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.

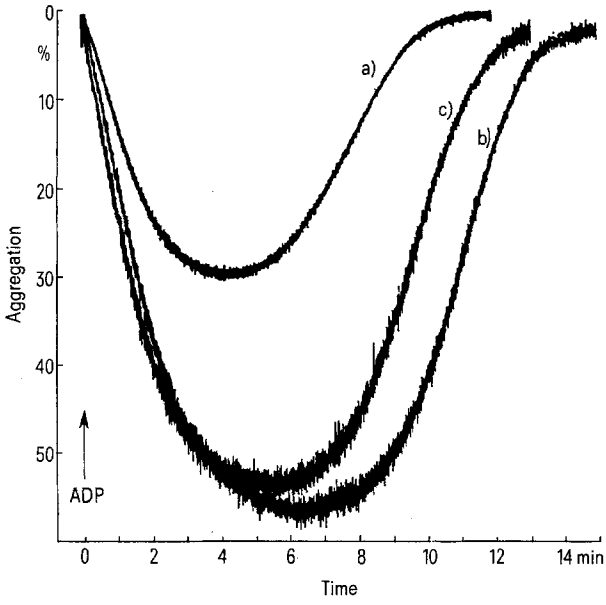

Fig. 2. Changes in O.D. induced by ADP (final concentration $9.2 \times 10^{-6} M$) in PRP (700,000 platelets/μl) obtained from right ventricle (c) and from left ventricle in open-chest animals submitted (a) or not (b) to artificial ventilation.

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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lected by light microscopy were thin sectioned parallel to the surface from which the coverslip had been removed.

Results. Intercellular lacunae are shown in sections at three different levels (Figures 1–3) of a 24-h PHA-stimulated cluster of lymphoid cells. A cytoplasmic projection (Pro) from one cell extends into the intercellular space (ISp) between lymphoid cells L_1 and L_2. A lacuna (*) is present between this projection and cell L_2 in all 3 sections.

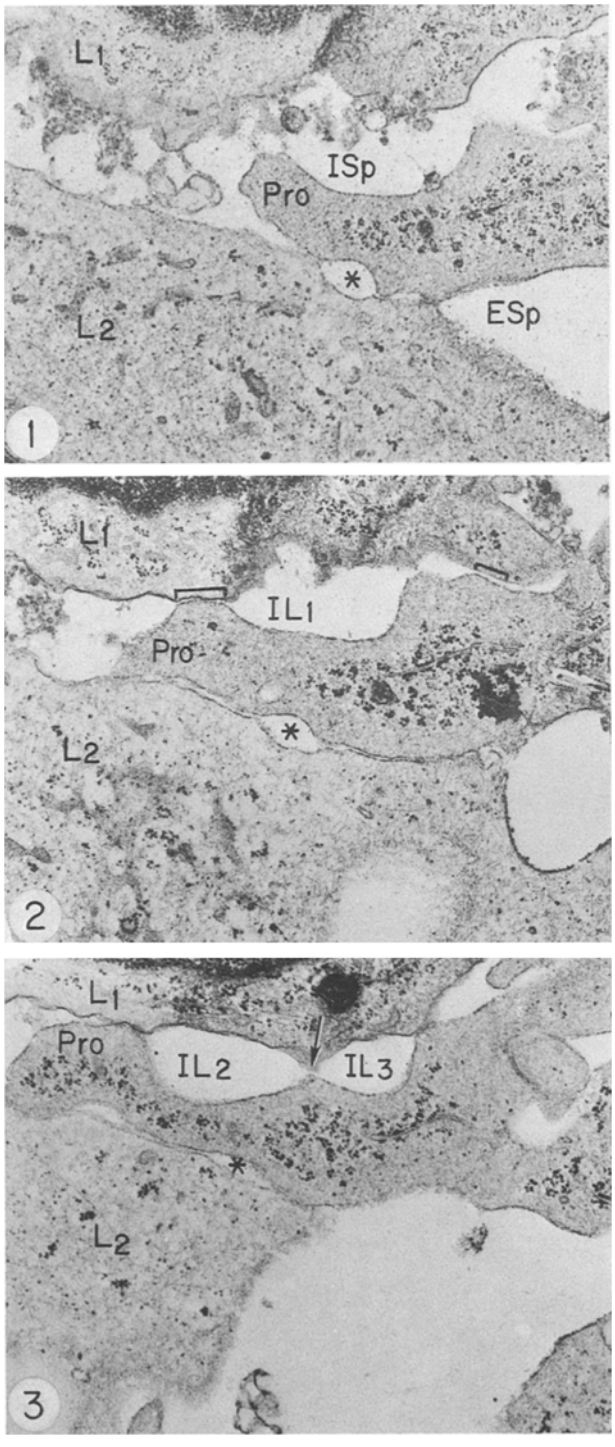

Fig. 1–3. Intercellular lacunae and interactions between lymphoid cells at different levels of a cell cluster from a 24-h PHA-stimulated culture. Details in text. ×22,500.

At the levels of these sections the passage of its contents into the larger pools (ISp and ESp) of culture fluid will be prevented because the lacuna is separated from these pools by the intercellular contacts formed between the projection and cell L_2.

Figures 1–3 show also the formation of anastomosing lacunae. Part of the intercellular space shown in Figure 1 becomes isolated from the remaining intercellular space by the contacts (indicated by the brackets) in the section of Figure 2 and is thereby converted into an intercellular lacuna, IL_1. In Figure 3 it can be seen that intercellular lacuna IL_1 separates into two other intercellular lacunae, IL_2 and IL_3, at their anastomosis (⇓).

Intercellular lacunae such as those illustrated between one lymphoid cell and another were also found between lymphoid cells and macrophages in these PHA-stimulated cultures. Similar lacunae between lymphoid cells and between lymphoid cells and marcophages were observed also in tuberculin-stimulated cultures. In contrast, however, coverslips from unstimulated cultures had very few cell clusters, and extensive intercellular lacuna formation was rarely observed.

Discussion. We have previously reported[12] that in cell clusters from PHA- and tuberculin-stimulated cultures numerous contacts developed during the first 24 h between lymphoid cells and between lymphoid cells and macrophages. Whenever these contacts could be adequately visualized, e.g., to the right of the lacuna (*) in Figure 2, no intercytoplasmic bridges or accumulations of cell organelles were observed. The contacts were found to consist of apposed unit membranes separated by an electron-lucent space approximately 150 Å wide. In this electron-lucent space the surface coats of the apposed cells may be in direct contact with each other, thereby providing an ultrastructural basis for a direct transfer of material in the surface coat of one cell to the surface coat of another. However, our present studies indicate that these intercellular contacts perform at least one additional function, viz., conversion of the intercellular space between juxtaposed cells into a network of anastomosing intercellular lacunae. The contents of these lacunae are only indirectly in communication with, and are therefore relatively isolated from, the large extracellular pool of culture fluid. Consequently, substances released by the cells into this network of sequestered microenvironments would reach the juxtaposed cell in an effectively higher concentration than if secreted in the large pool of extracellular culture fluid. Furthermore, these intercellular lacunae would make it possible for a substance released therein, perhaps from an as yet unidentified secretory site, to travel without excessive dilution within the lacunae to receptor sites on the juxtaposed cell.

Zusammenfassung. In Zellkulturen menschlicher Leukocyten wurden nach Stimulation zur Blasten-Transformation elektronenmikroskopische Verbindungen zwischen sich berührenden Zellen nachgewiesen, die wahrscheinlich dem Übertritt spezifischer Plasmabestand dienen.

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