Blood-Forming Tissues in Orthopteran Insects: An Analogue to Vertebrate Hemopoietic Organs

The blood of insects (hemolymph) contains numerous small circulating cells, termed hemocytes, the origins of which have been a matter of controversy for a long period. In several morphological studies[1–3] we have shown that the 'phagocytic' tissues and organs situated in the vicinity of the dorsal vessel in Orthopteran insects (review by NUTTING[4]) have the classical features of hemopoietic organs. Reticular cells, loosely aggregated in vast networks show the same characteristics as the reticular cells of the vertebrate lymph tissue or spleen for example. They divide actively and some of them undergo differentiation leading to the formation of hemocytopoietic islands with young blood cells of the same cell type at the same stage of maturation. In both *Locusta* and *Gryllus*, the differentiation of the 3 main circulating hemocytes was easily ascertained in these tissues.

As in vertebrate hemopoietic tissues, the reticular cells of Orthopteran blood-forming tissues and organs are also concerned with removing debris from the circulation (which property accounted for the restrictive term of 'phagocytic tissues'). The stimulation of the macrophagic capacity can easily be induced by injecting inert powders into the hemocoele. Such a stimulation results in a temporary blockage of the hemocytopoietic function, as the reticular cells become macrophages; their hemocytopoietic differentiation is thus prevented for at least some time. In *Locusta*, the injection into the hemocoele of a strong dose of iron saccharate actually has a dramatic effect on hemocytopoiesis: the number of circulating hemocytes decreases by some 85% during the 3 days following the injection[5].

In Orthopteran insects, the circulating hemocytes are able to divide, although such divisions are not very frequent. An important question, not answered by the preceeding observations, is that of the functional importance of the hemocytopoietic tissue under normal conditions. Do these tissues produce and release hemocytes during the entire life-span of a normally developing insect, or is their activity restricted to periods of agression, such as hemorrhages, wound-healing, encapsulation of parasites, etc., where stringent requirements for intense and rapid hemocyte production exist? – Attempting to answer this question we irradiated selectively the hemocytopoietic tissue of fourth and fifth instar larvae and male and female adults of *Locusta* (25,000 R administered over a 5 sec period), the anatomical situation of this tissue in *Locusta* allowing selective X-irradiations which do not affect other radiosensitive tissues, such as the digestive tract. – The results, the details of which are given elsewhere[6], show that 24 h after the single X-ray treatment, the hemocyte count has fallen by some 50% (Figure 2). All hemocyte types are affected by this strong decrease. Sham-irradiations of similar surface areas on other body regions do not affect the hemogramme. The hemocytopoietic tissue thus appears to play a primary role in the production of differentiated blood cells during the life history of this insect.

The very strong decrease in the number of hemocytes after the X-ray treatment of the hemocytopoietic tissue is followed 3 days later by a recovery period during which the hemocyte count increases progressively (Figure 2). During this period the number ot circulating hemocyte divisions does not rise significantly: the recovery is essentially due to the intensification of the hemocytopoietic functioning of those stem cells which were not affected at the time of the short X-irradiation of the hemocytopoietic tissue. A microscopic study of this tissue during the

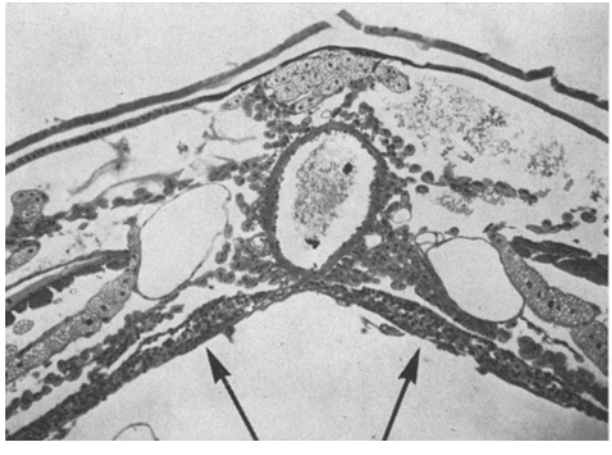

Fig. 1. Paraffin section through the pericardial sinus of a normal male adult of *Locusta migratoria*. The hemocytopoietic tissue consists of a large band of loosely aggregated cells, extending on the upper surface of the dorsal diaphragm (arrows) from the 1st to the 5th abdominal segment. Note the absence of connective layers around the hemocytopoietic tissue, the cell clusters being directly in contact with the hemolymph. The section also shows the cardiac vessel, 2 tracheal trunks on both sides of the vessel, as well as pericardial cells, fat body and fragments of the cuticle and epidermis. ×130.

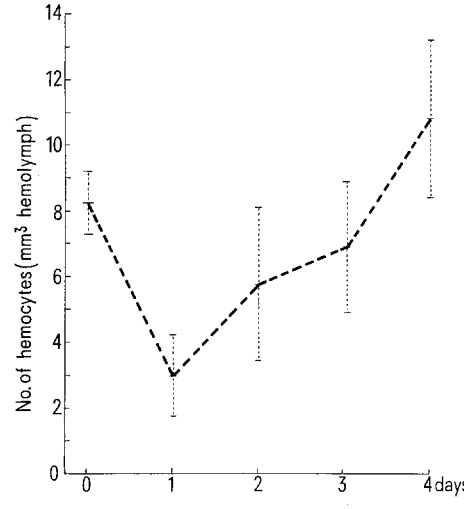

Fig. 2. Modifications of the hemogramme of 5th larval instar larvae of *Locusta migratoria* after selective irradiation of the hemocytopoietic tissue. Abscissa: 0, 1, 2, 3, 4 days after the single administration of 25,000 R; ordinate: number of hemocytes per mm³ of hemolymph (in thousands).

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recovery period of the hemogramme reveals obvious signs of a functional stimulation: the intense proliferation of the highly polymorphous reticular cells and the great increase in maturating blood cell clusters results in a marked hypertrophy of this tissue which invades large portions of the pericardial sinus, and even, to a lesser extent, penetrates into the perivisceral sinus.

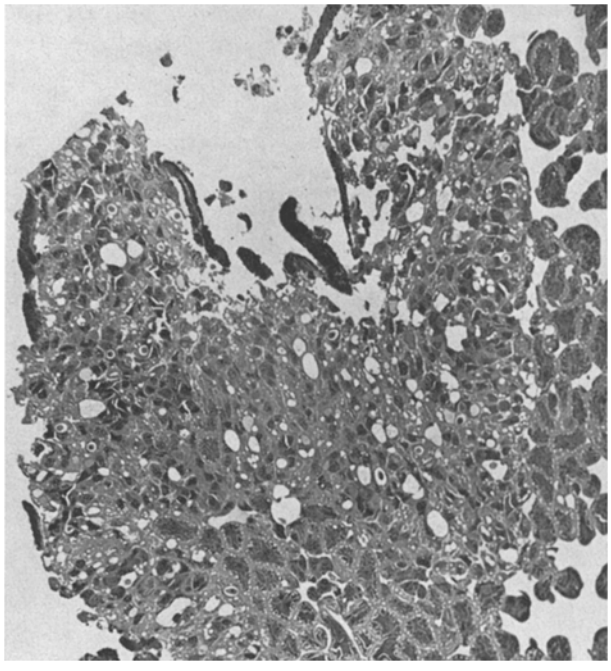

Fig. 3. Intensely proliferating hemocytopoietic tissue of a male adult of *Locusta migratoria*, 5 days after selective X-irradiation (time of recovery of the hemogramme). Glutaraldehyde-osmium tetroxyde fixation; araldite embedding medium, 1 µm thick section. ×160.

It appears now that there exist in Orthopteran insects reticular cells showing both macrophagic and hemocytopoietic potentialitites. These cells are grouped together either in diffuse tissues or more closely integrated in far more complex organs (*Gryllus*, see[3]). The normal functioning of these tissues and the reactivity in abnormal and experimental conditions are both remarkably close to those of the Vertebrate hemopoietic organs. While their importance in the continuous production of mature hemocytes is essential, the macrophagic capacity of these tissues enables them to play an important role in eliminating worn-out hemocytes and various debris, and especially in contributing to the defense reactions and to resistance to bacterial infection. What is more, selective X-ray-provoked lesions of this tissue affect the normal evolution of the proteinemia, or the humoral determinant of moulting[7] and ovarian maturation[8] as recent results have shown in *Locusta*. These different functional aspects are at present under investigation.

Résumé. Des cellules sanguines différenciées sont produites tout au long de la vie larvaire et imaginale des Insectes Orthoptères par des tissus hématopoïétiques spécialisés. Nos études morphologiques et expérimentales soulignent l'analogie que présentent ces tissus avec les organes hématopoïétiques des Vertébrés, à la fois sur le plan structural et sur la plan fonctionnel.

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Thermo-regulatory Responses to Hypothalamic Heating in Dehydrated Rabbits

Thermal panting and sweating are known to be reduced in hot environment in dehydrated steers[1,2] and desert animals[3-5]. Similarly, the rate of sweating is diminished in men during thermal or exercise dehydration[6-8].

One of the reasons for the above phenomena may be a decreased reactivity of the hypothalamic thermoregulatory system. In order to check this possibility, thermoregulatory responses to a direct heating of the heat loss center were investigated in water-deprived and in normally hydrated rabbits.

Material and methods. A thermode and a copper-constantan thermocouple were inserted stereotaxically under hexobarbital anesthesia into the preoptic anterior hypothalamic area (POA) of 12 male rabbits. The thermode consisted of a miniature carbon resistor heated electrically by passing a direct current. The temperature sensor was placed at a distance of 1,5 mm from the heater.

The experiments started not earlier than 14 days following surgery. The animals were placed in a 45 × 15 × 15 cm cage and were not restrained. Hypothalamic and rectal temperatures as well as the temperature of the outer surface of the ear pinna were taken by means of copper-constantan sensors and displayed on a Motor-Kompensator 2 mV type EKN (VEB Messgerätewerk E. Weinert, Magdeburg). The accuracy of measurements was within the range of 0.2°C. Respiratory movements were transformed to voltage oscillations using a resistance transducer fastened around the animal's chest and, after amplification, were recorded continuously on a polygraph. Ambient air temperature was 23 ± 3°C.

Under control conditions the rabbits were fed with dry pellets and obtained water ad libitum. Dehydration was achieved by depriving the animals of water for 3 days, with no restriction of food intake.

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