with approximatively 80% lymphocytes. This is in accordance with the data of MORSE[7] and with our own findings[13]. After i.v. injection of PO the blood leukocyte counts increased rapidly, and reached a peak on the days 4 and 5. This was found to be due to a multiplication of both lymphocytes and granulocytes (Table II). 15 days after treatment, normal leukocyte counts were determined again. Pronounced leukocytosis became also detectable after s.c. injection of PO. But in comparison with the i.v. route, the development of leukocytosis appeared to be considerably delayed (Table II). This may explain why MORSE[7] did not demonstrate blood leukocytosis after the s.c. injection of PO, since he measured the response 1, 4 and 7 days after treatment.

On the basis of hematocrits and reticulocyte counts determined in the peripheral blood of NCS mice, the injection of PO was not found to be followed by a significant change in cells of the erythrocyte series[7]. This is not in full accordance with the findings of FRUHMAN[14] who demonstrated a doubling in reticulocyte percentages 7 days after the injection of PO into CF No. 1 mice, although the concentrations of circulating erythrocytes at this time were in the normal range. On the basis of further cytologic and ferrokinetic studies, the conclusion was drawn that the injection of *B. pertussis* leads to marked increase in splenic erythropoiesis accompanied by a decrease in bone marrow erythropoiesis[14]. Our data show that both the i.v. and the s.c. injection of PO results in marked decrease in the erythrocyte concentrations, whereby the lowest values were obtained at the 5th day returning to normal within the following 5 days (Table II). The decrease in the erythrocyte counts is apparently due to the damage of erythrocytes, and is attributed to the toxicity of *B. pertussis*[1,2,7,13].

Zusammenfassung. Sowohl die i.v. als auch die s.c. Injektion abgetöteter Zellen von *Bordetella pertussis* führt bei Mäusen zu Splenomegalie und ausgeprägter Blut-Leukozytose, an der Lymphozyten und Granulozyten beteiligt sind. Zudem bewirkte die Injektion von *B. pertussis* eine deutliche Verminderung der Erythrocyten mit Minimalwerten am 5. Tag. Am 10. Tag und danach wurden wieder Normalwerte gefunden.

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The Fate of Polymorphonuclear Neutrophils and Mononuclear Cells During Allograft Rejection in the Urodele *Pleurodeles waltlii* Michah.

Allograft rejection in Urodeles is actually a wellknown phenomenon and has been studied in various genus and species. Chronic rejection of skin grafts described by COHEN[1-3] in *Triturus viridescens* and in other species[4], is a general phenomenon. Acute rejection remains possible in some cases. Second set grafts are rejected in an accelerated fashion. In the Urodele, *Pleurodeles waltlii*[5], survival times of skin allografts are variables: rejection occurs in 50% of the grafts 15 to 20 days after grafting in a 'sub-acute' manner and is chronic for the other grafts (20–130 days after grafting). In genetically related newts, some cases of definitive tolerance sometimes occur.

In Urodeles, the hematopoietic system is primitive. Bone marrow and lymph nodes are absent. Erythropoiesis and thrombocytopoiesis occur exclusively in the spleen, lymphocytopoiesis in the thymus and in the spleen and granulocytopoiesis in the capsular layer of the liver[6,7].

In *Pleurodeles*, thymectomy performed at the larval stages 51–52[8], 1 month before metamorphosis, produces definitive tolerance to allografts in adults[9]. Irregulars and

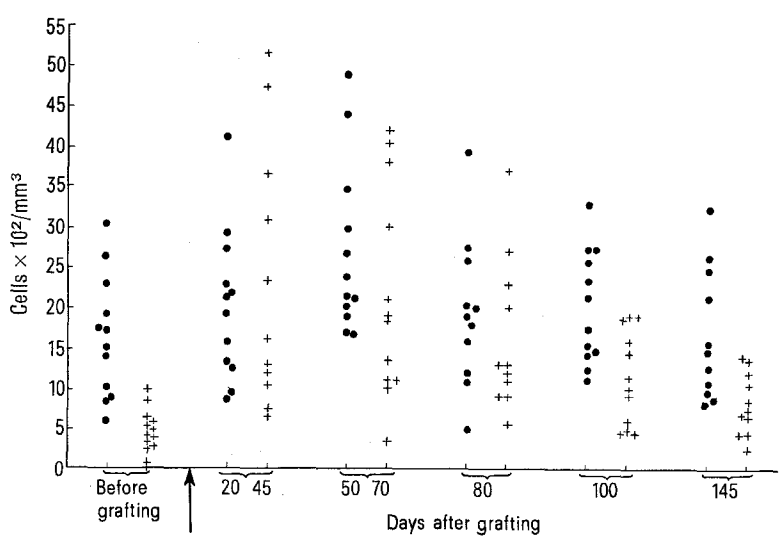

Fig. 1. The changes in the absolute numbers per mm³ of blood mononuclear cells (•) and polymorphonuclear neutrophils (+) during allograft rejection in *Pleurodeles waltlii* (12 animals).

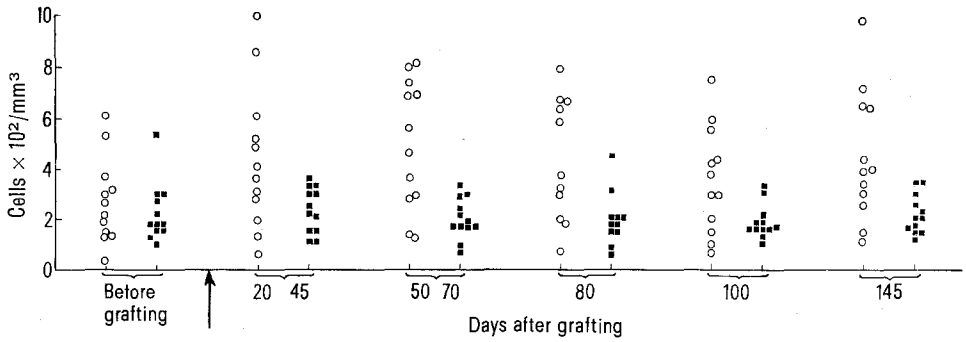

Fig. 2. The changes in the abolute numbers per mm³ of blood polymorphonuclear eosinophils (○) and basophils (■) during allograft rejection in *Pleurodeles waltlii* (12 animals).

discrete wasting diseases sometimes occur; females are more often attacked than males.

The present studies describes in adult *Pleurodeles* the numerical changes of circulating white blood cells (WBC) during allograft rejection.

Materials and methods. Two-year-old adult *Pleurodeles*, 6 males and 6 females, were used. All these animals were bred from one single offspring in our Laboratory. They were bred in individuals plastic containers, each with a small volume of tap water (20 ± 2°C) and fed with *Chironomus* larvae and hashed meat. Each animal was anesthetized with MS 222 (Sandoz) and blood punctured from a femoral artery with heparinized glass pipettes. Absolute numbers by mm³ of erythrocytes, thrombocytes and leukocytes were determined in a hemocytometer. Blood smears were stained with May-Grunwald-Giemsa buffered at pH 6.7. Microscopical observation of smears gives the relative percent of mononuclear cells (lymphocytes and plasmocytoids cells) and polymorphonuclear cells (neutrophils, eosinophils and basophils). At least 500 cells were counted on each smear. Absolute number by mm³ for each type of WBC was determined. 3 months later, each *Pleurodeles* received 3 allografts from 3 differents donors. During the rejection phase of these allografts, the animals were bled 5 more times (20–45; 50–70; 80; 100 and 145 days after grafting) and WBC counts were made.

Controls. 6 *Pleurodeles* received sham grafts: grafts beds were made but without grafting. Complete healing occurs 8 to 10 days later. Animals were bled 40 days later for WBC counts. 6 *Pleurodeles* were bled 2 or more times at appropriate intervals (20 to 40 days). At each bleeding, WBC counts were made. 6 *Pleurodeles* received autografts. WBC counts were made 40,80 and 145 days after grafting.

Results. Two groups of cells undergo quantitative modifications during the rejection phenomenon: polymorphonuclear neutrophils, and a group of cells we call 'mononuclear cells' including little, middle and large lymphocytes, blast cells and plasmocytoid cells. Before grafting, the absolute number of neutrophils varies from 50 to 850 cells by mm³ and the absolute number of mononuclear cells from 650 to 3000 cells by mm³.

During the rejection phase of the grafts, 20 to 45 days after grafting, a perceptible increase of mononuclear cells and an important increase of neutrophils are observed. These 2 groups of cells reach maximum values 50 to 70 days after grafting. From 70 to 145 days, the absolute numbers progressively decrease and statistically return to the initial values (Figure 1). Basophils do not undergo quantitative modifications. A slightly increase of eosinophils is possible (Figure 2).

In the three groups of controls, no significant differences in the absolute and relative numbers of WBC were observed with respect to intacts animals: healing, successive bleedings or autografts do not induce any changes in the WBC counts.

Discussion. In *Pleurodeles*, during the rejection of skin allografts, important modifications of the number of WBC occurs. The number of neutrophils increases at the time of the first rejection symptoms (vasodilatation, eodema) and reach important values during the rejection phase (hemostasis, hemorrhage, necrosis). 70 to 100 days after grafting, when complete destruction of the grafts and healing of the graft beds are accomplished, the number of neutrophils remains high. 5 months after grafting, the number of neutrophils falls to normal values.

In mammals, during allograft rejection, polymorphonuclear cell proliferation in peripheral blood is not a usual phenomenon. The role of neutrophils in cellular immunity is still hypothetical. Neutrophils possess various proteolytic enzymes and have phagocytic and pynocytic properties. They have membrane receptors for C3 complement component, IgA and IgG immunoglobulins[10] and the stimulation of these receptors by adherence to aggregated immunoglobulins induced the degranulation and release processes[11]. In *Pleurodeles*, thymectomy performed at a larval stage, sometimes induced wasting disease. In wasting animals, the granulocytopoietic capsular layer of the liver and also all circulating neutrophils completly disapeared. Similar observations were made by Du Pasquier[12] in larval thymectomyzed *Alytes obstetricans*.

The quantitative increase of mononuclear cells is accompanied by morphological modifications: new stimulated forms appear, specially enlarged lymphocytes, blasts and plasmocytoid cells.

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The results suggested that in Urodeles polymorphonuclear neutrophils have an important role in the immune reaction against allografts. Further research must be done to specify possible interactions with lymphocytic series and the ways which initiate the proliferative response.

Résumé. Chez l'Amphibien Urodèle *Pleurodeles waltlii*, la formule sanguine subit d'importantes varitions quantitatives au cours du rejet d'allogreffe. L'abondance particulière des polynucléaires neutrophiles dans la circulation pourrait être un phénomène non spécifique (élimination des complexes immuns) qui n'aurait pas d'équivalent chez les Vertébrés supérieurs. L'augmentation du nombre des cellules mononucléées est accompagné de certaines modifications morphologiques: on observe une multiplication de formes stimulées, en paticulier des cellules blastiques et des cellules de la lignée plasmocytaire.

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Seasonal Variation of Reserpine Pseudopregnancy in the Rat

Several authors have demonstrated that reserpine[1-3], as well as other catecholamine depletors[4], induce pseudopregnancy when administered to the rat. VAN MAANEN and SMELIK[5] have demonstrated that topical application of reserpine to the median eminence also results in pseudopregnancy, so implying that the catecholamine fibers and cells that have been described in the area[6,7] are responsible for the phenomenon. While studying the effects of various treatments on the appearance of reserpine-induced pseudopregnancy, we found a variability in the duration of the diestrus following reserpine in animals treated at different times of the year that suggested a spontaneous seasonal variation. To clarify this point, we devised the following experiment.

Material and methods. 139 female albino rats, 6 months old at the date of treatment, were used. The animals were individually caged and kept in an animal house with temperature maintained $\geqslant$ 24°C with natural illumination. The vaginal cytology was assessed daily by lavage during 4 weeks prior to treatment and animals showing irregular cycles were discarded. Reserpine (Serpasil CIBA) was administered on the day of estrus at the dose of 6 mg/kg by the i.p. route. Vaginal cytology was assessed daily thereafter to determine the length of the diestrus period that followed the reserpine treatment. This was taken as an index of pseudopregnancy duration. The study was performed for 24 months, from March 1968 until March 1970. During this period, groups of animals were injected each month with reserpine as stated above. The animals were treated only once and discarded. In order to determine the possible influence of ambient temperature, a number of rats were not housed in the animal quarters during the months of January–February and August–September of the 2nd year of the study. Fertility was calculated for 340 rats from the same colony as the experimental animals, by use of the coefficient $FC = \frac{7\,n}{d-21}$ where n = litter number and d = days of pairing with male[8]. During 1971, reserpine pseudopregnancy was induced in 3 groups of animals the months of February–March, July and August. Deciduomata were produced and measured accordingly to DE FEO[9].

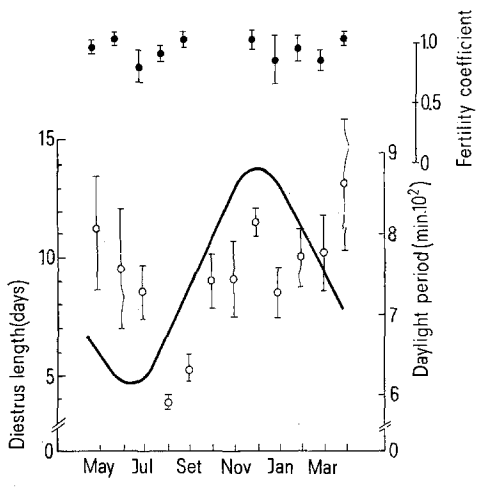

Fig. 1. Mean duration of diestrus following reserpine injection every month of the year. Each point represents pooled data from 2 years (mean $\pm$ S.E.). The continuous line represents mean day duration along the year at the lattitude of the laboratory. Fert. Coef. = fertility coefficient.

Results and discussion. The results obtained from the period 1968–70 are summarized in the Figure. A peak of minimum duration of the diestrus was observed that followed treatment in the months of August–September. Duration of diestrus after reserpine in August (3.90 ± 0.13) was significantly longer than pretreatment diestrus during the same month (2.17 ± 0.08). There were no differences in the pretreatment diestrus length throughout the year nor in the fertility coefficient as is shown in the Figure. Since the seasonal variation in reserpine-induced pseudopregnancy was seen in animals within and out of a temperature-controlled environnent but with natural illumination (Table I), this phenomenon might be related to the duration of daylight periods. In fact, the peak of minimum response followed the winter solstice (Figure).

The results of the experiments performed during 1971 confirmed the seasonal variation showing a significantly lower weight of deciduomata in August when compared to deciduomata induced in February–March (Table II, $p < 0.02$).

The step in reserpine-induced pseudopregnancy which is seasonally modulated might be located anywhere in the chain of events that lead to catecholamine depletion or

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