fine aluminium wire mesh, washed twice in minimum essential medium and resuspended in MEM plus 10% fetal calf serum (Grand Island Biologic Co.). 3 suspensions were prepared for each spleen and 1.0 μCi of thymidine-methyl-^{3}H (New England Nuclear) was added per 10^6 cells. Cells were incubated at 37 °C in 5% CO_2, humidified air atmosphere for 8 h, then solubilized with 0.1 *N* NaOH. DNA was precipitated with cold (4 °C) 20% trichloracetic acid (TCA), and filtered onto Whatman glass fibre paper (GF/A) in a Millipore Filter apparatus with cold 5% TCA. Filter papers were dried overnight, transferred into scintillation fluid (toluene containing Liquifluor, New England Nuclear), and counted in a Beckman Model LS-230 liquid scintillation counter. The final values, means of triplicate samples, are recorded in cpm per 10^6 splenocytes (cpm/10^6) plus or minus the standard error.

Results and discussion. Daily tryptophol administrations significantly depressed both primary and secondary hemagglutinin responses of *M. montanus* to human erythrocytes (Table I). However, the increases in antibody levels after secondary immunizations rose similarly (3-fold) in both control and tryptophol-treated animals. In mice after a single antigen exposure (top of Table I), tryptophol-treated animals responded with hemagglutinin titers significantly below controls. Immune responses at the cellular level, measured by immunocytoadherence of erythrocytes by spleen cells, were also depressed in tryptophol-treated mice. In a separate experiment (bottom of Table II), tryptophol-treated mice responded to both primary and secondary antigen administrations with significantly depressed responses. However, the increases in average hemagglutinin titers following secondary immunizations were nearly identical (5-fold) in both control and tryptophol-treated mice.

Tryptophol administrations did not seem to impair the cell-mediated, contact hypersensitivity responses of mice to oxazalone (Table III). After primary, secondary and tertiary oxazalone treatments there were no significant differences observed between tryptophol-treated and control mice. However, it was found that in preliminary investigations in mice, daily administrations of tryptophol depressed ($p < 0.1$) survival times of animals challenged with Ehrlich's ascites tumor cells compared to controls. Tryptophol-treated mice survived an average of 12.9 ± 1.1 days following tumor challenge, whereas controls survived an average of 15.5 ± 0.9 days.

Table IV depicts the effects of i.p. injections of tryptophol upon the in vitro incorporation of tritiated-thymidine into DNA by spleen cells of *M. montanus*. In two separate experiments after 5, 15 and 23 days of tryptophol-treatment, the uptake of thymidine over an 8-h period was significantly lower in tryptophol-treated animals than in controls. However, the degree of depression in thymidine-incorporation into DNA did not seem to be enhanced by longer periods of tryptophol-treatment (see the ratio of means).

The results of this investigation suggest that daily i.p. administrations of tryptophol in mice and *Microtus* depress humoral responses to heterologous erythrocytes, but do not affect cell-mediated reactions to oxazalone. The mechanism of this immunosuppressive effect is not fully understood. In a series of papers DEVOINO et al.[11–13] report that tryptophan metabolites, such as serotonin or 5-hydroxytryptophan, depressed immune function, possibly by suppressing protein synthesis or cell division. In this investigation it was noted that spleen cells of tryptophol-treated animals incroporated lower amounts of thymidine into DNA, than did controls. This suggests that tryptophol administrations may depress rates of cellular replication. Hence, it may be postulated that one cause for the observed immunosuppressive effects of tryptophol may be the inhibition of clonal proliferation following antigenic stimulation.

Further, in preliminary experiments it was suggested that tryptophol administrations decreased the mean survival times of mice challenged with Ehrlich's tumor cells. A similar result was seen in *Microtus* infected with *Trypanosoma brucei gambiense*[4]. It is suggested that in both of these situations, enhanced pathogenesis of the cancerous growth may be due to depressed immune activity in the host. Tryptophol has been slown to be metabolized from tryptophan in *T.b. gambiense*[9] and in trypanosome-infected laboratory animals[10]. Furthermore, parasite production of high levels of tryptophol in the host have been postulated do account for the characteristic neurologic abnormalities (sleep, depression, torpor and convulsions) seen in African trypanosomiasis[9]. The results of this investigation suggest that trypanosome production of tryptophol may account for the immunosuppression observed during infection[14].

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Cortisone Sensitive T-Cells in Peyer's Patches[1]

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Summary. Pretreatment of donor lymphoid cells with cortisone has been shown to depress the T-cell subpopulation responsible for cellular proliferation in the GVH reaction. A quantitative assay as well as the histological criteria of the GVH reaction have been used in this study to demonstrate the presence of cortisone-sensitive T-cells within the Peyer's patches as well as in the spleen and mesenteric lymph nodes in the rat.

The presence of T-cells within the Peyer's patches was demonstrated first by the use of T-cells markers[2–5] and subsequently by the study of migratory patterns of thymocytes in the small bowel[6,7]. There have been conflicting reports about the immunocompetence of these T-cells[8–10]. Nevertheless recent data involving in vitro culture with specific mitogens[11], the mixed lymphocyte reaction[12], and graft versus host (GVH) reactivity[12–14] have confirmed the thymus-dependent function of this gut associated lymphoid tissue. CANTOR, TIGELAAR and ASOFSKY[15,16] have shown that the GVH reactions are mediated by an interaction among different subpopulations of T-lymphocytes with different sensitivities to corticosteroid treatment, anti-lymphocyte serum or

adult thymectomy[17,18]. Therefore the discrepancies in previous results concerning the GVH reaction induced by Peyer's patches might be explained by a distribution of T-cell subsets different from that observed in other peripheral tissues. The aim of the present study was to test the presence of cortisone sensitive T-cells within the Peyer's patches and to compare any activity of such cells if present with those of the spleen, thymus and lymph nodes using the histological criteria of the GVH reaction and a quantitative assay, the paravascular infiltration of the liver.

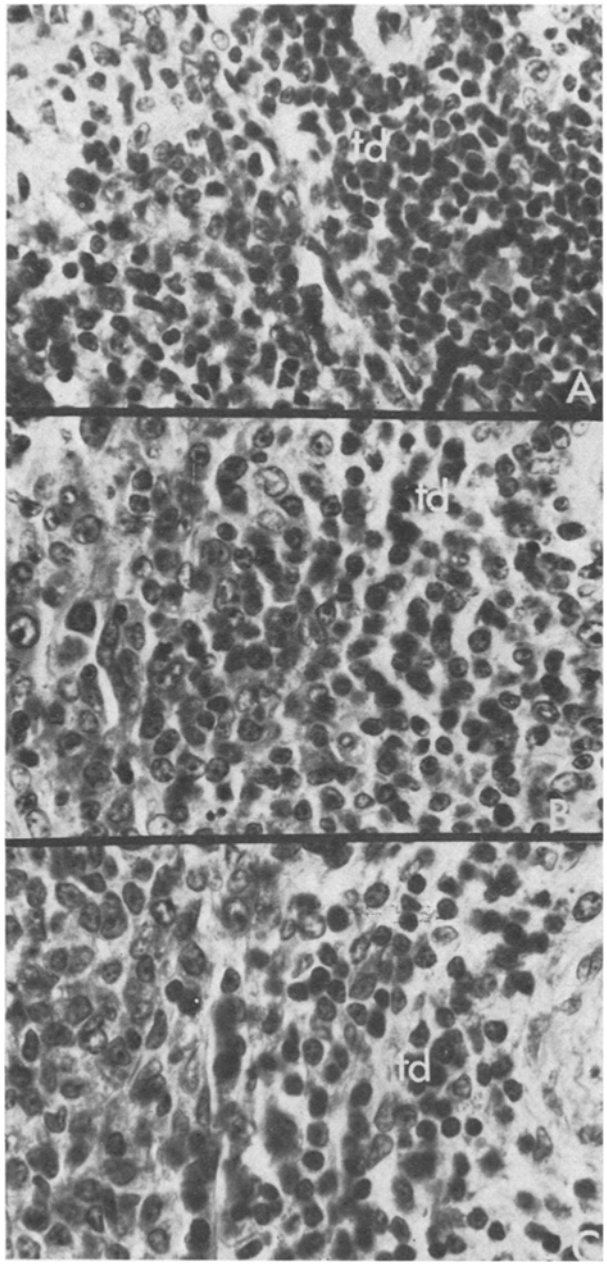

Fig. 1. Spleen section of the recipient 15 days after implantation of Peyer's patches (Hematoxylin Eosin × 350).
A) Implantation of isogeneic Peyer's patches.
B) Implantation of semiallogeneic non-treated Peyer's patches.
C) Implantation of semiallogeneic cortisone-treated-Peyer's patches. Note the cellular depletion observed in the thymus dependent (td) periarteriolar area in cases B) and C).

Material and methods. According to a previously described procedure[13], lymphoid tissues were implanted into the anterior hepatic lobe of F1 Hybrid rats (AUG × LEW). The Peyer's patches, the spleen, the mesenteric lymph nodes and the thymus were transplanted according to 3 different experimental schedules: a) hybrid lymphoid tissues in F1 Hybrid recipients (50 animals), b) parental August lymphoid tissues in F1 Hybrid recipients (55 animals), c) cortisone treated (CT) parental August lymphoid tissues in F1 Hybrid recipients (60 animals). In the latter the donor animals received an i.m. injection of a suspension containing 125 mg/kg body weight cortisone acetate (Roussel, France) 48 h before sacrifice. For the non-cortisone-treated (NCT) donors, the mean number of lymphoid cells for each implantation exceeded 10^7 cells. Cortisone treatment of the donor, however, reduced the number of the transplanted lymphoid cells to $4 \pm 0.005 \times 10^6$ for the Peyer's patches, $8 \pm 0.016 \times 10^6$ for the thymus and $4.6 \pm 0.034 \times 10^6$ for the lymph nodes. Cortisone treatment did not change the number of lymphoid cells present in the spleen transplant. All recipients were examined daily for weight change and general appearance. They were killed by ether in groups of 3 to 5 post-operatively on days, 8, 15 and 30. The spleen and liver were removed for histological study and the extent of the paravascular infiltrates (PVI) within the liver was estimated in the non-implanted hepatic lobes according to BAIN's criteria[19].

Results. In control animals with an isogeneic implantation of lymphoid tissue no clinical and no histological changes (Figure 1 A) were observed. The hepatic lobes contained a similar number of PVI whether they had been the site of implantation or not. This averaged 0.1/10 mm² for each section in all the post-operative examinations from 8 to 30 days. When F1 Hybrids were implanted with non-treated parental lymphoid tissue, no lethal GVH syndrome was observed whichever tissue was used. The clinical course of experimental animals with CT Peyer's patches was similar to that observed after implantation of non-treated Peyer's patches. However, 4 recipients of parental CT lymph nodes died between the 5th and 30th post-operative day with a clinical picture of GVH disease. The CT spleen and thymus did not induce any mortality

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in F1 Hybrid recipients. Histological examination of the spleen sections after implantation of Peyer's patches whether CT (Figure 1C) or not (Figure 1B) showed cellular depletion located in the thymus-dependent area of the follicle. On the other hand, after CT Peyer's patches, the cellular blastic proliferation within the red pulp was less marked than after NCT parental Peyer's patches implantation, but was always associated with the presence of numerous pleomophic macrophages. Comparison of the PVI between CT and NCT animals at different post-operative times is illustrated in Figure 2. Between the 8th and 30th day, there was no change in PVI count after CT Peyer's patches implantation. On the other hand, a significant decrease of PVI was noted at the 15th after implantation of CT spleen ($p < 0.05$) and at the 30th day after CT thymus and lymph node implantation, whereas at this time an increase of the PVI count was noted after CT spleen implantation. The numbers of PVI produced by the tested lymphoid tissues from cortisone-treated animals were compared at the different post-operative times. A significant difference ($p < 0.05$) appeared only at the 30th day when PVI producing activity was greatest for the spleen. Comparison of the PVI count observed after implantation of CT or NCT lymphoid tissues showed that except for CT spleen at the 8th day, the PVI was significantly lower after each CT tissue implantation. It was noted that NCT lymphoid tissue produced significantly different numbers of PVI at the 15th day, as follows:

$$\text{PVI (thymus)} < \text{PVI} \begin{cases} \text{Peyer's patches} \\ \\ \text{lymph nodes} \end{cases} < \text{PVI (spleen)} \quad (p < 0.01)$$

whereas no significant difference between the PVI responses of the different CT lymphoid tissues was observed at the 15th day. Thus the cortisone treatment of the donor reduced the PVI producing capacity of the Peyer's patches, as well as that of the spleen and lymph nodes, to the level of the non-treated thymus capacity.

Discussion. In the rat acute glucocorticosteroid administration reduced quantitatively the cell population of the different lymphoid tissues under study. The reduction of circulating thymus and spleen lymphocytes has been regulary noted by others[20–23]. Similarly in the case of CT Peyer's patches, a reduction of the lymphocyte cell population has been noted. It has been previously reported in vivo and in vitro that despite a marked increase in GVH reactivity on a cell for cell basis, cortisone treatment of the donors gave a reduction in the splenomegaly induced by thymus or spleen cells[16, 21, 22, 24]. Our histological observations suggest that the cell proliferation in the spleen of the recipient during the GVH reaction to implanted Peyer's patches is reduced by treatment of the donor with cortisone. Similarly, the cortisone treatment of Peyer's patches of the donor reduced significantly their PVI forming capacity in the GVH reaction, as compared to non-treated Peyer's patches. This reduction is of the same range as that observed after CT spleen or lymph nodes implantation. However, the cortisone sensitive cell population of Peyer's patches appears more closely related to that of mesenteric lymph nodes. The cortisone sensitivity of lymph nodes and Peyer's patches cells on one hand, and spleen cells on the other hand, is slightly different, the latter being only temporarily inactivated and thus corresponding to the functional escape observed in the PVI count after CT spleen implantation. Bain[25] suggested that the cells which produce paravascular infiltration might belong to the same class of cells which cooperate in splenic enlargement. Histological examinations of the spleen sections in this study confirm this assessment. Our results lead to the conclusion that this T-cell subpopulation is destroyed or inactivated by cortisone treatment. Moreover, the reduction in number of the PVI observed in this study after CT Peyer's patches implantation suggest that steroid sensitive T-cells exist in Peyer's patches.

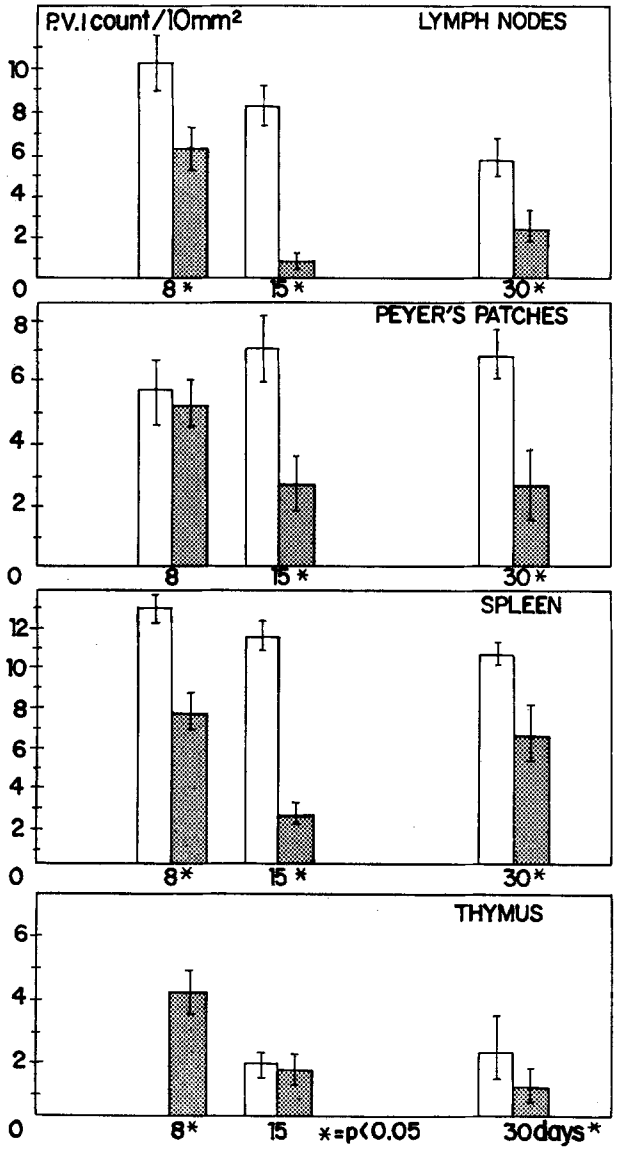

Fig. 2. Course of the paravascular infiltrate enumeration after the different cortisone treated (hatched areas) and non-treated (non-hatched areas) parental lymphoid tissues implantations.

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