mine positively whether the response observed in the blue crab is truly an adaptive immune phenomenon. Since both a heterologous antigen (chicken RBC) and a sterile saline evoked a slight increase in agglutinin titer, comparable to that of homologous antigen (rabbit RBC), this response may represent a simple variation of the normal titer range due to any stress. It is certainly not unusual to find an adaptive immune response completely lacking in invertebrates[18,19]. The current work indicates at best a very weak adaptive immune response and one without specificity, similar to that of other invertebrates. Studies of the blue crab agglutinin are now underway to ascertain its physicochemical properties and its function, since it has recently been shown that invertebrate agglutinins may play a significant role in the animals' defence by acting as opsonins[20].

Résumé. Nous avons essayé d'augmenter le titre de l'hémagglutinine naturelle qui se trouve dans le sérum du crabe bleu, *Callinectes sapidus*, par l'inoculation d'hématies de vertébrés. On a noté une augmentation légère de ce titre après 48 h, mais elle n'était apparemment pas spécifique, puisque on peut la provoquer avec l'antigène hétérologique et une solution saline ainsi que l'antigène homologique. Il appert qu'il s'agit là d'une réponse intérieure défensive et non-spécifique due à quelque force extérieure.

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Artero-Venous Differences in Blood Platelet Clumping by ADP

The contribution of the vessel wall (like that of different tissues) to the blood haemostatic and coagulative properties has as yet been scarcely studied[1–5]. The literature in this field is very conflicting; consequently we undertook experiments to verify whether there are some differences between arterial and venous blood in platelet aggregation induced, in vitro, by ADP.

Methods. Blood samples were collected from adult rats lightly anaesthetized with ether and then with urethan (Carlo Erba, Milano, 400 mg/kg body wt.) and Na nembutal (Abbot, Aprilia, 30 mg/kg body wt.) by a plastic syringe from different vessels: femoral vein, renal vein, abdominal aorta. Collection from the heart was performed from the right and left ventricle in openchest animals submitted or not to artificial ventilation. Blood was immediately added to the sodium citrate solution in a siliconized centrifuge tube (1 vol of 3.8% sodium citrate to 9 vol. of blood). The samples were centrifuged for 13 min at $100 \times g$. The supernatant platelet-rich plasma (PRP) was collected by siliconized Pasteur pipets and transferred into a plastic container; the platelet count was then estimated according to Rees and Ecker[6]. The remainder of the samples was centrifuged at $700 \times g$ for 15 min to obtain platelet-poor plasma (PPP).

PRP was diluted with PPP to a standard number of 700,000 platelets/μl. This PRP was divided into polystyrene tubes, stored at room temperature, and then ADP (Na_3ADP- C.F. Boehringer and Soehne H-Mannheim – final concentration $9.2 \times 10^{-6} M$) was added for the examination of the optical density (O.D.) by a 169 Platelet Aggregation Meter (Evans Electroseleniun Ltd), at a stirring rate of 2,800 rpm. The O.D. variations were recorded by Speedomax XL 690 Series Recorder (Lees and Northrup, North Wales and Philadelphia). PPP served as a blank to adjust the 100 point and PRP served to adjust the zero point on the optical density scale for each assay.

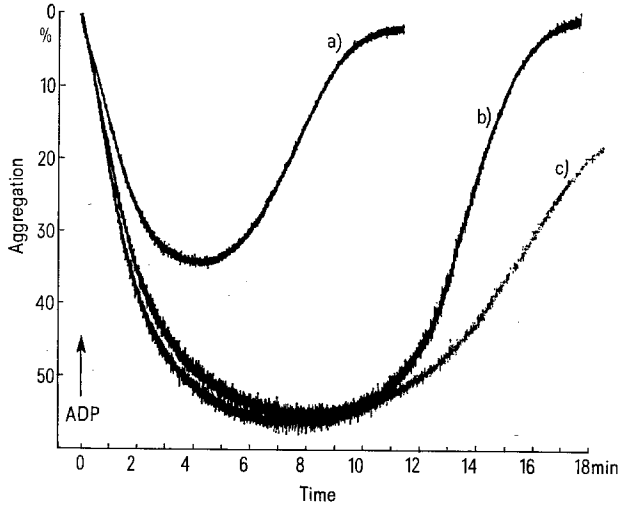

Fig. 1. Changes in O. D. induced by ADP (final concentration $9.2 \times 10^{-6} M$) in PRP (700,000 platelets/μl) obtained from aorta (a), femoral vein (b), renal vein (c).

Results. Figures 1 and 2 show the O.D. changes observed in PRP obtained from aorta (Figure 1a), femoral vein (Figure 1b) and renal vein (Figure 1c), from left ventricle of rats submitted to artificial ventilation (Figure 2a) or not (Figure 2b), and from right ventricle (Figure 2c).

The number of experiments was: right ventricle 18; left ventricle with ventilation 19; left ventricle without ventilation 18; aorta 15; femoral vein 9; renal vein 12.

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There is evidence that 'arterial' platelets show less marked responsiveness to ADP than 'venous' ones; and that the behaviour of the 'venous' platelets is quite similar whatever the blood source (renal vein, femoral vein, right ventricle). On the contrary, the behaviour of the platelet obtained from blood of the left ventricle turns to a 'venous type' as the animal is not ventilated.

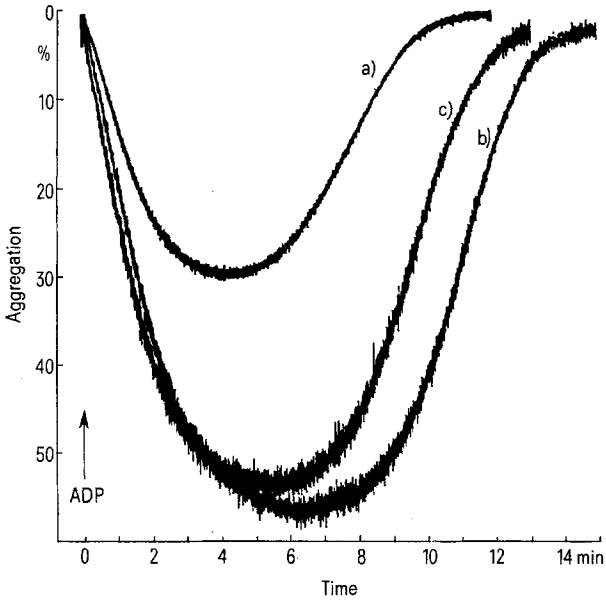

Fig. 2. Changes in O.D. induced by ADP (final concentration $9.2 \times 10^{-6} M$) in PRP (700,000 platelets/μl) obtained from right ventricle (c) and from left ventricle in open-chest animals submitted (a) or not (b) to artificial ventilation.

As the size of the rats was small, we could not contemporaneously withdraw blood from different parts. However, sometimes it was possible in pairs (e.g. aorta and femoral vein, aorta and renal vein). The results obtained under these conditions confirmed those found by a single withdrawal.

Moreover all the data were elaborated for their statistical significance. The t-values obtained comparing arterial and venous blood by the 2 sample-t-test were always greater than the limiting value for 0.05 probability, while those obtained comparing the blood from different venous sources were not.

Discussion. Present results provide evidence that platelet clumping power is effectively more marked in venous than in arterial PRP. Moreover there is no suggestion for a peculiar contribution of any vascular area to this phenomenon, as platelet aggregation is quite similar in PRP obtained from the right ventricle, renal vein and femoral vein. The latter results seem interesting notwithstanding the different characteristics of circulation through kidney and muscular and cutaneous tissues.

The different behaviour of arterial and venous platelets suggests that the lungs play an important role in this regard.

Riassunto. È stata studiata con aggregometro l'aggregazione delle piastrine di ratto in PRP ottenuto da sangue arterioso e da diversi vasi venosi. È risultato che l'effetto aggregante dell'ADP è molto più marcato sulle piastrine venose che su quelle arteriose.

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Intercellular Lacunae: Sequestered Microenvironments in Stimulated Leukocyte Cultures

The presence of other leukocytes is required in order for lymphocytes to undergo blastogenesis in vitro when stimulated by phytohemagglutinin (PHA)[1], antigens[2–5] or allogeneic cells[2,3,6–8]. Several types of biologically active substances, including soluble mediators of cellular immunity, are released in these leukocyte cultures[9–11]. In order to search for the ultrastructural basis for this cellular cooperation and for the transfer of substances between cells, electron microscopic studies were performed on human leukocytes which were cultured on glass coverslips and then fixed and embedded in situ in order to preserve the spatial relationship and contacts between cells. Three-dimensional reconstruction of cell clusters observed in electron micrographs of stimulated cultures reveals a morphologic entity, the intercellular lacuna, which, with its sequestered microenvironment, may serve important functions in immune reactions.

Materials and methods. Culture fluid containing 10^6 leukocytes/ml was prepared by adding 4 parts of Medium 199 (containing penicillin and streptomycin) to 1 part of leukocyte-rich supernatant plasma obtained after a 5-min, 300 g centrifugation of a donor's heparinized peripheral venous blood. The cells were grown on coverslips at 38 °C in stoppered Leighton culture tubes containing 0.4–ml aliquots of culture fluid with or without a blastogenic factor (1.0 μg of PHA or 0.5 μg of PPD). The cells were fixed for 1 h by replacing the 38 °C culture fluid with iced 6% glutaraldehyde in pH 7.4, 0.1 M phosphate buffer. The coverslips were then washed overnight in pH 7.4, 0.1 M phosphate-sucrose buffer, post-fixed for 1 h in osmium tetroxide diluted to 1% in a pH 7.4, 0.1 M phosphate solution, and dehydrated in graded alcohols. An Epon-filled gelatin capsule was inverted over each coverslip to embed the cells in situ, and the Epon-embedded cells were cured for 48 h at 60 °C. The differing expansion coefficients of the Epon and glass made it possible to remove the coverslip by alternating 30-sec immersions of the Epon block in boiling water and liquid nitrogen, respectively. Areas se-

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