that a removable coat on lymphocytes, presumably the attached blocking factor(s), may be responsible for their decreased reactivity in pregnancy. This factor is probably lacking or is present only in lower quantities on lymphocytes derived from habitually aborting women.

Lymphocytes (LC-1) of 20 pregnant and 20 nonpregnant women were tested for cytotoxic activity. Our cytotoxicity test, which has been described elsewhere[10], utilized the endogeneous enzyme activity of residual target cells. Lymphocytes of the IUD group showed a significantly higher cytotoxic activity ($p 0.001$) than those of PR group (fig. 2).

20 sera (1 ml each) from women in their 16th week of pregnancy were dialyzed for 48 h against 500 ml of PBS at 4 °C. Dialysis tubing (SPECTRA/Por 3 Berghof GmbH FRG) with an exclusion limit of 3500 daltons was used. An aliquot of each serum kept at 4 °C for 48 h served as control. LC-5 of 6 nonpregnant women were incubated overnight at 37 °C in 1 ml of dialyzed or nondialyzed pregnancy serum, which was then removed by washing. Cytotoxic activity of lymphocytes incubated in dialyzed sera was significantly higher ($p < 0.001$) than that of lymphocytes incubated in nondialyzed pregnancy sera (fig. 2). Residual blocking activity remaining in pregnancy sera after dialysis did not exceed 15%. These results suggest that the blocking factor is dialyzable, consequently its molecular weight is possibly under 3500 daltons. This finding is at variance with identification of the blocking factors as IgG molecules[5] or immune complexes[11]. A possible explanation for this apparent discrepancy is, that in vivo these small molecular size blocking factors may be attached to one or the other fraction of human serum.

Our data suggest that an increased level of serum factor(s) with a relatively small molecular weight is responsible for decreased cellular reactivity in pregnancy against an embryonic antigen (HEF). Perhaps it contributes to the nonrejection of the fetus. Increased lymphocyte reactivity in AB women might be a result of a lower level of blocking factor(s), or a decreased attachment of the factor(s) to the lymphocytes or both. The lack of an appropriate depression of lymphocyte reactivity might be one of the causative factors of idiopathic spontaneous abortion.

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Nonspecific acid-esterase activity in lymphoid cells of *Bufo bufo*

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Summary. E-rosette formation and nonspecific acid-esterase (ANAE) patterns were assayed, in parallel, to identify lymphocyte populations of *Bufo bufo*. The ANAE technique appeared to be a reliable method to study the immune system of *Bufo*.

Amphibia play an important role in the study of the phylogeny and evolution of immunity[1-3]. Although several studies on humoral and cell-mediated immune responses of amphibia have been carried out in recent years[4-7], little is available on the detection of lymphocyte populations.

In mammals, the nonspecific acid α-naphthyl esterase technique appeared to be practical to identify T-lymphocytes in addition to the traditional E-rosette assay and specific T-cell heteroantisera techniques[8-10].

The purpose of this study was to investigate whether acid esterase staining can be considered a valid technique in amphibia also, for identification of T-cells and for localization of these elements in lymphoid organs.

Materials and methods. Larvae at stages 1 and 2 according to the morphological criteria of Schreiber's table[11] and adults of *Bufo bufo* were employed in this investigation. The thymus and spleen were removed and transferred into PBS (pH 7.2) to make single cell suspensions. Every cell suspension was used to determine, in parallel, the E-rosette-forming cell (RFC) response with sheep erythrocytes (SRBC) and the nonspecific acid α-naphthyl esterase (ANAE) activity.

Immuno-cytoadherence assays were performed with the method used in a preceding study on the RFC response of *Triturus cristatus*[12]. To demonstrate ANAE activity, smears of cell suspensions were fixed and stained by the method described by Mueller et al.[13] using alpha-naphthyl-acetate and hexazotized pararosanilin. A group of larvae, before RFC and ANAE activity assays, were injected i.p. with 3 µl 75% SRBC in saline to test the immunological responsiveness of thymus and spleen. For a topographic tissue localization of identifiable lymphoid cells, cryostat sections of adult thymus and spleen were prepared and stained by the method of Mueller et al.[13]. It is known that in mammals T-lymphocytes show a localized brown nodular $ANAE^+$ reaction (T-pattern) that can be distinguished from the diffuse cytoplasmatic staining of macrophages (M-pattern). In this study $ANAE^+$ cells were considered to be those exhibiting the T-pattern described for mammals.

Results and discussion. For each animal the RFC and $ANAE^+$ cell numbers were determined in cell suspensions of thymus and spleen. The results, expressed as the means, are reported in the table.

The major finding from these data is the remarkable difference between the RFC and $ANAE^+$ cell numbers. Nevertheless, the RFC results are in agreement with those obtained by other authors in amphibia[5,14]. As to the $ANAE^+$ cells, it can see that the so-called 'T-pattern' in *Bufo* is identical to that of mammals (fig. 1). In addition the number of $ANAE^+$ cells in *Bufo* larva thymus is not very dissimilar to the number of $ANAE^+$ cells in mammalian thymus[15]. The data for the larvae at stage 1 show that $ANAE^+$ cells are present in the thymus and that some thymus cells have the capacity to form rosettes even when

the spleen is very small and does not present lymphocytic differentiation. The data for the larvae at stage 2 show that the spleen, undergoing lymphoid maturation, is populated by ANAE$^+$ cells and RFCs. Adult data, compared to those of larvae, show that in the adult, ANAE$^+$ cells and RFCs are prevalently in the spleen. Data for immunized larvae show that the thymus and spleen, even during larval stages, are capable of generating an immunological response. All data in the table indicate that in larvae and adults, each organ has a characteristic constitution of ANAE$^+$ cells and RFCs.

The histochemical demonstration of ANAE activity in tissue sections permitted localization of the ANAE$^+$ cells (fig. 2). Cryostat sections of adult thymus showed T-pattern ANAE$^+$ cells in low numbers scattered evenly in the cortical and medullary areas. Cryostat spleen sections showed ANAE$^+$ cells within the red-pulp and within the white-pulp area, where many M-pattern ANAE$^+$ macrophages were present.

Given the findings that plaque-forming cells have been observed in the thymus of immunized amphibia[16] and that B-cells of mammals are either ANAE$^-$ cells or show a granular pattern, it is of interest to observe in the thymus of immunized larvae some cells with a granular ANAE activity (fig. 1) similar to that of mammalian B-cells.

Although no functional studies were performed to ascertain the immunological potentials of ANAE$^+$ cells of *Bufo*, the ANAE$^+$ cell pattern that was identical to the mammalian

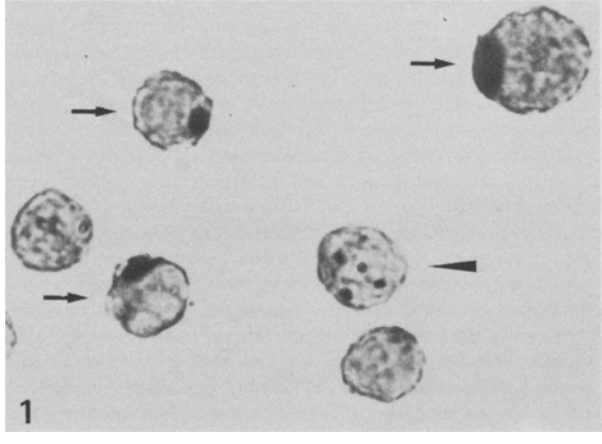

Fig. 1. ANAE activity in a smear of thymus cell suspension from an immunized stage 2 larva. 3 cells (arrows) show the T-pattern, whereas 1 cell (arrowhead) shows a granular pattern. × 2000.

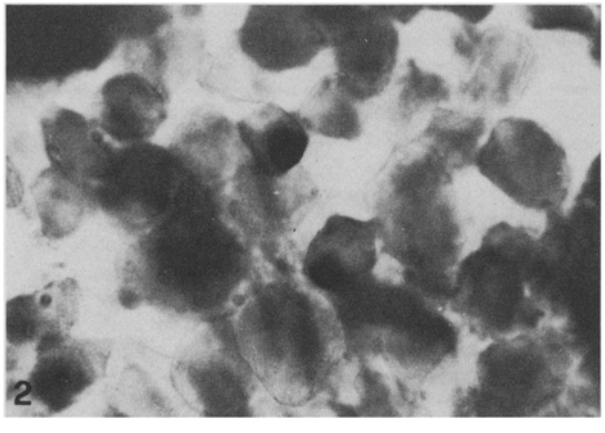

Fig. 2. ANAE activity in cryostat section of adult thymus. 2 ANAE$^+$ cells (T-pattern) appear in the middle of figure. × 2000.

Number of RFCs/10^6 white cells and number of ANAE$^+$ cells/100 white cells in thymus and spleen cell suspension of larvae and adults of *Bufo bufo*

Animals	Organs	RFCs/10^6 white cells	ANAE$^+$ cells/100 white cells
Stage 1 larvae	Thymus	13.95 ± 2.52	46.10 ± 4.24
	Spleen	-	-
Stage 2 larvae	Thymus	11.03 ± 1.87	35.01 ± 4.30
	Spleen	123.75 ± 13.19	25.02 ± 5.47
Immunized stage 2 larvae	Thymus	41.99 ± 3.78	33.11 ± 2.50
	Spleen	968.60 ± 37.13	25.85 ± 4.35
Adults	Thymus	1.24 ± 0.65	2.45 ± 0.96
	Spleen	560.80 ± 66.43	12.08 ± 3.04

Each value is the mean ± SD.

T-pattern and the ANAE$^+$ cell percentage in the larval thymus indicate that these cells presumably correspond to a T-lymphocyte subpopulation. A previous study of human rosette-forming T-cells from peripheral blood[9] showed that a T-pattern is a characteristic of T_M cells, which are a distinct subpopulation of human T-cells judged by their ability to bind the Fc portion of IgM. On the basis of these results, it appears difficult to establish a correlation between the high number of ANAE$^+$ cells and the low number of RFCs in the thymus and spleen of *Bufo*. One explanation would be that heterologous red cell response indicates a maturation pattern of ANAE$^+$ cells. One could also entertain the possibility that, in *Bufo*, cells bearing heterologous erythrocyte receptors are only a small population of the ANAE$^+$ cells. It can be postulated that the rosette-forming capacity is not a characteristic of all T-lymphocytes of *Bufo*. A study on peripheral blood lymphocytes of *Bufo* should permit a useful comparison between *Bufo* and mammalian lymphocytes.

On the basis of the fact that all the pattern types of ANAE activity in mammals were observed in *Bufo*, the technique employed appears to be a reliable method for identification of lymphoid populations and for their localization in *Bufo* tissues. It is clear that further investigation is required to delineate the role of distinct lymphoid populations and to elucidate the functional significance of ANAE activity in the immune system of *Bufo*.

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