nuclei (nuclei in mitotic phases ranging from mid-prophase to telophase), and (b) the total number of nuclei. This was repeated on a series of fields in a given preparation until a total of 1000 nuclei had been counted.

Results and Discussion.–The results of our determinations of mitotic frequencies are summarized in the Table and the Figure. They show that in the case of the normal (nonregenerating) tail tip the mitotic frequency is at first low, then rises to a sharp peak at stage 22, and subsequently sinks again to a low level. When the tail is amputated at stage 18 the result is quite different. The mitotic frequency of the regenerate increases slowly during stages 19 to 24, displaying a plateau at stages 21 to 23. Then at stage 25 mitotic frequency increases suddenly, and subsequently is reduced again to a low level. Amputation at stage 19 also results in a plateau at stages 21 to 23 and displaces the frequency peak from stage 22 to stage 25 (see Table).

Stage	Age (days)	Number of mitoses per 1000 cells		
		Normal (non-regenerating) $N = 5$	Regenerates (amputation at stage 18) $N = 8$	Regenerates (amputation at stage 19) $N = 2$
18	4	*29* (21–35)		
19	5	*25* (15–32)	*13* (10–17)	
20	6	*13* (8–23)	*26* (17–36)	*23* (21–24)
21	7	*27* (19–37)	*40* (32–45)	*42* (40–44)
22	9–	*91* (84–101)	*42* (33–49)	*43* (42–44)
23	9+	*47* (41–55)	*41* (35–47)	*52* (50–54)
24	10	*19* (14–20)	*55* (51–59)	*63* (62–63)
25	12	*17* (13–20)	*109* (95–120)	*78* (77–78)
25+	13	*13* (12–15)	*23* (21–25)	*69* (68–70)
25+	14	*13* (9–16)	*13* (9–18)	*44* (41–46)
25+	15	*9* (8–10)	*7* (5–11); $N = 7$	*25* (23–26)

The *italic* values give the mean number of mitoses per 1,000 cells; the values in parentheses give the range. N = number of tail tips upon which each mean is based.

Another experiment, not included in the Table, gave the same type of result as that described in the preceding paragraph. In this experiment tail tips of approximately uniform size were squashed at all stages from 18 to 25 (normal embryos, 3 to 5 per stage); and from 19 or 20 to 25 (regenerates, 2 to 4 per stage). In each squash we counted all the mitoses, but did not count the total number of nuclei. In the case of the normal embryos the mean number of mitoses per squash increased from 1 at stage 18 to 123 at stage 22, and then decreased to 26 at stage 25. In the regenerates the number of mitoses increased gradually from 1 at stage 19 to 43 at stage 24, with a plateau at stages 21 to 23. Then at stage 25 the number of mitoses increased suddenly to 134. Thus, in this experiment as well as in the one previously described we find for the normal embryos a distinct peak in number of mitoses at stage 22, and for the regenerates a displacement of this peak to stage 25.

We should now consider briefly the question of how to account for these changes in mitotic frequency. The most obvious factors that might affect mitotic activity are (a) the development of the tail fin circulation, and (b) changes in the yolk supply. At first sight it seems reasonable to attribute the mitotic frequency peak found in normal embryos at stage 22 to the fact that tail fin circulation is established at this time, and the subsequent decline in mitotic activity to the diminution in the

[1] R. RUGH, *Experimental Embryology* (Burgess Publishing Co., Minneapolis, Minn., 1948), p. 102.

[2] Although the embryos were exposed to M.S. 222 for only a very brief period, and developed normally thereafter, it is still necessary to consider the possibility of there being effects of the chemical on mitosis. According to D. P. COSTELLO and C. HENLEY [Proc. Amer. Phil. Soc. *93*, 428 (1949)], M.S. 222 used as an anaesthetic in a concentration of 1:2000 (0·05%) has no noticeable effect on mitosis in regenerating tail tips of Triturus torosus larvae. Also, in this laboratory the anaesthetic has been used on young tadpoles without detectable effect on the mitotic mechanism during subsequent regeneration. Finally, it may be noted from data presented in this paper that the mitotic frequency peak in regenerating tips occurs at stage 25, whether the anaesthesia and amputation occurred at stage 18 or at stage 19. If there were a significant inhibiting effect of the chemical on mitosis one would expect embryos exposed to it at stage 19 to exhibit the peak at a later stage than those exposed at stage 18. Considering these points, we think it unlikely that the M.S. 222, as used in these experiments, has significant effects on mitosis.

[3] L. LACOUR, Stain Techn. *16*, 169 (1941).

yolk supply during the later embryonic stages. However, observations which we have made on tail tip whole mounts indicate (a) that the tail tip circulation is established in both the normal and the regenerating tail at about the same time (stage 22); and (b) that the subsequent rate of utilization of yolk is also about the same in the normal and regenerating tail tips. Thus, if these factors are affecting mitotic activity in the normal tail, the effects are easily superseded by other and unknown influences which are operating in the course of regeneration in such a way as to eliminate the mitotic peak at stage 22 and to stimulate mitotic activity considerably later on, at stage 25.

Acknowledgement.–The author wishes to thank Dr. ROBERT W. BRIGGS for his guidance throughout the course of this investigation.

MARIE DIBERARDINO

Institute for Cancer Research and the Lankenau Hospital Research Institute, Philadelphia 11, Pa., November 19, 1954.

Résumé

L'auteur détermine la fréquence mitotique au cours du développement normal et de la régénération caudale chez *Rana pipiens*. Les observations s'étendent du stade 18 (embryon de 4 mm) au stade 25 (jeune têtard). Les numérations sont faites sur des préparations par écrasement de l'extrémité de la queue.

Développement normal: la fréquence est tout d'abord basse (18–21) puis atteint son maximum au stade 22 et s'abaisse ensuite jusqu'à un faible niveau (23–25).

Régénération: si la queue a été amputée au stade 18, la fréquence mitotique augmente lentement du stade 19 au stade 24, la courbe étant horizontale du stade 21 au stade 23. Au stade 25, la fréquence augmente subitement et retombe ensuite. L'amputation au stade 19 déplace aussi la fréquence maximum du stade 22 au stade 25.

Die Regeneration heteroplastischer chimärischer Extremitäten bei *Triturus vulgaris* und *Triturus cristatus*[1]

Zur Klärung der Frage nach der Herkunft und den Potenzen des Blastemmaterials bei der Extremitätenregeneration der Urodelen wurden heteroplastische chimärische Extremitäten zwischen *Tr. vulgaris* und *Tr. cristatus* hergestellt und später durch Amputation Regeneration ausgelöst (Abb. 1).

Dazu erfolgte im frühen Schwanzknospenstadium (Harr. 27, 28) ein Austausch ganzer Extremitätenanlagen zwischen den beiden Arten[2]. Zu diesem Zeitpunkt ist das Ganglienleistenmaterial noch nicht in die Extremitätenanlage eingewandert. Man erhält also Extremitäten, deren mesodermaler und ektodermaler Anteil von der Spenderart stammen, deren Corium einschliesslich Pigmentzellen jedoch aus dem Wirt eingewandert ist[3]. Form und Grösse dieser Extremitäten sind spenderartgemäss, die Ausbildung des Pigmentmusters während der Metamorphose aber erfolgt wirtsgemäss. *Cristatus*-Extremitäten auf *Tr. vulgaris* färben sich wie der Wirt bräunlichgelb und besitzen wie dieser nur wenige schwarze Pigmentflecken. In den *vulgaris*-Extremitäten auf *Tr. cristatus* bildet sich ihrem Träger entsprechend eine dichte Schicht schwarzen Pigmentes (Abb. 2). Aus dem Regenerationsverlauf solcher Extremitäten ist deutlich ersichtlich, welchen Anteil die beiden artverschiedenen Komponenten im Stumpf der alten Extremität am Aufbau des Regenerates haben, und zu welchen Leistungen sie befähigt sind.

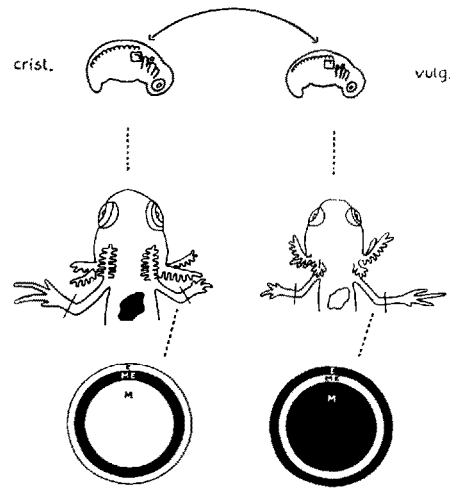

Abb. 1. Schematische Darstellung der beiden Operationsphasen und des Aufbaues der chimärischen Extremitäten vor und nach der Regeneration (*E* = Ektoderm, *ME* = Mesektoderm, *M* = Mesoderm).

Durch Voruntersuchungen wurde festgestellt, dass der Regenerationsvorgang in der Larvenzeit bei beiden Arten morphologisch der embryonalen Extremitätenentwicklung[1] entspricht. Die durch heteroplastische Transplantation entstandenen Extremitäten verhalten sich hinsichtlich Regenerationsgeschwindigkeit, Form und Grösse ebenso. Die auf *Tr. vulgaris* regenerierende *cristatus*-Extremität durchläuft alle Regenerationsstadien in der für ihre Art typischen Weise. Im Vergleich zu der als Kontrolle regenerierenden *vulgaris*-Extremität auf der anderen Seite erfolgt die Blastembildung später, die Wachstumsrate der jungen Regenerationsknospe ist relativ geringer, und die Gliederung des Autopodiums setzt zeitlich später ein. Das chimärische Regenerat wird aber der Herkunft seines Materials gemäss grösser als die *vulgaris*-Kontrolle. Charakteristisch ist bei den *cristatus*-Regeneraten die frühzeitige starke Streckung des ersten und zweiten Fingers und ein noch schnelleres Wachstum des dritten Fingers unmittelbar nach seinem Erscheinen. Umgekehrt verläuft die Regeneration der *vulgaris*-Extremitäten auf *Tr. cristatus* schneller als die der *cristatus*-Kontrollen. Auch jene lassen, obwohl sie von der kleineren Art stammen, keinerlei Wirtseinfluss erkennen; sich schneller entwickelnd, durchlaufen sie die ihrer Art entsprechenden Stadien. Im fertigen Zustand gleichen sie völlig nichtverpflanzten, normalen *vulgaris*-Extremitäten.

Dadurch ist nachgewiesen, dass ein Extremitätenregenerat aus Material des Extremitätenstumpfes gebildet wird, und dass die genetische Qualität des Blastemmaterials Regenerationsgeschwindigkeit und Ausgestaltung der Neubildung bestimmen. Die wirtsgemässe

[1] Die vorliegenden Untersuchungen sind auf Anregung von E. ROTMANN begonnen worden und wurden nach dessen Tod im September 1950 unter der Förderung von O. KUHN weitergeführt.

[2] E. ROTMANN, *Die Rolle des Ektoderms und Mesoderms bei der Formbildung der Kiemen und Extremitäten von Triton, I. Operationen im Gastrulastadium.* Roux' Arch. *124*, 747 (1931); *II. Operationen im Gastrula- und Schwanzknospenstadium*, Roux' Arch. *129*, 85 (1933). – V. C. TWITTY and J. L. SCHWIND, J. exper. Zool. *59*, 61 (1931).

[3] CHR. P. RAVEN, Roux' Arch. *134*, 122 (1936).

[1] S. GLÜCKSOHN, Roux' Arch. *125*, 341 (1932).