incorporation of thymidine by erythrocyte suspensions from rheumatoid patients shows a significantly greater variability than that from normal subjects. The control tests show that the incorporation is unlikely to be due to culture medium, contaminating platelets or leucocytes, or accidental microbial contamination, while the considerable reduction of incorporation by deoxyribonuclease very strongly suggests that incorporation is into DNA, almost certainly microbial, although the possibility that it is residual erythrocyte DNA cannot be absolutely excluded. We had anticipated that, in the event of incorporation of thymidine, this might correlate with the clinical activity of the rheumatoid patients, but this was not the case. However, the observation of a negative correlation with IgG and a positive correlation with the patients' white cell count are of considerable interest in view of the concept, often advanced, of rheumatoid arthritis being the result of inappropriate immunological responses to an infective agent.

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Dialyzable serum factors alter cellular immunity in pregnancy

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Summary. Lymphocyte responsiveness was found to be decreased in pregnant and increased in habitually aborting women. This is attributed to a dialyzable serum factor(s), that can be removed from the surface of lymphocytes by repeated washing.

Decreased cellular immunity in pregnancy may be due to either alteration in the proportion of lymphocyte subpopulations[1,2] or to certain blocking factors present in pregnancy sera[3,4] and absent from the serum of women with habitual abortions[5]. In the present work we attempted to obtain data about the approximate molecular size of the blocking factor, and investigated whether depressed reactivity depends entirely on blocking factors, and/or whether it has its basis in an intrinsic property of the lymphocytes.

Lymphocyte reactivity against embryonic antigen was tested in of 60 women (20–36 years old) including 20 women with a previous history of two or more idiopathic spontaneous abortions, without having successful pregnancies (AB group), 20 women pregnant for 13–30 weeks (PR group) and 20 nonpregnant women wearing intrauterine devices (IUD group). Venous blood was drawn from every subject into heparinized tubes. Lymphocytes were separated as described by Boyum[6], and divided into 2 parts; one part (LC-1) was washed once, the other part (LC-5) 5 times in 0.5 M phosphate buffered saline (PBS) and suspended in Hepes buffered minimal essential medium (MEM). Human embryonic fibroblast (HEF) cells, derived from 12 to 14 weeks old fetuses as described by Youngner[7] were used as target cells in the cytotoxicity assay and as stimulator cells in the experiments on production of migration inhibitory factor (MIF). MIF production by LC-1 and LC-5 of the 3 groups was compared. The method described by Rocklin et al.[8] was followed.

For testing MIF produced by lymphocytes, we used the guinea-pig macrophage migration inhibition assay according to Clausen[9]. It was found that LC-1 lymphocytes of the AB group produced significantly more MIF ($p < 0.02$) and those of PR group significantly less MIF ($p < 0.001$) than did LC-1 of nonpregnant women. In the case of LC-5, MIF production in all 3 groups was high, and no significant differences could be detected (fig. 1). These results indicate

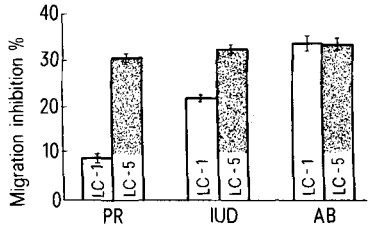

Fig. 1. Influence of exhaustive washing on MIF production by lymphocytes pregnant (PR), nonpregnant (IUD) and habitually aborting (AB) women. Lymphocytes were washed once (LC-1) or 5 times (LC-5) before stimulation. Each pair of columns indicates mean ± SE of 20 individuals.

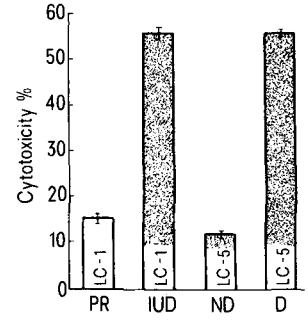

Fig. 2. Cytotoxicity of lymphocytes. The 1st 2 columns compare cytotoxic activity of lymphocytes from pregnant (PR) and nonpregnant (IUD) women. Cells were washed once (LC-1). Cytotoxic activity of lymphocytes from nonpregnant women (IUD), washed 5 times (LC-5) and than incubated in nondialyzed (ND) or dialyzed (D) pregnancy serum in shown in the last 2 columns. Means ± SE of 20 tests are indicated by each column.

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