tion and, after washing with isotonic saline solution, they are resuspended in a volume of saline similar to that of the initial plasma.

To effect the extraction of phospholipids, an acidified organic solvent is used once the platelets have been homogenized with perchloric acid 0.6 *M* (V/V). Then, it is centrifuged at 3000 rpm for 15 min ($\simeq$ 1500 $\times g$) and the resulting precipitate is treated with a mixture of chloroform, methanol, hydrochloric acid 0.1 *N* (20/10/0.1). After being agitated, 8 ml of hydrochloric acid 0.1 *N* is added and then centrifuged. The supernate is taken away and the platelet button undergoes another extraction following the same method.

Thin layer chromatography is used to evaluate the phospholipids using silical-gel at 250 μ. Previous to their use the chromatographic plates are washed with a mixture of chloroform, methanol, acetic, water (50/30/8/4), and immediately after they are dried at 110 °C for 1 h. The same mixture used to wash the plates is used as a solvent. After the application of the sample (0.1 ml), it is left running for 45 min, then dried, and finally developed with phosphomolybdic at 20% or rhodamine 6G at 0.1% in ethanol at 96%.

To quantify fractions obtained, they are separated by scraping and their contents of phosphorus multiplied by 25 to obtain phospholipids.

Results and discussion. In our experiences 0.9 ml of a normal plasma, with its platelets adjusted to 270,000 mm^3, was incubated with a dipyridamole solution in isotonic saline solution, giving a final concentration of 4 μg/l. After 10 min incubation at room temperature, the platelets were separated by differential centrifugation and the concentrations of the total phospholipids and the major phospholipid fractions were evaluated. In the control sample the 0.1 ml of dipyridamole solution was substituted by 0.1 ml of saline solution. The assays with and without dipyridamole have been performed on aliquots of the same platelet rich plasma.

It was observed (Table) that, after the platelet incubation with dipyridamole, a 38% reduction in the concentration of the sphingomyelin and a 21% increase, which is likely to be compensatory for the reduction, in the phosphatidyl choline, were produced. Undoubtedly, an alteration of the platelet phospholipids could influence the maintainance of the ideal conditions for the normal functioning of the platelet membranes[12]. All this is more suggestive if it is taken into account that, as recently described by SCHICK[13], small hydrolysis of the platelet phospholipids can notably influence the release reaction.

It has also been possible to assert in our laboratory that aspirin, a drug with an antitrombotic action as well, reduces in vitro as well as in vivo the sphingomyelin rate[14]. The possible correlation between both actions is, for the moment, unknown to us.

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The Effects of Tryptophol on Immune Responses and its Implications Toward Trypanosome-Induced Immunosuppression

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Summary. Daily administrations of tryptophol to laboratory rodents resulted in significantly depressed antibody production to heterologous red blood cell challenge, but did not alter cellular-mediated responses to oxazalone. These results suggest that trypanosome-produced tryptophol may account for the immunodepression observed during trypanosomiasis.

The phenomenon of trypanosome-induced immunodepression has been reported to occur in experimental rodent infections[3–5], and in natural human infections[6]. The mechanisms of such immunologic abberations are ill-understood. One suggestion is that the trypanosomes elaborate a product which is directly suppressive to the host's immune apparatus[7]. Indeed, LONGSTAFFE[8] reported that live trypanosomes added to cultures of normal lymphocytes depressed mitogenic responses to phytohemagglutinin.

This paper reports upon an investigation into the immunosuppressive properties of tryptophol (indole-3-ethanol). This substance has been demonstrated to be synthesized by *Trypanosoma brucei gambiense*[9] and it has been reported that the metabolism of tryptophan is significantly increased in *T.b. gambiense* infected rats[10]. Laboratory animals, mice and field voles, were given repeated injections of tryptophol and the following immunologic parameters were investigated: 1. humoral levels and cellular production of antibodies to heterologous erythrocytes; 2. cell-mediated responses to oxazalone; 3. spleen cell thymidine-uptake; and 4. the course of Ehrlich's ascites tumor growth.

Materials and methods. The laboratory animals used in this investigation consisted of the field vole, *Microtus montanus*, and white mice. *M. montanus* were obtained from our colony maintained at Tulane University in New Orleans, Louisiana. CD-1 mice were obtained from Charles River Laboratories, Wilmington, Massachusetts. Daily

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Table I. The effects of tryptophol upon humoral responses of *Microtus montanus* to human type B+ erythrocytes

Group	No. of animals	Immunization	Hemagglutinin titer[a]	Significance
Control	12	Primary	1.8 ± 0.5	
Tryptophol	10	Primary	0.9 ± 0.4	$p < 0.01$
Control	11	Primary + secondary	2.7 ± 0.4	
Tryptophol	12	Primary + secondary	1.6 ± 0.2	$p < 0.005$

[a] Titers are expressed as $\log_{10}$ values of the geometric means $\pm$ SE.

doses of 150 mg tryptophol (Sigma Chemical Co.) per kg body weight were administered via i.p. injections 8 days prior to and throughout the immunization schedule. Tryptophol was suspended in a 1:1 mixture of propylene glycol:water (PG:W) and controls received equivalent amounts of PG:W without tryptophol. Other animals received daily injections of either 0.25 ml PG:W (controls) or 100 mg tryptophol per kg body weight in 0.25 ml PG:W for 5, 15 or 23 days prior to sacrifice for the thymidine-uptake study and for 5 days prior to tumor cell challenge for Ehrlich's ascites tumor study.

Antibody responses were measured in responses to either a single i.p. injection or primary and secondary i.p. injections (separated by 7 days) of 10^8 thrice washed human type B+ erythrocytes suspended in phosphate buffered saline, pH 7.2. The procedures for hemagglutination test, immunocytoadherence investigations, and the techniques for stimulating and measuring contact hypersensitivity responses to oxazalone have been reported by ACKERMAN and SEED[4]. All statistical analyses were performed utilizing the students *t*-test and were considered significantly different at the 95% confidence level.

For the spleen cell thymidine-uptake study spleens from tryptophol-treated and control *Microtus* were rapidly removed from cervically dislocated animals and placed in cold (4 °C) Eagle's minimum essential medium (MEM, Microbiologic Associates). Spleens were ground through

Table II. The effects of tryptophol upon immune responses of mice to human type B+ erythrocytes.

Experiment 1: Primary immunization only

Group	No. of animals	Hemagglutinin titer[a]	Significance	Immunocytoadhering cells per 5000 splenocytes	Significance
Control	15	1.8 ± 0.4		27.0 ± 4.3	
Tryptophol	15	1.2 ± 0.2	$p < 0.05$	12.8 ± 6.1	$p < 0.005$

Experiment 2: Primary and booster immunizations

Group	No. of animals	Immunization	Hemagglutinin titer	Significance
Control	4	Primary	1.8 ± 0.2	
Tryptophol	4	Primary	1.2 ± 0.2	$p < 0.05$
Control	7	Primary + secondary	3.3 ± 0.4	
Tryptophol	7	Primary + secondary	2.6 ± 0.2	$p < 0.05$

[a] See Legend to Table I.

Table III. The effects of tryptophol upon contact hypersensitivity to oxazalone sensitization in mice: Changes in ear thickness $\pm$ SE (in mm)

Oxazalone sensitization	Control	Tryptophol-treated	Significance
1°	0.11 ± 0.10 (12)[a]	0.28 ± 0.18 (12)	none
2°	1.38 ± 0.13 (12)	1.20 ± 0.18 (12)	none
3°	1.08 ± 0.11 (12)	1.06 ± 0.17 (12)	none

[a] Numbers in brackets represent number of animals tested.

Table IV. The effects of tryptophol upon 8-H thymidine-uptake by splenocytes of *Microtus montanus:* Thymidine-uptake $\pm$ SE (cpm/10^6 splenocytes)

Experiment	Days treated	Control	Tryptophol-treated	Ratio of means	Significance
I	5	3864.2 ± 1182.5 (3)[a]	911.2 ± 130.2 (4)	4.2	$p < 0.05$
II	15	1409.5 ± 152.9 (6)	775.8 ± 50.3 (7)	1.8	$p < 0.005$
	23	1487.8 ± 189.6 (7)	694.1 ± 52.2 (7)	2.1	$p < 0.001$

[a] Numbers in brackets represent number of spleens examined.

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