2/3rds of the urethra although none was observed in the distal portion below the level of the sphincter urethrae muscle. In the male, fluorescent cells occurred in that region of the prostatic urethra extending from just below the internal urethral meatus to the point of entry of the ejaculatory ducts. In all specimens examined no fluorescent cells were observed in distal parts of the urethra.

Electron microscopy of female urethral epithelium revealed numerous cells which could be distinguished by their content of dense, cytoplasmic granules (Figure 2). The granules varied in diameter from 80 nm to 100 nm and each was bound by a membrane and contained a central electron dense core. Thus cells similar to those described in the male urethra[4] have now been defined electron microscopically in the human female. However, further study is required to establish whether two types of granule-containing cell (as described by CASANOVA et al.[4]) also occur in the female.

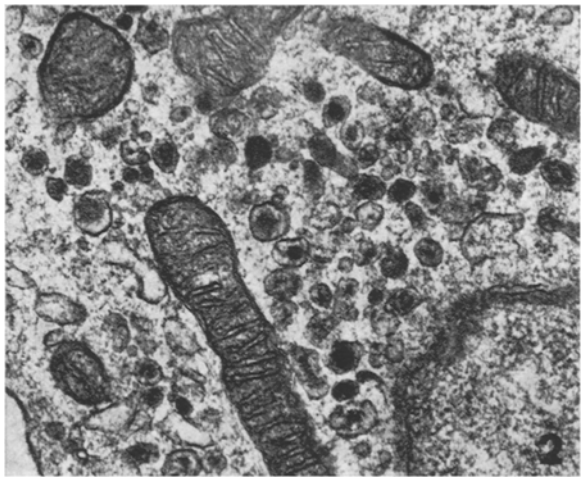

Fig. 2. Electron micrograph of an endocrine-like cell in human female urethral epithelium. The cell is characterized by the presence of numerous electron dense cytoplasmic granules (80–100 nm diameter). ×36,000.

Collectively, the present observations have confirmed endocrine-like 'APUD' cells[6] in the human urethral epithelium and have also established the distribution of these cells in both male and female specimens. Their similarity to enterochromaffin cells of the alimentary tract raises the possibility that the embryological development of the region may explain the presence of this cell type in the urethral epithelium. On the assumption that the epithelium of the cloaca normally includes enterochromaffin cell precursors, septation of the cloaca by the urorectal septum would, therefore, result in the inclusion of this type of cell in the epithelium of the primitive urogenital sinus. Thus, the distribution of such cells in the adult might indicate those parts of the urinary tract to which the cloaca directly contributes. Interestingly, most embryologists consider as developmentally analogous those parts of the male and female urethra which have now been shown to possess endocrine-like cells. Clearly more work is required to verify this hypothesis and further study using foetal material is currently in progress in this laboratory.

Finally, the occurrence of this type of cell in normal urethral epithelium should be borne in mind when examining the cytology of urethral tumours. The possibility exists that these endocrine-like cells might undergo neoplastic change similar to that known to occur in carcinoid and related conditions of the alimentary tract.

[6] A. G. E. PEARSE, J. Histochem. Cytochem. *17*, 303 (1969).

Differentiation in Renal Homografts of Isolated Parts of Rat Embryonic Ectoderm[1]

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Summary. The central and the peripheral areas of the head-fold stage rat embryo ectoderm develop into both the neural tissue and the epidermis when grafted under the kidney capsule of adult rats.

During gastrulation, the primitive or primary ectoderm of the rat embryo undergoes a restriction of developmental potentialities. At the pre-primitive streak, and at the early primitive streak stages, it contains presumptive cells of all three definitive germ layers. At the head-fold stage, however, it contains only the definitive ectodermal and the presumptive mesodermal cells[2,3].

The purpose of the present pilot experiment has been to show whether the central and the peripheral parts of the head-fold stage rat embryonic ectoderm differ in their capacity to differentiate into two major ectodermal derivatives: the neural tissue and the epidermis. We have previously shown that at this developmental stage the endoderm + mesoderm combination already displays a regionally restricted capacity to differentiate into different segments of the primitive gut, when transplanted under the kidney capsule of adult animals[4].

Pregnant females of the inbred Fischer strain albino rat were anaesthesized with ether on the gestation day 9 (at 08.00 h on the 10th day). Whole embryos (embryonic shield + extra-embryonic membranes + ectoplacental cone) were isolated from the uteri. They belonged to the

[1] This investigation was supported by the grant No. IV/3 from the Research Foundation of S.R. Croatia, and in part by NIH PL 480 Agreement No. 02-038-1.

[2] B. LEVAK-ŠVAJGER and A. ŠVAJGER, Experientia *27*, 683 (1971).

[3] B. LEVAK-ŠVAJGER and A. ŠVAJGER, J. Embryol. exp. Morph. *32*, 445 (1974).

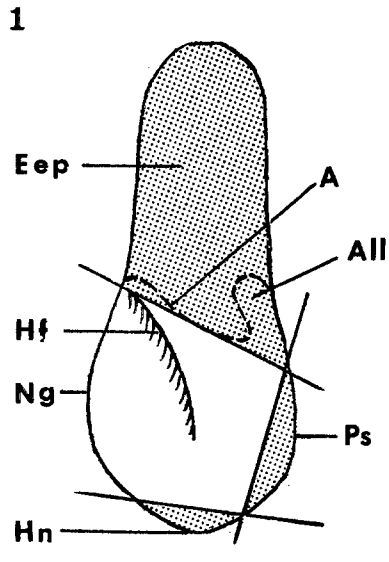
[4] A. ŠVAJGER and B. LEVAK-ŠVAJGER, J. Embryol. exp. Morph. *32*, 461 (1974).

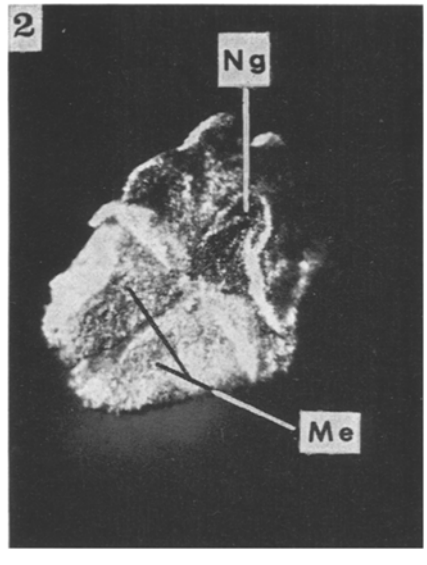
Differentiation of mature tissues in renal homografts of isolated parts of rat embryonic ectoderm

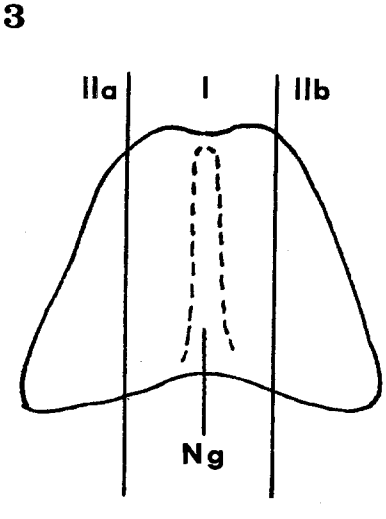
Series	Graft	No. of cases	Neural tissue	Epidermis and derivatives	Cartilage	Bone	Skeletal muscle
I	Central area	16	16	11	12	8	6
IIa + IIb	Peripheral area	16	16	14	8	2	5

stage 15 of NICHOLAS[5], or the stage 14 of WITSCHI[6]. The extra-embryonic part, the primitive streak and the Hensen's node areas were cut off (Figure 1). The previously cup-shaped egg-cylinder was transformed into a flat, bi-lobated shield. In this form the embryos were treated with enzymes: 0.5% trypsin (crystallized, lyophilized, Worthington) + 2.5% pancreatin (Difco) in the calcium- and magnesium-free Tyrode's saline, at +4 °C for 30 min[7]. After this treatment, the ectoderm was removed from the underlying layers of the embryonic shield (endoderm + mesoderm). The neural groove was clearly visible as a median invagination of the ectoderm (Figure 2). Subsequently, the ectoderm was divided into 3 parts by 2 cuts parallel with the neural groove: the central (anteromedian) area (I) comprizing the neural groove, and 2 peripheral areas (IIa and IIb) comprizing the ectoderm lateral to the neural groove (Figure 3.) (For better orientation, the corresponding areas on the more familiar chick epiblast are depicted in Figure 4). The area I of each embryo was grafted alone, and the areas IIa and IIb were grafted close to one another under the kidney capsule of syngeneic adult males. After 15 days, the host animals were killed and the teratomas were

[5] J. S. NICHOLAS, in *The Rat in Laboratory Investigation* (Eds. E. J. PARRIS and J. A. GRIFFITH; Hafner, New York 1962), p. 51.
[6] D. A. T. NEW, *The Culture of Vertebrate Embryos* (Logos Press, London 1966).
[7] B. LEVAK-ŠVAJGER, A. ŠVAJGER and N. ŠKREB, Experientia *25*, 1311 (1969).







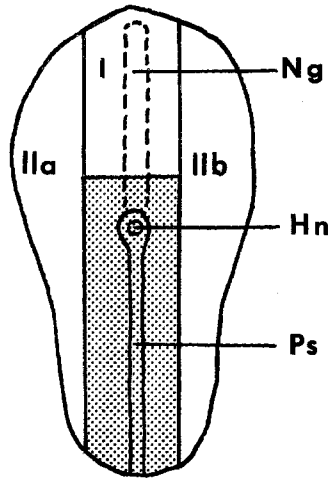


Fig. 1. Profile view of the head-fold stage rat embryo. Shadowed areas were cut off.

Fig. 2. Photograph of an embryonic shield with the ectoderm almost completely separated from the underlying mesoderm + endoderm.

Fig. 3. Diagram of the operation on the isolated rat embryonic ectoderm.

Fig. 4. Diagram of a head-process stage chick epiblast. To be compared with Figures 1 and 3 for better understanding of more intricate topographical relationships on the rat embryonic shield. A, amnion; All, allantois; Eep, extra embryonic part; Hf, head-fold; Hn, Hensen's node; Me, mesoderm; Ng, neural groove; Ps, primitive streak; I, central (anteromedian) area of ectoderm; IIa and b, peripheral (lateral) areas of the ectoderm.

isolated, subjected to the routine histological procedure, and examined for the presence of mature tissues.

The results of the histological examination are summarized in the Table. It is obvious that, in the present experimental conditions, the two areas of the ectoderm do not significantly differ in their capacity to differentiate into the neural tissue and the epidermis and its derivatives. Quantitatively, the differentiation of neural tissue was predominant in grafts of both series. The possible explanations of this feature (to be further analyzed in a more extensive study) are: a) the cuts, by which the ectoderm was divided into 3 areas, did not correspond to the demarcation between the presumptive neurectoderm and the presumptive epidermis; b) the 2 developmental capacities of the ectodermal cells are not yet strictly regionally restricted at this stage; c) the competences of different areas of the ectoderm are not yet definitively stabilized; d) the atypical environment has led to the partial neuralization of the lateral areas of the ectoderm (presumptive epidermis?); e) different combinations of the above-mentioned possibilities.

We have previously shown that the head-fold stage rat embryonic ectoderm still contains some prospective mesodermal cells[3]. In the present experiment, mesodermal tissues appeared quite regularly in both series, although the grafted areas of the ectoderm were lacking the regions of in situ immigration of prospective mesodermal cells (primitive streak, Hensen's node). This can be explained by at least 2 possibilities: a) some cartilage and bone may have originated from the neural crest cells ('mesectoderm'); and b) in experimental conditions, the prospective mesodermal cells can segregate and differentiate in any area of the ectoderm ('regeneration' of the primitive streak).

Cytochemical Evidence for Stage-Specific Changes of Nuclear RNA and Nonhistone Protein Content During Early Development of *Triturus vulgaris*

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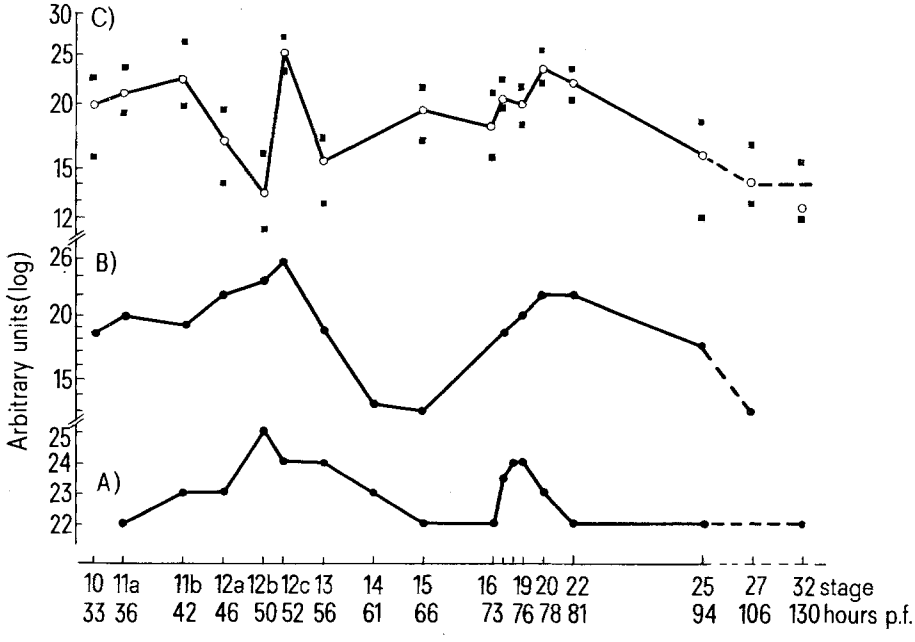
Summary. During early embryogenesis of *Triturus vulgaris*, RNA and nonhistone protein contents of neuroectoderm nuclei change with stage specifically. Maximum values were found in the late gastrula after embryonic induction, and in the late neurula with the formation of the neural tube. The stage-specific increases of RNA and nonhistone protein are correlated with a preceding increase of Feulgen-DNA content.

In recent years much evidence has been accumulated to show that nonhistone proteins participate in regulation of transcription[1]. On the other hand, studies on synthesis and turnover of nuclear precursor-RNA led to new hypotheses and models about the mechanism of gene expression in eukaryotic cells[2].

From cytophotometric measurements of Feulgen-DNA content in various regions and developmental stages of *Triturus vulgaris*, which did not agree with the theory of DNA constancy, we made the assumption that the phase-specific increase of DNA might cause an enhancement of gene activity in definite regions of the embryo[3–5].

Materials and methods. From small pieces of neuroectoderm (from early gastrula to tailbud) nuclei smears were prepared as described earlier[4]. Nonhistone proteins were stained for 30 min with 0.1% fast green FCF at pH 2.0, according to the procedure of KAYE and MCMASTER-KAYE[6]. Before staining nucleic acids were removed with hot TCA (5%, 90 °C, 15 min), and then histones were extracted by immersion of the slides in 0.1 *N* HCl



Changes of DNA (curve A), RNA (curve B) and nonhistone protein (curve C) content of neuroectoderm nuclei during development of *Triturus vulgaris*. Ordinate: relative dye content in arbitrary units. Abscissa: developmental stages (Harrison) and h after fertilization (18 °C). The points in curve C illustrate the medians with the 95% confidence limits.