Since several heavy metals, drugs and toxins, as well as other factors, such as hemodynamic alterations, damage the renal tubules it is attractive to speculate that such injury may lead to release of one or more tubular antigens resulting in antibody formation. Antibody may induce tubular damage resulting in tubulointerstitial disease or form immune complexes with antigen in the circulation. Deposition of antigen-antibody complexes may result in an immune complex glomerulonephritis. The morphologic and immunohistologic alterations apparently are related to the nature of the tubular antigen or antigens involved.

In these studies, mercuric chloride administration was associated with an immune complex nephritis apparently secondary to deposition of RTE-anti-RTE immune complexes. Although antibodies to TBM were detected in the serum of these animals, tubulointerstitial disease usually associated with this antigen was not prominent and TBM could not be implicated in the pathogenesis of the glomerular lesions.

These studies support the hypothesis that tubular injury induced by mercuric chloride may be followed by release of renal proximal tubular antigen, subsequent formation of antibody to this antigen, followed by formation and glomerular deposition of immune complexes of RTE-anti-RTE resulting in an autologous immune complex glomerulonephritis. This etiopathogenic mechanism may occur in man and may also be a factor in the development of several cases of nephritis associated with agents which produce tubular alterations. The significance of TBM in this study is unclear.

Ultrastructural Identification of Human Tonsil T-Lymphocytes by Peroxidase-Conjugated Anti-HTLA Serum[1]

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Summary. A horse anti-serum rendered specific for human T-lymphocytes was conjugated with peroxidase and used for ultrastructural identification of human tonsil T-lymhocytes. With T- and B-enriched suspensions, virtually all T-lymphocytes were labelled with Po-anti-HTLA, whereas no B-cells were stained with this conjugate. The labelling was found to be irregularly distributed on the plasma membrane of T-cells. Direct identification with specific Po-anti-HTLA conjugate confirm the ultrastructural characteristics of thymus-dependant cells.

Human T-lymphocytes are often identified by the formation of rosettes with sheep erythrocytes[5] (E), although the mechanisms and specificity of the binding are still unclear. Demonstration of an antigen specific for the T-cell lineage by using specific antisera[6] provides a new approach for enumeration and characterization of T-lymphocytes in cell suspensions of various origins. We have prepared a horse antiserum specific for human T-lymphocyte antigen (HTLA). Criteria of specificity of this serum are described in another publication[7]. We report here its use for ultrastructural studies of T-lymphocytes after conjugation with peroxidase.

Material and Methods. Lymphocyte suspensions: tonsils freshly obtained from operation were teased with forceps into Hanks, washed twice, centrifuged (20 min, 400 *g*, 4 °C) on Ficoll-Isopaque (6.3%–9.9% final concentration; density 1.080) and washed 3 times. T- and B-enriched suspensions were prepared by centrifugation on Ficoll-Isopaque after T-cells had been allowed to form E rosettes: T-enriched cells were recovered at the bottom and used after E lysis by NH_4Cl (0.87%, 10 min) and B-enriched cells at the interface.

Anti-HTLA serum: anti-HTLA serum was prepared from anti-human thymocyte globulins (Institut Mérieux, Lyon) sequentially absorbed on AB red cells, polymerized Ig, placental tissue and lymphoblastoid cell lines[7]. Cytotoxicity tests were performed as previously described[8].

Peroxidase-labelling of anti-HTLA Ig: peroxidase-conjugated anti-HTLA (Po-anti-HTLA) Ig were prepared according to AVRAMEAS' technique[9]. Lymphocytes were fixed in 2% paraformaldehyde (20 min, 4 °C), washed with PBS and incubated with Po-anti-HTLA Ig for 3 h at 37 °C. After washing with PBS, cells were fixed again with 2% glutaraldehyde, washed with PBS and incubated with Graham-Karnowsky medium for 30 min at 37 °C. Cells were washed with *tris*-HCl buffer (0.2 *M*, pH 7.6), post-fixed with OSO_4 1% in cacodylate buffer, and included in epoxy medium. Sections were observed unstained under the electron microscope (Philips EM 300).

Other techniques for T- and B-cell identification: T-cells were also characterized by the E rosette test[10] and B-lymphocytes by the detection of membrane Ig (S Ig) with a fluoresceinated anti-human Ig (Behring). In addition a B-lymphocyte and monocyte common antigen (HBLMCA) was identified by mean of a rabbit antiserum as previously described[7]. In electron microscopy studies, B-cells were identified by reaction with peroxidase-conjugated Fab anti-human Ig (Institut Pasteur, Paris) according to the technique described above.

Table I. Percent of T- and B-cells in tonsillar lymphocytes

	Anti-HTLA	E rosettes	Anti-HBLMCA	S Ig
No. of donors	47	14	40	7
Cells ± SE (%)	47.1 ± 1.7	42.8 ± 4.0	47.4 ± 1.8	40.7 ± 5.4

Table II. Percent of T- and B-lymphocytes in purified suspensions

	Anti-HTLA	Po-anti-HTLA	Anti-HBLMCA	Po-Fab anti-Ig
T-enriched (%)	91	96	4	5
B-enriched (%)	5	3	93	90

Results. Distribution of T- and B-lymphocytes in tonsils (Table I) showed an approximately equal number of B- and T-cells and a few percent of null cells depending on the techniques used for identification, some of these null cells possibly being monocytes. Both Po-anti-HTLA and Po-Fab anti-Ig preparations stained nearly 50% of cells; when they were mixed and allowed to react with tonsillar lymphocytes, virtually all cells were stained. Similar studies were undertaken using B- and T-enriched suspensions: each suspension was contaminated by about 5% of the other type of cells (Table II). Very similar results were observed using peroxidase conjugates.

Polymorphonuclear cells and monocytes represented less than 3% of the cells observed. B-cells were easily identified by the electron dense thin ring dispersed along the cytoplasmic membrane. In a few small lymphocytes, intracytoplasmic Ig as well as SIg were stained with Po-Fab anti-Ig. Typical plasma cells accounted for about 5% of all tonsillar cells. Virtually all T lymphocytes were labelled with Po-anti-HTLA (Table II), whereas no B-cells were stained with this conjugate (Figure 1). The staining was only found on the cytoplasmic membrane as irregular small spots (Figure 2) all over the cell surface. Most T-lymphocytes appeared as small round cells (5–8 μm) with dense nuclear heterochromatin and a cytoplasmic ring thinner than that of B cells.

Discussion. Anti-HTLA used in this study has already been shown to react specifically with T-lymphocytes[7]: using either cytotoxicity test or indirect immunofluorescence, it delineated to a plateau a subpopulation of lymphocytes which were found distinct from Ig-bearing cells and monocytes, which formed E but not EAC rosettes. This subpopulation was present in T-enriched and absent from B-enriched cell suspensions. The present report demonstrates that, after conjugation with peroxidase, this serum binds specifically to T-cells and, consequently, confirms its specificity. Similar studies have been done in the mouse with Po-anti-Ig[11] and Po-anti-0[12] sera. Our

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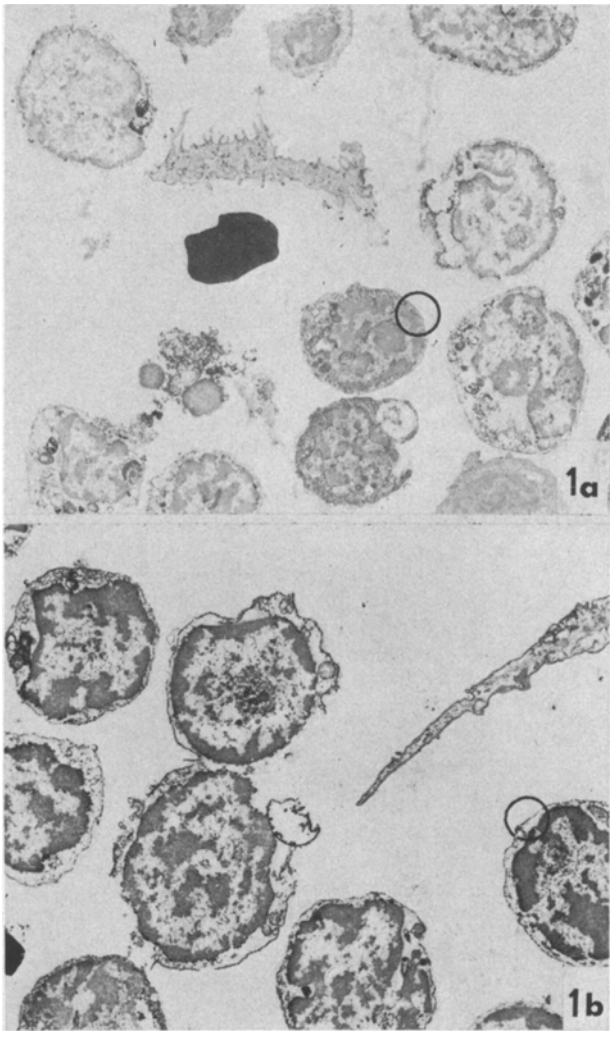

Fig. 1. Staining of the membrane tonsillar lymphocytes by Po-anti-HTLA serum (circle). a) B-enriched lymphocyte population. ×6, 400. b) T-enriched lymphocyte population. ×6, 400.

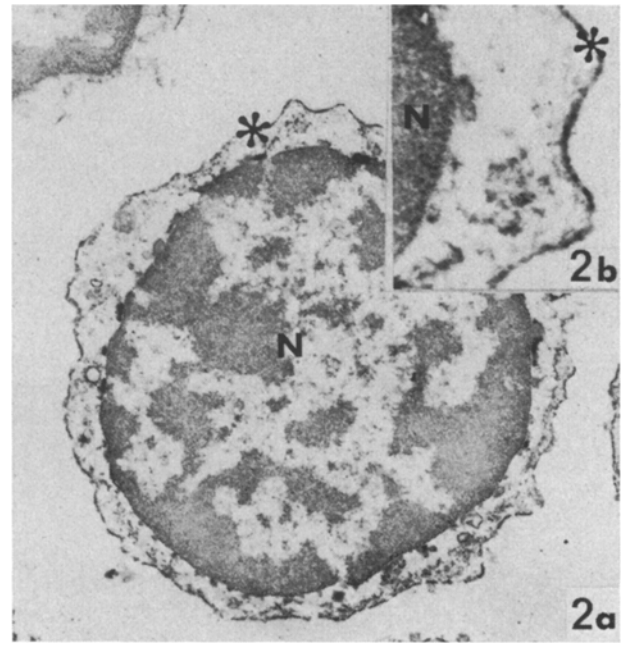

Fig. 2. Typical aspect of a T-lymphocyte stained by Po-anti-HTLA serum. N, nucleus. a) ×15,000; b) ×45,000.

preliminary results show that, similarly to mouse lymphocytes, the majority of human T-cells are characterized by a round shape and a high nucleoplasmic ratio. Po-anti-HTLA conjugate was found to be irregularly distributed on the plasma membrane, in small spots, contrasting with the continuous distribution of Po-Fab anti-Ig stain. Partial redistribution of HTLA does not account for this difference since cells were pre-fixed before addition of Po-anti-HTLA Ig. Moreover, no spontaneous capping could be evidenced by anti-HTLA serum unless a second antibody layer was added[13]. Direct identification of T-cells with specific Po-anti-HTLA conjugate will allow an extensive study of the ultrastructure of T-lymphocytes at different stages of maturation in various lymphoid tissues.

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Plasma Free and Total Tryptophan During the Oestrus Cycle, in Ovariectomized and in Male Rats

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Summary. Plasma free tryptophan was higher during prooestrus and early oestrus than at metoestrus or in ovariectomized or male rats. In contrast, total tryptophan was higher in ovariectomized and male rats than at any time in cycling females.

The rate-limiting step in the synthesis of 5-hydroxytryptamine (5-HT) appears to be tryptophan hydroxylation[1], the concentration of tryptophan (T) in the brain being much lower than the Michaelis constant of the enzyme for its substrate[2]. The availability of T in brain depends primarily on the rate of transport into the brain[3], but also on the availability of T in plasma in the free form, as most T is bound to plasma albumin[4]. Although under many experimental conditions changes in the amount of free T may influence brain T and 5HT turnover[5,6], changes in 5HT metabolism do not always lead to easily interpretable changes in free T[7], as would be expected in a 'closed loop' feedback system. The easy pharmacological[8,9] and dietary[10] manipulation of free T indicates that a study of changes in peripheral and central T metabolism during a 'natural' rhythm, such as the diurnal[11] or the oestrus cycle, may provide more information as to the regulatory role of free T in cerebral serotoninergic metabolism.

The present study reports changes in plasma free and total T at different times during the oestrus cycle. Parallel analyses of regional brain T, 5HT, catecholamines and monoamineoxidase activity were also carried out[12]. As a comparison to cycling rats, plasma free and total T was measured in ovariectomized and in male rats.

Experimental. Adult female albino Wistar rats (210–240 g) were maintained for at least 3 weeks in an animal room controlled for light (from 05.00 h to 19.00 h) and temperature (24 ± 1.5 °C), fed ad libitum, and observed by means of daily vaginal smears at 08.00 h for the presence of 2 consecutive 4-day oestrus cycles. Male rats (210–240 g) were studied after a similar 3 week period in the same light regimen, ovariectomized rats (250–280 g) 6–7 weeks after operation. Groups of 5 rats in the identical phase of their oestrus cycle were decapitated, and trunk blood collected in 1/10 volume 5% EDTA. Plasma was immediately separated. Free T was obtained by ultrafiltration of 4 ml plasma (combined from 2–3 rats) as previously described for human plasma[13]; both free and total tryptophan concentrations were determined fluorometrically[14].

Results and discussion. Under controlled conditions of light, temperature, and feeding ad libitum, both the gender and the endocrinological state of an animal were found to be associated with changes in free and total plasma T (Table).

A possible diurnal rhythm must be considered before defining the hormone-related changes. A preliminary investigation in male rats had indicated that plasma free T did not vary greatly between 10.00 h and 18.00 h, but doubled at night (light phase: 1.54 ± 0.08 µg/ml (mean ± SEM), $n = 6$; dark phase: 3.53 ± 0.68 µg/ml, $n = 7$; $p < 0.02$). A detailed study of the diurnal rhythm[11] has shown that free and total T in rats have a minimum at midday and a maximum at midnight. We have found a similar diurnal rhythm in free T in man[13].

Plasma tryptophan in various endocrinological states[a]

Endocrinological state		Free tryptophan (µg/ml plasma)	Free:total tryptophan (%)	Total tryptophan (µg/ml plasma)
Prooestrus	10.00 h (12)	2.70±0.33[c,g]	24.6±2.4[d,e]	10.77±0.61[d,e,g]
Prooestrus	15.00 h (6)	2.87±0.48[c,g]	22.9±3.5[c,e]	12.51±0.47[b,h]
Oestrus	10.00 h (6)	2.24±0.21[c]	25.0±1.7[d,f]	8.92±0.46[d,f]
Oestrus	15.00 h (10)	1.96±0.22	18.1±1.1[d]	10.67±0.74[d,e,g]
Metoestrus	10.00 h (9)	1.63±0.21	19.5±2.3[c]	8.54±0.59[d,f]
Ovariectomized	17.00 h (6)	1.92±0.12	14.5±1.4[b]	13.57±0.85
Male	17.00 h (8)	1.46±0.16	9.6±1.5	17.53±1.55

[a] Mean ± SEM for the number of determinations given in brackets. Significance calculated with Student's t-test: [b] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$ compared with male rats. [e] $p < 0.05$; [f] $p < 0.001$ compared with ovariectomized rats. [g] $p < 0.05$; [h] $p < 0.001$ compared with metoestrus.