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 erythematosus[1], and renal transplants[2], in lymphocytes of patients with chronic rheumatism[3], in lymphocytes in various syndromes associated with antibody deficiencies[4], 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 CHANDRA[6] and interpreted as pathological secretions of endoplasmic reticulum, or as an early response to injury[7].

On the other hand, because their characteristic morphology, it was suggested that they represent a special type of myxoviruses[1] and perhaps play a role in the etiology of some collagen diseases, such as systemic lupus erythematosus[1].

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 origin[8]. 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 patients[2], they are considered to be pathognomonic for these diseases[1]. Our findings, however, confirmed those of BESSIS[9] and showed that also apparently health donors may show in lymphocytes 'undulating tubules'.

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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 × CBA)F_1 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 PIKE[3], 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% CO_2 according to FIRKET[4], aggregates of at least 50 cells were counted as colonies under a dissecting micro-

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scope (magnification: × 40). If necessary, diluted acetic acid was added to the plates prior to counting to lyse erythrocytes.

Leukocytes were separated from heparinized blood on a methylcellulose-iodamide (Bracco, Milano) mixture[5,6], washed thrice with Hanks' solution and finally resuspended in supplemented McCoy's 5A medium.

Results. When bone marrow cells were plated with various amounts of erythrocytes prepared from heparinized blood, a remarkable increase in colony numbers was observed till 0.025 ml erythrocyte suspension per dish. At larger doses, however, a gradual decrease in colony formation was found with almost no colonies at 0.15 ml erythrocytes per dish (Figure). This inhibitory effect proved to be reproducible even with erythrocytes prepared from defibrinated blood (Table I).

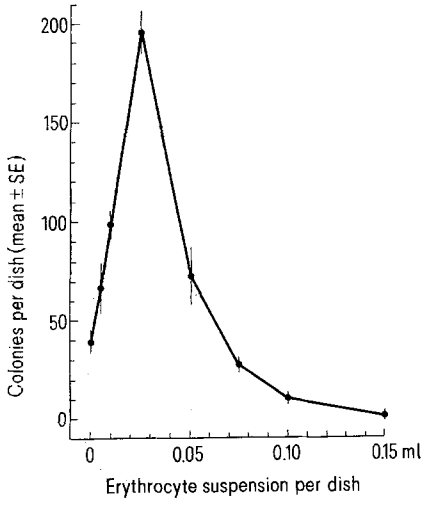
As some leukocytes always contaminated the erythrocyte suspensions, the effect of blood leukocytes on colony formation by bone marrow cells was studied in mixed cultures. Table II shows that leukocytes in numbers equal to those present in 0.1 ml of the fresh (unwashed) blood did not inhibit the growth of colonies.

The effect of mouse serum on bone marrow colony formation was studied by including 0.05 ml of serum (prepared from defibrinated blood) per dish in the mixture before plating. In 4 independent experiments, the number of colonies was 99.05 ± 4.6% (mean ± SE) of the controls set up simultaneously without mouse serum.

Table I. Effect of washed syngenic erythrocytes prepared from defibrinated blood on the in vitro growth of colonies from murine bone marrow cells

No. of bone marrow cells per dish	Number of colonies (mean ± SE)	
	Control (without erythrocytes)	With 0.15 ml 50% erythrocyte suspension per dish
5×10^4	39.3 ± 6.4	0
5×10^4	18.3 ± 3.4	0
10^5	41.0 ± 7.2	0
10^5	54.7 ± 2.4	1.0 ± 0.6

The cultures were set up in triplicate.



Number of colonies developing from 5×10^4 mouse bone marrow cells in cultures containing various amounts of syngenic 50% washed erythrocyte suspension prepared from blood anticoagulated with 10–15 IU/ml preservative free heparin. The total volume of a culture was kept constant at 1.2 ml.

Table II. Effect of syngenic peripheral blood leukocytes and erythrocytes on the number of in vitro colonies developing from 10^5 murine bone marrow (BM) cells

Mean number of colonies ± SE in cultures containing		
10^5 BM cells	10^5 BM cells + 0.1 ml leukocyte suspension[a]	10^5 BM cells + 0.1 ml erythrocyte suspension[a]
115 ± 9 (3)[b]	161 ± 9 (3)	0 (3)
48 ± 3 (6)	54 ± 12 (2)	7 ± 5 (2)

[a] The concentration of leukocytes and erythrocytes in the suspensions, respectively, was the same as in the fresh blood out of which the erythrocyte suspension was prepared. The same number of leukocytes did not give rise to more than 5 colonies when plated without bone marrow cells. [b] The number of cultures is shown in parentheses.

Discussion. It was found in the present experiments that syngenic washed erythrocyte suspensions present in soft agar cultures of mouse bone marrow inhibited the growth of cell colonies.

Leukocytes are known to inhibit the in vitro colony formation by mouse bone marrow cells[7,8]. Thus the question arises as to whether leukocytes, that were always contaminating the erythrocyte suspensions used in the present experiments, may have caused the observed inhibition of colony growth. However, this possibiity. can be excluded on the basis of data presented in Table II.

Serum of mice and humans may contain some substance(s) inhibiting haemopoietic colony growth[9–11]. As 0.05 ml mouse serum did not cause any appreciable inhibition in our experiments, it seems to be unlikely that traces of serum that may have contaminated the erythrocyte suspensions could cause the observed inhibition of colony growth. This inhibition, similarly, cannot be explained in terms of a nutritional deficiency due to the dilution of the medium, since the replacement of as much as 0.15 ml medium per dish by bidistilled water decreased the number of developing colonies to only about 40% (not shown in Results).

As other possibilities have been excluded, it seems likely that erythrocytes themselves cause the observed inhibition of colony growth. It appears relevant to note here that the phytohaemagglutinin induced transformation of murine peripheral blood lymphocytes was found also to be inhibited by the presence of more than 0.1 ml blood per ml medium[12]. Whether the phenomenon described in the present paper has any relevance to in vivo granulopoiesis and/or macrophage production cannot be judged at present.

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