Figure 2 gives the regression lines fitting the data concerning the motor activity levels (for each genotype and sex the means of the 15 repetitions are plotted as percentage of possible moves in function of time). For males as for females, the differences between the three genotypes are always significant or highly significant, the order of superiority being the same as for the sexual activity of the males. Perhaps the common level of agitation could be a stimulating factor for the courting initiatives of the males.

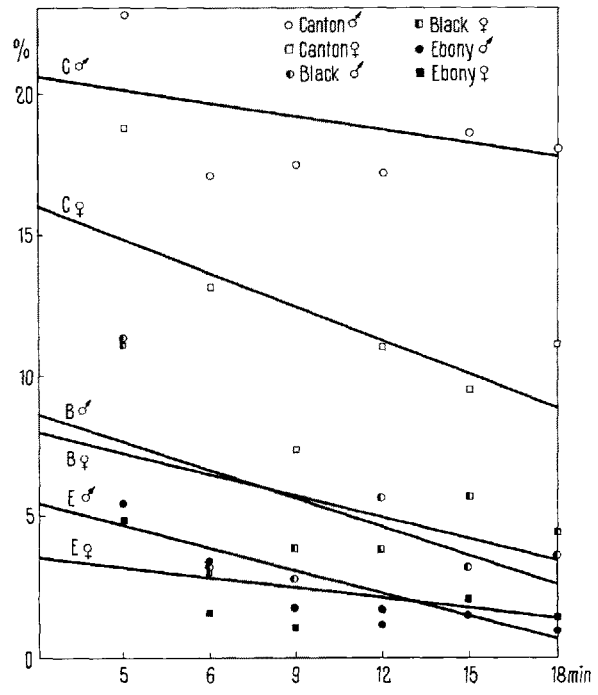

Fig. 2

The two melanistic mutants *ebony* and *black*, phenotypically very similar, are certainly different in selective value. The second one is rapidly eliminated in competition with its wild type allele, but, under the same conditions, *ebony* may be present in the population for many generations. The explanation of its maintenance can certainly not be found in the difference of activity: the *ebony* flies are the least active. But, in a previous paper, we have shown that the sexual activity of heterozygote flies from father *wild* and mother *ebony* is significantly higher than that of the *wild* ones[5].

Résumé. L'observation directe a montré qu'il n'y a pas d'isolement sexuel entre le type *sauvage* et les mutants mélaniques *ebony* et *black* de *Drosophila melanogaster*, mais il existe de notables différences entre l'activité sexuelle et motrice des mâles des trois génotypes. L'importance sélective de ces différences est soulignée.

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[5] A. A. ELENS, Exper. *14*, 274 (1958).

The Regeneration of Accessory Limb Parts Following Epidermal Cap Transplantation in Urodeles

Introduction. Recently, interest in the functions of the wound epidermis in amphibian limb regeneration has focused on the phagocytic and histolytic activities of the epithelial cells[1], and on their apparent ability to influence the aggregation of mesenchymatous cells to form the regeneration blastema[2,3]. The purpose of this communication is to supply new information on the influence of the apical epidermal cap on the formation and outgrowth of the limb blastema, and in particular, to describe the production of accessory limb outgrowths following transplantation of epidermal caps to the basal areas of limb blastemata.

Material and methods. Two species of urodele larvae were used in these experiments: *Ambystoma talpoideum* 70–80 mm long, and *A. mexicanum* 80–90 mm long. The right forelimbs were amputated through the distal condyles of the humerus. After 11 days (*A. talpoideum*) or 14 days (*A. mexicanum*) a mound-stage blastema had formed at the end of the limb stump. At this stage the larvae were anesthetized with MS 222 and placed on a pad of sterile gauze moistened in Ringer's solution. A section of epidermis, equivalent in area to the apical epidermal cap, was excised from the base of each blastema on its pre-axial surface. Immediately after the epidermis was removed the apical cap was cut away from the blastema tip, cleared of adhering blastema cells and placed autoplastically over the proximal wound in the blastema. Each larva was placed for 6 h in a refrigerator, held at 6°C, in order to insure healing of the edges of the apical cap with the cut edges of the blastemal epidermis. Within a week a new apical cap regenerated at the blastema tip to replace the one which had been removed and transplanted. In 27 of 51 blastemata both epidermal caps (the grafted and the regenerated) continued their individual growth. In these cases, therefore, the limb blastemata continued to develop in association with the regenerated epidermal caps while a secondary blastema formed beneath each of the grafted epidermal caps. These accessory blastemata differentiated supernumerary limb parts. In the remaining 24 cases the graft cap was suppressed or its outgrowth fused indistinguishably with that of the regenerated apically situated cap so that single, typical limbs were produced.

Control experiments consisted of the transplantation of whole head skin (dermis and epidermis – 10 cases) and

[1] M. SINGER and M. M. SALPETER, Basic Books Inc. (New York 1961).
[2] C. S. THORNTON, Devel. Biol. *2*, 551 (1960).
[3] T. P. STEEN and C. S. THORNTON, J. exp. Zool. *154*, 207 (1963).

regenerated head skin (epidermis alone – 10 cases, Figure 1) which were placed at the surface of the blastema to close an epidermal wound in a manner similar to that used for the grafted apical caps; and of the implantation of pieces of urodele liver (10 cases) and kidney (10 cases) which were placed just beneath the skin of the blastema by means of a slit in the skin of the limb stump adjacent to the blastema. Finally, a simple skin excision, similar to that made in preparation for the apical cap grafts, was performed in 10 cases. There were no instances of accessory outgrowths in any of the control experiments.

Results and discussion. Figure 2 is a photomicrograph of a part of a longitudinal section through a blastema showing the grafted cap and its small accessory blastema 6 days after the transplantation. A prominent basement membrane can be seen to underly the epidermis of the host blastema but is absent beneath the grafted cap. A dense cluster of blastema cells of which approximately 20% are undergoing mitosis lies in close contact with the under surface of the grafted cap. These cells appear to have been recruited from the blastema cells of the host regenerate. At this time, although it is not shown in Figure 2, cartilage differentiation is beginning in the central axis of the host blastema so that both proliferation and differentiation are proceeding in neighboring cell populations.

Figure 3 is a photomicrograph of an accessory blastema 10 days after epidermal cap transplantation. No basement membrane can yet be observed beneath the grafted cap. The accumulation of blastema cells has increased, largely by mitotic proliferation, to enlarge the volume of the accessory blastema. Continued growth of accessory blastemata is in no way unusual and by 3 weeks after transplantation of the apical cap, morphogenesis is nearly completed, as may be seen in Figure 4 which is an example of the differentiation of an accessory digit. An unexplained species difference in response to transplanted epidermal caps was discovered. The blastemata of the axolotl produced a higher percentage of accessory limb outgrowths following apical cap transplantations (14 of 23) but these were invariably accessory digits (whose skeletons fused proximally with the metacarpals). The blastemata of the *A. talpoideum* larvae produced fewer cases of accessory limb outgrowths (10 of 27) but all of the positive cases consisted of several limb segments. In 6 cases, the supernumerary limb consisted of the distal third of the humerus (fused proximally with the host humerus), the radius and ulna, the carpals and the metacarpals and phalanges associated with 3 digits (Figure 5). The remaining 4 cases were similar except only that 2 digits were developed in each.

In the blastemata of the *A. talpoideum* larvae the grafted epidermal cap remained at its original implantation site near the base of the blastema as morphogenesis proceeded. In the axolotl, on the other hand, as morphogenesis proceeded in the host blastema, the grafted cap, with its accessory blastema, was carried distally so that when histogenesis began the accessory blastema was

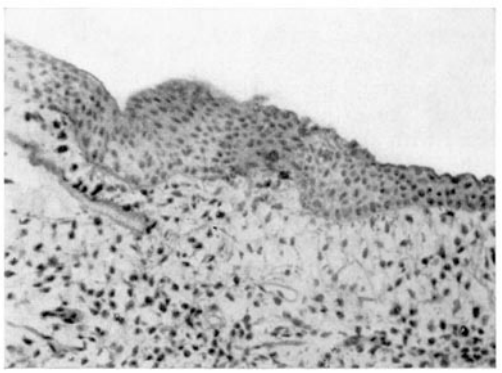

Fig. 1. Regenerated head skin (epidermis) grafted to base of host blastema (*A. mexicanum*). Blastema cells have not accumulated to form an accessory blastema at 12 days (× 195).

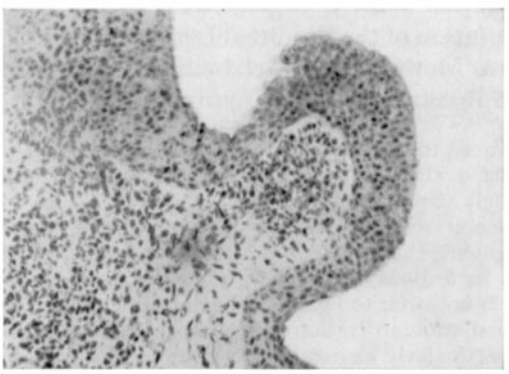

Fig. 3. Accessory blastema (*A. mexicanum*) developing on the preaxial surface of a limb blastema 10 days after transplantation of an epidermal cap (× 195).

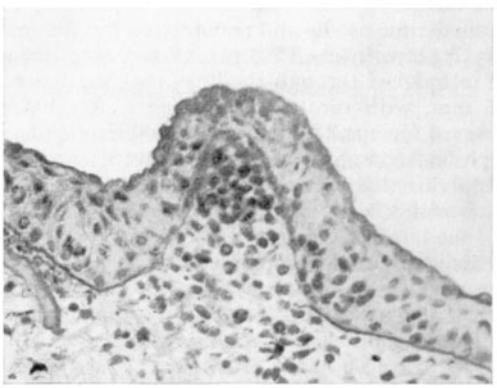

Fig. 2. Small accessory blastema (*A. mexicanum*) at 6 days following transplantation of an epidermal cap (× 325).

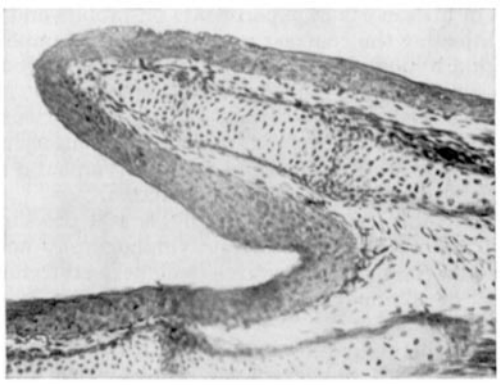

Fig. 4. Regenerated accessory digit (*A. mexicanum*) (× 136).

located at the metacarpal level of the host regenerate. Thus, in the two species of urodele used in these experiments the nature of morphogenesis of the accessory blastemata was directly correlated with their location along the proximo-distal axis of the host regenerate. It is concluded that the *stimulus* for the accumulation of the blastema cells of the accessory regenerates, as distinct from their *morphogenetic* induction, was provided by the transplanted epidermal cap[4].

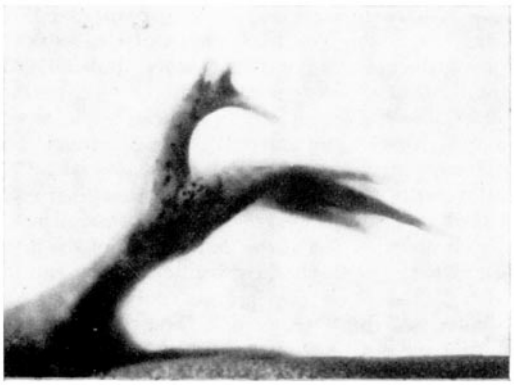

Fig. 5. Regenerated accessory 3-digit limb (*A. talpoideum*) (× 6).

Résumé. Les événements morphogénétiques qui suivent le greffage d'un blastème apical à la face préaxiale d'un membre antérieur en voie régénératrice, chez *Ambystoma*, sont tout à fait normaux, mais le niveau où se localise le régénérat accessoire est différent selon l'espèce utilisé. Ces résultats sont discutés au point de vue du stimulus d'agrégation des cellules appartenant au régénérat accessoire.

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Department of Zoology, Michigan State University, East Lansing (Michigan USA), August 28, 1964.

[4] This work was supported by a research grant (NB 04128) from the National Institute of Neurological Diseases and Blindness, National Institutes of Health.

Cannulation of the Popliteal Lymph Node in Rabbits: A Method for Introducing Radio-Opaque Substances into the Lymphatic System

The need to have available an experimental method for the first evaluation of lymphographic contrast media is much felt at present.

A useful method for this purpose must essentially comply with the following practical requirements: (1) be usable in ordinary laboratory animals, possibly of low cost; (2) not offer too great technical difficulties; (3) permit good standardization of the methods of administration, particularly as regards the rate; (4) ensure survival of the animal in order to allow long-term controls.

Two routes of introduction of the contrast medium – the lymphatic vessel and the lymph node – are known in direct lymphography. With the procedures now in use, each of the routes of introduction offers benefits and inconveniences which have been clearly shown by TJERNBERG[1] in his reports of experiments on rabbits and dogs.

On injecting the contrast medium into the lymph node through a hypodermic needle, TJERNBERG observed that the ease with which the substance flows from the site at which the needle is introduced, and the insufficient control of the flow, due to the 'manual' regulation, rather limit the possibilities of examining the lymphatic structures remote from the site of introduction.

He concludes, however, that this is still the simplest method for carrying out direct lymphography and expresses the hope that research will perfect techniques capable of eliminating the above-mentioned inconveniences.

Bearing this suggestion in mind, we have studied a procedure of direct lymphography in rabbits which, although using the lymphatic node as a route of introduction, also offers the advantages obtained with cannulization. This procedure is based on the use of a polyethylene catheter instead of the needle, and on the possibility of ligaturing the lymph node capsule around same.

Method. Adult rabbits of both sexes were used. After pentobarbital anaesthesia, the skin area is shaved and disinfected with alcohol. The skin is incised for 2 cm, following the fold between the femoral and semi-membranous biceps as closely as possible. In order to determine the exact point of the incision, it is always advisable to press the sides of the popliteal fossa to make the lymph nodes stand out better below the skin, forming a hernia between the margins of the above-mentioned muscles.

After incising the skin, we usually continue under a dissection microscope (magnification: 6 ×) in order to free the lymph node from the surrounding connective tissue. The lymph node is generally easily recognized, but it may be of help to make it stand out by subcutaneously injecting Patent blue peripherically, in the quantity of 1–2 ml of 5% solution.

Delicately holding the lymph node between two fingers, a hole is made in the capsule at the inferior pole, using a no. 1 hypodermic needle and penetrating for 2–3 mm into the pulp. A polyethylene PE 100 catheter, 40–50 mm long, is then introduced through the hole, making it penetrate for 4-5 mm, with rotatory movements. At this point, using curved forceps, the catheter is held firm and a circle of lymph node tissue is collected around it. At the same time, applying slight traction, an assistant can carry out a ligature with silk thread immediately below the holding point of the forceps. The ligature fixes the catheter to the lymph node and ensures a perfect fit.

After placing the animal under the radiological apparatus in the required position, the syringe containing the contrast medium is connected to the catheter fixed in

[1] B. TJERNBERG, Acta rad., Suppl. 214 (1962).