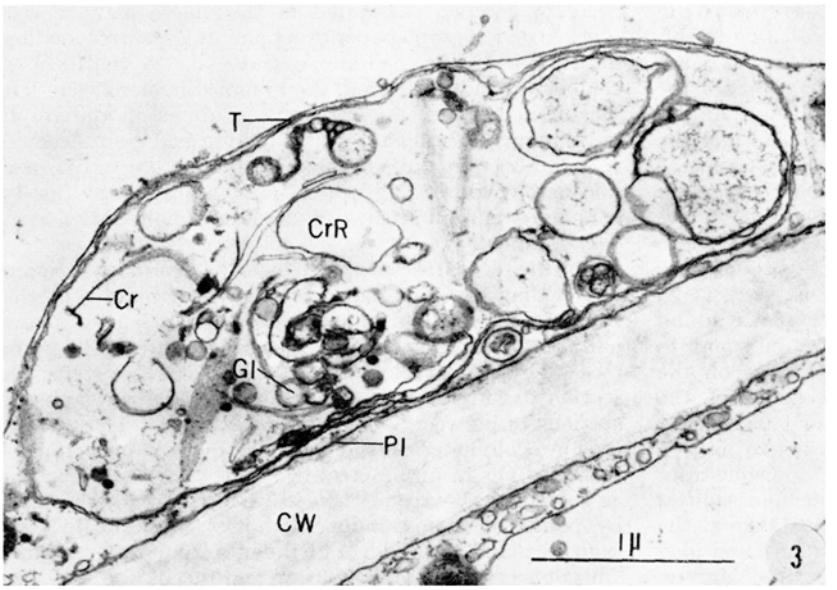


Fig. 3. Carrot root grown in Nemagon fumigated soil (3 gal/acre).

embedded in Epon 812[4]. The blocks were sectioned with a glass knife on a Sorvell Porter-Blum MT-2 ultramicrotome. Sections around 80 µm were mounted on the 200 mesh copper grids and stained with uranyl acetate and Reynold's lead, finally examined with a Zeiss EM-9A electron microscope.

Results and discussion. The preplanting soil fumigation with Telone and Nemagon significantly influenced the chromoplasts of carrot roots when compared with those from the non-fumigated plots (Figures 1–3), especially the size, shape, and organization of the chromoplasts. The chromoplasts of the carrots grown on the fumigated soil were larger and contained more globules and crystals than those grown on the non-fumigated soil. The chromoplasts of the carrot roots from Telone fumigated soil contained long needle crystals while those of the Nemagon fumigated contained short needle ones. It has been reported that the carotenes of carrot roots were located in the crystals and globules of chromoplasts[5]. It is possible that the increase in amount and size of crystals and globules might result in the increase in carotene content of the carrot roots. Evidence from the electron microscopic examinations substantiates our previous finding that the soil fumigation with Telone and Nemagon increased total carotenes and β-carotene of carrot roots[6].

Zusammenfassung. Wenn der Boden vor der Aussaat von *Daucus carota* L. mit Telon und Nemagon behandelt wurde, konnten Strukturveränderungen in den Chromoplasten der Karottenwurzeln festgestellt werden.

M. Wu and D. K. Salunkhe

Food Science Department, Utah State University, Logan (Utah, USA), 23 November 1970.

[4] J. H. Luft and R. L. Wood, J. Ultrastruct. Res. *12*, 22 (1965).
[5] K. Steffen and G. Reck, Planta *60*, 627 (1964).
[6] This study was supported by the Agricultural Research Service, U.S.D.A. Grant No. 12-14-100-9903 (61), administered by the Human Nutrition Division, Beltsville, Maryland.

PRO EXPERIMENTIS

A Simple Method for Reconstructing Serial Sections

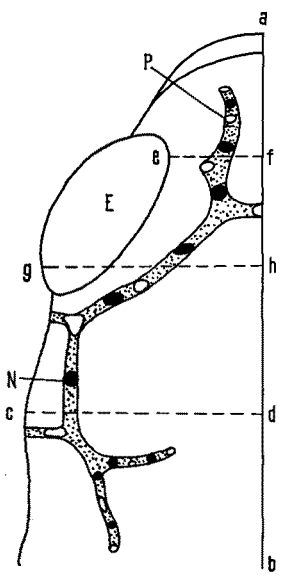
Reconstruction of serial sections is an important and sometimes indispensable step in studying the topography of various organ-systems and organs of the body of an animal. A number of methods for such reconstructions have been described by Peter[1]. The more important of these are the wax model reconstruction method of Born[2–4] using wax sheets, the graphic reconstruction method of Kastschenko[5] employing squared paper, the graphic reconstruction method of Kerr[6] using glass plates immersed in cedarwood oil, and Lewis's modification[7] of Born's method by substituting plaster of Paris for wax. De Beer[8] used blotting paper soaked in wax and Pusey[9] employed greaseproof 'Sketching Bank' paper immersed in xylol. Most of these methods are, however, tedious and necessitate the sketching of a large number of drawings. The author, while working on the development of latero-sensory canals and related dermal bones of the head in fishes, found that a simpler method could be equally useful. It is described below with the aid of a specific example.

A millimeter (Graph) paper was taken. The head of an 11 mm long larva of *Ophicephalus punctatus* (a teleostean fish) was cut in serial transverse sections of 12 µm thickness. The total number of sections obtained was 225. Since each section was of 12 µm or 0.012 mm thickness, the total length of the head to be reconstructed was $225 \times 0.012 = 2.7$ mm. Since it was considered desirable to plot a magnified reconstruction of this 2.7 mm long head on the graph paper, it was arbitrarily decided that the thickness of 1 section (which, in actual fact, was 0.012 mm) be deemed equivalent to 0.5 mm, and hence, be represented by $^1/_2$ of a 1 mm^2 division of the graph paper.

Consequently, the thickness of 225 sections (which, in actual fact, was $225 \times 0.012 = 2.7$ mm), according to this scale of magnification, was deemed equivalent to 112.5 mm and was represented by 112.5 one mm² divisions of the graph paper. This 'thickness' of 225 sections (2.7 mm), in actual fact, was the total length of the head and the magnification of this length in the reconstruction plotted on the graph paper was approximately 42 times (112.5mm/2.7 mm).

A graduated scale or the oculometer was slipped into the eye-piece and a 1 mm graduated slide or the slide micrometer was placed on the stage of a microscope. Using a 6X eye-piece and a 10X objective, it was found that 50 divisions of the oculometer were equivalent to the entire 1 mm length of the graduated scale on the slide micrometer. In other words, 1 division of the oculometer was found equivalent to 1/50 or 0.02 mm.

Now, let it be assumed that the aim was to draw a general outline of the head of the larva and to reconstruct within it the eye in a dorsal view. The median sagittal plane of the larva in transverse sections was taken as the landmark for taking all measurements. A 112.5 mm long line (Figure, a–b) was drawn to represent this plane on the graph paper (112.5 mm being the proposed length of the magnified reconstruction of the larval head). Using the oculometer, the distance between the median sagittal plane and the lateral margin (Figure, c–d) of every 5th of the 225 sections (as for example, 5th, 10th, 15th and so on) was measured, leaving 4 sections in between 2 consecutive readings. Every 5th section was taken up arbitrarily and it could as well have been every 3rd or every 4th section. The readings for these distances were obtained in oculometer divisions and were, therefore, converted into equivalent readings in millimeters. To exemplify: If the number of oculometer divisions for any reading was 25, it was multiplied by 0.02 (since one oculometer division was equal to 0.02 mm) and then by 42 (since the reconstruction was being magnified 42 times from the original). The resultant reading in millimeters in this case would be $25 \times 0.02 \times 42 = 21$.



Reconstruction of a dorsal view of the lateral half of the head of an 11 mm long fish (*Ophicephalus punctatus*) larva on a millimeter (graph) paper showing the eye (E) and the cephalic lateral line canals (dotted). The sense organs (N) and pores (P) of these canals are shown. ×42.

Every reading calculated in the above manner was marked on the graph paper by a point at the corresponding distance from the median sagittal plane at the level of the corresponding section. 2 one mm² divisions were left vacant in between 2 consecutive readings on the graph paper (since the thickness of 1 section had been deemed equal to 0.5 mm and since 4 sections were left in between 2 consecutive readings). All these points were finally connected and an outline of the lateral half of the head was obtained.

For the reconstruction of the eye, the distances between the median sagittal plane and the inner margin of the eye (Figure, e–f) and between the median sagittal plane and the outer margin of the eye (Figure, g–h) were measured with the help of the oculometer for every 5th section passing through the region of the eye (leaving 4 sections in between 2 consecutive readings). These readings in oculometer divisions were converted into equivalent readings in millimeters in exactly the same manner as described above and then marked on the graph paper by points at corresponding distances from the median sagittal plane at the level of the corresponding sections. On connecting these points, an outline of the eye was obtained.

The figure shows a reconstruction of the lateral half of the head of the larva obtained by this method. It shows the general outline of the head and the topography of the eye and the cephalic lateral line canals as seen in a dorsal view. The author made use of this method primarily for reconstructing the course of the lateral line sensory canals. It could, however, be usefully employed for reconstructing the topography of other parts of the body in different views – dorsal, ventral and lateral. What is important is that there must always be a landmark available in the serial sections from which all measurements can be made. If necessary, such a landmark or guideline can be introduced at the time of making the paraffin block by painting one surface parallel to the object to be sectioned with lampblack and recoating it with a thin coat of paraffin. Cross sections of the tissue will then also show cross sections of this lampblack layer in a constant relation to the tissue.

Short of giving a three dimensional image, the method described above is as useful as any other known method for studying the anatomy and interrelationships of various structures through a reconstruction of serial sections.

Résumé. On décrit une méthode simple de reconstruction des sections sériées qui évite l'exécution fastidieuse d'un grand nombre de dessins. Bien qu'elle ne donne pas une image à trois dimensions, cette méthode est aussi utile qu'une autre pour étudier l'anatomie et les relations structurales par reconstruction de sections sériées.

A. S. KAPOOR

Department of Zoology, University of Rajasthan, Jaipur (India), 9 October 1970.

[1] K. PETER, *Rekonstruktionsmethoden* (Greifswald 1922).
[2] G. BORN, Morph. Jb. *2*, 577 (1876).
[3] G. BORN, Arch. mikrosk. Anat. *22*, 584 (1883).
[4] G. BORN, Z. wiss. Mikrosk. *5*, 433 (1888).
[5] N. KASTSCHENKO, Anat. Anz. *2*, 426 (1887).
[6] J. G. KERR, Q. J. microsc. Sci. *45*, 1 (1902).
[7] W. H. LEWIS, Anat. Rec. *9*, 719 (1915).
[8] G. R. DE BEER, *The Development of the Vertebrate Skull* (Clarendon Press, 1937).
[9] H. K. PUSEY, J. R. Microsc. Soc. *59*, 535 (1939).