plusieurs enzymes SH en même temps, comme le pensent LUCAS et NEWHOUSE[11].

Plusieurs auteurs considèrent la dégénérescence pigmentaire expérimentale et celle de l'homme comme identiques, vu que le tableau clinique et les lésions fonctionelles sont très semblables. Sans vouloir prendre une position aussi affirmative, nos résultats expérimentaux soulèvent la question de savoir si certains types de dégénérescence pigmentaire humaine ne pourraient pas être considérés comme une enzymopathie familiale ou acquise.

Summary. The author established that, at the moment of the extinction of the electroretinographic response after i.v. injection of iodacetate, the glyceraldehydephosphate dehydrogenase activity is suppressed in the retina of the rabbit.

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On the Topographical Differences in the Localization of Certain Enzymes in Trigeminal Ganglion Cells of Rat[1]

Previously we have described the localization of a variety of enzymes in the spinal ganglion cells of rat[2]. The present study is concerned with the distribution of certain enzymes in trigeminal ganglion cells of the same animal. From the words of PEEL[3], 'the semilunar, or gasserian ganglion, comparable to the posterior root ganglion of a spinal nerve' we would expect the enzymatic distribution in trigeminal ganglia to be the same as that in spinal ganglia, yet we find that there are distinct topographical differences in localization of certain enzymes, between the two.

In the present experiments trigeminal ganglia, obtained from normal healthy rats, were subjected to the techniques for alkaline and acid phosphatases and simple esterase[4], succinic dehydrogenase[5], cytochrome oxidase[6], glucose-6-phosphatase[7], 5-nucleotidase[8], and specific cholinesterase[9].

Alkaline phosphatase has been exclusively localized at the periphery of the ganglion cells, similar to the pattern found in spinal ganglion cells. There is no reaction in the nuclei or central cytoplasm (Figure 1). Adenosine triphosphatase activity is similar in reaction to the above, although a moderate amount of activity is also present in the cytoplasm (Figure 2). Although in spinal ganglia, some cells show a peripheral concentration of adenosine triphosphatase, in none of them has the activity been seen in the pericellular area. However, all the cells of the trigeminal ganglion have invariably shown a heavy concentration of adenosine triphosphatase activity in the pericellular zones.

5-Nucleotidase preparations of these cells have given a positive reaction only among small cells though not restricted to the periphery of the cytoplasm (cells a, Figure 7), while most of the large cells are negative (cells b, Figure 7). This is again in sharp contrast to the condition seen in spinal ganglion cells which have shown 5-nucleotidase activity to some degree or other in all the cells.

Succinic dehydrogenase preparations (Figure 4) as well as cytochrome oxidase preparations show a cytoplasmic reaction, some cells demonstrating a perinuclear concentration of the enzyme (arrows P, Figure 4) while others show a general distribution (arrows, Figure 4). The former pattern has been seen in those cases where the nucleoli touch the nuclear wall, whereas the latter arrangement is observed when nucleoli are lying in the center of the nuclei. It should be mentioned that in oxidative preparations some cells show a more intense cytoplasmic reaction than others. Unlike alkaline phosphatase and adenosine triphosphatase preparations no peripheral accumulation of oxidative enzymes is seen.

It is interesting that glucose-6-phosphatase preparations (Figure 5) have given a positive reaction in all the trigeminal ganglion cells. No peripheral concentration of the enzyme was noticed, as occurs in adenosine triphosphatase reactions, although the cytoplasmic reaction in other parts of the cell bears an identical topographical relationship with the distribution of adenosine triphosphatase[10] and also with acid phosphatase and the oxidative enzymes.

It is of interest that in spinal ganglion cells[2], Purkinje cells[11], and in cerebral neurons[12], we have already shown that whenever the nucleolus touches the nuclear wall there is a concentration of mitochondria around the nucleus. However, when the nucleolus shifts to the central part of the nucleus the mitochondria are spread uniformly throughout the cytoplasm. Since it is well known that oxidative enzymes are exclusively located in mitochondria[13], there is every likelihood that the perinuclear concentration and general distribution of oxidative enzymes, as seen in the present observations, represent the localization of the enzymes in mitochondria distributed in these patterns.

There is some difficulty in associating the perinuclear and general distribution of acid phosphatase with particular organelles which may be concentrated or distributed

[1] Supported by Grant B-1914 from the National Institute of Neurological Diseases and Blindness.

[2] H. B. TEWARI and G. H. BOURNE, J. Histochem. Cytochem. *10*, 42 (1962).

[3] T. L. PEEL, *The Neuroanatomical Basis for Clinical Neurology* (The Blakiston Division, McGraw-Hill, New York 1954).

[4] M. S. BURSTONE, J. Histochem. Cytochem. *6*, 322 (1958).

[5] M. NACHLAS, K. C. TSOU, E. DE SOUZA, C. S. CHENG, and A. M. SELIGMAN, J. Histochem. Cytochem. *5*, 420 (1957).

[6] M. S. BURSTONE, J. Histochem. Cytochem. *7*, 112 (1959).

[7] M. WACHSTEIN and E. MEISEL technique for glucose-6-phosphatase. See PEARSE, *Histochemistry* (Little, Brown and Co., Boston 1960).

[8] M. WACHSTEIN and E. MEISEL technique for 5-nucleotidase. See PEARSE, *Histochemistry* (Little, Brown and Co., Boston 1960).

[9] GOMORI's modification of KOELLE's technique for specific cholinesterase. See PEARSE, *Histochemistry* (Little, Brown and Co., Boston 1960).

[10] H. B. TEWARI and G. H. BOURNE, J. Histochem. Cytochem., in press (1962).

[11] H. B. TEWARI and G. H. BOURNE, in preparation.

[12] H. B. TEWARI and G. H. BOURNE, J. Histochem. Cytochem. *11*, 121 (1963).
press (1962).

[13] G. H. HOGEBOOM, A. CLAUDE, and R. D. HOTCHKISS, J. biol. Chem. *165*, 615 (1946).

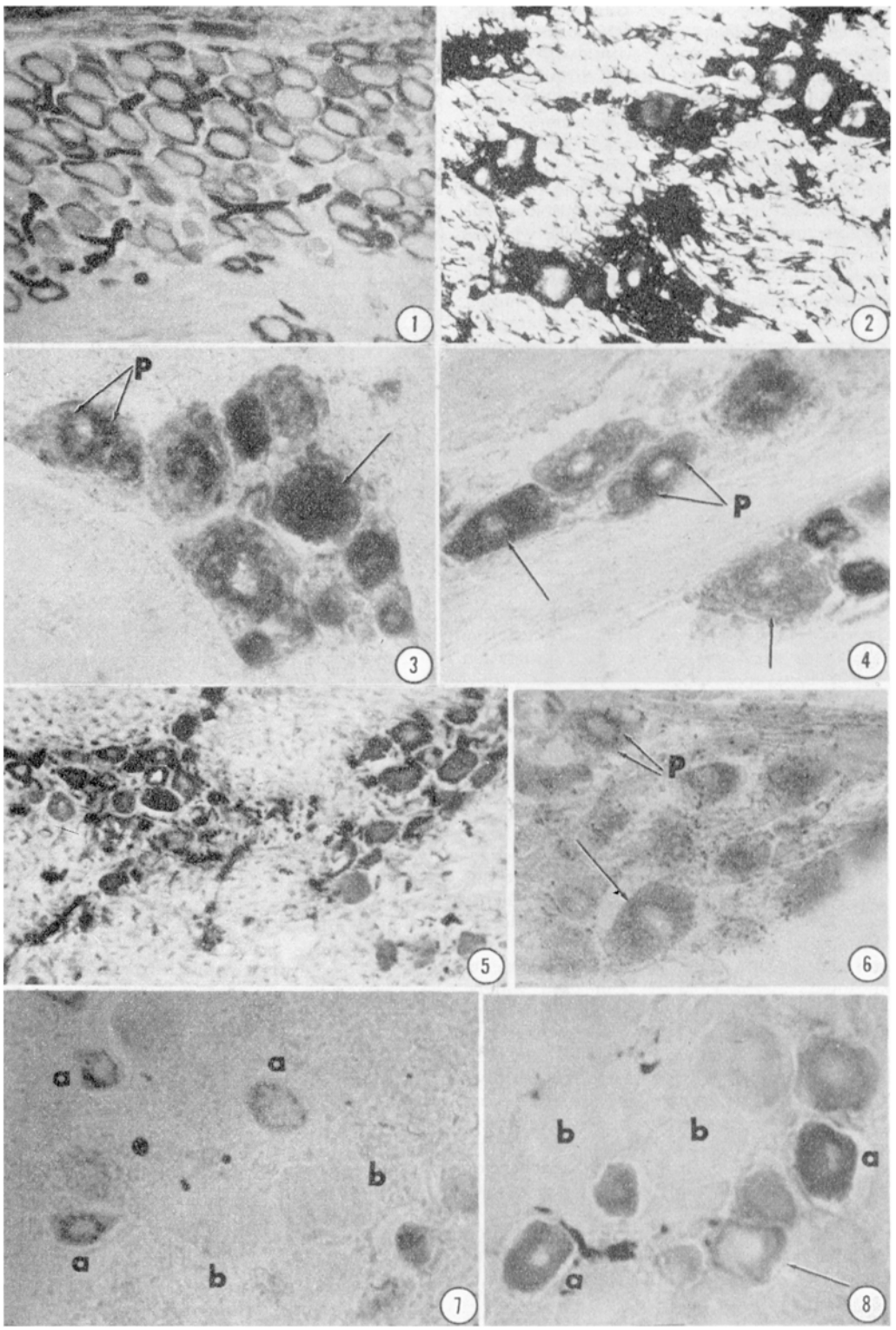

Fig. 1. Note the peripheral localization of alkaline phosphatase in all the ganglion cells. ×136. Fig. 2. Intense activity of adenosine triphosphatase in the peripheral and pericellular areas of the cells. ×340.—Fig. 3. Note the general distribution (unlabelled arrow) and the perinuclear concentration (arrows P) of acid phosphatase. ×340.—Fig. 4. Identify the general distribution (unlabelled arrows) and perinuclear concentration (arrows P) of succinic dehydrogenase among various cells. ×340.—Fig. 5. Positive cytoplasmic reaction for glucose-6-phosphatase among different cells. ×136.—Fig. 6. Distinguish the perinuclear concentration (arrows Pc) and general distribution (unlabelled arrow) of simple esterase among ganglion cells. ×340.—Fig. 7. Identify the positive (a) and negative (b) cells in 5-nucleotidase preparation. ×340.—Fig. 8. Note the three patterns of specific cholinesterase reaction among different cells: cytoplasmic (cells a), negative (cells b) and peripheral (arrow). ×340.

in similar patterns. BECKER et al.[14] working with neurons, have suggested that acid phosphatase is associated with lysosomes. Recently OGAWA and SHINONAGA[15], from electron microscopical studies of cultured fibroblasts of chick embryo origin, have suggested that acid phosphatase is localized in the membranes of lysosomes. They remark that 'the endoplasmic reticulum and perhaps the Golgi apparatus are involved in the formation of lysosomes'. It is of interest to mention here that in spinal ganglion cells we have described the localization of the Golgi bodies in perinuclear areas in some cells, whereas there is a general distribution in others[16]. In their topographical arrangement these stages are identical with acid phosphatase distribution seen in the present studies. Therefore, it seems possible that acid phosphatase is either localized in the Golgi bodies or in lysosomes derived from the Golgi bodies.

Simple esterase preparations have demonstrated intracytoplasmic distribution of the enzyme in two patterns: perinuclear (arrows P, Figure 6) and general (arrows, Figure 6). In this respect the enzymatic localization resembles that of acid phosphatase and oxidative enzymes, and differs from the peripheral localization of alkaline phosphatase and adenosine triphosphatase. All the cells of the trigeminal ganglion are positive for simple esterase to some degree or other. In this respect they differ from specific cholinesterase preparations where only a few trigeminal ganglion cells show enzymatic activity (cells a, Figure 8). The remaining cells are either totally negative cells b, Figure 8) or show only peripheral localization (arrow, Figure 8). In spinal ganglion of rat[2] none of the cells showed exclusive peripheral localization of specific cholinesterase.

A detailed paper discussing the significance of the enzymes active in various parts of ganglion cells will be published elsewhere.

Zusammenfassung. Es wird über die topographische Verschiedenheit der Verbreitung diverser Enzyme in den Trigeminus-Ganglien von Ratten berichtet. Die Bedeutung der peripheren Verteilung von alkalischer Phosphatase und adenosiner Triphosphatase, der perinukleären und allgemeinen Verteilung von Cytochrom-Oxydase, Succinodehydrase und saurer Phosphotase in den Neuronen wird besprochen, ebenfalls die Verteilung spezifisch cholinesterase-positiver Zellen und die cyclische Verteilung von 5-Nukleotidase in einigen Zellen.

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Anatomy Department, Emory University, Atlanta (Georgia, U.S.A), November 12, 1962.

[14] N. H. BECKER, S. GOLDFISCHER, W. Y. SHIN, and A. B. NOVIKOFF, J. Biophys. Biochem. Cytol. *8*, 649 (1960).
[15] K. OGAWA and Y. SHINONAGA, Abstr. Amer. Soc. Cell Biol. (1962).
[16] H. B. TEWARI and G. H. BOURNE, La Cellule, *63*, 25 (1962).

Sex Chromatin as a Marker in some Rabbit Cells

Introduction. In the course of a quantitative investigation on the role of monocytes in wound repair tissue (HULLIGER and ALLGÖWER[1]), a cell marker was needed to follow the fate of leucocytes for 2 to 3 weeks in a model experiment of inflammatory repair tissue.

Cell markers such as radioactive isotopes, and more recently chromosomal markers, have been used widely. Most of these have certain limitations which made them useless for our purpose. Radioactive isotopes such as tritiated thymidine or phosphorous were not considered suitable since the label diminishes with successive cell divisions. In an actively proliferating tissue the label disappears within 2 to 3 weeks, unless highly radioactive tracer material is used. In this case interference with normal cellular functions may result. Chromosomal markers do not allow morphological identification of the cell investigated.

The sex chromatin body seemed suitable as a marker with which the fate of a certain cell type could be followed in a mixed population *in vitro* or in diffusion chambers *in vivo*. The fact that sex chromatin occurs naturally in cells seemed to have an advantage over artificial markers.

In order to test the usefulness of sex chromatin as a marker, we attempted to determine whether it can be identified in rabbit fibrocytes and leucocytes.

Materials and Methods

Subcutaneous connective tissue was obtained from the abdominal region of 6 male and 6 female rabbits. Part of this tissue was fixed and sectioned, from some whole mounts were made, and the rest was explanted in small pieces on coverglasses in plasma clots (50% rabbit plasma, 50% Hanks salt solution) and incubated for 10 to 16 days in roller tubes with 25% rabbit serum and 75% Eagle's solution.

Leucocytes from the same animals were concentrated from cannulated carotid artery blood by centrifugation, explanted as buffy coat pieces in plasma clots and incubated for 6 to 20 days.

Cytological methods. Blood smears, whole mounts of connective tissue and cultures of leucocytes and fibrocytes were fixed in 95% alcohol and stained according to the Feulgen method. Sex chromatin was counted using a 95× objective and a 10× ocular. At least 100 nuclei were examined, often 500. Only non-pycnotic nuclei which were not folded or overlapping were considered. Oval nuclei with finely distributed chromatin were considered as belonging to fibrocytes. The position of the sex chromatin, whether at the periphery of the nucleus and adjacent to the membrane or free in the cytoplasm was noted and nuclei with two sex chromatin bodies were recorded separately.

Results

Connective tissue. In freshly isolated connective tissue of the rabbit, the nuclei are folded and densely stained so that sex chromatin cannot be identified (Figure 1). Fibrocytes growing *in vitro* from an excised piece of connective tissue have flat oval nuclei with finely distributed chromatin. The sex chromatin can be identified in 84–93% (average = 91.5%) of all female cells. Combined sex chromatin frequencies for all cultures from female (Figure 2) and male (Figure 3) connective tissue are given in Table I. Little difference was obtained if 500 instead of 100 nuclei were counted.

[1] L. HULLIGER and M. ALLGÖWER, in preparation (1963).