If homogenization was carried out with a minimum of isotonic sucrose, protein concentrations adequate for electrophoresis could be obtained, omitting dialysis and freezing-drying; in this case the results were similar to those observed with the usual procedure.

Electrophoresis was carried out with a constant potential of about 6–8 V/cm for periods variable from 4 to 12 h. After drying in an oven at 120°C, the strips were stained with amido-schwarz 10 B according to GRASSMAN, HANNIG, and KNEDEL[3]. The relative amounts of the components were determined cutting the corresponding portions of the stained paper, eluting the dye with 5 ml of 5% solution of phenol in water and reading the optical density at 640 mμ.

The Figure shows the patterns of the soluble cerebellum proteins after 12 h of migration in borate buffer. 7 components are indicated with the numbers from 1 to 7 in the order of their increasing mobility. Some material is detectable ahead of fraction No. 7 indicating the presence of proteins with higher mobility, which represent 16·2% ($\pm$ 1·1) of the total amount.

After runs lasting only 6–8 h, this material appeared distributed in 2 fairly definite bands. The band visible at the point of application of the protein solution on the paper is considered to represent particulate matter not sedimented by the centrifugal force employed.

The Table shows the relative amounts of the separated fractions obtained by dye elution.

Relative amounts of electrophoretic components of cerebellum proteins, obtained by dye elution, after 12 h of electrophoresis (means $\pm$ S.E.M.)*.

Fraction No	% Content	S.E.M.
1	6·61	0·71
2	8·33	0·81
3	11·27	0·59
4	7·33	1·45
5	16·45	0·37
6	17·97	1·04
7	15·13	1·43
Fast moving material	16·20	1·10

* The immobile material located at the point of application of the protein solution on the paper corresponds to 5% of the total amount of the soluble proteins.

Similar results were obtained with proteins extracted from the brain.

As resulting from the researches of NAKAMURA *et al.*[2] and from the present results, the patterns of the soluble proteins of the central nervous tissue appear differently from those of the nerve[1] and from patterns observed in other organs (liver[4]; muscle[5]).

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Istituto di Patologia Generale and Istituto di Semeiotica Medica, Università di Pisa, November 10, 1955.

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[4] G. ADJUTANTIS, Nature *173*, 539 (1954); *174*, 1504 (1954).

[5] G. TOSCHI and A. MARIANI, R. C. Accad. Lincei, Cl. Sci. fis. mat. e nat. [8] *16*, 365 (1954).

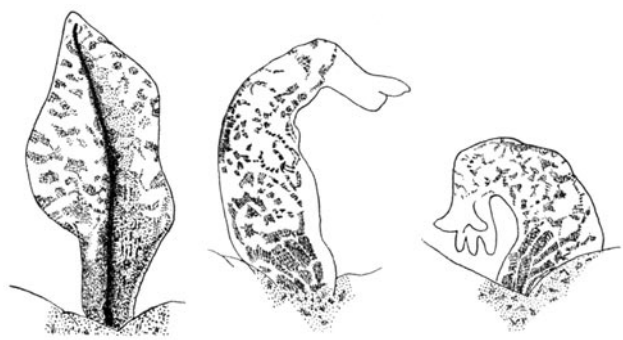
Riassunto

Le proteine idrosolubili estratte dal sistema nervoso di ratti sono state separate mediante elettroforesi su carta.

Il quadro elettroforetico appare costantemente riproducibile nelle condizioni sperimentali impiegate.

The Transformation of a Tail into Limb After Xenoplastic Transplantation

(1) One unexpected result obtained from the graft of a tail bud of a Urodele (*Triton cristatus; Axolotl*) on to an Anuran embryo at the neurula stage (*Discoglossus pictus*) is that the tail which differentiates from the transplant, may become transformed into a limb[1]. This, however, only occurs if the graft is made into a limb field of the host, and starts at, or close to, the time at which the metamorphosis of the host is initiated. This transformation occurred in 15 of 180 *Triton* grafts, and in 2 of 120 *Axolotl* transplants.



1 *2* *3*

Figs. 1, 2 and 3.–Axolotl tail bud grafted on to Discoglossus, respectively 13, 28 and 38 days after transplantation.

The stages of the transformation can be set out as follows:

(a) The graft develops into a normal tail, complete with fin, nerve cord, notochord and symmetrically arranged myotomes. It is slender and lanceolate in form (Fig. 1). Chromatophores, arranged in the pattern typical of the donor, are to be observed. The tail moves both spontaneously and upon stimulation.

(b) When the initial process of morphological and histological differentiation is complete, the tail undergoes regression, a process which particularly affects the fin. The tail ceases to be lanceolate, and instead assumes a cylindrical shape (Fig. 2). Concurrently it becomes fleshy and turgid and develops an intensely black pigmentation. The peduncle bends at right angles and at the distal extremity appears a variable number of digits (Fig. 3). It is, however, neither sensitive nor motile.

The limb persists for a considerable time. Meanwhile at its base a normal limb grows from the limb field of the host. In some cases an additional limb develops, which may fuse with the transplant, thus producing a chimera. A study of the histology of the transformed tail shows the nerve cord to be degenerating; in most specimens, remains of the notochord are present, but the musculature is almost completely lacking, a few sporadically

[1] N. FARINELLA-FERRUZZA, Boll. Zool. *17*, 113 (1950); Riv. Biol. *45*, 523 (1953).

distributed striated muscle fibres alone remaining. The bulk of the transplant is composed of mesenchyme probably derived from the de-differentiation of the tail tissues. Among them are also to be found many pigmented cells. Where externally the appearance of the transplant most resembles a limb, internally nodular or elongated masses of healthy cartilage cells are found in the process of organization to form skeletal structures. In those specimens in which such organization has reached a maximum, it is possible to distinguish the appropriate parts of the pectoral girdle, stilo- and zeugopodia, carpus and digital cartilages. Surrounding the existing parts of limb skeleton are bundles of muscle fibres, almost certainly the developing limb musculature. More often, however, the girdle is absent, the skeletal components are reduced in size and number, and the musculature may be lacking.

(2) What can be said of the mechanism by which these changes are effected? As has been said previously, the progressive transformation outlined above can only occur if the graft is made into the limb field of the host. From its first appearance the limb field possesses capacity for induction, as has been shown by the present work. Probably chemical substances elaborated by the field pass into the graft. In most specimens no mesodermal cells from the host were to be found in the transplant; in the remainder they were present in extremely small numbers. Under the influence of these substances the first stages of degeneration are perhaps initiated in the donor tail, and subsequently the processes of reorganization which result in the formation of the limb from the tail. In corroboration, it should be noted that when the graft is made into a region other than the limb field, tail degeneration does not occur. Instead the tail maintains its shape and internal structure well beyond the period of metamorphosis of the Anuran host[1].

From what material is the limb formed? A study of histology directs attention to the differentiated mesenchymal cells among which are some that would naturally form the vertebral cartilages. It is probable that the limb skeleton differentiates from such cells under the inductive influence of the limb field. The caudal musculature probably redifferentiates to form the limb musculature.

(3) Recently HOLTFRETER[2] has obtained similar results to those outlined above. He has observed that tails obtained by induction (from transplants of pieces of mouse kidney into the blastocoel of *Triton alpestris*) and which find themselves in the host limb field, are transformed into limbs. He did not obtain the same results from direct transplants of the tail buds. It is possible that the explanation of the latter result is that the direct transplants were homoplastic. Xenoplastic grafts perhaps increase the inductive capacities of the limb field, as is shown by the frequent appearance of supplementary limbs.

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Riassunto

Bottoni caudali di Triton e di Axolotl trapiantati sul campo dell'arto di Discoglossus (allo stadio di neurula) si differenziano in code, le quali talvolta, all'epoca della metamorfosi dell'ospite, si trasformano in arti. Viene discusso il meccanismo di tale trasformazione.

[2] J. HOLTFRETER, J. exper. Zool. *129*, 623 (1955).

On the Interpretation of the Low-Angle Scatter of X-Rays from Bone Tissue

ENGSTRÖM and FINEAN[1] demonstrated that, in addition to the wide-angle X-ray diffraction pattern, bone tissues also give a diffuse low-angle scatter. The same authors[2] assumed that the low-angle scatter could be treated as a particle scatter pertaining to the inorganic or mineral fraction. In this way they concluded that the particles are rod-shaped, the long axis of the rods being aligned in the direction of the longitudinal axis of the bone, and parallel to the collagen fibres. In the intact human bone these particles appear to have a diameter of about 73 Å, and a length of about 210 Å.

Recently ROBINSON and WATSON[3] have criticized the conclusions of ENGSTRÖM and FINEAN, because observations with the electron microscope do not support the view that the inorganic particles are rod-shaped.

The findings which one of us published in this journal (see ASCENZI and CHIOZZOTTO)[4] on the electron microscopy of the organic bone substance, using the pseudo-replica technique, induced us to repeat and possibly to improve on the investigations made by ENGSTRÖM and FINEAN in order to find a more adequate interpretation of the low-angle diffraction pattern.

Material and method.— Longitudinal and cross sections (0·2 mm thick) of the femoral diaphysis of cattle were prepared by grinding. The low-angle scatter was recorded from the untreated sections as well as from similar sections from which the ossein had been removed according to GABRIEL's method (boiling in glycerol with 6% KOH). This procedure is unable to produce any change in the crystalline structure of the inorganic bone fraction[5] or increase in the size of the crystallites[6].

A low-angle scatter apparatus, somewhat similar to that employed by FINEAN[7] was used. The scatter was recorded using Ni filtered CuKα radiation ($\lambda = 1{\cdot}54$ Å)[8]. The maximal recorded scattering space corresponded to 250 Å. The intensity variation of the low-angle scatter was measured using a Leeds and Northrup automatic recording microphotometer.

Results and discussion.— The low-angle scatter of X-rays from longitudinal sections of bone shows a marked asymmetry (Fig. *a*), indicating an elongation of the elements or units responsible for the scatter along the longitudinal axis of the bone. On the contrary, the low-angle scatter obtained from cross-sections of bone (Fig. *b*) reveals no appreciable orientation.

This behaviour suggests, in agreement with ENGSTRÖM and FINEAN, that the scattering elements are well aligned and symmetrical around their long axes. Therefore the same elements appear as if they were like ellipsoids of revolution, the long axes being

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[5] M. J. DALLEMAGNE, J. Physiol. *43*, 425 (1951).

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[7] J. B. FINEAN, J. sci. Instr. *30*, 60 (1953).

[8] J. B. FINEAN and A. ENGSTRÖM have observed that with a system of the type existing in the bone tissue, the use of Ni-filtered radiation (CuKα) does not entail more substantial errors than the use of monochromatic radiation.