served in the cells of proximal convoluted tubule (Figures 1 and 2).

The total number of the renal corpuscles and the number of those corpuscles containing the TLC have been counted in the sections stained with H. E. The results demonstrated an average of 97 ± 8 renal corpuscles for each section calculated on 100 kidneys, 28 ± 3% of which showed the TLC. Further significant variations of the TLC were present according to the phase of the sexual cycle. Statistically we counted 31 ± 3% of TLC in proestrum, 29 ± 2% in oestrum, 24 ± 2% in metaestrum and finally 25 ± 3% in diestrum (Figure 3).

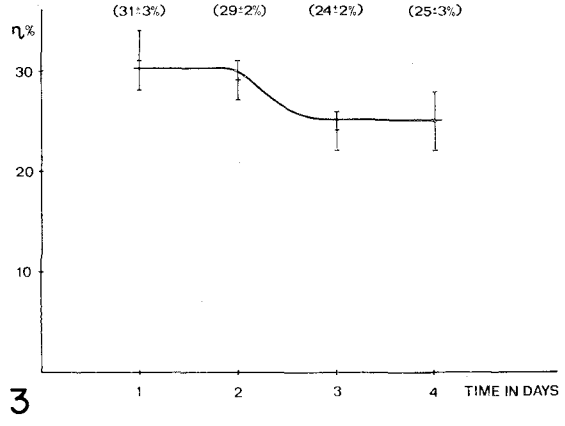

Fig. 3. Percentage of TLC (n%) in the phases of the sexual cycle. 1. Proestrum; 2. oestrum; 3. metaestrum; 4. diestrum.

Discussion. Previous reports demonstrated that in the male mice kidney the TLC are about 38 ± 5%[6,7] and a particular relationship was described between the presence of TLC and the testosterone secretion in these animals[5]. The present findings demonstrated 1) that in the female mice kidney about 28 ± 3% of the renal corpuscles showed the TLC and 2) that these corpuscles with these cells increase of about 5% in proestrum and oestrum; this increase may be related to the increase of the oestrogen secretion or to the fall of the progesteron secretion in the above phases. Further experimenal data are needed in order to clarify this relation.

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Nephrocompensatory Growth Following Thymectomy[1]

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3 May 1976.

Summary. Thymectomy performed 28 days before unilateral nephrectomy produced significant inhibition in compensatory renal growth (CRG) in 3–6-month-old rats. Sera from thymectomized animals are not deprived of their renotrophic activity, but thymectomy of serum recipients almost abolished the capability of renotrophic serum to produce CRG.

Following thymectomy, a multifacet physiological distress usually appears. Wasting disease and impaired immune competence could be considered as most evident dramatic outcome[2]. Body growth retardation, being a major sign of wasting disease, could be considered indicative for a more general role of the thymus in the control of growth.

Another aspect of growth, the compensatory enlargement of an organ, following removal of its portion, could also be thought as being controlled by the thymus. In the present study, we investigated the influence of thymectomy on the enlargement of the remaining kidney after unilateral nephrectomy. In addition, the renotrophic features of the serum from unilaterally nephrectomized animals[3] were investigated in conditions altered by the thymectomy of serum donor or serum recipient.

Material and methods. In all experiments, outbred albino male rats were used. At nephrectomy the animals were grouped according to their age as follows: 1–2, 2–3, 3–4 and 6 months old. Since, according to our experience, age exerts a major influence on the compensatory renal growth (CRG)[4], age selection was performed with particular care.

Unilateral nephrectomy was performed on the right side and the remaining left kidney was removed 48 h afterwards. The wet and dry weight increase of the remaining kidney was expressed in percentage. Thymectomy was always performed 28 days before unilateral nephrectomy.

The donors of serum were 3.5-month-old rats. All were unilaterally nephrectomized, and some of them were also thymectomized 28 days before unilateral nephrectomy. The sera were obtained from blood taken by abdominal aorta puncture, 48 h after unilateral nephrectomy, and were stored at − 25 °C.

Serum recipients were also unilaterally nephrectomized, and some of them were thymectomized 1 month before uninephrectomy. The first 2 ml injection of serum was

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given i.v. immediately after nephrectomy. 5 h later a second injection of 2 ml serum was given i.p. The animals were sacrified by bleeding 48 h after nephrectomy.

The statistical analysis of results was performed by the Student *t*-test[5]. The statistical significance was calculated by comparing the gain in kidney weight in mg.

Results. As shown in Figure 1, the rate of the compensatory growth in control animals (kidney dry weight) was influenced by the age of animals. The maximal kidney weight increase was observed in the 3–4-month-old rats, diminishing with age gradually thereafter. When thymectomy preceded unilateral nephrectomy, the decrease of the kidney compensatory enlargement with age was much more pronounced. Maximal inhibition was noted if the thymectomy was performed at 2–3 months of age, and nephrectomy at 3–4 months. Curiously enough, this depressive effect of thymectomy on the compensatory enlargement of the left kidney was absent in the age group of 2–3 months.

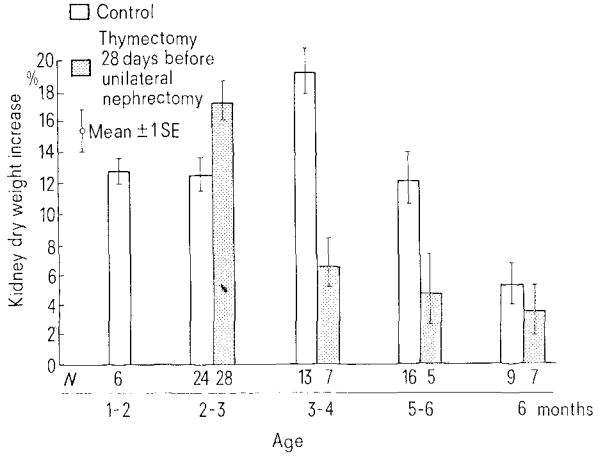

Fig. 1. The influence of age and thymectomy on the compensatory renal growth in rats. Kidney dry weight increase (%) = percentage of the dry weight increase of the left kidney over the dry weight of the right kidney, 48 h following right nephrectomy. Age (months) = age of rats in months at the time of unilateral nephrectomy. N = number of animals.

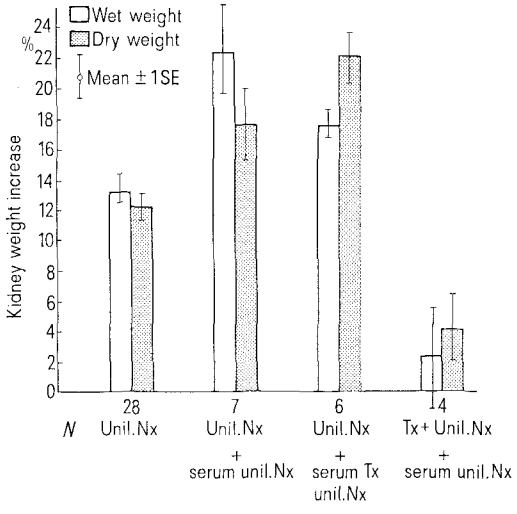

Fig. 2. The influence of thymectomy on the compensatory renal growth produced by renotrophic serum. Kidney weight increase (%) and N = same as on Figure 1. Abbreviations: Unil. Nx., unilaterally nephrectomized rats; Unil. Nx + serum unil. Nx, unilaterally nephrectomized rats injected with serum from unilaterally nephrectomized donor rats; Unil. Nx + serum Tx unil. Nx, unilaterally nephrectomized rats, injected with serum from thymectomized and unilaterally nephrectomized donor rats (donor rats were thymectomized 28 days before unilateral nephrectomy); Tx + Unil. Nx + serum Unil. Nx, thymectomized rats, unilaterally nephrectomized 28 days later, injected with serum from unilaterally nephrectomized donor rats.

Passively transferred sera from unilaterally nephrectomized animals stimulated not only normal kidney growth[3] but also produced an overcompensation in the unilaterally nephrectomized recipient rats (Figure 2). The percentage of this compensatory overgrowth is significant ($p < 0.01$) for both wet and dry kidney weight. The influence of thymectomy on the compensatory growth was tested following thymectomy of serum donors or following thymectomy of serum recipients. As shown in Figure 2, thymectomy of serum donors (group III) has not significantly influenced the capability of the serum to produce overcompensation in uninephrectomized recipients. On the contrary, thymectomy of the recipient almost annihilated the renotrophic features of sera from normal, unilaterally nephrectomized rats ($p < 0.01$, group IV).

Discussion. The experiments described here offer a substantial justification for postulating a role for the thymus in the mechanism of the renal compensatory growth. Thymectomy, performed 28 days before unilateral nephrectomy, produces a pronounced defect in the CRG of 3–6-month-old rats. In 1–2-month-old rats, thymectomy did not produce an inhibitory effect.

The influence of thymus on the CRG could be associated with its hormonal function. The hormones might have a direct effect on the growing tissue[6]. It is also possible to postulate the influence of the thymic hormones on maturation and competence of lymphocytes which might thus acquire a 'trephocytic' function[7]. Accordingly, lymphocytes should have important functions other than those associated with immunological tasks[8]. In fact FABRIS et al[9] have found that lymphocytes from peripheral lymph nodes could prevent the aging symptoms in dwarf Snell-Bagg mice[10]. According to BURCH and BURWELL[11], the basic function of the lymphatic system is a central regulation of tissue and organ growth. Tissue specific factors should have an inhibitory action upon growth-promoting functions of lymphocytes. Thus, unilateral nephrectomy would deplete the number of tissue factors and allow the lymphocytes to perform their growth-promoting action in the remaining kidney[11].

Partial hepatectomy produced an increase in the mitotic activity of the remaining liver tissue simultaneously with the depletion of thymus cellularity[12], indicating again a possible role of thymus in the process of compensatory growth.

Our results obtained with the transfer of sera from the unilaterally nephrectomized animals would indicate the particular importance of thymus in CRG. It is evident that sera from thymectomized animals are not deprived

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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