Immuno-Cyto-Adherence Tests in *Xenopus laevis* Daud.

As the next step in studies on the nature of antibodies and antibody production mechanisms in Amphibia[1,2], it was decided to investigate the nature of the immunological cells concerned, adopting an immuno-cyto-adherence technique[3,4]. This method reveals whether an antibody-producing cell can synthesize just 1 specific antibody, or several types.

Materials and methods. Four adult *Xenopus laevis* Daud. females were used, kept at a constant temperature of 25 °C, and fed on alternate days with ox liver. Antigens adopted were: red cells of the rabbit in a concentration of about 1.5×10^7/ml and red cells of the chicken in a concentration of 10^7/ml.

Three *Xenopus* were given 3 injections, 1 a week, of 0.4 ml red cell suspension in physiological solution containing 1 part chicken red cells and 1 part rabbit red cells. The fourth *Xenopus* received only 2 injections. No adjuvant was used[5]. 7 days after the last injection, bleeding from the back limb marginal vein was performed, and the agglutinines were titraded in an ordinary agglutination test. The animals were then killed and the lymphatic cells of the spleen were isolated, using HOWARD's technique[6]. Each spleen was homogenized with 2 ml of Hanks solution. The lymphocytes obtained were washed 3 times and centrifuged (at 4 °C) and then resuspended in 1 ml of cold Hanks solution. The cells were then forced several times through a microsyringe so as to obtain a suspension of isolated cells. The lymphocytes were counted in a cell counter. For the immuno-cyto-adherence test, 1 ml of chicken red cells and 1 ml of rabbit red cells, each in 10^6/ml concentrations were added to 1 ml of the spleen cell suspension (about 10^7 cells). This suspension was shaken at regular intervals for 30 min and then left to stand at 4 °C for 12 h. The sedimented cells were resuspended by shaking the tubes very slowly for 5 min.

Results. The sera of the *Xenopus* treated with 3 injections gave positive agglutination up to a 1:640 dilution, while the sera of the *Xenopus* treated with 2 injections gave positive results up to a 1:320 dilution.

Optical microscope (× 400) observation of the cell suspensions revealed a positive response to the immuno-cyto-adherence. 'Rosettes', i.e. corona-shaped formations of red cells around single lymphocytes, were seen in all the cases. The 'rosettes' were pure, i.e., composed of only 1 of the 2 types of red cells (Figures 1 and 2) and mixed, i.e., of the 2 red cell types (Figure 3).

The Table suggests above all, that the number of cells capable of synthesizing more than 1 antibody is considerably greater in amphibians than in mammals. In fact, 29% of cells in the *Xenopus* are capable of synthesizing antibodies, both against red cells of the chicken and of the rabbit, while the percentage in mammals is only 0.8 to 10%[4,7,8]. As the antigens of the corresponding red cells gave no cross-reaction in the amphibian tested, this fact would seem to indicate that the antibody production in

Percentage of the types 'rosettes' observed

	Pure rosettes rabbit erythrocytes	Pure rosettes chick erythrocytes	Mixed rosettes
Xenopus immunized 3 injections	38.9 ± 2.5	32.1 ± 1.0	29.0 ± 2.3
Xenopus immunized 2 injections	35.7	32.7	31.6

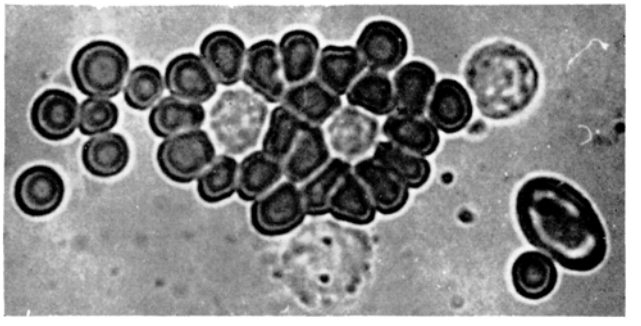

Fig. 1. Lymphocytes of *X. laevis* Daud. surrounded by red cells of rabbit (pure 'rosette'). × 400.

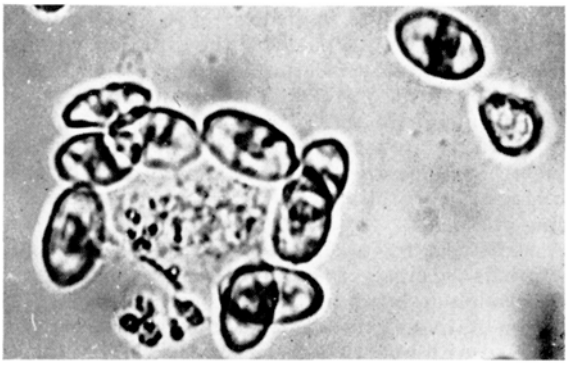

Fig. 2. A pure 'rosette' of chick red cells. × 400.

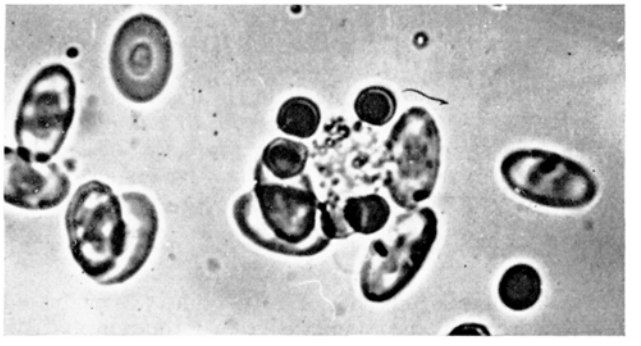

Fig. 3. A mixed 'rosette' formed by rabbit (small) and chick (large) red cells. × 400.

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these animals is not as developed or specialized as in the mammal.

It is also worthy of note that, while in animals treated with 3 antigen doses, the number of antibodies circulating becomes greater than in those treated with 2 doses, the ratio between the various types of antibody producing cells does not change.

Research is in progress to establish the percentage of antibody producing cells out of the total number of cells extracted from the spleen, and whether this percentage varies with the antigen dose. Moreover, by using other pairs of antigens (red cells of newts and lizards, of lampreys and trout etc.), it will be interesting to see if the mixed ‚rosette' percentage varies.

Riassunto. Si è studiata la natura delle cellule immunologicamente competenti della milza di *X. laevis* Daud. Mediante la tecnica della immuno-cito-aderenza, si è potuto constatare che tali cellule estratte da milze di animali immunizzati con globuli rossi di pollo e coniglio, rispondono ad uno solo o ad ambedue gli antigeni, che la percentuale delle cellule che reagiscono sia contro le emazie di coniglio che di pollo è di molto superiore a quella dei mammiferi e che tale percentuale non varia al variare delle dosi di antigene somministrato.

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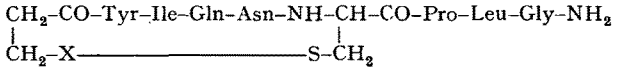
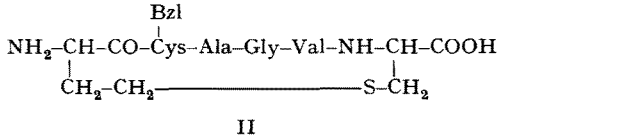
'Insulin-Like' Action of an Oxytocin Analogue Lacking a Disulphide Group, and of a Cystathionine Peptide Related to a Sequence of Insulin, on Rat Epididymal Adipose Tissue in vitro

Like insulin, oxytocin and some synthetic analogues of the neurohypophysial hormones stimulate glucose uptake[1], glucose oxidation[2–7], lipid[2–5] and protein[8] synthesis by rat epididymal adipose tissue in vitro. MIRSKY and PERISUTTI[4] have shown that the action of oxytocin on adipose tissue in vitro is inhibited by pretreatment with sulphydryl-blocking reagents and concluded that the disulphide bond is a necessary (though not a sufficient[3]) requirement for 'insulin-like' action on this tissue[4]. This was in line with earlier conclusions about the functional role of the disulphide bond in the action of neurohypophysial hormones on the amphibian bladder and mammalian kidney[9]. However, it has recently been shown[10–13] that a synthetic oxytocin analogue[14] Ib isosteric with deamino-oxytocin[15] (Ia), but with the disulphide group replaced by a methylene thioether grouping, shows the typical effects of oxytocin on the uterus[10,12], mammary gland[12], vascular smooth muscle[12], mammalian kidney[11–13] and amphibian membranes[11,13]. We have now used this analogue to examine the functional significance of the disulphide bond for the action of oxytocin on rat epididymal adipose tissue.

A structural feature shared by the insulins and by oxytocin is the presence of a hexapeptide sequence bridged by a disulphide bond, and it has been suggested[3,6] that their qualitatively similar effects on adipose tissue may be associated with this particular feature. We have therefore extended our study to a cystathionine peptide[16], II, related to the disulphide-bridged sequence A (6–11) of ovine insulin.



Ia X = S Ib, X = CH_2



II

The 'insulin-like' activity of the peptides was evaluated from their ability to stimulate $^{14}CO_2$ formation and ^{14}C incorporation into total lipids from [1-^{14}C] glucose by rat epididymal tissue pieces in vitro. The tissue was taken from male Wistar rats (100–120 g body weight) after an overnight fast. Tissue samples (80–100 mg) were incubated in 5 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 12.5 μmoles of unlabelled glucose, 1 μCi of [1-^{14}C] glucose (Radiochemical Centre, Amersham, England), and 10 mg of bovine serum albumin (Fraction V, Armour, Chicago, USA) at 37 °C in a Dubnoff-type metabolic shaker for 2 h. The buffer was equilibrated with 95% O_2 and 5% CO_2. At the end of the incubation period, 0.5 ml of 50% aqueous ethanolamine[17] was injected through the stopper into the central compartment and 0.5 ml 2.5 *N* H_2SO_4 into the incubation medium in the main compartment of the incubation vessel. The $^{14}CO_2$ evolved was

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