which are undergoing mitosis. According to MARREC[16] and DEMAL[17], these are 'stem cells' of the hemopoietic lines; the ophthalmic artery and the hepatopancreas are the places where some lines migrate and differentiate.

In the reticular connective tissue, around the ophthalmic artery and in the spaces between the caeca of the hepatopancreas, several very large eosinophilic, PAS positive cells are present together with granulated hemocytes and fagocytes.

By light and electron microscopy several stages of differentiation of the eosinophilic cells have been identified as cells characterized by the presence of one or few compact inclusions which fill most of the cellular body. The inclusions are not contained in vacuoles, although sometimes membrane profiles are seen around them (Figure 1); the material has a granular structure and in several cases shows a number of crystalline bodies formed by a succession of parallel dark and clear bands repeating every 130 Å (Figures 2 and 3). The dark bands are apparently composed of a regular alignement of dense granules with a periodicity of 120 Å. These dimensions correspond to the 16 S component of the crustacean Hcy[3]. In the last stages of maturation, the cells show a small peripheric nucleus and no longer contain cytoplasmic organelles except for a few reticulum profiles. Supposedly they disrupt and discharge their content in the nearby circulatory sinuses.

On the basis of the dimensions of the granular and the crystalline material, these cells are homologous to the cyanoblasts and the cyanocytes described in *Limulus*. The coloured reaction with rubeanic acid is very faint on account of the low copper concentration of crustacean Hcy (0,17%) whereas the PAS positive reaction is probably due to the polysaccharide fraction of the Hcy molecule[3,17]. As demonstrated by the fluorescent reaction, only the cells which have been identified by light and electron microscopy as cyanoblasts and cyanocytes in the hemopoietic tissue of *Carcinus maenas* react with the specific antibody for *Carcinus* Hcy (Figure 4)[18].

The ferritin conjugated specific antibody will be employed for the identification of the crystalline material with Hcy. This technique will also be used with the aim of establishing the cell lineage. The analysis by optical diffraction of the crystalline bodies is under study.

Riassunto. Nel tessuto emopoietico di *Carcinus maenas* sono state identificate le cellule che sintetizzano l'emocianina. Queste cellule contengono materiale che al microscopio elettronico presenta un aspetto granulare con particelle di dimensioni costanti simili a quelle dell'emocianina circolante e che in molti punti sono organizzate in una struttura cristallina. L'identità di questo materiale con l'emocianina è stata dimostrata col metodo dell'immunofluorescenza.

ANNA GHIRETTI-MAGALDI, CARLA MILANESI and B. SALVATO[19]

Centro per lo studio della Fisiologia e Biochimica delle Emocianine del Consiglio Nazionale delle Ricerche, Istituto di Biologia Animale dell'Università di Padova. Via Loredan 10, I-35100 Padova (Italy), 5 April 1973.

[16] M. MARREC, Bull. Inst. Océanogr. Monaco *867*, 1 (1944).
[17] J. DEMAL, Céllule *56*, 87 (1953).
[18] V. ALBERGONI, A. CASSINI and B. SALVATO, Comp. Biochem Physiol. *41 B*, 445 (1972).
[19] Thanks are due to Mr. G. TOGNON for technical assistance; to Prof. P. OMODEO for advice and criticism and to Prof. V. ALBERGONI and Dr. P. BURIGHEL for help and discussion.

The Occurrence of a Band of Nuclei in Primary Neural Induction in the Chick Embryo

Primary neural induction has been previously deduced by HARA[1] to occur in the very early stage 5 chick embryo[2]. At this point in development the presumptive head mesenchyme cells are present as a mass anterior to Hensen's node but as yet have not formed any notochord. The ectoderm overlying these mesenchyme cells has thickened. Neural folds are not present.

When the very early stage 5 embryo is fixed in Bouin's or Carnoy's fluid and sectioned transversely at 10 µm a band of nuclei can be seen in the ectoderm overlying the presumptive notochord cells (Figure 1). This appearance lasts for approximately one half hour. The band of nuclei appears at the time at which HARA found induction to occur. It is thus a natural marker indicating that induction is occurring.

This band of nuclei is also visible when induction is brought about experimentally. A 'pocket' was formed in a stage 3.3/4 host embryo by inserting a scalpel between the ectoderm and endoderm near the area opaca border on a level with Hensen's node. A stage 4 Hensen's node (0.3 mm × 0.4 mm) was excised from a donor chick embryo and then transferred by pipette to the host embryo. The graft was then inserted into the 'pocket' so that the graft endoderm was adjacent to the host ectoderm. The host embryo was incubated at 38 °C. 72 embryos were prepared in this manner. 6 were fixed at 0 min, and further groups of 6 at 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 16–24 h. Both the ectoderm of the graft and host were examined to determine: 1. the length of time between implanting the graft of Hensen's node into a host and the corresponding neural induction in the host ectoderm and 2. the time between the node graft forming a band of nuclei and the host ectoderm forming a band of nuclei.

A band of nuclei appeared in the graft ectoderm by 3 h (Figure 2). The host ectoderm responded similarly by

[1] K. HARA, Ph. D. Thesis, University of Utrecht (1961).
[2] V. HAMBURGER and H. L. HAMILTON, J. Morph. *88*, 49 (1951).

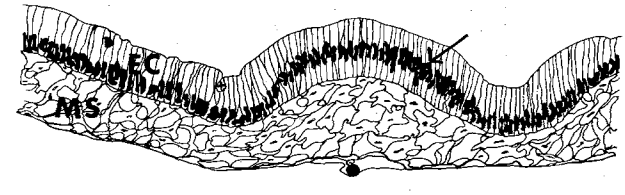

Fig. 1. Transverse section anterior to Hensen's node of the normal early stage 5 chick embryo. Note the band of nuclei (arrow) in the ectoderm. EC, ectoderm; MS, mesoderm.

6 to 8 h (Figure 3). In one instance however, the nuclear band formed by 3 h. The appearance of the nuclear band at 6 to 8 h agrees with the conclusion by GALLERA[3], who showed that host ectoderm differentiated into a neural plate after only 6–8 1/2 h of contact with the inductor.

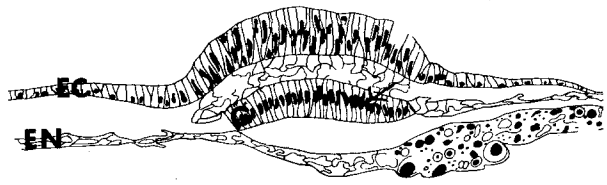

Fig. 2. Transverse section of a graft after 6 h incubation in a chick embryo host. Note the band of nuclei (arrow) in the graft ectoderm. G, graft ectoderm; EC, host ectoderm; EN, endoderm.

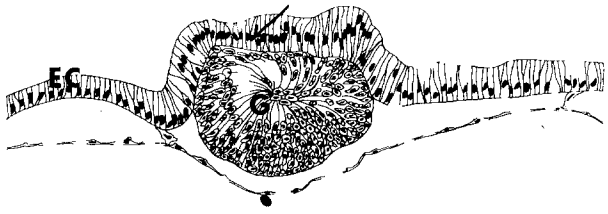

Fig. 3. Transverse section of a graft after 10 h incubation in a chick embryo host. Note the band of nuclei (arrow) in the host ectoderm. EC, host ectoderm. The graft ectoderm has formed a neural tube. G, graft neural tube.

The nuclear band in the graft ectoderm appears approximately 3 to 5 h before that in the host ectoderm. This band in the graft is comparable to the one seen in the unoperated embryo at the time of induction. Thus, it is concluded that in experimental specimens the presence of a nuclear band in graft ectoderm is an indication that the host ectoderm is being induced at this time. This suggests that primary neural induction takes place approximately 3–5 h after the graft is induced, even though the morphological results are not visible in the host ectoderm for another 3–5 h.

Résumé. L'induction primaire du système nerveux est étudiée chez l'embryon normal de poulet. Un nouveau marqueur morphologique de cette induction est découvert dans l'ectoderme. Au moment de l'induction (étape −5), une bande de noyaux apparaît dans l'ectoderme en avant du nœud de Hensen. Dans une autre série d'expériences, on a implanté un greffon de nœud de Hensen dans un autre embryon. Une bande de noyaux est aussi apparue dans l'ectoderme du greffon 3 h plus tard et dans l'ectoderme de l'hôte, 6 à 8 h après l'opération.

MARJORIE A. ENGLAND[4]

Department of Anatomy and Embryology
University College, London, W.C. 1, (England),
9 April 1973.

[3] J. GALLERA, Experientia *21*, 218 (1965).
[4] Present address: Department of Anatomy, Royal Free Hospital School of Medicine, 8 Hunter Street, London W.C.1. (England).

Structural Basis for Resetting of Baroreceptor Regulation in Spontaneously Hypertensive Rats (SHR)

A large variety of mechanisms have been considered which may account for the high pressure level of genetic hypertensive rats[1,2]. Among these we mention hypertrophy of the vascular wall of the resistance vessels[2] and insufficiency of the sympathoinhibitory role of a noradrenergic mechanism in the brain stem[3]. These mechanisms tend to override or to diminish the baroreceptor regulatory mechanisms. However, it must be admitted that the baroreceptor reflexes still function very effectively in SHR. They have been shifted to a higher set-point of the blood pressure[4,5]. This type of functional modification can be expected if the input signal at the level of the stretch receptors has been damped. Therefore, our investigation was focused on the possibility of structural differences of the vessel wall in the stretch receptor areas of the carotid sinus and the aortic arch.

Material and methods. Spontaneously hypertensive rats of the Okamoto-strain[6] and normotensive Wistar rats of our own permanent laboratory colony established in 1956, were studied. The rats were transiently anaesthetized with ether and the femoral artery was catheterized under local anaesthesia with lidocaine. The animals were transferred in a specially designed cage[7], allowing continuous blood pressure measurement. The femoral blood pressure of the awake rats was recorded during a minimum of 30 min.

Subsequently, the animals were anaesthetized with Hypnorm® (haloanisone 10 mg/kg and fentanyl 0.1 mg/kg, s.c.). The abdominal caval vein was sectioned and the abdominal aorta was perfused retrogradely at 140 mm Hg with heparinized Ringer solution followed by 2% glutaraldehyde in 0.1 *M* cacodylate buffer (5 min). Sampling of artery segments was done at the following topographical spots: the common, internal and external carotid arteries at 2 mm from bifurcation; the aortic arch at 1 cm from the heart and the abdominal aorta 1 cm before the offspring of both external iliac arteries. These segments were post-fixed in 1.5% OsO_4 and embedded in Epon.

Transversely cut, semi-thick sections were prepared and stained using Masson's tri-chrome technique, adapted for Epon embedded tissue. Electron microscopic examination of the same material is in progress.

[1] K. OKAMOTO, in *International Review of Experimental Pathology* (Ed. G. W. RICHTER and M. A. EPSTEIN; Academic Press, New York 1969), vol. 7, p. 22.7.
[2] B. FOLKOW, M. HALLBÄCK, Y. LUNDGREN, R. SIVERTSSON and L. WEISS, in *Spontaneous Hypertension* (Ed. K. OKAMOTO; Igaka Skoin Ltd. Tokyo 1972), p. 103.
[3] Y. YAMORI, W. LOVENBERG and A. SJOERDSMA, Science *170*, 544 (1970).
[4] S. NOSAKA and S. C. WANG, *Spontaneous Hypertension* (Ed. K. OKAMOTO; Igaka Skoin Ltd., Tokyo 1972), p. 79.
[5] S. NOSAKA and S. C. WANG, Am. J. Physiol. *222*, 1079 (1972).
[6] We are indebted to Dr. T. PRUSS (McNeil Laboratories, Fort Washington USA) for donation of breeder animals.
[7] J. L. BOLLMAN, J. Lab. clin. Med. *33*, 1348 (1948).