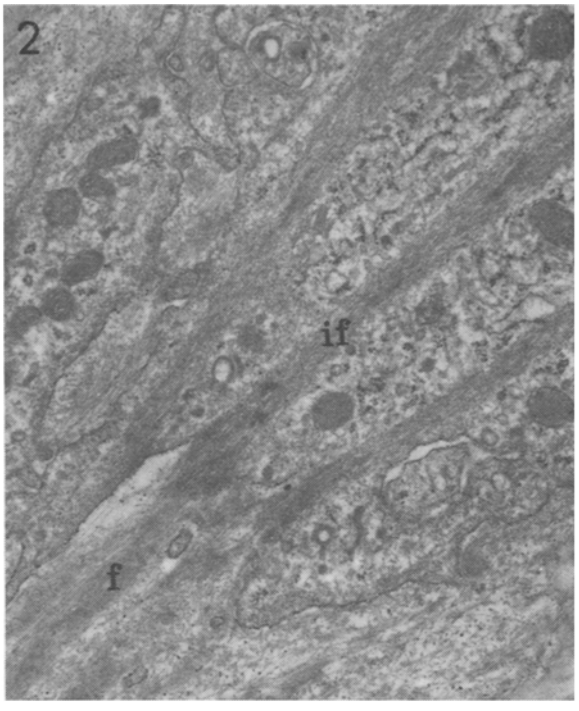
Light- and Electron-Microscopic Observations on the Presence of Pre-Elastic (Oxytalan) Fibres Around the Mature Cartilage in the External Ear of the Rat

The oxytalan fibres[1] are defined by their stainability with some of the common elastica-stains (acid orcein, resorcin-fuchsin, aldehyde fuchsin) after previous oxidation[1–3]. Several data support the hypothesis that at least some of these fibres represent immature elastic fibres ('pre-elastic fibres'[3]). We have recently shown that the appearance of these fibres precedes by several days the appearance of mature elastic fibres in the developing cartilage of the rat external ear[4]. Some relevant light- and electron microscopic observations on the external ear cartilage of adult rats will be briefly discussed in this communication.

Mature (3–5 months) albino rats of the inbred Fischer strain were used. Pieces of cartilage from the external ear were isolated by dissection. They were fixed for light microscopy in Zenker's fluid for 4 h. The dehydrated specimens were embedded in parafin wax and sectioned at 7–10 μm. The deparafinized sections were digested with 0.01% elastase (ESFF, 1FB, Worthington) in Holmes' 0.2 *M* borate buffer, pH 8.8 at 37 °C for 30 min[5], oxydized with peracetic acid for 20 min[2] and stained with the acid orcein[6] and Gomori's aldehyde fuchsin[7]. For electron microscopy, small pieces of cartilage were fixed at 4 °C for 1–2 h in 1% osmium tetroxyde (alone or with 6.25% glutaraldehyde) buffered in 0.06 *N* cacodylate buffer. The specimens were embedded in Durcopan (Fluka), sectioned with glass knives in the Reichert's ultramicro-



[1] H. M. Fullmer and R. D. Lillie, J. Histochem. Cytochem. *6*, 425 (1958).
[2] H. M. Fullmer, Science *127*, 1240 (1958).
[3] H. M. Fullmer, J. Histochem Cytochem. *8*, 290 (1960).
[4] Ž. Bradamante and A. Švajger, in press (1975).
[5] H. D. Boadley and R. L. Wood, Anat. Rec. *172*, 71 (1972).
[6] B. Romeis, *Mikroskopische Technik*, 15th edn. (Leibnitz, München 1948).

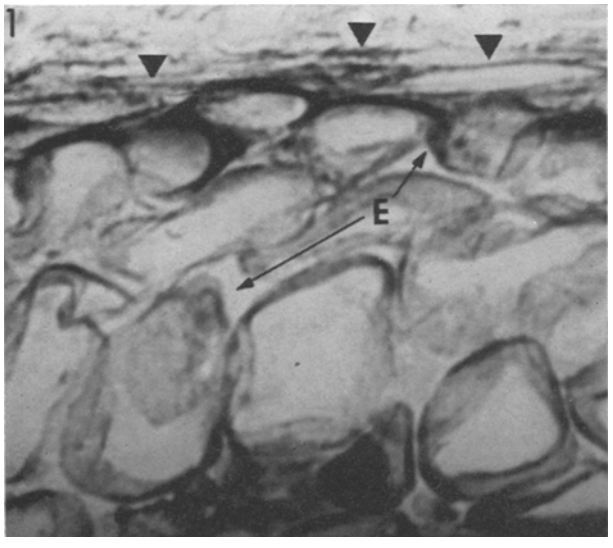


Fig. 1. Periphery of the ear cartilage after digestion with elastase, oxidation with peracetic acid and staining with acid orcein. E, digested elastic fibres; arrows, oxytalan fibres. ×1000.

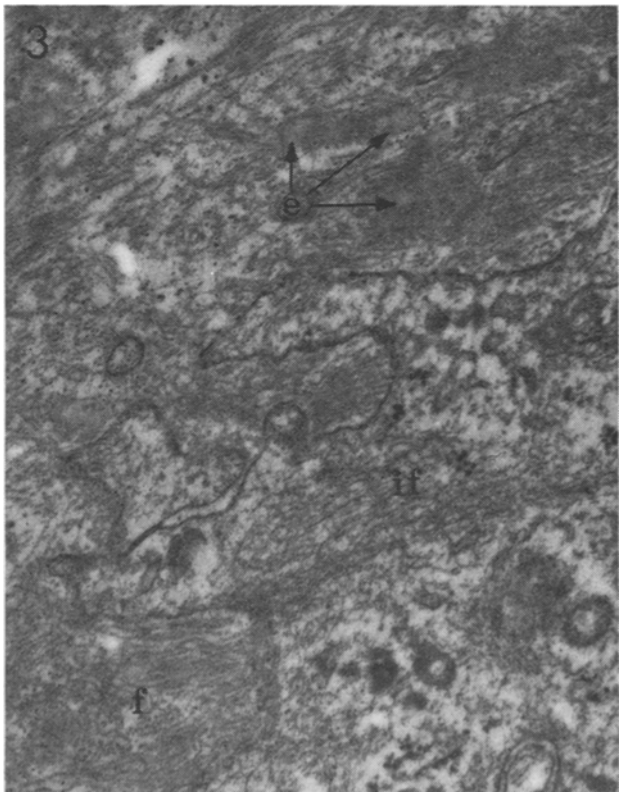


Fig. 2 and 3. Periphery of chondroblasts in the perichondrium of the ear cartilage. f, bundle of extracellular fibrils; if, bundle of cytoplasmic filaments; e, initial deposition of amorphous elastin. ×17,000 (Figure 2); ×37,000 (Figure 3).

tome, stained with lead citrate and uranil acetate and examined with Siemens Elmiskop I.

In sections treated with elastase, oxidized with peracetic acid and stained with acid orcein or aldehyde fuchsin, the spaces between the neighbouring chondrocyte capsules remained unstained (Figure 1, E). These spaces are normally occupied by elastic fibres which can be demonstrated with both these dyes in both oxidized and non-oxidized sections. Parallel with the periphery of the cartilage plate, however, around the perichondral chondroblasts, elastase-resistant fibres have been demonstrated with acid orcein or aldehyde fuchsin following oxidation with peracetic acid (Figure 1, arrows). These fibres are absent in sections stained with the same dyes, without previous oxidation, and therefore are not mature elastic fibres.

In electron micrographs of the innermost layer of the perichondrium, bundles of fine fibriles are seen which run parallel with the long axis of chondroblasts (Figures 2 and 3, f). Some of these bundles seem to be continuous with the bundles of intracytoplasmic filaments (Figures 2 and 3, if). The thickness of fibrils varies from 80 to 120 Å. These findings are consistent with the previous ultrastructural descriptions of oxytalan fibres as bundles of 50–150 Å thick microfibrils, which are similar to those which make part of the mature elastic fibres[8,9]. Also in our electron micrographs, small foci of deposition of the amorphous elastin can be observed within bundles of extracellular microfibrils (Figure 3, e).

We have recently shown that, during the chondrogenesis in the rat external ear, the appearance of oxytalan fibres precedes the appearance of mature elastic fibres[4]. The present investigation has revealed the presence of these fibres in the germinative layer of the perichondrium around the mature cartilage. We consider these findings as additional arguments in favour of the hypothesis that the so-called oxytalan fibres are regular precursors of mature elastic fibres. They very probably represent the early microfibrillar 'matrix' in which elastin has to be laid down during the formation of elastic fibres[10]. This is consistent with FULLMER's[3] claim that oxytalan fibres may be designated as pre-elastic fibres in organs, in which elastic fibres are normal constituents of mature tissues.

Zusammenfassung. Im Perichondrium des Ohrknorpels erwachsener Ratten wurden licht- und elektronenmikroskopisch die sogenannten Oxytalanfasern beschrieben (Elastase-resistent), die mit saurem Orcein und Aldehydfuchsin nach der Oxydation färbbar sind (Bündel von 50–150 Å dicken Mikrofibrillen). Dieser Befund spricht für die Annahme, dass die Oxytalanfasern in elastischen Geweben als normale Vorstufen reifer elastischer Fasern zu betrachten sind.

Ž. BRADAMANTE, LJ. KOSTOVIĆ-KNEŽEVIĆ and A. ŠVAJGER

Institute of Histology and Embryology, Faculty of Medicine, University Zagreb, Šalata 3, P.O. Box 166, Yu–41001 Zagreb (Yugoslavia), 20 February 1975.

[7] G. GOMORI, Am. J. clin. Path. *20*, 665 (1950).
[8] G. G. CARMICHAEL and H. M. FULLMER, J. Cell Biol. *28*, 33 (1966).
[9] C. J. GRIFFIN and R. HARRIS, Arch. oral Biol. *12*, 971 (1967).
[10] R. Ross and P. BORNSTEIN, J. Cell Biol. *40*, 366 (1969).

T and B Lymphocytes in Patients with Chronic Renal Disease on Hemodialysis

Human lymphocytes consist of at least two subpopulations; thymus-derived or T cells, which are responsible for cell-mediated immunity, and bone-marrow-derived or B cells, which are responsible for antibody-mediated immunity[1]. T lymphocytes can be identified by their ability to form rosettes with sheep erythrocytes[2], and B lymphocytes have surface immunoglobulins, detectable with fluorescent anti-immunoglobulin serum[3]. The study of T and B cells in man utilizing these markers has provided basic information in understanding various disease states[4]. The present communication reports our observations of relative and absolute numbers of T and B lymphocytes in the peripheral blood of patients undergoing maintenance hemodialysis.

Materials and methods. The study was carried out on 23 peripheral blood specimens from 11 patients between 17 and 67 years old with chronic renal failure who were undergoing stable maintenance hemodialysis. All specimens were drawn before the start of a dialysis. The renal failure was due to glomerulonephritides of diverse origins; systemic lupus erythematosus (SLE) in 5 patients; diffuse chronic glomerulonephritis in 5; and Goodpasture's syndrome in one. Tissue diagnosis was available in 9 of 11 patients. 24 peripheral blood specimens from 13 men and 5 women were used as controls. They were free of any serious or chronic diseases at the time of study. Patients and controls were studied simultaneously, and the samples were coded until the analyses were completed.

Lymphocytes were separated from peripheral blood as previously described[5]. T lymphocytes were determined by rosette formation and B lymphocytes by surface immunofluorescence[5].

Results and discussion. The total number of lymphocytes per mm³ was significantly reduced in the maintenance hemodialysis patients as compared to normal individuals ($p < 0.0125$). Similarly, the percent and absolute numbers of T and B lymphocytes were reduced in the patients (Table). The mean percent of 'null' lymphocytes (defined as the lymphocytes that could not be identified as either T or B cells) was 37.5 ± 3.4 in controls compared to 55.5 ± 4.6 in the patients. This difference is statistically significant ($p < 0.0025$).

Our observations in patients with lupus erythematosus are in agreement with those of SCHEINBERG and CATHCART[6]. Lymphocytotoxins have been detected in the

[1] M. C. RAFF, Nature, Lond. *242*, 19 (1973).
[2] M. JONDAL, G. HOLM and H. WIGZELL, J. exp. Med. *136*, 207 (1972).
[3] E. R. UNANUE, H. M. GREY, E. RABELLINO, P. CAMPBELL and J. SCHMIDTKE, J. exp. Med. *133*, 1188 (1971).
[4] M. SELIGMANN, N. Engl. J. Med. *290*, 1483 (1974).
[5] M. M. REDDY, K. O. GOH and L. H. HEMPELMANN, Proc. 14th Hanford Biol. Symp. USAEC, in press.
[6] M. A. SCHEINBERG and E. S. CATHCART, Cell. Immun. *12*, 309 (1974).