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).

Distribution of T, B and 'null' lymphocytes in maintenance hemodialysis patients

Groups	Total lymphocytes per mm³ (mean ± SE)	T lymphocytes (mean ± SE) %	per mm³ [a]	B lymphocytes (mean ± SE) %	per mm³	'Null' lymphocytes (mean ± SE) %	per mm³
Control ($n = 24$)	2577 ± 124	38.4 ± 1.9	974 ± 51	24.1 ± 2.4	611 ± 71	37.5 ± 3.4	992 ± 115
Nephritis ($n = 23$)	1691 ± 361	26.2 ± 4.0	664 ± 216	18.3 ± 2.5	329 ± 86	55.5 ± 4.6	697 ± 120
P-value [b]	< 0.0125	< 0.005	< 0.10	< 0.05	< 0.01	< 0.0025	< 0.05

[a] The mean was calculated from each individual's value. [b] According to Student's t-test.

sera of patients with SLE[7], lipoid nephrosis, and glomerulonephritis[8]. LIES et al.[9] demonstrated that these lymphocytotoxic antibodies were directly formed against T cells.

It has been postulated that infection by a latent virus may alter the T cells in such a way as to trigger the formation of T cell specific cytotoxins in SLE[10]. These toxins can destroy the T cells. This destruction resulted a decrease in the T cells as observed in our patients. On the other hand, the decrease in T cells may be associated with a deficiency in suppressor T cells[11]. This deficiency allows the B cells to produce a variety of autoantibodies. These autoantibodies, such as antinuclear antibody, could participate in the production of immune complex disease such as glomerulonephritis[12]. This interpretation is supported by a recent report of HUSTED et al.[13] who observed an increased incidence of antinuclear antibodies in chronic dialysis patients.

In conclusion, we observed a decrease in the circulating T and B lymphocytes in a group of patients with chronic renal failure who were being maintained on hemodialysis. Accompanied by the decrease in the T and B cells, there was an increase in the percentage of the 'null' cells. The cause of the renal disease in these patients was the result of various forms of immunologic glomerulonephritis. Therefore, additional studies are needed to determine whether similar changes in T, B, and 'null' cells also occur in renal failure due to non-immunologic causes.

Summary. The T and B lymphocytes in peripheral blood were reduced in patients with glomerulonephritis treated with hemodialysis as compared to normals. Although the absolute number of 'null' cells was also decreased, the percentage of 'null' cells was increased in these patients.

M. M. REDDY, K. O. GOH and R. V. M. CESTERO[14]

University of Rochester and Monroe Community Hospital, Rochester (New York, 14620 USA), 26 March 1975.

[7] K. K. MITTAL, R. D. ROSSEN, J. T. SHARP, M. D. LIDSKY and W. T. BUTLER, Nature, Lond. *225*, 1255 (1970).
[8] B. S. OOI, A. R. ORLINA and L. MASAITIS, Lancet *2*, 1348 (1974).
[9] R. B. LIES, R. P. MESSNER and R. C. WILLIAMS JR., Arth. Rheum. *16*, 369 (1973).
[10] R. P. MESSNER, F. D. LINDSTROM and R. C. WILLIAMS, JR., J. clin. Invest. *52*, 3046 (1973).
[11] A. C. ALLISON, A. M. DENMAN and R. D. BARNES, Lancet *2*, 135 (1971).
[12] R. P. MESSNER, Arth. Rheum. *17*, 339 (1974).
[13] F. C. HUSTED, K. D. NOLPH and G. C. SHARP, abstracts 7th Ann. Meeting Am. Soc. Nephrology (1974), p. 40.
[14] This work was supported in part by a Grant, No. CA 14876, from the National Cancer Institute. Ms. RITA GIULIANO gave valuable technical assistance.

Fetal Calf Serum Alters Cyclic Adenosine 3′,5′-Monophosphate's Effect on Heme Synthesis in vitro[1]

Reports are often contradictory about the effect of cyclic adenosine 3′,5′-monophosphate (cAMP) on heme synthesis in vitro. GORSHEIN and GARDNER[2], for example, showed that cAMP stimulates radioiron incorporation into heme of human marrow cells. DUKES[3] illustrated that dibutyryl cAMP potentiates the effect of erythropoietin (EPO) on heme synthesis of rat marrow cells. BYRON[4] showed that cAMP increases the sensitivity of mouse marrow cells to tritiated thymidine suicide. Among those finding no effect are GRABER et al.[5] and BOTTOMLEY et al.[6].

This paper presents new data on the role of cAMP in erythropoiesis in vitro. It also highlights the importance of GOLDWASSER's[7] advice about independently evaluating each lot of fetal calf serum used in erythropoietic studies, perhaps explaining or reconciling such inconsistencies in the literature.

Materials and methods. Rat marrow cells were cultured by a variation of the technique described by GOLDWASSER and GROSS[8]. Details of the procedure are presented by OLANDER[9]. Standard medium comprised 65% National Collection Type Culture 109 (Micro Biological Associates) containing 100 units penicillin and 100 µg streptomycin per ml, 30% fetal calf serum (Lot R2230U or Lot C2220L, Grand Island Biological) and 5% isologous rat serum.

[1] This work was performed at the Laboratory of Experimental Hematology, Department of Zoology, the University of Nebraska-Lincoln, Lincoln, Nebraska 68508, USA.
[2] D. GORSHEIN and F. H. GARDNER, Blood *36*, 847 (1970).
[3] P. P. DUKES, Blood *38*, 822 (1971).
[4] J. W. BYRON, Nature, Lond. *234*, 39 (1971).
[5] S. E. GRABER, M. CARRILLO and S. B. KRANTZ, Proc. Soc. exp. Biol. Med. *141*, 206 (1972).
[6] S. S. BOTTOMLEY, W. H. WHITCOMB, G. A. SMITHEE and M. Z. MOORE, J. Lab. clin. Med. *77*, 793 (1971).
[7] E. GOLDWASSER, in *Peptide Hormones* (Eds. S. A. BERSON and R. S. YALOW; North-Holland Publishing Company, Amsterdam 1973), p. 1091.
[8] E. GOLDWASSER and M. GROSS, in *Hemic Cells in Vitro* (Ed. P. FARNES; Williams and Wilkins, Baltimore 1969), p. 36.
[9] C. P. OLANDER, Effects of Cyclic Adenosine 3′,5′-Monophosphate on Erythropoiesis in Vitro (Ph. D. dissertation, University of Nebraska-Lincoln, 1974), p. 14.