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).

Leucocytes. In freshly isolated leucocytes sex chromatin cannot be detected with certainty, because monocyte and lymphocyte nuclei appear distorted and folded and generally contain multiple chromatin clumps (Figures 4 and 5). After a few days in culture, a gradual change takes place in the appearance of the large mononuclear cells. They enlarge and their nuclei flatten. Mitoses begin around the third or fourth day of incubation. The number of granulocytes and lymphocytes diminish. After 10 to 14 days a large cell population has developed in which three different cell-types can be distinguished: (a) Macrophage-like cells with distorted and indented, mostly elongated, nuclei and a coarse chromatin structure (Figure 6). There is no consistent difference between male and female cells. (b) Histiocyte-like cells with round nuclei in which heterochromatin is distributed in several small centres. No definite difference between male and female cells can be seen (Figure 7). (c) Fibroblast-like cells with oval nuclei and finely distributed chromatin. Sexual dimorphism is very evident in these cells (Figures 8 and 9).

The distribution of the different cell types varies from one culture to the next. One cell type often forms a cluster; mitotic figures could be seen in the clusters of all three cell types. In fibroblast-like cells sex chromatin is recognizable in 82–90% (average 89%) of female nuclei. Male cultures had 0 to 1% nuclei with sex chromatin-like bodies (average 0.3%). The sex chromatin incidence in two cultures with actively growing fibroblast-like cells is given in Table II.

Discussion. ASHLEY[2] claimed to be able to identify the sex chromatin bodies in freshly isolated human female

[2] D. J. B. ASHLEY, Nature *179*, 969 (1957).

Fibrocytes and leucocytes from the rabbit. Magnification: 1425 ×, Feulgen stain

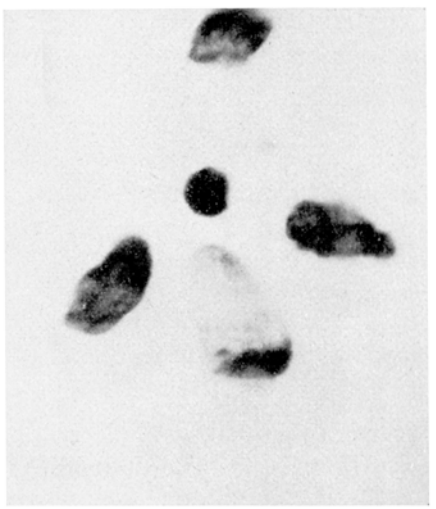

Fig. 1. Freshly isolated female subcutaneous connective tissue. Fibrocyte nuclei.

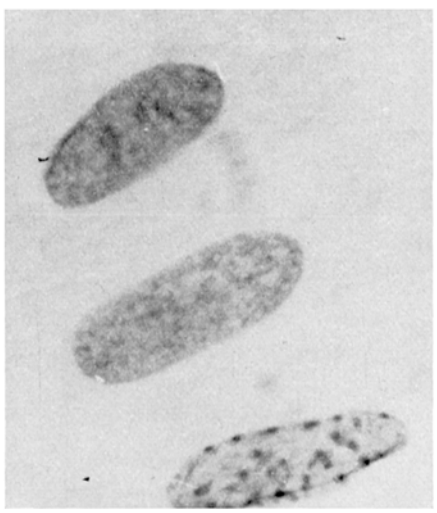

Fig. 3. Sex chromatin negative connective tissue nuclei of a male after 10 days in culture.

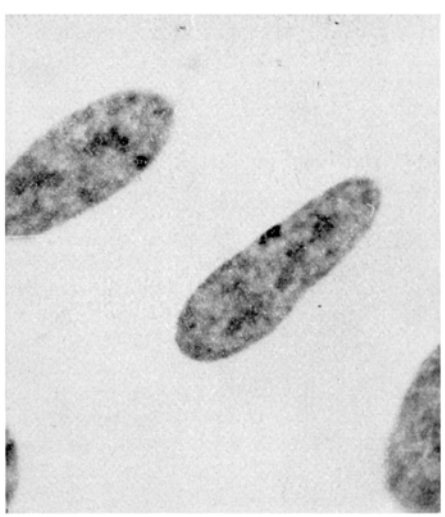

Fig. 2. Sex chromatin positive connective tissue nuclei of a female after 10 days in culture.

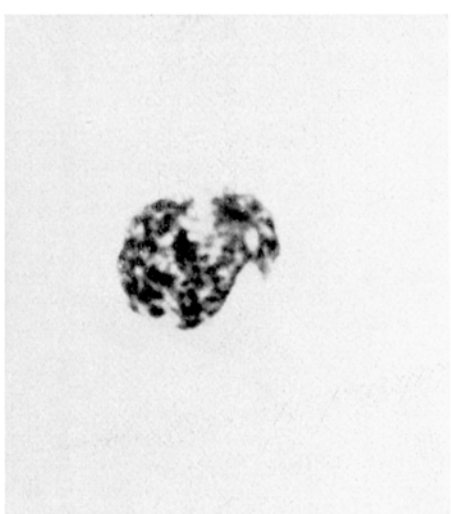

Fig. 4. Freshly isolated female leucocyte (monocytic).

Table I

Sex	Number of cultures	Sex chromatin negative (%)	Sex chromatin positive (%) one peripheral	one central	two bodies	Standard deviation of negative
♂	14	99.5	0.3	0.2	—	1.0
♀	20	8.5	74.0	16.5	1.0	4.8

leucocytes. RIIS[3] on the other hand, could not differentiate between the heterochromatic structures and the sex chromatin body of freshly isolated human leucocytes. We can confirm this for human leucocytes (unpublished observations). RIIS, however, describes a difference in the chromatin structure of human lymphocytes after 24 h incubation *in vitro*, although he does not give the percentage of cells with sex chromatin. After 24 h incubation of rabbit leucocytes we were not able to identify male and female cells. We first find a difference after 3 to 4 days of incubation when cell proliferation starts and a fibroblast-like cell type appears in larger numbers; in this cell type nuclear chromatin is finely distributed.

Earlier investigations have indicated that among rabbit leucocytes only large mononuclear cells (monocytes and large lymphocytes) are responsible for cell division and differentiation into fibroblasts (HULLIGER[4]). In human leucocytes, BOND et al.[5] showed that the same cell type takes up tritiated thymidine *in vitro*. RIIS' suggestion that small lymphocytes also transform into macrophages, does not appear to apply for rabbit lymphocytes.

The fibrocytic nature of the fibroblast-like cells arising in leucocyte cultures has been indicated by the demon-

[3] P. RIIS, Acta haematol. *18*, 168 (1957).
[4] L. HULLIGER, Virchow's Archiv *329*, 289 (1956).
[5] V. P. BOND, E. P. CRONKITE, T. M. FLIEDNER, and B. SCHORK, Science *128*, 208 (1958).

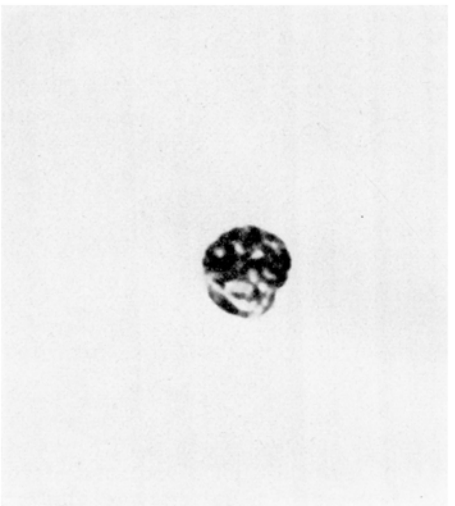

Fig. 5. Freshly isolated female leucocyte (lymphocytic).

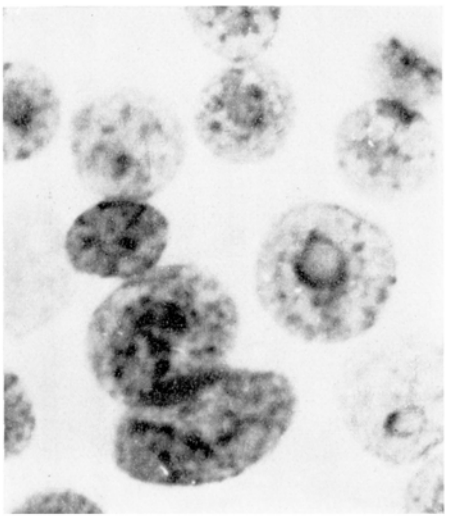

Fig. 7. Male leucocyte nuclei (histiocyte-like) after 10 days in culture.

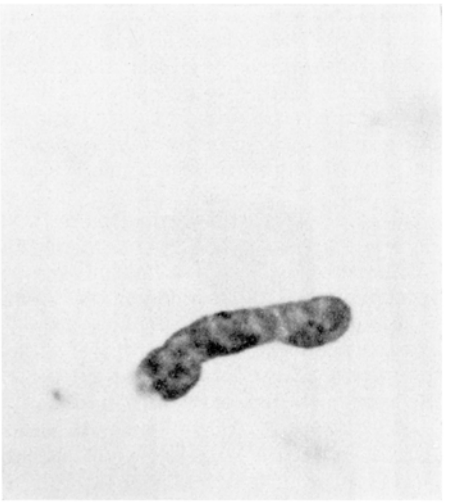

Fig. 6. Female leucocyte nucleus (macrophage-like) after 10 days in culture.

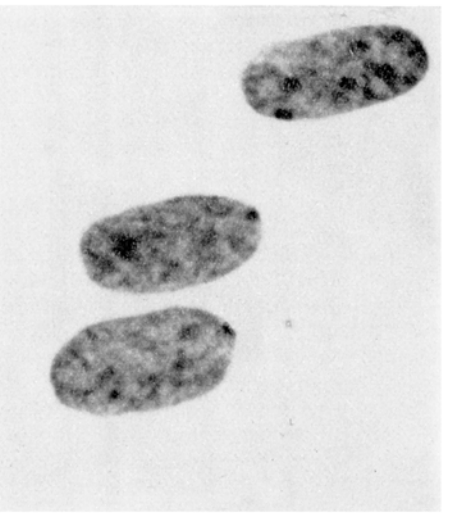

Fig. 8. Sex chromatin positive nuclei from fibrocyte-like female cells in leucocyte culture after 10 days.

stration of collagen production (ALLGÖWER and HULLIGER[6]). If the fate of only one cell type is to be investigated, it is adequate to take these cells from a female animal and mix them with or transplant them to a male[7].

Zusammenfassung. In frisch isolierten Kaninchenfibroblasten- und Leukocyten kann kein Geschlechtsdimorphismus festgestellt werden. Nach einigen Tagen Zellkultivierung findet man in 84–93% (durchschnittlich 91,5%) der weiblichen Fibroblasten den Geschlechtschromatinkörper. Nur 0–2% (durchschnittlich 0,5%) der männlichen Fibroblasten weisen ein dem Geschlechtschromatinkörper ähnliches Gebilde auf. In Leukocytenkulturen sind die entsprechenden Zahlen 82–90% (durchschnittlich 89%) für weibliche, 0–1% (durchschnittlich 0,3%) für männliche Zellen. Diese Daten lassen den Geschlechtschromatinkörper als brauchbaren Zellmarkierer beim Kaninchen erscheinen.

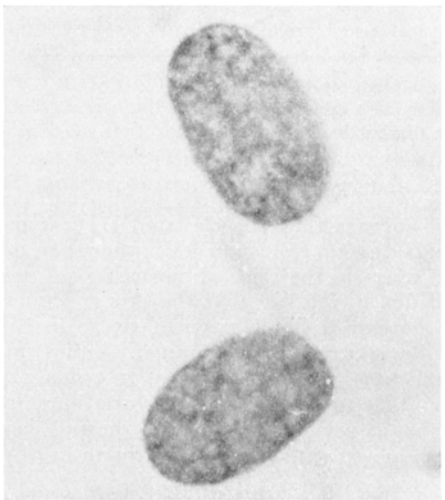

Fig. 9. Sex chromatin negative nuclei from fibrocyte-like male cells in leucocyte culture after 10 days.

Table II

Sex	Sex chromatin negative (%)	Sex chromatin positive (%) one peripheral	one central	two bodies
♂	100	—	—	—
♀	10	73.0	16.0	1.0

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[6] M. ALLGÖWER and L. HULLIGER, Surgery *47*, 603 (1960).

[7] *Acknowledgments.* This research was supported by a grant of the Schweizerischer Nationalfond. The authors are indebted to Miss A. ARCON for her skilled assistance.

Histamine Formation in Human Wound Tissue

The discovery of a singularly high rate of histamine formation in rat pregnancy[1] encouraged studies on a possible connection between histamine formation and certain kinds of rapid tissue growth characterized by a high rate of cell multiplication. Subsequently, a high histamine-forming capacity (HFC), i.e. high rate of decarboxylation of histidine, was found in embryonic rat[2] and mouse[3] tissues, and in wound and granulation tissues in rat skin wounds[4]. A high correlation between HFC and mitotic index of multiplying cells was seen in the Landschütz I ascites tumor in the mouse[5]. The rat bone marrow also has a spectacularly high HFC[6].

In vitro histamine formation by human tissues, including skin, was studied by WATON[7], who reported no histidine decarboxylase activity. However, *in vitro* histamine formation has been demonstrated in human basophil blood cells[8,9] and skin[10] by isotopic technique.

The cooperation of Dr. GÖSTA JÖNSSON, Head of the Urology Clinic of the University of Lund, made possible the examination of the HFC in human skin wounds. An incision, 2–3 cm in length, was made through the entire thickness of the skin of the lower abdominal region and closed by sutures. Circular pieces of skin, including the full length of the incision, were excised at 24 or 48 h after wounding. Normal tissue to serve as controls was obtained from the excised piece at sites maximally distant from the incision. Specimens are defined as follows. Skin: epidermis and dermis at control sites excised to the same depth as wound tissue; subcutis: subcutaneous layer of control sites excised at the same depth as granulation tissue; wound tissue: tissue immediately surrounding the incision to a depth of 2–3 mm; granulation tissue: tissue from the depth of the wound. The HFC was measured in terms of ^{14}C-histamine formed on incubating minced tissue with ^{14}C-histidine for 3 h. The results in Table I are expressed in counts per min per g tissue. With the ^{14}C-histidine used, 1 μg ^{14}C-histamine formed corresponded to about 5000 counts per min. The isotopic technique employed in this laboratory has been described elsewhere[11]. The results summarized in Table I indicate that in the majority of

[1] G. KAHLSON, E. ROSENGREN, and H. WESTLING, J. Physiol. *143*, 91 (1958).
[2] G. KAHLSON, E. ROSENGREN, and T. WHITE, J. Physiol. *151*, 131 (1960).
[3] E. ROSENGREN, Exper. *18*, 176 (1962).
[4] G. KAHLSON, K. NILSSON, E. ROSENGREN, and B. ZEDERFELDT, Lancet II, 230 (1960).
[5] G. KAHLSON, E. ROSENGREN, and C. STEINHARDT, Nature *194*, 380 (1962).
[6] G. KAHLSON and E. ROSENGREN, Exper., in press (1963).
[7] N. G. WATON, Brit. J. Pharmacol. *11*, 119 (1956).
[8] W. J. HARTMAN, W. G. CLARK, and S. D. CYR, Proc. Soc. exp. Biol. Med. *107*, 123 (1961).
[9] S. E. LINDELL, H. RORSMAN, and H. WESTLING, Acta Allerg., Kbh. *16*, 216 (1961).
[10] J. DEMIS, M. WALTON, D. WOOLEY, N. WILNER, and G. MCNEIL, J. invest. Derm. *37*, 513 (1961).
[11] G. KAHLSON, E. ROSENGREN, H. WESTLING, and T. WHITE, J. Physiol. *144*, 337 (1958).