and these could not be identified as histotypical osteoblasts or osteoclasts. This pattern was typical of femoral, tibiofibular, metatarsal, and phalangeal cartilages.

PTE-treated newts. After PTE-treatment, there was a marked increase in the numbers of osteoclasts throughout the skeletons of the newts. Yet the pattern and timing of resorption in the different bone was variable and restricted almost entirely to the cartilaginous ends. The earliest changes were observed in the carpals. Serial sections revealed numerous foci of resorption after 24 to 48 h, but histotypical multinucleated osteoclasts were not invariably present within the spaces. Changes in the long bones of the foot were first evident at 14 days. Resorptive cells eroded the cartilages centrifugally, leaving a narrow zone of tissue adhering to the endosteum as the bones grew in length (Figure 3). In the metatarsals and phalanges, however, chondroclasts frequently dissected away a part of the calcified zone of cartilage, and the spaces were filled with loose connective tissue (Figure 4). In the bones most severely affected, the epiphysis was effectively amputated. Fibrotic changes in the marrow were never observed during PTE treatment, and they were rare even after 14–21 days. At no time did PTE induce cortical bone remodelling.

Discussion. The results of this study appear to explain the curious staining properties with methylene blue observed in cleared whole mounts of bone from PTE-treated anurans and urodeles described by one of us[2]. They are all consequences of hormone-induced resorptive activity. While this process in the tibiofibula primarily thins the cartilages, it leaves a shell of matrix laterally at the epiphyseal-diaphyseal junction, and under conditions of optimum cartilage growth, gross specimens stained for cartilage would appear to have thicker epiphyses than normal. The peculiar mode of intracartilaginous matrix resorption mediated by chondroclasts and subsequent connective tissue formation in the epiphyses of the metatarsals and phalanges could produce double bands of stainable matrix. All of these findings are consistent with the observation of increased numbers of resorptive cells in amphibian bone following the administration of parathyroid extract[4,5]. Although resorptive osteoclasts were apparent within 48 h after initiating PTE therapy, their origin is in question. In parallel studies with a pulse label of ^{3}HTdr (unpublished observations), labeling of marrow elements did not occur until the 3rd day after injection, and osteoclasts were not labeled until the 7th day[7].

Résumé. L'extrait parathyroïdien a augmenté le nombre des ostéoclastes dans tous les os des jambes des salamandres *Triturus sp.* Cependant, dans ces éléments, les modèles résorptifs et le moment de leur apparition sont variables. Ces modèles de résorption expliquent leur apparition charactéristique dans les préparations teintées graduellement, en particulier lors du redoublement des cartilages terminaux des phalanges.

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Kynurenine Localization in the Egg of *Drosophila melanogaster*

Studies on the genetics of eye color in *Drosophila* have demonstrated that the pathway of xanthommatin synthesis involves tryptophan, kynurenine and hydroxykynurenine (see[1] for a review). The mutation vermilion is known to block the formation of kynurenine from tryptophan via formylkynurenine and the mutant cinnabar the formation of hydroxykynurenine from kynurenine[1]. These two mutants therefore can provide convenient tools for the study of kynurenine metabolism during the life cycle and in different organs of the fruit fly. The accumulation of kynurenine in the larval fat body of *Drosophila*[2–5] and its presence in the Malpighian tubules has been clearly documented[6–9]. Earlier reports on the presence of kynurenine in the ovary[10] and eggs of *Drosophila*[11] suggested that a study of the ovaries combining chromatography and fluorescence microscopy should reveal the location of kynurenine in the developing and mature egg. This study provides such a demonstration.

Materials and methods. Descending paper chromatograms of ovaries were prepared by squashing the ovaries from a 2-day-old fly on Whatman No. 1 filter paper. Solvent systems used consisted of *n*-butanol:acetic acid:water (20:3:7) or *n*-propanol:1% ammonia (2:1).

For studies with the fluorescence microscope ovaries were prepared in one of two ways: 1. They were dissected out of 2-day-old flies in *Drosophila* Ringer's solution and smeared on a slide with a drop of fluid so that individual ovarioles and eggs could be viewed. Preparations were then air dried and viewed uncovered or mounted in Zeiss mounting medium ($L25 - n_D = 1.525$) or paraffin oil. 2. Some ovaries were dissected out and the egg chambers separated in paraffin oil on a slide.

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A Zeiss Photomicroscope equipped with an HBO 200 W mercury source was used for the study. One of the exciter filters BG12, BG3, UG5 or UG1 was used in combination with different barrier filters, Zeiss 41, 44, 47, or 50, to analyze the egg fluorescence.

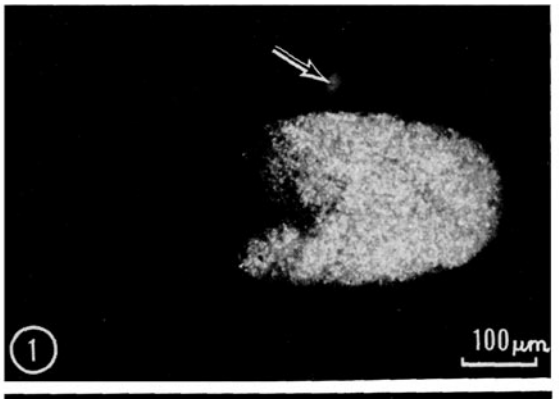
Results and discussion. Evidence from chromatography confirms the fact that the main fluorescing component of the egg is kynurenine. The main fluorescing spot on each chromatogram has the same Rf value as commercial (Calbiochem) L-kynurenine sulfate with the solvents used and compares with those obtained by WESSING and EICHELBERG[9] for kynurenine from Malpighian tubules of *Drosophila*. Absence of the kynurenine spot in chromatograms prepared from ovaries of vermilion females and its presence in those made from ovaries of the cinnabar mutant demonstrates that the material is indeed kynurenine.

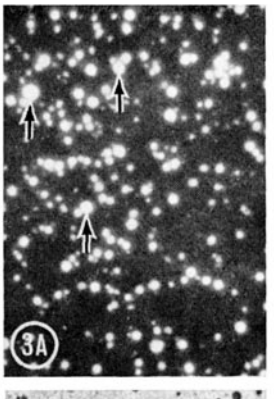
Observation of different stages (eggs were staged according to KING, RUBINSON and SMITH[10]) shows that the vitellogenic and mature eggs of wild type *Drosophila* exhibit a brilliant blue-white or light blue fluorescence of the ooplasm (Figures 1 and 2). The fluorescence appears initially at the onset of vitellogenesis and its increase in intensity tends to parallel the growth of the ooplasm. It is possible to see in the ooplasm of stage 8 egg chambers that the main fluorescing material of the egg is localized in particles which correspond in size to the protein yolk spheres. The parallel between growth of the fluorescing particles during vitellogenesis and growth of protein yolk spheres indicates that the kynurenine is located in these spheres. The amount of fluorescence of an individual sphere must increase as the yolk sphere increases in size since the brilliance of each sphere does not seem to diminish as the sphere grows in size. By stage 10 the fluorescence of the yolk spheres contrasts strongly with the absorbing material of the nurse cell cytoplasm as seen with the BG12–50 filter combination (Figure 1). With the UG1 exciter and 41 barrier filter combination the fluorescence of the particles appears blue-white – the kind of fluorescence exhibited by kynurenine. Mature eggs show an intense fluorescence of the yolk spheres with the relative concentration of yolk spheres in the ooplasm less than that at stage 10 (Figure 2). Resistance of the main fluorescent material to fading is shown by the fact that the fluorescence persists even with prolonged exposure to UV-light.

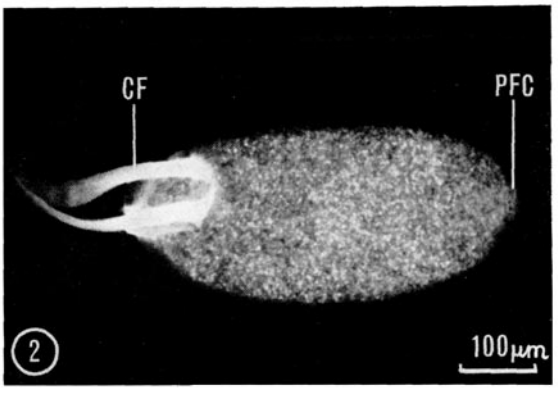
Two other components of the mature egg which exhibit a clear autofluorescence are the chorionic filaments and a group of cells at the posterior end of the egg (Figure 2). Observation of these components with different filter combinations shows that the fluorescing component(s) here differ(s) from the ooplasmic fluorescence.

Comparison of fluorescence and phase contrast pictures gives added evidence for the identity of the fluorescing spheres in the ooplasm and the protein yolk spheres. Preparations mounted in Zeiss mounting medium L25 (n_D 1.525) make it apparent that the particles with a high refractive index as seen with phase contrast microscopy (i.e. protein yolk spheres) correspond to the fluorescing bodies (Figures 3 A and B).

Microscopic observation reveals that the protein yolk of the vermilion mutant contains at least one fluorescent component (greenish-yellow) which fades during observation. This fluorescence, which is most clearly seen in egg chambers of stages 9 and 10, is masked in the wild type fly by the much more intense kynurenine fluorescence. However, the fluorescence of the egg from the vermilion mutant is only a fraction of that from the wild type egg as suggested by GRAF[11]. It is apparent that the main fluorescing component of the wild type is not present







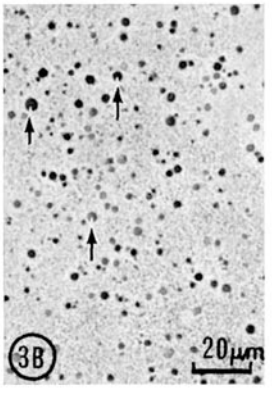


Fig. 1. Fluorescence photomicrograph of egg chambers dissected from a 2-day-old wild type *Drosophila* and mounted in paraffin oil. A small amount of fluorescence is seen in the ooplasm of a stage 8 egg chamber (arrow) whereas a much greater amount is present in the ooplasm of the later stage 10 chamber. Filter combination: Zeiss BG12 exciter and 50 barrier.

Fig. 2. Fluorescence photomicrograph of a mature wild type *Drosophila* egg handled as above. Fluorescence is apparent in the chorionic filaments (CF) and in some cells at the posterior end of the egg (PFC) as well as in the ooplasm. Filter combination same as above.

Fig. 3. A) Fluorescence photomicrograph of ooplasm from a mature egg spread on a slide and covered with Zeiss L 25 mounting medium (h_D = 1.525). Filter combination: Zeiss UG 1 exciter and 41 barrier. B) Phase contrast photomicrograph of same field as A) to show yolk spheres (some indicated by arrows) which contain fluorescing material seen in A).

here. This is consistent with the fact that the vermilion mutant is either lacking in or has a very small quantity of tryptophan pyrrolase[12-14] and therefore would not be expected to accumulate kynurenine anywhere in the body. We can conclude therefore that the protein yolk spheres contain at least 2 different fluorescing substances and that the more brilliant stable component is kynurenine[15].

Zusammenfassung. Papierchromatographische und fluoreszensmikroskopische Untesuchungen von Ovarien der Wildtypen und der Vermilion- und Cinnabar-Mutanten von *Drosophila* zeigen, dass sich Kynurenin (der Hauptbestandteil der fluoreszierenden Komponente des Eies) in den proteinhaltigen Dotterschollen des reifenden und des reifen Eies befindet.

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Differentiation of Chick Embryo Brain Cells in Culture

Previous investigations have demonstrated that neurons separated from their glial cells can be maintained in tissue culture and are able to regenerate processes. Neurons have been isolated from spinal and trigeminal ganglia[1-4], from the spinal cord[5-7] and from the cerebral hemisphere[8] of chick embryos of more than 6 days of age. In all these conditions the neurons were separated when nerve fibres already existed. During the dissociation procedures all cell processes were destroyed and subsequently reappeared during the cultivation. Thus, the growth of the fibers can be considered to involve regeneration rather than differentiation.

We attempted to cultivate undifferentiated cells dissociated from cerebral hemispheres during the initial stages of differentiation when there were no nerve fibres.

Material and methods. Cerebral hemispheres denuded of their connective tissue covering, from 25-somite chick embryos (4.5–5 days of incubation), were passed through a nylon sieve (82 μ pore size) into chick embryo extract. One drop of the cell suspension was then placed onto a collagen coated coverslip, supplemented with one drop of cockerel plasma and cultivated in the Maximow's double-coverslip assembly. Phase contrast microscopy was employed for observation of the cells during their subsequent growth. Some cultures were fixed and stained by the Bodian's method.

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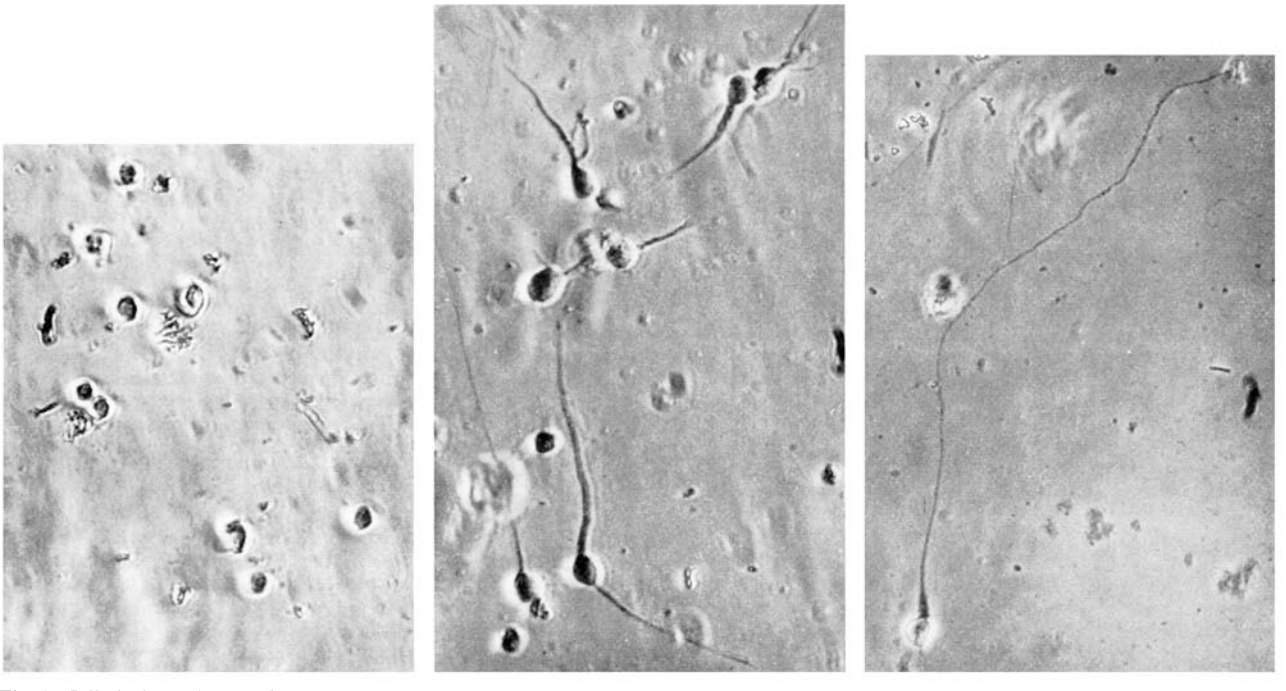

Fig. 1. Cells isolated from 5-day-old chick embryo cerebral hemispheres, photographed with phase contrast microscope. ×283. a) Freshly isolated cells without processes. b) After 24 h in culture, cells with several processes. c) After 48 h in culture, a cell with a long process.