Ammoniacal silver staining of normal and inflamed rat synovial membrane

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Summary. An ammoniacal silver technique was used to detect changes in histone profiles in normal rats and rats with adjuvant-induced arthritis. Under these conditions histone staining reactions change at times when synovial cells are becoming more metabolically active.

Histones, the basic proteins of chromatin, have been the subject of much interest since the suggestion was made that they might function as modifiers of gene activity[2,3]. More recently, histones have been found to be intimately involved in the structure of the nucleosome, a repeating unit composed of protein and DNA found in a wide spectrum of eukaryotic cell nuclei[4]. A post-formalin ammoniacal silver staining reaction (ASR) which demonstrates color differences between arginine-rich and lysine-rich histones with the light microscope was developed by Black and Ansley[5]. Lysine-rich histones were reported to stain yellow, while arginine-rich histones stained dark brown or black. Specificity was established by staining electrophoretically fractionated histones, and purified histone extracts. These color-specific reactions have been repeated and confirmed by others (Meetz, unpublished observations). Meetz and MacRae[6] utilized this staining procedure in a study of the chick erythropoietic system which consists of a cell population that ceases DNA-dependent RNA synthesis during differentiation. The results of this investigation showed that as maturation progressed, the nuclei of these cells demonstrated increased dark brown staining characteristics, indicating a change in the nucleohistone component. This change paralleled the cessation of DNA-dependent RNA synthesis. In further investigations with the ASR using the electron microscope, the dark brown stain was found to consist of an intra-nuclear deposit of electron-opaque particles[7]. Blastoid cells showed a few finely dispersed granules while mature forms had fewer, larger silver deposits. In the present investigation, we have used the ASR to determine the ultrastructural histone profile in normal rat synovium and in synovium of rats with intraarticular adjuvant arthritis. It is known that the metabolism of synovial cells changes markedly when arthritis is induced and data presented here indicate that histone profiles change as well.

Adult (440 g) male Sprague-Dawley rats, maintained on standard laboratory diet and water ad libitum were injected intraarticularly with 0.05 ml complete Freund's adjuvant (CFA) through the patellar ligament under general anesthesia. Control animals were age and sex matched uninjected rats maintained under identical conditions. 2 animals each were sacrificed at 7 and 14 days post-injection and synovial membranes removed from each knee joint. Membranes were immediately immersed in 10% formalin neutralized with sodium acetate (2 g/100 ml formalin) at pH 6.8–7.1, and cut into small pieces. Tissue was then processed[7], dehydrated and embedded in Spurr embedding medium. Thin sections were cut with glass knives on a Porter-Blum Mt-2B ultramicrotome and examined with an RCA EMU-3F electron microscope. All sections evaluated in this study were of the same thickness. No fixation other than formalin was used. Electron microscopic examination of ASR-treated normal rat synovial cells shows a heavy deposition of discrete electron-dense particles within the nucleus (figs 1 und 2). These particles are localized primarily over areas of heterochromatin with only occasional granules in areas of euchromatin. The particles average 50 nm in diameter and may aggregate to sizes of 75 nm. Membranes and cytoplasmic organelles are not well preserved because of formalin fixation. Occasionally, however,

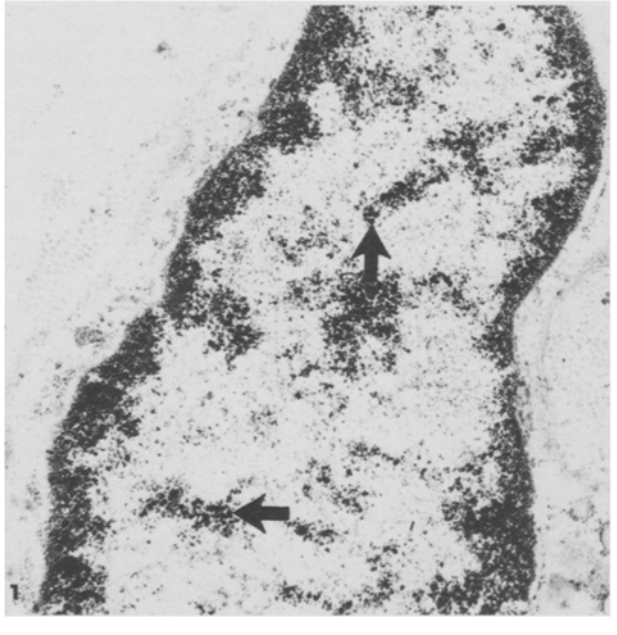

Figure 1. Electron micrograph of a formalin-fixed normal rat synovial cell stained with ammoniacal silver. Note the heavy silver deposit in areas of heterochromatin (arrows). × 18,000.

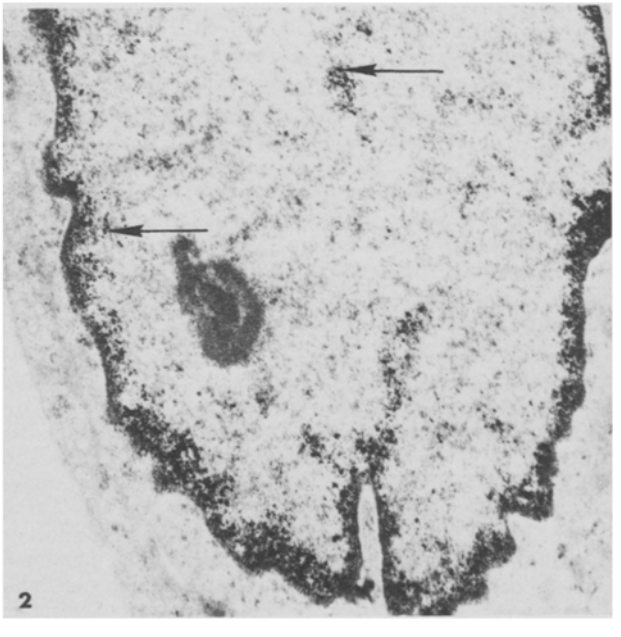

Figure 2. Electron micrograph of formalin-fixed normal rat synovial cell stained with ammoniacal silver. Note heavy silver deposit in areas of heterochromatin (arrows). × 14,800.

silver reaction product may be found in the cytplasm probably associated with ribosomal proteins or other cytoplasmic basic proteins[7]. Synovial cells at 7 days post-injection (fig. 3) show a considerable reduction in the number of granules in their nuclei. The deposit is, like that in normal synovial tissue, localized over areas of heterochromatin. However, many areas are completely free of silver. The granules are of distinctly larger size (100 nm), and appear in some cases to be formed from a coalescence of several adjacent smaller particles. At 14 days post-injection (fig. 4) there is a further reduction in the number of silver granules deposited in nuclei. Extensive areas of heterochromatin are completely free of granules and there is a further increase in size (300 nm) over that noted in the silver particles at 7 days.

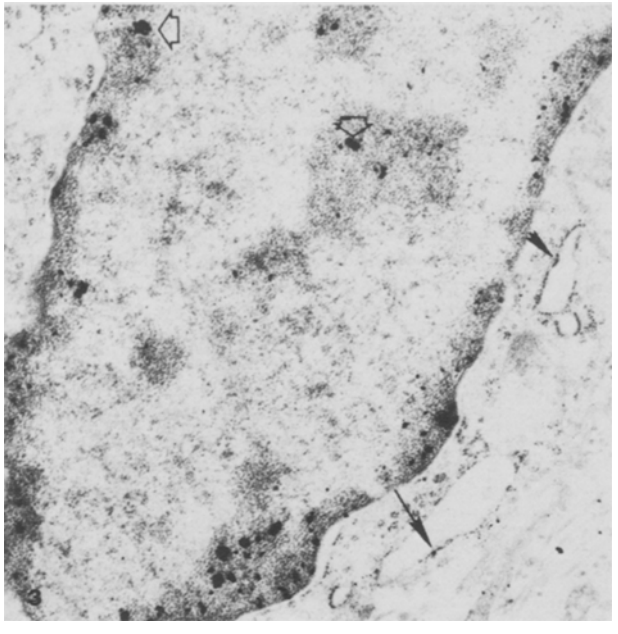

Figure 3. Electron micrograph of formalin-fixed rat synovial cell 7 days after intraarticular injection of complete Freund's adjuvant. × 16,200.

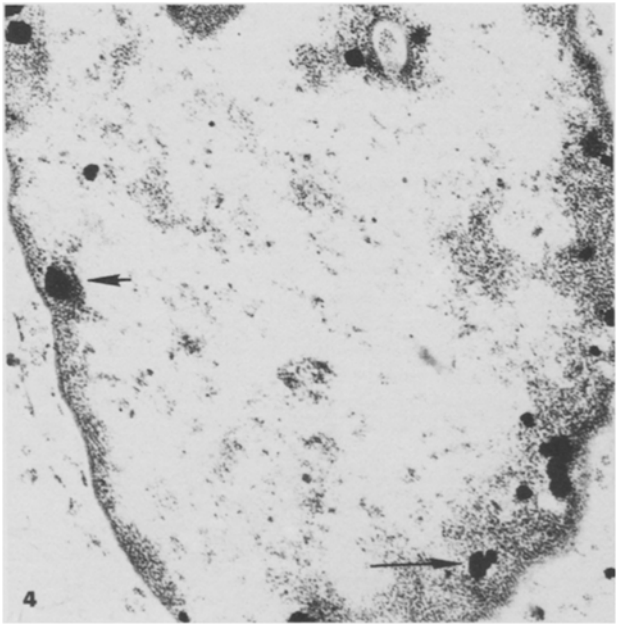

Figure 4. Electron micrograph of formalin fixed rat synovial cell 14 days after intraarticular injection of complete Freund's adjuvant. × 20,000.

When CFA is introduced into a joint cavity, a constellation of inflammatory changes is produced which is common to a number of conditions with a variety of etiologies including osteo- and rheumatoid arthritis[8–10]. Histopathological descriptions may be found in Pearson and Wood[9] and Hammerman et al.[10]. Ultrastructural changes in cells of inflamed synovial membrane are consistent with an increase in protein synthesis[11], and a general increase in other metabolic processes[12–14]. These changes must be preceded by synthesis of new or increased amounts of various DNA-dependent RNA species. The results of the present study show that the nucleohistone component of synovial cells, as revealed by the ASR, undergoes substantial changes following injection of CFA. These changes, which are revealed by a conversion in staining reaction of histones of these cells, occur during a period of time when synovial cells are changing metabolically from a relatively inactive to a relatively active state. The findings reported here are in agreement with previous studies in which similar histone staining changes have been noted in cells undergoing similar metabolic conversions.

Recent evidence indicates that proposing a conversion from lysine-rich to arginine-rich histones as a control mechanism in cells which are undergoing changes in template activity is an oversimplification of the process[15], since histone content of nuclei from different sources is nearly identical. What the mechanism for control of gene function in eukaryotic cells may be and what, if any, role histones may play in this control mechanism is not clear. However, changes in the ASR at times when cells are changing metabolically indicate that some structural or other alteration is occurring in histone polypeptides and that these changes may serve as indications of nuclear activity. Histochemical procedures have been used for years to study nuclear behavior with the light microscope. Although the precise mechanism involved awaits further clarification, it appears that the ASR may provide a useful histochemical procedure for the study of nuclear activity with both light and electron microscopes.

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2 E. Stedman and E. Stedman, Nature *166*, 780 (1950).
3 T.C. Spellsberg and L.S. Hnilica, Biochim. biophys. Acta *195*, 55 (1969).
4 R.D. Kornberg, A. Rev. Biochem. *49*, 931 (1977).
5 M.M. Black and H.R. Ansley, J. Histochem. Cytochem. *14*, 177 (1966).
6 E.K. MacRae and G.D. Meetz, J. Cell Biol. *45*, 235 (1970).
7 E.K. MacRae and G.D. Meetz, J. Cell Biol. *45*, 235 (1970).
8 M. Sherman, J. Bone Jt Surg. *12*, 603 (1930).
9 C.W. Castor, Arthritis Rheum. *3*, 140 (1960).
10 C.M. Pearson and F.D. Wood, Am. J. Path. *42*, 73 (1963).
11 D. Hammerman, P. Barland and R. Janis, in: The Biological Basis of Medicine, vol. 3, p. 269. Academic Press, New York 1969.
12 K.D. Muirden and G. Peace, Ann. rheum. Dis. *28*, 392 (1969).
13 J.T.M. Dingle and D.P. Page-Thomas, Br. J. exp. Path. *37*, 318 (1956).
14 J.E. Roberts, B.O. McLees and G.D. Kerby, J. Lab. clin. Med. *70*, 503 (1967).
15 J. Uitto, S. Lindy, H. Turto and K. Uainio, J. Lab. clin. Med. *79*, 960 (1972).