mutation *ac* du Pleurodèle. L'étude histologique et ultrastructurale déjà entreprise des embryons mutants, libres ou associés, permettra de préciser les caractères de l'expression de la mutation *u* au niveau cellulaire.

Summary. The autonomous expression of a recessive lethal mutation ('ulcère' *u*), isolated in the salamander *Pleurodeles waltlii* Michah. is demonstrated by the way of parabiotic and telobiotic associations, allogenic chimaeras, heterotopic grafts of organ primordia and of anterior or posterior parts from lethal embryos.

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Ultrastructure of Cell-Mediated Immunity. I. Comparison between E and EA Rosettes

In previous studies, the ultrastructural appearance of interaction between human lymphocytes and chicken red cells in the course of phytohemagglutinin-induced[1] and antibody-mediated[2] cytotoxicity were described. In both types of cytotoxicity, interdigitations between some lymphocytes and target cells were observed.

The present study deals with the ultrastructure of the interaction of human lymphocytes and erythrocytes in E rosettes, considered to be specific for T lymphocytes[3] and EA rosettes, in which the rosetting lymphocyte is supposed to be a B lymphocyte with receptors for the Fc fragment of IgG[4].

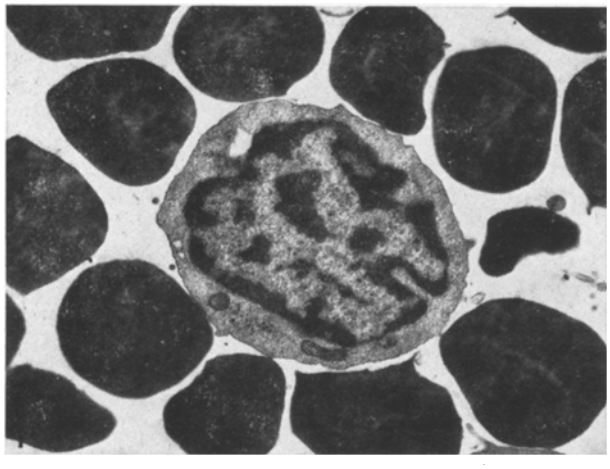

Fig. 1. E Rosette. Sheep red cells are regularly arranged around a lymphocyte, grossly round in shape. Limited areas of membrane contact between lymphocyte and erythrocytes are visible. × 8,500.

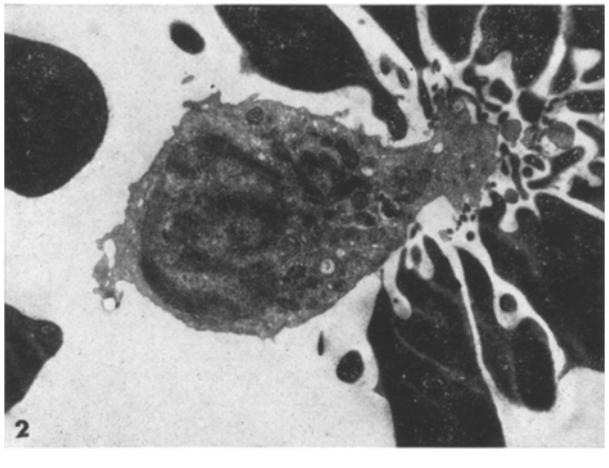

Fig. 2. EA rosette. Sensitized ox red cells are visible sorrounding one pole of a lymphocyte elongated in shape. Numerous cytoplasmic pseudopods protrude from the red cells and come into contact with the lymphocyte membrane. × 8,500.

Materials and methods. The technique for separation of lymphocytes, obtained from 8 healthy volunteers, has already been described[1]. Preparation of E rosettes was performed according to JONDALL et al.[5]. Sheep red cells stored in Alsever at 4 °C were washed 3 times in Hanks and resuspended in the same medium to a final concentration of 0.5%; 0.25 ml of lymphocytes suspension (4×10^6 cells/ml) were mixed with 0.25 ml of sheep red cells suspension, incubated for 5 min at 37 °C, centrifuged at 800 rpm for 5 min and then incubated again for 12 h at 4 °C. At the end of the incubation, the cell mixture was gently resuspended and a drop was put on a microscope slide and observed in the light microscope. The percentage of lymphocytes binding more than 3 sheep erythrocytes over a total of 300 lymphocytes was recorded.

Preparation of EA rosettes was performed according to HAEGERT et al.[4]. Ox red cells were washed 3 times in *Tris*-Hanks and resuspended at a final concentration of 2%. The suspension was sensitized with rabbit IgG anti-ox antiserum at a subagglutinating dose, for 1 h at room remperature. The sensitized cells were then washed 3 times in *Tris*-Hanks and a 0.8% suspension was prepared. For testing, 1 drop of sensitized ox red cells and 1 drop of lymphocytes suspension (2×10^6 cells/ml) were mixed in a test tube and centrifuged at 300 *g* at 4 °C. Counting of rosettes was performed as described above.

For each experiment, samples of cells mixtures of E and EA rosettes were processed for electron microscopy.

Results. The percentage of E rosettes varied in the 8 subjects studied from 58 to 72, that of EA rosettes from 22 to 26. The ultrastructural observation of E and EA rosettes showed differences in the 2 types of rosetting lymphocytes. Those of E rosettes were grossly round in shape (Figure 1), whereas those of EA rosettes were almost invariably in the shape of uropods[6] (Figure 2).

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However, the most evident morphological differences between the 2 types of rosettes were seen in the interaction between lymphocytes and erythrocytes. In E rosettes, this consisted only of limited areas of membrane attachment between lymphocytes and sheep red cells (Figure 1). In EA rosettes, on the contrary, sensitized ox red cells appeared gathered around one pole of the lymphocyte, and numerous cytoplasmic pseudopods protruded from the red cells and came into contact with that part of the lymphocyte (Figure 2). Ox red cells which were not part of a rosette did not show pseudopods and non-rosetting lymphocytes had not the shape of uropods.

Discussion. Our observations indicate that relevant morphological differences exist between rosetting lymphocytes and lymphocytes-erythrocytes interaction in E and EA rosettes. In E rosettes, lymphocytes are grossly round in shape and the interaction between them and sheep erythrocytes consists only of limited areas of membrane attachment, as already described by others[7]. In EA rosettes, lymphocytes are mostly in the shape of uropods[6] and an evident interaction occurs between one of their poles and sensitized ox red cells. We do not yet know the significance of these morphological differences between E and EA rosettes. The morphological phenomenon of lymphocyte-erythrocyte interaction observed in EA rosettes is very similar to that described in the course of antibody-mediated cytotoxicity[2]. It is tempting to speculate that the same population of lymphocytes is responsible for the same phenomenon in the two experimental models. This population could be composed of lymphocytes with receptors for the Fc fragment of IgG. The different manifestations of the interaction between lymphocytes and erythrocytes, i.e. EA rosettes formation and antibody-mediated cytotoxicity, would depend only on the experimental conditions in which the interaction occurs. Our results confirm, on morphological grounds, that the formation of different types of rosettes is not due in all cases to the same type of lymphocyte-erythrocyte interaction. In fact, it seems likely that different morphological types of interaction are indicative of different types of links between the two types of cells.

Summary. The ultrastructural comparison between E and EA rosettes showed that, in the former, the rosetting lymphocytes are mostly round in shape and their interaction with sheep erythrocytes only consists of limited areas of membrane contact, in the latter, rosetting lymphocytes are mostly in the shape of uropods and surrounding ox red cells show pseudopods protruding towards the lymphocyte and coming into contact with it.

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The Electrophoretic Mobility of Serum Lysozyme

Since the publication of the original studies of Osserman and Lawlor[1], the unique cathodal electrophoretic mobility of human lysozyme has been considered as one of its most important characteristics. Distinct lysozyme fractions have been observed in conventional electrophoretic separations of the urinary proteins from patients with monocytic leukemia[1], but have never been found in separations of serum proteins from the same patients, even in the presence of considerably increased levels of the enzyme. The present report demonstrates that serum lysozyme has a different mobility from urinary lysozyme, perhaps as a consequence of complex formation with serum glycoproteins.

Material and methods. Serum and urine were obtained from patients with monomyelocytic leukemia, monocytosis, and hypogammaglobulinemia[2]. Lysozyme levels were determined with the lysoplate method[1] using egg white lysozyme[3] as standard.

Human lysozyme was isolated from the urine of a patient with monomyelocytic leukemia by ion-exchange chromatography in DE-52 (Whatmann) using sodium phosphate buffer, pH 6.5, 0.01 *M* for the elution of the enzyme. Lysozyme obtained by this procedure appeared to be pure by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, and to have a molecular weight of 15,000 daltons[4,5].

The electrophoretic mobility of serum lysozyme was studied by obtaining imprints of the proteins separated on cellulose acetate membranes in agar plates to which a suspension of killed *Micrococcus lysodeikticus*[3] had been incorporated. For this purpose, unfixed cellulose acetate membranes were laid over the surface of the agar plates, and diffusion of proteins was allowed to proceed overnight. At the end of the incubation period, the membranes were removed and the agar plates inspected for lytic areas. All electrophoretic studies were performed with Sartorius cellulose acetate strips, using the barbital-boric acid described in a previous publication[6] and Ponceau S[3] for total protein staining, according to Neremberg[7].

Results and discussion. Routine cellulose acetate electrophoresis of concentrated urine from a patient with suspected mono-myelocytic leukemia (Z.V.) showed a distinct post-gamma fraction that raised the suspicion of lysozymuria. Lysozyme assays were consistent with this interpretation: 19 mg/ml in 65× concentrated urine (corresponding to a lysozymuria of 318 mg/24 h) and 360 µg/ml in serum. Trying to be completely certain about the identity of the urinary post-gamma fraction, we attempted to obtain an imprint of the proteins separated from serum and urine samples of this patient on a *M.*

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