

regions of 206–248 nm. The boundaries of this energy region are purely empirical. There are several exceptions to this rule in the vicinity of the border wavelengths.

From table 1, one can see how the above rule solves the problem of structural isomerism, which cannot be taken into account by the criterion based on the average quasi-valence number.

The other, more important benefit from the established correlation could be connected with the mechanism of carcinogenesis. The discovery of correlation gives support to the basic assumption made on the mechanism of carcinogenesis. It proves the similarity of action for different carcinogenic agents. Additional facts in favour of postulated mechanism are found in table 3, where relevant spectral data and carcinogenic properties of some steroid hormones are given. Most of the listed hormones are carcinogenic, in agreement with the rule stated above. On the other hand, it is well known that hormones play an important role in differentiation, in process of deblocking the relevant part of DNA. Such a deblocking represents decisive step in carcinogenesis, as envisaged by the proposed mechanism.

By combining findings from this paper with those of preceding work¹, we can state the following rule: if the organic substance has the average quasi-valence number less than 3.20, and if its spectrum shows absorption peak(s) in the wavelength region of 206–248 nm, it should be

considered (with high probability) as carcinogenic. The organic substance which does not satisfy the above requirements should be noncarcinogenic.

On the basis of the analyzed organic substances considered in IARC Monographs², we found that the above rule is fulfilled in 91% of the cases. While our finding could be used for preselection of organic substances with respect to carcinogenicity, its main importance is, however, related to the mechanism of carcinogenesis.

The 'island' of chemical carcinogenicity, encompassed by the average quasi-valence number and the UV-spectral requirement, could be taken as a guide for the further studies of the basic cancer mechanism.

- 1 V. Veljković and D.I. Lalović, *Experientia* 33, 1228 (1977).
- 2 IARC Monographs, Evaluation of Carcinogenic Risk, vol. 1–16. Lyon, 1972/1978.
- 3 H. Busch, in: *Molecular Biology of the Cancer*. Academic Press, New York 1974.
- 4 Similar hypothesis already exists, see B.J. Culliton, *Science* 177, 44 (1972).
- 5 H.F. Blum, in: *Carcinogenesis by Ultraviolet Light*. Princeton University Press, Princeton 1959.
- 6 A.I. Scott, in: *Interpretation of the UV-Spectra of Natural Products*, p. 7. Pergamon Press, London 1964.

Quantitative aspects of structural changes in chorioallantoic placenta of the rat during its development¹

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Summary. Volume analysis of chorioallantoic placenta of the rat from day 12 through day 22 of fetal development shows quantitatively the changes in volume density of fetal and maternal parts, and changes of volume fractions of structural components along with the increase of absolute volume of the placenta.

The placenta differs from other organs in its origin. It develops from maternal and fetal tissue into an integral morphologically differentiated organ. Considerable changes in the quantitative relation between the parts of maternal and fetal origin have been observed in the course of complex developmental processes of chorioallantoic placenta of the rat. The structure of that placenta is also subjected to various changes, and characteristic histomorphological features arise in both parts of the placenta during its development². The kinetic of these developmental processes is also manifested by constant changes in quantitative relations of these structural components³. The quantitative changes of maternal and fetal parts, as well as those of their individual structural components, were investigated by volumetric analysis⁴ in the chorioallantoic placenta of the rat from the initial stage of its development until parturition.

Material and methods. Placentae of Wistar rats were taken on each day from the 12th to the 22nd day of embryonic development. They were fixed in Gendre's solution and embedded in paraffin. The 7 μ m thick serial sections were stained with hematoxylin eosin and Masson's trichrome stain. In addition PAS reaction and PAS reaction with the diastasis test were made.

Weibel's multipurpose test system was used for point counting volumetry^{5,6}. For each day of embryonic development, the relative volume density of the following components was determined: a) maternal and fetal parts, b) their integral parts: decidua basalis and its blood vessels, labyrinth, basophil, giant and glycogen cells, and spaces

arising from cytolysis of glycogen cells. By the same method the absolute volume of placenta was determined on days 13, 16, 19 and 22 of embryonic development. Statistical evaluation of the data included calculation of arithmetic means of results obtained for each component by day of observation, and the SE.

Results and discussion. The results of volumetric analysis display numerically the constant changes in the quantitative relations of integral parts of the placenta from the beginning of its formation to parturition. In the initial developmental stage, the chorioallantoic placenta consists almost entirely of maternal tissue ($V_{vm}0.94, \pm 0.02$) with a minimum volume participation of fetal tissue ($V_{vf}0.0$), (figure 1). In the following days, this relation rapidly changes in favour of the fetal part, so that on the 14th day the fetal part ($V_{vf}0.53, \pm 0.03$) exceeds the maternal ($V_{vm}0.47, \pm 0.03$). The ratio between fetal and maternal elements continues to change in the same direction. From the 16th day of embryonic development until parturition, the relation between fetal and maternal parts does not show any essential changes. The volume density of the fetal part then amounts to $V_{vf}0.96, \pm 0.03$. These data indicate that, at the end of embryonic development, the relation between structural elements of maternal and fetal origin is inversely proportional to the relation of these elements in the beginning of placental development.

The volume analysis of structural elements of maternal origin shows (figure 2) that, in the initial stage of placental development, the decidua basalis has the greatest share ($V_{vd}0.79, \pm 0.02$) and the decidual blood vessels the smaller

part ($V_{vd}, 0.15, \pm 0.01$). In the diminution of the maternal part, both components are involved but the decidua basalis to a greater extent than its blood vessels. After the 17th day, they are represented in the placenta with approximately the same small quantities ($V_v 0.03, \pm 0.00$).

The volume analysis of structural elements of fetal origin shows that the labyrinth, after its differentiation, i.e. from the 13th day onward, constantly grows and attains at the end of embryonic development the greatest volume participation in the placenta ($V_{vl} 0.86, \pm 0.06$).

The volume analysis of structures of fetal origin shows an intensive kinetic of development of this part of placenta. In the beginning of placental development, the fetal part consists of basophil and giant cells in about equal small volume proportions ($V_v 0.03, \pm 0.00$). These values increase in the days to follow. The volume density of basophil cells attains the maximum value on the 17th day ($V_{vb} 0.22, \pm 0.03$). The volume density of giant cells begins to decrease on the 13th day ($V_{vg} 0.09, \pm 0.01$) and from the 17th day the decrease

becomes faster ($V_{vg} 0.06, \pm 0.01$). The labyrinth has a share in the volume of the fetal part of placenta from the 13th day on. Its volume density continuously increases, so that as early as on the 16th day the labyrinth makes half of the relative volume values ($V_{vl} 0.51, \pm 0.02$) and on the 22nd day it represents with its $V_{vl} 0.86, \pm 0.06$ by far the greatest part of fetal components of the placenta, and the greatest part of the placenta as a whole. With the differentiation of the fetal part of placenta, some new elements appear. Glycogen cells appear on the 15th day. Their maximum volume density ($V_{vg} 0.19, \pm 0.01$) diminishes after the 16th day because these cells undergo cytolysis. The volume density of cytolitic spaces increases from day 17 ($V_{vc} 0.03, \pm 0.00$) through 20 ($V_{vc} 0.08, \pm 0.01$) and then this value too decreases.

The absolute volume of the placenta determined by volumetric analysis was 0.98 mm^3 on day 13, 4.75 mm^3 on day 16, 7.20 mm^3 on day 19 and 9.53 mm^3 on day 22. The increase of absolute volumen of the placenta, which becomes almost 10fold, does not proceed evenly. The

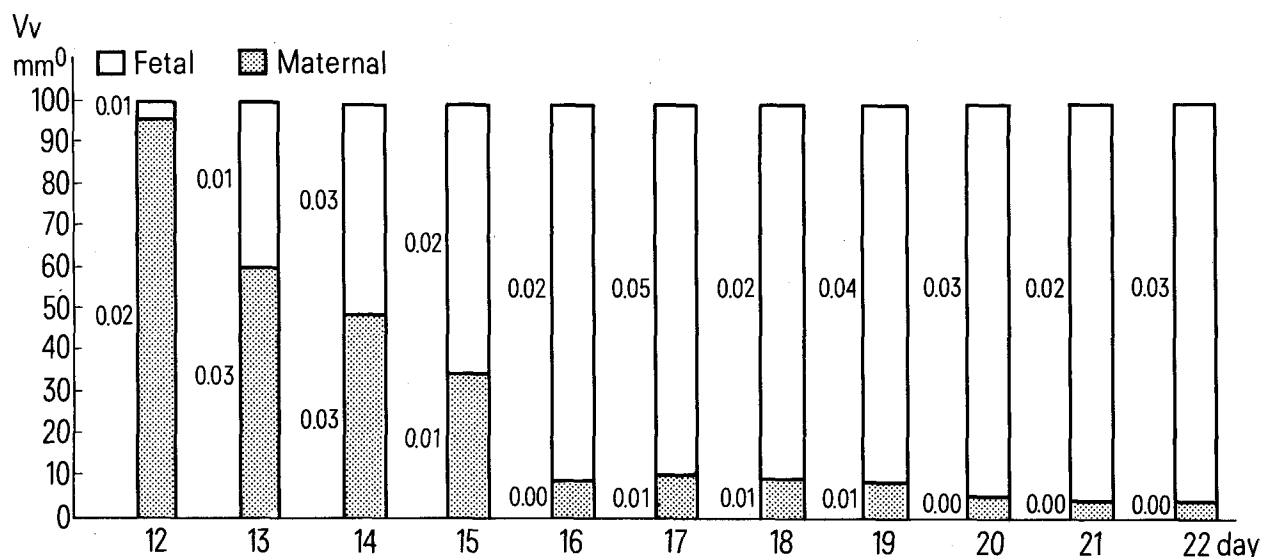


Fig. 1. Mean arithmetic volume densities of maternal (V_{vm}) and fetal (V_{vl}) origin in chorioallantoic placenta of the rat. The number beside the column indicates the SE.

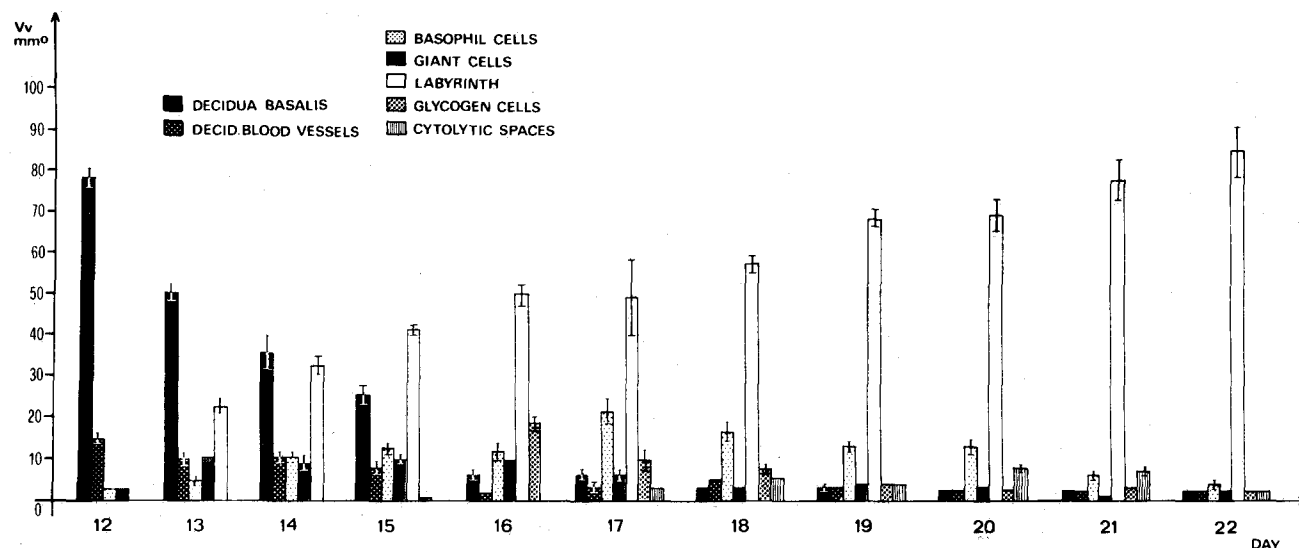


Fig. 2. Volume density (arithmetic means and SE) of decidua basalis (V_{vd}), decidual blood vessels (V_{vdv}), basophil cells (V_{vb}), giant cells (V_{vg}), labyrinth (V_{vl}), glycogen cells (V_{vgi}) and cytolitic spaces (V_{vc}).

greatest rise was observed between the 13th and 16th day (3.77 mm^3). Thereafter the increase in volume gradually slowed down, so that in the period from day 16 through 19 the volume increased for 2.45 mm^3 and from day 20 through 22 for 2.33 mm^3 .

Normal development of the placenta ensures its adequate exchange function and is a condition for normal growth and development of the fetus. By comparing the findings of my earlier study⁷ concerning the quantitative analysis of fetal growth with those of the present study, it becomes evident that the intensive increase of absolute volume of the placenta in the course of its development precedes the period of the greatest fetal weight gain. In the labyrinth the fetal blood vessels intertwined with maternal sinuses form the morphological base of the placenta as an exchange

organ. The constant increase of volume density of the labyrinth, and its greatest share in the volume of the completely developed placenta, is a quantitative morphological manifestation of intensive feto-maternal exchange function.

- 1 This work was supported by a grant of the Republic Research fund of Croatia No. 18-04-06/19-1977.
- 2 J. Davies and S.R. Glasser, *Acta anat.* 69, 542 (1968).
- 3 M.A. Kenney, *Nutr. Rep. int.* 11, 141 (1975).
- 4 R. Baur, *Acta anat.*, suppl. 86, 75 (1973).
- 5 M. Kališnik, *Osnove stereologije*. Prir. društvo, Ljubljana 1976.
- 6 E.R. Weibel, *J. Microsc.* 100, 261 (1974).
- 7 B. Durst-Živković, *Experientia* 33, 1371 (1977).

Anatomical identification of the presumed electroreceptors of two air breathing catfishes, *Clarias batrachus* and *Heteropneustes fossilis*¹

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Summary. Histological preparations for light microscopy have revealed for the first time the structure of the electroreceptor of *Clarias batrachus* and *Heteropneustes fossilis*, the 2 Indian air-breathing catfishes. These sensory organs are found to resemble the ampullary organs of many weakly electric and nonelectric electroreceptive teleost.

Histological studies of the skin of *Clarias batrachus* and that of *Heteropneustes fossilis* reveal the presence of a category of cutaneous receptors which are different from either the taste buds or the ordinary lateral line organs so far reported for these fishes^{2,3}. These receptors present a number of morphological features which are similar to those of the ampullary organs of certain teleosts^{4,5}, on the basis of which these receptors will henceforth be called the ampullary organs. The description of the ampullary organ given below applies to both *Clarias batrachus* and *Heteropneustes fossilis*, unless stated otherwise.

Material and method. *Clarias batrachus* and *Heteropneustes fossilis* are locally available in plenty all the year round. They are also sold alive in the market, as these catfishes are airbreathing and hardy to keep. The fishes were kept in aquaria when necessary for long periods in good health; they are fed on minced liver.

Ampullary organs are easily detectable in the skin of the back under a binocular. Pieces of the skin containing ampullary organs were removed and fixed in Bouin's fluid. Paraffin sections were cut 6 to 8 μm thick and stained with iron haematoxylin and delafield haematoxylin eosin. Likewise pieces of proximal part of nerve dorsal ramus were prepared to locate the ganglion cells. Ampullary organs were also denervated by transectioning the nerve a few mm away from its ganglion, and removed after varying periods ranging from 15 days to 45 days post operative and prepared as above.

For central connection of this nerve, the nerve was transected in region lying between its ganglion and brain stem, in fish anesthetized with MS 222 (Sandoz). Brain was removed after 1-2 months post operative and processed according to Fink and Heimer technique.

Results and discussion. Each ampullary organ consists of a single ampulla or a group of 2 or 3 ampullae, bearing receptor cells in the epithelium (figure 1). The ampulla or the group of ampullae opens to the exterior by means of a single duct, having a well-defined wall of its own (figure 3). The duct is short and intraepidermal, whereas the ampulla

bulges into the dermis though separated from it by the basement membrane. The receptor cells are large, ovoid and hypertrophied. An appreciable apical part of the receptor cells is exposed to the lumen of the ampulla (figure 4).

The ampullary organs are easily contrasted from taste buds or ordinary lateral line organs by surface examination. They appear in the dark pigmented skin as white spots which are largest in diameter and show a central opening. The ampullary organs are distributed over the dorsal surface of the body, occurring singly in rows or in groups of a few. Those on the hinder part of the head and on the trunk and tail are innervated by a special nerve, one on each side of the body (figure 5). This nerve – ramus dorsalis, a branch of VIIIth cranial nerve – reaches the hind brain just where the anterior, cranial branches of the VIIth nerve enter. However, the ramus dorsalis is found to retain its individuality inside the brain by way of its specific central pathways, as revealed by degeneration studies on this nerve according to Fink and Heimer technique, and outside the brain by the presence of its ganglion. Transection of this nerve leads, in course of 18 days, to degeneration of the ampullary organs in general and receptor cells in particular (figure 2).

The skin around the ampullary organs is the same as elsewhere. The epidermis appears ordinary, though thick and rich in club cells (Bhatti⁶, quoting Rauther 1907). The dermis, however, presents a specialized, thick, subepidermal layer composed of closely set bundles of collagen fibres.

The ampullary organs of the electric and nonelectric teleosts, so far investigated, are held to be electroreceptors⁵ involved in their well-demonstrated electroreception mechanism. *Clarias* and *Heteropneustes* are known to respond to moving magnets⁷, and *Clarias* is one of the few fishes in which electrosensitivity was early demonstrated⁷. However, so far no anatomical identification of the likely electroreceptors was forthcoming for these 2 fishes. The ampullary organs of these fishes, here described for the first