of their renotrophic activity. On the contrary, thymectomy of the serum recipient almost abolished the capability of the renotrophic serum to produce CRG. The renotrophic serum activity could be ascribed to the presence of a growth stimulator[10], or to the deficiency of an inhibitor[13–15].

According to the results described above, the following mechanism of the CRG might be postulated. The renotrophic activity of the serum appears after removal of 1 kidney[3]. Recently it was claimed that also deazotized serum from bilaterally nephrectomized rats might have renotrophic features[16]. The renotrophic activity is directed toward the thymus, wherefrom a stimulation of the remaining kidney occurs. This stimulation, presumably humoral, could be direct, or indirect through the 'trephocytic' action of lymphocytes.

The proposed mechanism did not take into account the two distinct processes that take part in the compensatory organ enlargement: the hypertrophy and hyperplasia. According to Fox and WAHMAN[17], hypertrophy is an early process regulated humorally, and hyperplasia is a later one, most probably mediated by lymphocytes. In fact both processes could be controlled by the thymus.

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In vitro Culture of Larval Amphibian Erythroblasts

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Summary. A larval erythroblast culture method is described. By this method, it is possible to cultivate for several weeks a homogeneous population of cells ($5 \cdot 10^5$ cells/ml medium on average after 4 or 5 days of culture), which are relatively synchronous with regard to their state of differentiation.

Among the numerous biological systems that allow the study of factors interfering in cell differentiation, the erythropoietic cell line constitutes an excellent biological model.

The differentiation of erythroid cells can be envisaged in several ways: the maturation of a cell line, the evolution of haemoglobins, the role of humoral factors intervening in this ontogenesis and so on. The study in vitro of the switch of synthetized haemoglobins in the course of the different stages of development, from the embryo to the adult ought to make it possible to tackle other aspects of this problem.

Several authors[1–7] have described procedures for studying steps of erythropoiesis in amphibian or chick cultures, but in all cases the length of culture time is short (few hours or few days). To study these phenomena in the amphibians, it was necessary in the first experimental stage to perfect a technique of larval erythroblast culture that would allow normal cell life to continue for a sufficient length of time (several weeks) so that the haemoglobin switch could be followed or induced, and at a later stage the molecular study of this switch, as well as other related problems, could be undertaken.

Materials. 1. Biological material. The erythroid cells are taken from spleen of tadpoles of *Pleurodeles waltlii* (Amphibian, Urodela) recovered at different stages preceding the metamorphosis (50–53 of the GALLIEN and DUROCHER[8] chronological table). – 2. Culture medium. Many tests of different culture media were carried out: 50% LEIBOVITZ[9], purely mineral BARTH[10], Barth enriched amino acids and vitamins, as well as WOLF and QUIMBY[11]. This latter medium of Wolf and Quimby gives the best results, along with enriched Barth medium, in which the absence of heterologous serum enables to absence of exogeneous thyroxine to be better controlled. – 3. Culture chambers. Various culture chambers were also tested: glass chambers, polystyrene flasks (Nunclon or Falcon) and 0.5 ml transparent plastic trays (disposotrays, Block, Strasbourg, France), which proved to be the most suitable for our experiments.

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Average number of cultured erythroblasts

Culture duration	20 h	40 h	5 days	9 days	12 days	15 days	27 days
Average number of erythroblasts (cells/ml)	425,600	473,350	498,350	291,200	187,200	165,600	174,400

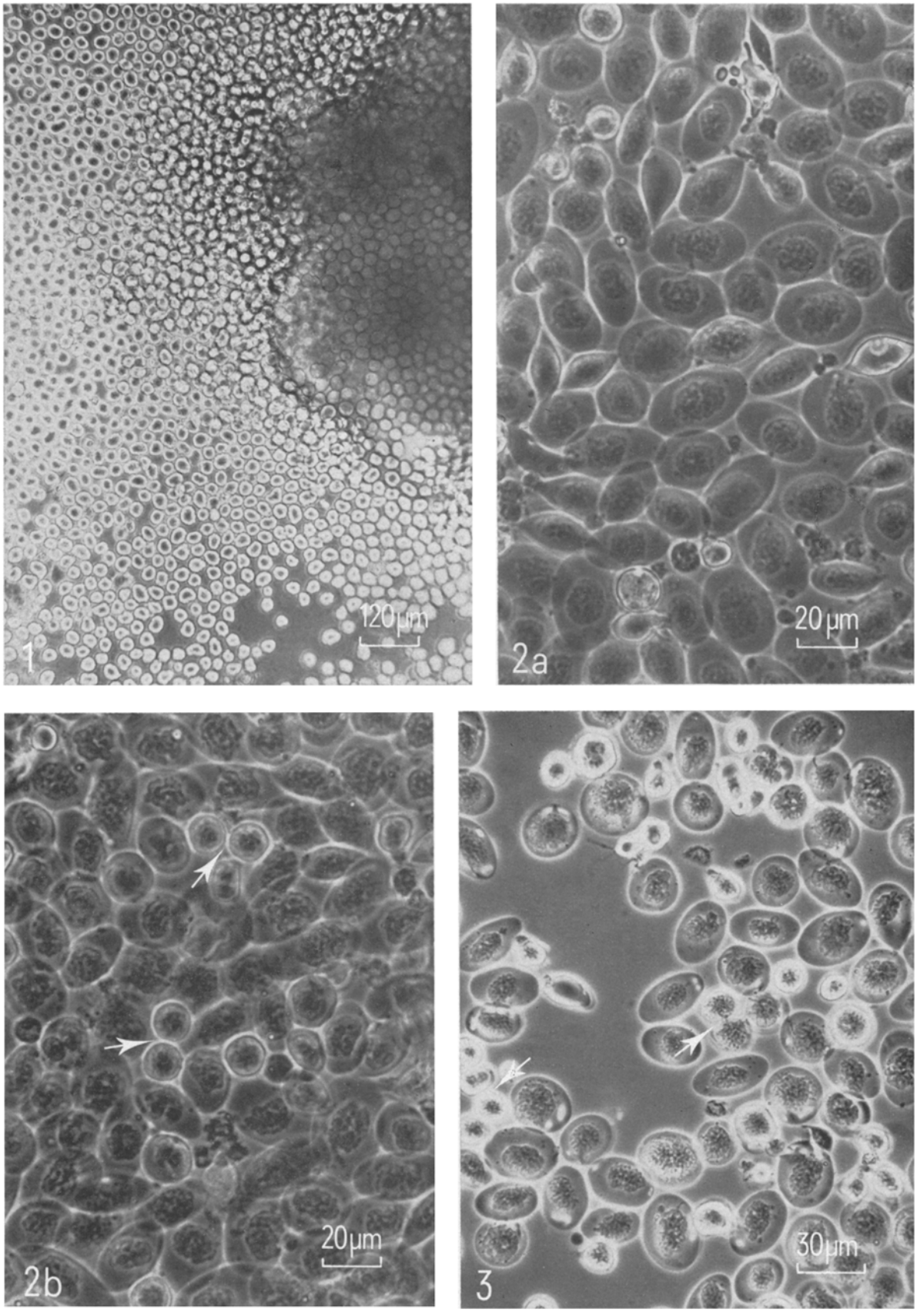

Fig. 1. Phase contrast micrograph of erythroblasts released from a fragment of spleen. 1 h culture.
Fig. 2a and b. Phase contrast micrographs of 4 days cultures. Homogeneous population of cells. Numerous mitosis (→).
Fig. 3. Phase contrast micrograph of 15 days culture. Presence of mitosis (→).

Methods. Before the experiment the tadpoles are kept for more than 24 h in sterilized well water to which antibiotics (penicillin: 100000 μ/l and streptomycin: 100 mg/l) are added. The speens are removed and placed, 2 or 3 at a time, in the disposo-trays which have been previously filled with 0.5 ml of medium. The erythroblasts are freed by fragmentation of the speen with small pincers. Then the chambers are hermetically sealed with a thin sheet of coverslip. Qualitatively and quantitatively similar results were obtained with cultures incubated in air and without air. The cultures are kept at a temperature of 18 °C and the medium is renewed every 4 or 5 days.

Observations. This culture technique allows continuous observation during the entire life of the cells. At the larval stages of development at which they are removed, the spleens are simple pouches filled with erythroid cells (Figure 1). As soon as spleen fragmentation into explants takes place, the erythroblasts spread over the bottom of the chamber. 30 minutes after the beginning of the culture, the release of a significant quantity of erythroblasts was observed.

After 15–20 h of culture, the erythroblasts which were packed in the spleen (Figure 1) have aquired a characteristic morphology (Figure 2a). It is quite remarkable that the cells thus isolated are highly homogeneous (Figure 2b). Indeed we have a majority of polychromatophil and acidophil erythroblasts. There are always cases of mitosis. The *average number* of erythroblasts thus obtained is about $5 \cdot 10^5$ cells/medium ml for 4 or 5 days of culture. It is important to note that after about 8 days of culture a part of the 'mature cells' degenerate. At 9 or 10 days, the average number of erythroblasts is about $3 \cdot 10^5$ cells/medium ml. After 12 days this number remains constant, about $2 \cdot 10^5$ cells/medium ml (table). Actually it is not possible to say whether 'mature cells' degenerate because they reach a degree of differentiation for which the conditions of culture are not adequate, or whether they must die and in vivo they would have degenerated in the same way.

The morphology, the behaviour of the remaining erythroblasts (about $2 \cdot 10^5$ cells/medium ml) continues to be normal (Figure 3). These erythroblasts thus cultivated for 4 weeks with frequent renewals of the medium, actively incorporate labelled elements in the proteins (leucine 3H) and the RNA (uridine 3H).

Conclusion. The larval erythroblast culture described above is an original for amphibians. The cells thus obtained constitute a homogeneous population, and are relatively synchronous with regard to their state of differentiation. Their numbers ($5 \cdot 10^5$ cells/ml medium on average after 4 or 5 days of culture) are sufficiently great to be biochemically studied. It should be underlined that this method of erythroblast culture makes it possible to follow their evolution during a period of several weeks.

This biological system should therefore be particularly suitable for studying the different problems raised in the introduction concerning the simultaneous analysis of biochemical and morphological red line cell differentiation.

Alternative Complement Pathway: Activity Levels in Allogeneic Pregnancy

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Summary. Classical and alternative complement pathway activities have been evaluated in sera of women in progressive stages of gestation and in pregnant mice belonging to outbred or inbred matings, as compared to suitable controls. While classical C pathway was found to be unmodified, the alternative one attained in pregnancy significantly higher activity levels. Results are discussed in the light of mother-conceptus relationships.

Foetal allografts survive in the uterus and are normally delivered notwithstanding their burden of histocompatibility antigens which partly differ from those of the mother[1,2]. Several mechanisms have been postulated which might account for this unexpected immunological behaviour, but up to date no conclusive evidence has been presented. Nevertheless, immune reactions do occur in pregnancy, as has been demonstrated either in normal or in pathologic conditions[3-5]. Among the factors which are possibly involved, the complement system is receiving increasing attention[6-10]. Thus, the early finding[6] of a significantly lower level of hemolytic activity in the sera of pregnant women has been confirmed in serum samples from late pregnancy[7], while a slight but steady increase in hemolytic potency has been recently reported in a prospective study of uncomplicated human pregnancies[8,9]. Propp and Alper[10] found augmented amounts of C3 in the serum of pregnant women at the time of parturition and their results have been widely confirmed[8,9].

Many features of the complement system have recently been clarified[11]. It is now well established that besides the classical pathway of complement activation, initiated by the assembly of the C1q–C1r–C1s complex on altered sites of the immunoglobulin Fc region, other pathways do exist which bypass the so-called early components (C1,

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