We essentially agree with the hypothesis that the polyclonal activation of B-cells in the presence of a continuous trypanosome infection is likely to result in a progressive depletion of antigen-reactive B-lymphocytes[10], since these cells would then be activated to change into secretor cells, perhaps with little or no accompanying proliferation. In this way the immunosuppression in trypanosome infections may be explained by the depletion of B-cells capable of recognizing the introduced antigen. Indeed, a number of B-cell-mitogens have recently been shown to completely suppress primary immune responses in vivo to sheep erythrocytes[19]. Our demonstration of the in vitro mitogenic activity of these trypanosome products lends concrete support to the above hypothesis. Finally, if both activation and immunosuppression are polyclonal, it would be expected that the antibody responses to later-appearing trypanosome antigenic variants would also be impaired. Recent findings[10] lend some support to this view.

Other theories, such as enhancement of T-suppressor cell function during trypanosomiasis[7], must be considered in the light of recent compelling evidence that suppressor cells were involved in the immunological hyporesponsiveness observed in African trypanosomiasis[20]. Yet still another relevant and possible mechanism of immunosuppression could be that cells stimulated in vivo with the mitogen could release lymphokines[21] (e.g. interferon[22]), which would then block (or stimulate) other essential cell lines likely to play a prominent role in primary immune response, as has indeed recently been postulated[23] to explain the complex phenomenon of immunosuppression. We have, however, recently failed to detect increased interferon levels in *T. congolense* infected calves (Dr B. Rouse, personal communication).

In conclusion, our findings indicate that, under optimal conditions of culture, the *T. congolense*-derived factors are potently mitogenic in vitro for splenic lymphocytes from normal and congenitally athymic mice. We feel that it would also be worthwhile to investigate the possibility of demonstrating this mitogenic effect on the lymphoid cells of cattle and man, the 2 species in which the natural disease is most important. We would also like to know which fraction of the trypanosomal factor produces this effect and its role in the pathogenesis of the natural disease. We are currently investigating all these possibilities.

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Small lymphocyte production and lymphoid cell proliferation in mouse bone marrow[1]

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Summary. In mice given ^{3}H-thymidine systemically during temporary circulatory occlusion of one hind limb, comparison of the labeling of rapidly-renewing small lymphocytes in the tibial marrows demonstrated that these cells were locally produced. Labeling by ^{3}H-thymidine infusion revealed that many marrow large lymphoid cells, presumptive small lymphocyte progenitors, had a marked proliferative activity and rapid turnover which varied according to cell size, was maximal in young mice and declined with increasing age.

Inbred mice have been used widely in immunological studies and the bone marrow has been strongly implicated as a primary site of production of rapidly-renewing, virgin B small lymphocytes[3,4], but the myelogenous origin of such cells has not been formally proven. The present studies, utilizing radioautography with ^{3}H-thymidine, were therefore designed to demonstrate whether or not the rapidly-renewing small lymphocytes in mouse marrow are locally produced, and to examine the proliferation of presumptive progenitor cells in this species.

^{3}H-thymidine was administered systemically to C3H mice while being excluded from the tissues of one hind limb by temporary circulatory occlusion (figure 1). The appearance of labeled cells in the tibial marrow of both hind limbs was then compared. At all sampling intervals after ^{3}H-thymidine injection, a marked disparity existed between the small lymphocyte labeling index in the marrow of the 2 limbs. The customary rapid increase in small lymphocyte labeling was shown only in the marrow that had initial access to the injected ^{3}H-thymidine (figure 1), indicating that the accumulation of labeled small lymphocytes was dependent upon the initial labeling of locally situated precursor cells. If the labeled marrow small lymphocytes were blood-borne immigrants they would be expected in similar numbers and labeling intensities in both tibial marrows, because each limb had access to labeled, circulating cells for up to 3 days. A transitory appearance of small numbers of weakly labeled small lymphocytes in the initially occluded marrow (figure 1) can be attributed to the local reutilization of circulating ^{3}H-thymidine labeled products. That the temporary vascular stasis had not, in itself, impaired the local renewal of marrow small lymphocytes was demonstrated by subjecting mice to the standard limb occlusion procedure shortly after ^{3}H-thymidine had been administered and cleared from the circulation: 48 h after ^{3}H-thymidine injection the labeling index of

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small lymphocytes in the tibial marrow was 47.8 ± 3.7%, closely similar to that in normal non-occluded limbs (figure 1). These results demonstrate that in the mouse, as shown previously in the guinea-pig[5,8], the newly-formed small lymphocytes in the marrow are locally produced.

Yoshida and Osmond[5] have demonstrated in the guinea-pig that the immediate precursors of indigenous marrow small lymphocytes are contained in a population of large lymphoid cells. Mouse marrow also contains large lymphoid cells[6] many of which, unlike small lymphocytes, undergo DNA-synthesis, as shown by ^{3}H-thymidine incorporation[3]. Probably, such large lymphoid cells are small lymphocyte progenitors in mouse marrow, as shown in the guinea-pig, because they are the only population of otherwise uncommitted marrow cells which are adequate in numbers to fulfil this role[6]. To study their proliferative behaviour a continuous infusion of ^{3}H-thymidine was given to mice aged 4, 8 and 16 weeks, thus labeling all DNA-synthesizing cells and their progeny for periods up to 5 days. Many large lymphoid cells showed immediate (1 h) uptake of ^{3}H-thymidine, the proportion of such labeled DNA-synthesizing cells being higher in younger animals than in older ones (60%, 4 weeks; 42%, 8 weeks; 36%, 16 weeks) (figure 2a). The labeling of large lymphoid cells increased rapidly during ^{3}H-thymidine infusion (figure 2a). By 1 day, 94%, 88% and 85% of marrow large lymphoid cells were labeled in mice aged 4, 8 and 16 weeks, respectively. From 2 days onward, the rate of appearance of labeled cells declined markedly, but the labeling indices remained highest in young animals (figure 2a). When subdivided according to cell diameter the proportions of large lymphoid cells in each of 3 size groups were similar in mice of all ages studied (8.0–9.9 μm; 86.3 ± 0.2%; 10.0–11.9 μm; 10.1 ± 0.1%; > 12.0 μm; 3.7 ± 0.3%). The labeling index was higher among larger cells than smaller ones from 1 h onward (figures 2b, c, d). The largest cells (> 12.0 μm) showed 100% labeling throughout. For 2 other size categories (8.0–9.9 μm; 10.0–11.9 μm) the levels of both the initial labeling indices and subsequent labeling curves were related to the age of the mice (figures 2b, c, d). By 2 days, 95%, 90% and 88% of the cells measuring 8.0–9.0 μm were labeled in mice aged 4, 8 and 16 weeks, respectively. After 4 days, while nearly all (98%) such cells were labeled in 4–8-week-mice, 10% still remained unlabeled in 16-week-mice (figures 2b, c, d).

The active proliferation of many large lymphoid cells in mouse marrow, revealed in the present work by the high proportion of cells in DNA-synthesis and their rapid increase in labeling at early intervals of ^{3}H-thymidine infusion, is consistent with a postulated lymphocyte progenitor function. The decline with age in the fraction of large lymphoid cells in DNA-synthesis and in their ^{3}H-thymidine labeling curves accords with previously demonstrated age-related changes in the magnitude of small lymphocyte renewal[4] and in the number of lymphoid cells of various sizes in mouse marrow[6]. Thus, both the proliferation of large lymphoid cells and the production of small lymphocytes are maximal in young (4 weeks) mice, declining thereafter until adulthood. While this is the first study of age-related changes in large lymphoid cell kinetics in the marrow, the correlation between proliferative activity and cell size matches that observed previously in young guinea-pigs[7–9]. In other respects, the findings in mice and guinea-pigs show some differences. In the guinea-pig, large lymphoid cells exhibit a range of cytoplasmic basophilia, generally correlated with cell size, most of the DNA-synthesizing cells being basophilic[7,8]. No such range of basophilia has been observed

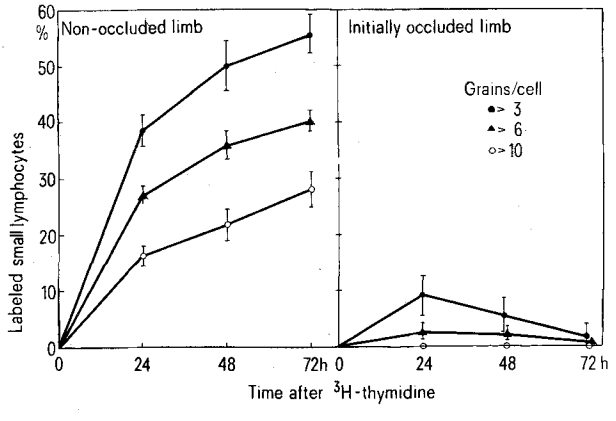

Fig. 1. Percentage of labeled small lymphocytes in bone marrow after ^{3}H-thymidine injection. 8, male, 12 weeks old, C3H/HeJ mice (Jackson Laboratories, Bar Harbor, Me., USA), were injected via the lateral tail vein with ^{3}H-thymidine (25 μCi; spec. act. 6.7 Ci/mM; New England Nuclear Corp., Boston, Mass., USA), while the circulation to the left hind limb was occluded by a rubber tourniquet tied around the proximal portion of the thigh for 20 min. After 24, 48 and 72 h, radioautographs of bone marrow smears from both tibiae were prepared as described elsewhere[4].

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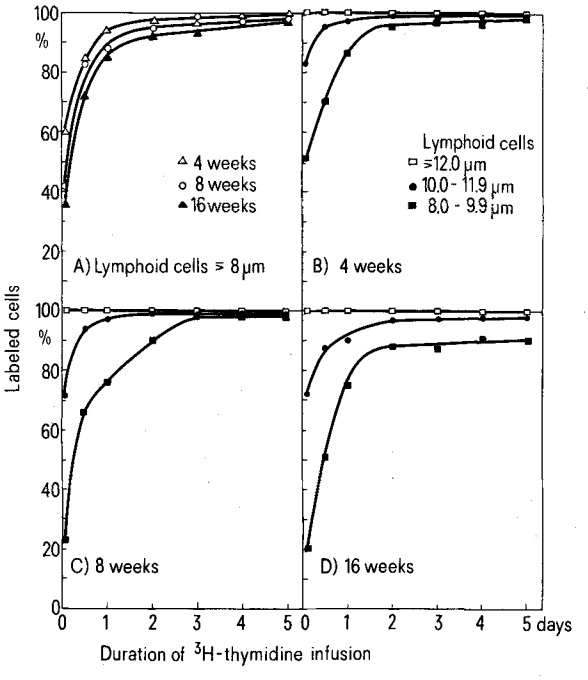

Fig. 2. Percentage of labeled large lymphoid cells in bone marrow during ^{3}H-thymidine infusion. 18 mice, comprising 3 equal groups aged 4, 8 and 16 weeks, respectively, were infused s.c. with ^{3}H-thymidine, as described previously[4]. Animals were killed at 12 h and at daily intervals from 1 to 5 days after beginning isotope infusion. Radioautographic smears of femoral marrow were prepared, as described[4]. From each animal, approximately 800 large lymphoid cells (> 8.0 μm nuclear diameter) were measured noting cells labeled with 3 or more grains. In each experiment, 3 grains indicated positive labeling by comparison with counts of background grains over 500 erythrocytes. Values at 1 h represent the mean from 2 to 3 mice, each given a single dose of ^{3}H-thymidine (1 μCi/g b. wt) 1 h before sampling.

among mouse large lymphoid cells, possibly reflecting a species difference in their potential. Large lymphoid cells account for approximately 20% of all lymphoid cells in mouse marrow[6], but 45% of all lymphoid cells in guinea-pig marrow[7]. Some cells in guinea-pig marrow, classified morphologically as large lymphoid cells, may be destined to produce cells other than small lymphocytes, accounting for the relatively high proportion of large lymphoid cells. On the other hand, the observation in mice of all ages that some large lymphoid cells remain unlabeled after 4–5 days continuous ^{3}H-thymidine infusion suggests that, even in mouse marrow, not all large lymphoid cells are engaged in the continuous production of small lymphocytes. Some may comprise a noncycling subpopulation of cells, possibly 'resting' progenitor cells, as postulated for the guinea-pig[9].

The attenuation of prostaglandin-induced luteolysis in decidual tissue-bearing pseudopregnant rats[1,2]

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Summary. Decidual tissue (DT)-bearing pseudopregnant (PSP) rats, in contrast to hysterectomized PSP rats, were resistant to a luteolytic regimen of either $PGF_2\alpha$ or PGE_2 when examined for the duration of PSP diestrus. The PG treatments, however, caused a marked fall in the serum progesterone levels on days 10 and 12, although the levels in DT-bearing rats on day 10 were significantly higher than in the hysterectomized rats.

Melampy et al.[4] suggested that the decidualized uterus prolonged pseudopregnancy (PSP) in the rat in the same manner as hysterectomy, that is, by preventing the production of a uterine luteolysin. Recent evidence has suggested that this luteolysin in the rat, as in some other species, may be a prostaglandin (PG)[5-7]. Reports from this laboratory[8-10] and from Melampy's[11] have indicated that decidual tissue (DT) may have a luteotrophic effect which is distinct from whatever ability it may have to prevent luteolysis. Other reports have shown that decidualization does not cause a decrease in the production of PGs by the uterus[12-14]. To see whether DT interferes with PG-induced luteolysis, the effect of PG treatment on progesterone secretion in DT-bearing PSP rats was compared with its effect in hysterectomized PSP rats. The duration of vaginal diestrus was used as a measure of the maintenance of progesterone secretion and the change in peripheral serum progesterone levels as a measure of change in rate of progesterone secretion since, in the rat, a change in the former closely parallels one in the latter[15].

Materials and methods. Regularly cyclic, 250–300 g female Holtzman (Sprague-Dawley) rats were maintained at 24.5 °C under a 14:10 light:dark schedule, with free access to Purina rat chow and water. Pseudopregnancy was induced by mechanical stimulation of the cervix with a glass rod on the days of vaginal proestrus and estrus. Day 1 of PSP was the day of ovulation (estrus).

On day 5 of the PSP, rats were divided into 2 groups, at which time 1 group was hysterectomized, and the other had their uteri scratched with a barbed needle to induce DT formation[13]. On the morning of day 9, each rat was laparotomized; this was done primarily to confirm the formation of DT in the latter rats, and as a control procedure, therefore, in the hysterectomized ones. Each group was then divided into 2 subgroups. In one, the rats were injected with PGE_2 (1 mg) twice daily on days 9 and 10, and in the other, with the tromethamine salt of $PGE_{2\alpha}$ (400 µg) each morning, on days 9 and 10. Each dose was given s.c. in 0.2 ml of 25% ethanol:saline. Vehicle controls were not included, but data from uninjected controls were available from other studies done at the same time[9,17].

Blood was collected by direct jugular venipuncture from each rat just before the PG injection on days 9 and 10, and again on day 12. Light ether anesthesia was used to facilitate the blood sampling. The blood samples were allowed to clot, and were centrifuged at 4 °C; the serum was then stored at − 20 °C until assayed for progesterone. Vaginal smears were recorded daily for each rat for the duration of the experiment to determine the length of PSP; in all the rats, an estrous cycle of normal duration followed the end of PSP.

Progesterone was assayed at first by the protein binding displacement (PBD) method, as used in this laboratory[18], and in later experiments by radioimmunoassay (RIA) with a sheep antiserum to 11β-hydroxyprogesterone complexed to BSA (GDN-337). The details of the assay, evidence for its specificity, sensitivity, and variability, and a comparison with the PBD method, are described

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