

of wood shavings. Purina laboratory chow and tap water were available to the mice ad libitum. Room temperature was constant at 22°C and a 12-h photoperiod was maintained.

Nematospiroides dubius exposure was accomplished by stomach intubation of the desired number of larvae. Eggs of *N. dubius* were cultured to infective larvae from the fecal pellets of 'source' mice according to the method of Cross and Scott⁹.

Prednisolone acetate administered i.p. at a dose of 1 mg/day for 4 days prior to exposure with virulent *N. dubius* larvae was used to immunosuppress the mice. After exposure, prednisolone (1 mg) was administered every other day for 10 days to maintain immunosuppression.

Results and discussion. Fecal floats for eggs, using NaCl-specific gravity of 1.20, were positive on day 10 post-exposure and remained positive as long as steroid

treatment continued (see Table). However, fecal floats became negative within 10 days after the termination of steroid treatment.

From this work and that of previously mentioned investigators, we conclude that *P. maniculatus* is not a natural host for *N. dubius*. Indeed, it is the only mouse strain of the many tested in our laboratory that demonstrated this state of increased resistance. In this regard, *P. maniculatus* very much resembles the rat, as infection in this rodent can only be established with the use of steroids¹⁰. The exact mechanism of steroid action is unknown; however the mouse is categorized as a steroid sensitive animal¹¹. Also, a sharp decrease in circulating lymphocytes, half that of normal levels, was seen in *P. maniculatus* mice immunosuppressed by prednisolone. These histological events are very similar to those seen in some viral infections of mice¹². This evidence leads us to speculate that *N. dubius* infection occurs naturally only when the *Peromyscus* mouse is sufficiently immunosuppressed, perhaps by a concurrent viral or parasitic infection. Support for the latter speculation comes from the work of COLWELL and WESCOTT¹³ who showed that *N. brasiliensis* infection of mice was prolonged by a concurrent infection of *N. dubius*. Apparently any natural resistance of the mouse against infection with *N. brasiliensis* was suppressed by the presence of *N. dubius*.

We believe that the inability to infect *P. maniculatus* with *N. dubius* is due to the increased natural resistance of the mouse and not to an adaptation of the parasite because of laboratory passage as speculated by some authors. One possible reason for the increased native resistance of this mouse is the tendency to avoid inbreeding as evidenced by our work and that of others¹⁴. We have already demonstrated that inbred strains have a decreased capability for immunization¹⁵; therefore, the lack of inbreeding in *P. maniculatus* may account for the high degree of native resistance to *N. dubius* infection.

Infectivity of *N. dubius* for steroid-treated and non-steroid-treated *P. maniculatus*

Group	No. of mice	Treatment	Results
12-weeks	5	Exposure with 200 infective larvae	No infection
12-Weeks	5	Exposure with 500 infective larvae	No infection
12-Weeks	4	Exposure with 800 infective larvae	No infection
8-Weeks*	10	Exposure with 200 infective larvae	No infection
8-Weeks*	10	Steroid treatment-exposure ^b with 200 infective larvae	Infection established range (120-180 worms recovered as adults)
12-Weeks*	10	Steroid treatment-exposure with 200 infective larvae	Infection established range (120-180 worms recovered as adults)

*Groups were repeated with the same results. ^b1mg/day i.p. for 4 days prior to challenge, then on alternate days for a period of 10 days.

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¹³ D. A. COLWELL and R. B. WESCOTT, *J. Parasit.* 59, 216 (1973).
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Undulating Tubules in Lymphocytes of an Apparently Healthy Human Donor

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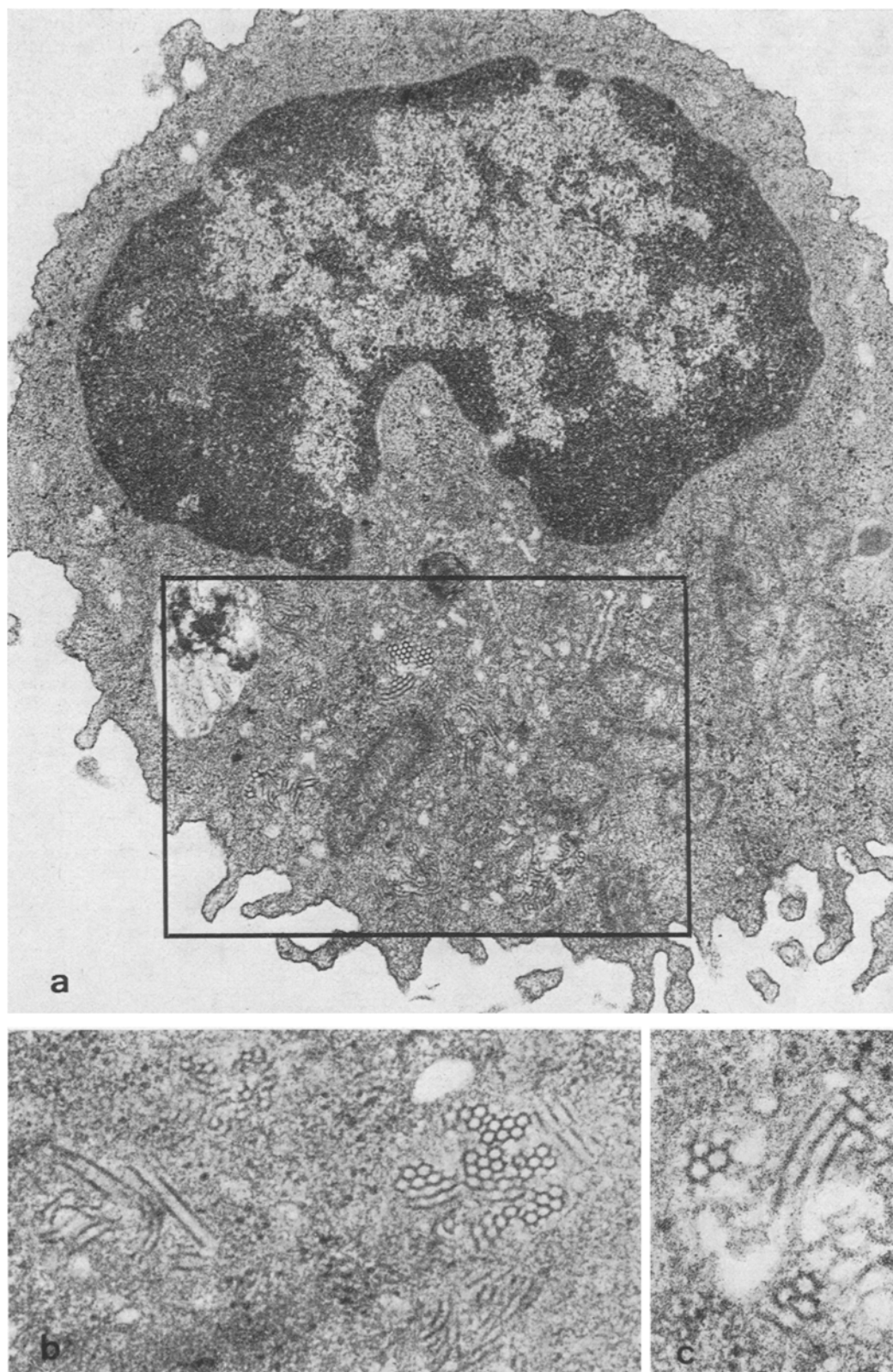
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Summary. So- called 'undulating tubules' were found in the blood lymphocytes of an apparently healthy 33-year-old male. Undulating tubules have been noted to occur frequently in kidney cells and blood lymphocytes of patients suffering from collagen diseases and especially from SLE. They have been suggested to be a possibly pathognomonic finding in such diseases. Our result seems to contradict such an association.

In the course of systemic electron microscopic examinations of blood of patients with leukemia, we observed unusual tubular inclusions in the circulating lymphocytes of a seemingly healthy control patient. Since similar structures have been noted in lymphocytes or other cells in several diseases, especially in collagenosis, our finding seems to merit a brief report.

Peripheral blood was drawn from the cubital vein of a 33-year-old man, who was seemingly in good health and had no history of collagenosis or recent viral diseases. Blood smear examination was normal.

The lymphocytes were separated as follows: 1. defibrination of the blood by stirring for 10 min at room temperature; 2. 4-fold dilution with phosphate-buffered



a) Circulating lymphocyte of an apparently healthy donor. In square: tubular structures occupying large cytoplasmic area. $\times 35,800$.
b) and c) Undulating tubules in cross and longitudinal sections. Note circular profiles of cross-section tubules. b) $\times 78,000$; c) $\times 120,800$.

saline (pH 7.2) containing 1% bovine serum albumin; 3. layer 6 ml of this blood solution on top of 2 ml of a Ficoll-Hypaque gradient (density 1.08); 4. centrifugation at $2,000 \times g$ and 4°C ; for 20 min; 5. collection of the lymphocytes from the interface with a Pasteur pipette. Thereafter the cells were fixed in 2.5% glutaraldehyde buffered with sodium cacodylate for 2 h at 4°C followed by an osmification in 1% buffered osmiumtetroxide. The specimen were dehydrated with graded ethanol and embedded in Spurr embedding medium. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips electron microscope, model EM 201. Lymphocyte yields were higher than 80%. Remaining cells were platelets and some granulocytes. The majority of these cells were morphologically normal, however about 2% of lymphocytes contained unusual inclusions. These structures were lying free in the cytoplasmic matrix (Figure, a and b). They were undulating or elongated, tubular and frequently closely packed, (Figure, a and b).

Dense circular outlines representing cross sections of the tubules had an outer diameter of about 300 Å. The thickness of the wall was approximately 50 Å. The tubules generally contained a substance of low electron density (Figure, a, b and c). There was no relationship between these tubules and the endoplasmic reticulum or other cellular structures.

Inclusions, similar to those here described, have frequently been reported to occur in lymphocytes and renal cells of patients with disseminated lupus erythematosus¹, and renal transplants², in lymphocytes of patients with chronic rheumatism³, in lymphocytes in various syndromes associated with antibody deficiencies⁴, and other diseases as reviewed by ANDRES et al.⁵. The true nature of these tubular structures is at present not known.

The inclusions resemble closely the 'undulating tubules' of cells in a variety of tissues in culture described by CHANDRA⁶ and interpreted as pathological secretions of endoplasmic reticulum, or as an early response to injury⁷.

On the other hand, because their characteristic morphology, it was suggested that they represent a special type of myxoviruses¹ and perhaps play a role in the etiology of some collagen diseases, such as systemic lupus erythematosus¹.

The induction of similar inclusions in cultured human lymphoid cells after administration of halogenated pyrimidines, substances known to activate latent viruses, seems furthermore to support their viral origin⁸. Since 'undulating tubules' have been found to occur in high frequency in biopsy material from patients with lupus erythematosus, and in blood lymphocytes of renal transplant patients², they are considered to be pathognomonic for these diseases¹. Our findings, however, confirmed those of BESSIS⁹ and showed that also apparently healthy donors may show in lymphocytes 'undulating tubules'.

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⁹ M. BESSIS, *Living Blood Cells and their Ultrastructure* (Springer Verlag, Berlin/Heidelberg 1973), p. 456.

Inhibition by Syngenic Erythrocytes of in vitro Growth of Colonies from Murine Bone Marrow Cells

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Summary. The growth of colonies from murine bone marrow cells in soft agar cultures was found to be inhibited by 0.1 ml or more 50% washed syngenic erythrocyte suspension per 1.2 ml culture volume.

It has been observed by BRADLEY et al.^{1,2} that the addition of syngenic or xenogenic erythrocytes to soft agar cultures of murine haemopoietic cells increased the number and size of granulocytic and/or macrophage colonies. Enhancement of colony formation was found even at the maximal concentration tested, i.e. at about 0.03 ml 50% erythrocyte suspension per ml culture medium. The present experiments show that syngenic erythrocytes, when added to soft agar cultures of murine bone marrow in quantities of about 0.1 ml or more 50% erythrocyte suspension per ml culture volume, inhibit the growth of colonies.

Materials and methods. 2- to 4-month-old (BALB/c \times CBA)F₁ mice of both sexes were used. Bone marrow cells were washed out of the femur with, and suspended in McCoy's 5A medium (see later). Blood was taken by cardiac puncture and anticoagulated with preservative free heparin or defibrinated. The erythrocytes were washed thrice with Hanks' solution with an attempt to remove buffy coat cells after each centrifugation. The packed erythrocytes were resuspended finally in an equal volume of McCoy's 5A medium.

For culturing haemopoietic colonies, McCoy's 5A modified medium (Grand Island Biological Co., Grand Island, N.Y.) was supplemented, according to ROBINSON and PIKE³, with the only modification that 20% horse serum was used instead of 15% fetal calf serum. Bone marrow cells, agar to a final concentration of 0.3%, and the materials to be tested (erythrocytes, etc.) were added to the above medium. 1 ml aliquots of this mixture were placed into 40 mm glass Petri dishes, to which 0.2 ml per dish L cell conditioned medium had been added as source of an optimal amount of colony stimulating activity. After 7 days of incubation at 37°C in humidified air with 3% CO₂ according to FIRKET⁴, aggregates of at least 50 cells were counted as colonies under a dissecting micro-

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