

et al.<sup>5</sup>, dans une étude des «réseaux admirables» du rein chez le rat, décrivent une intense activité estérasiqne dans l'endothélium des artérioles droites fausses. ARVY<sup>6</sup>, démontre une intense activité cholinestérasiqne dans la paroi des artères interlobulaires, des artères afférentes et des glomérules mais l'attribue à l'acétylcholinestérase.

D'après nos constatations personnelles, les butyrylcholinestérases ont une localisation très particulière dans le rein du rat, puisque cette activité enzymatique n'apparaît que dans la paroi de certains vaisseaux.

La présence d'une importante activité butyrylcholinestérasiqne dans les fibres musculaires lisses de la média des artères lobulaires et arquées et dans la paroi des artères interlobulaires et afférentes laisse supposer que les butyrylcholinestérases pourraient intervenir dans la régulation du calibre de l'artère et par conséquent du débit sanguin dans le parenchyme rénal.

Dans les glomérules, les butyrylcholinestérases pourraient intervenir dans la filtration glomérulaire. Dans l'endothélium des artérioles droites fausses, elles pourraient jouer un rôle dans les modifications de perméabilité capillaire qui interviennent dans la régulation du mouvement des molécules et des ions soumis aux échanges par «contre-courant», par passage direct vers les veinules

droites fausses, cheminant parallèlement au contact des artérioles droites fausses et constituant avec elles un «réseau admirable». Le passage direct de certaines de ces substances vers les veinules droites fausses, du fait de «la multiplication de concentration par contre-courant» pourrait contribuer à la formation et à l'entretien de l'hyperosmolarité de la papille rénale.

**Summary.** A pseudocholinesterase activity was found in the wall of some vessels of the rat's kidney. The pseudocholinesterases could interfere in blood flow regulation and in capillary permeability.

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<sup>5</sup> J. B. LONGLEY, W. G. BANFIELD and D. C. BRINDLEY, J. biophys. biochem. Cytol. 7, 103 (1960).

<sup>6</sup> L. ARVY, C. r. Acad. Sci., Paris 256, 1162 (1963).

## Cell Proliferation and Cell Function of the Mesenchymal Cells of Lathyrotic Rats, as Revealed by the Nuclear Size

Several studies have been done in the past to examine the effect of lathyrism on the periodontal ligament of the rats<sup>1-3</sup>. All these investigations mainly dealt with the weanling or adult rats kept on a lathyrus odoratus diet for various periods of time. So far no attempt has been made to produce lathyrism in rats during intrauterine life and to study the effect on the mesenchymal cells of the periodontal ligament after birth. The present study was initiated to study the differences between the lathyrotic and the normal control mesenchymal cells at a particular cellular level. A special technique was used to determine the changes in the size of cell nuclei.

**Materials and methods.** A total of 30 rats were used for this investigation. 10 belonged to the control and 20 to the experimental group. The experimental group was subdivided into 2; 10 rats were recovered from mothers who had received 2 mg of aminoacetonitrile from day 9 to 20 of gestation and the other 10 from mothers who had received 5 mg of aminoacetonitrile from 9 to 20 days of gestation. The rats were sacrificed on day 28, after birth.

The molar teeth were fixed in Susa (according to Heidenhain) decalcified in buffered EDTA and embedded in paraffin. For the determination of the nuclear size 7 µm thick sections were stained according to Goldner. A Leitz projection microscope, built for horizontal projection, was mounted above a plane-table and the beam was bent vertically on the table by means of a prism. With a magnification of 2000:1 the nuclei were drawn from the projected contours and their size was determined with a planimeter. The measured values were classified in a logarithmically divided scale of rhythmic doubling intervals according to HINTZSCHE<sup>4</sup>. Each doubling interval (log 2), which represents the double nuclear volume, was divided into 4 subclasses (1a, b, c). Each subclass differed from the other and from the main class by a factor 1/4 log 2. The nuclear volumes were given in relative units (1-8). 100 nuclei of mesenchymal cells of the peri-

odontal ligament of 28-day-old rats from each of the 2 experimental groups and from the control group were drawn and measured by planimetry.

**Findings.** (Figure). In normal animals the distribution of the nuclear size is unlike a regular Gauss-distribution, because nuclei of a given size were more frequent (1c, 2a, 4, 4b, 8, 8b and c) than other sizes, thus several maxima were seen to spring up. However, the maxima are not distributed in 'rhythmic' doubling intervals. From this it can be inferred that in the normal animals the mesenchymal cells consist of at least 6 cell classes, with the nuclear size varying by about factor 10.

In the experimental animals the mean nuclear volume was distinctly larger than the controls, because in both experimental groups the small-sized nuclei were absent and the percentage of large-sized nuclei was higher. However, only in animals which received 5 mg of aminoacetonitrile a small number (4%) of nuclei belonged to a class which was never observed in the controls (class 16). The base of the curve of 3 nuclear size distributions was smaller than in the normal animals, thus, the nuclear size varied only by the factor 4 to 5. Furthermore, only 2 or 3 maxima were distinguishable, but these maxima were found to be arranged within the scope of 'rhythmic' doubling intervals (4b, 8b, and 2c, 8, 16).

**Discussion.** JACOB<sup>5,6</sup> showed that in tissues and organs with a low rate of physiological regeneration the curve of nuclear volume distribution was unlike a Gauss-distribution, because 'maxima', that means accumulations of nuclei exhibiting nearly the same volume, were discernible.

<sup>1</sup> G. R. CASWELL, Iowa dent. Bull. 42, 193 (1956).

<sup>2</sup> A. F. GARDNER, J. Periodont. 30, 253 (1959).

<sup>3</sup> G. A. KRIKOS, J. dent. Res. 38, 27 (1959).

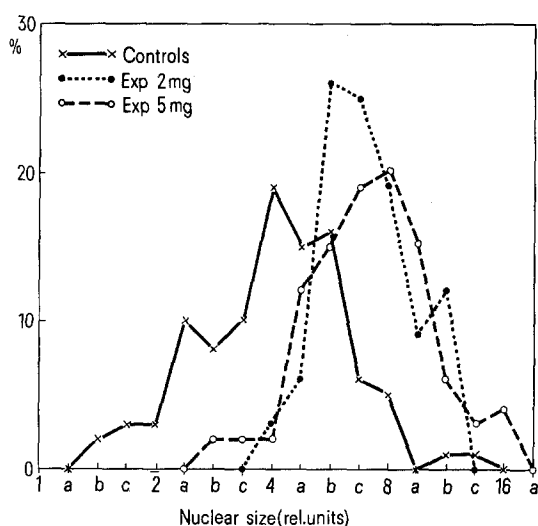
<sup>4</sup> E. HINTZSCHE, Experientia 7, 103 (1945).

<sup>5</sup> W. JACOB, Wilhelm Roux Arch. Entw. Mech. Org. 106, 124 (1925).

<sup>6</sup> W. JACOB, Z. mikrosk.-anat. Forsch. 38, 161 (1935).

The mean nuclear volumes of these maxima are in the ratio of 1:2:4:4 etc. Deviations from this rule were observed when the normal function of the cells changed. BENNINGHOFF<sup>7</sup> found, in instances of increased cell function, an increase of the nuclear volume, and vice-versa a decrease of the nuclear volume when the cell function decreased ('Functional swelling and shrinking'). JERUSALEM<sup>8</sup> pointed out that an increase of the nuclear volume can be a symptom of a cellular lesion e.g. in the kidneys after administration of alloxan, trypan blue and in instances of lower nephron nephrosis ('dystrophic' swelling). In both functional and dystrophic changes, the increase and decrease of the nuclear volume is gradual and does not appear stepwise and particularly not in doubling intervals. Moreover, there was no increase in the number of existing 'maxima'.

According to JERUSALEM and ZAKI<sup>9</sup> the occurrence of new cell populations with different nuclear sizes, which manifests in new 'maxima', is a characteristic sign for tissues showing a high rate of cell proliferation. They further added that the nuclei synthesize DNA which does not result in a gradual but stepwise increase of the nuclear volume. They also observed that, on the other hand, the high rate of mitosis leads to a quick reduction of the nuclear volume when the cell has finished the DNA reduplication phase.



Percentage of nuclear size distribution of mesenchymal cells of the periodontal ligament of 28-day-old control and lathyrus rats.

From the distribution of the nuclear sizes in the experimental animals, it can be concluded that the proliferation tendency of the mesenchymal cells is distinctly lower than in normals because the number of 'maxima' was reduced and the mean nuclear volume of these 'maxima' was in the ratio of about 4:8:16. The accumulation of large sized nuclei points to the assumption that the ability of the mesenchymal cells to enter into mitosis and thus reduce the nuclear volume, might have been delayed. However, it is not a conclusive supposition that all large sized cells are of tetra- and octoploid type.

According to LEUCHTENBERGER and SCHRADER<sup>10</sup> the nuclear volume can increase even in doubling intervals though a reduplication of DNA fails to appear. It is also impossible to exclude with any degree of certainty regarding a toxic nuclear oedema though it is found only in stages of acute intoxication (JERUSALEM<sup>8</sup>). Lathyrus agents may also cause lasting alteration of membrane functions e.g. of ion transport and thus causing a 'dystrophic' nuclear swelling. All the above mentioned deviations from the normal characteristics point to the fact that in the experimental animals both the normal mitotic cycle and the function of the mesenchymal cells was distinctly altered.

**Zusammenfassung.** Es wurden biometrisch mesenchymale Zellen des Paradontiums als Parameter der Zellproliferation und -funktion untersucht. Durch Verfütterung von Lathyrus odoratus wurde ein Lathyrismus erzeugt mit Veränderungen der Membranfunktion und des Ionenaustausches. Die Kerne der Versuchstiere waren wesentlich grösser als bei den Kontrollen (Dosisabhängigkeit).

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<sup>8</sup> C. JERUSALEM, Anat. Anz. 111, 141 (1962).

<sup>9</sup> C. JERUSALEM and F. G. ZAKI, Anat. Forsch. 38, 161 (1958).

<sup>10</sup> C. LEUCHTENBERGER and F. SCHRADER, Biol. Bull. 101, 95 (1951).

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## H-Y Antigens on Mouse Spermatozoa

Using epididymal spermatozoa injected i.p. as a source of Y antigen, KATSH et al.<sup>1</sup> reported that they were able to accelerate or delay the rejection of syngeneic male skin in the C57BL/6 mouse. In addition, it was possible to induce tolerance to such grafts with the appropriate doses of spermatozoa and time interval before grafting. Further, the authors concluded from their data that spermatozoa were a richer source of Y antigen than spleen cells. Because of current interest in histocompatibility antigens and their relation to cell development and differentiation<sup>2, 3</sup>, the expression of the Y antigen on spermatozoa would appear to provide an extremely

interesting example to study, particularly since it should be possible to study any changes in Y antigen expressivity following fertilization, and the subsequent incorporation into a diploid genome. Thus, as a first step in such an investigation, it was considered important to repeat

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<sup>2</sup> H. E. GOLDBERG, T. AOKI, E. A. BOYSE and D. BENNETT, Nature, Lond. 228, 570 (1970).

<sup>3</sup> J. PALM, S. HEYNER and R. L. BRINSTER, J. exp. Med. 33, 1282 (1971).