the junctions had aggregated, and presumably distorted the adjacent plasma membranes, to form quite large deposits (Figure 1); usually they were quite small and discrete (Figures 1 and 2). In the heart the tracer had passed through the connective tissues and into the lymphatics. No tracer was found in unexpected, and possibly artefactual places, such as the cytoplasmic matrix, the nucleus, etc.

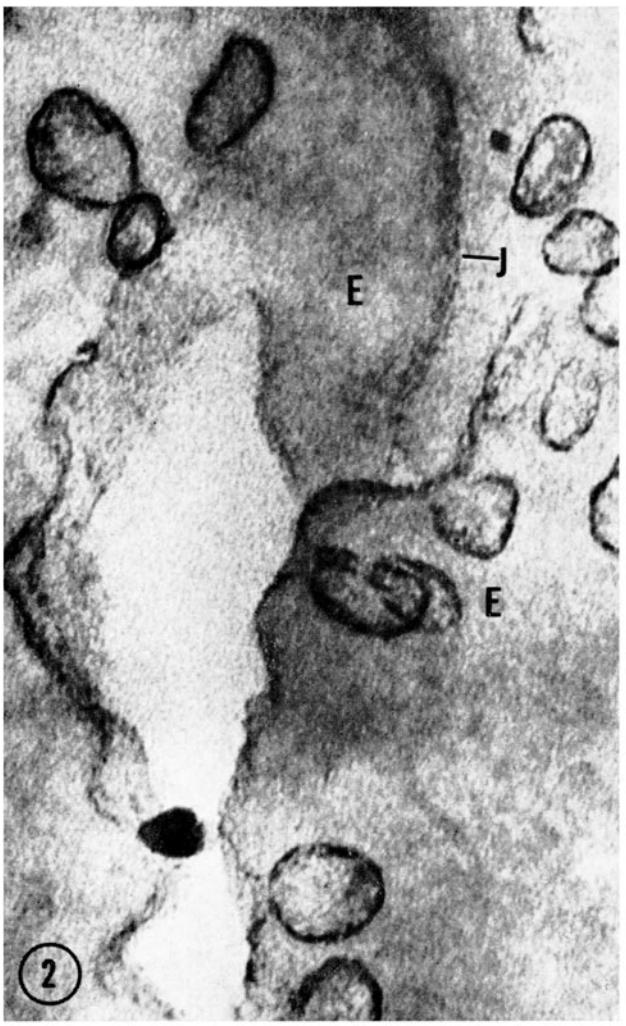

Fig. 2. Heart capillary. Small particles of tracer can be seen in a zonula occludens in a junction (J) and on the membranes of small vesicles in the cells. Unstained. ×170,000.

The bladder showed a similar localization of tracer to that of the blood vessels, i.e. in continuous lines down the junctions from the lumen, through the zonulae occludentes, to the connective tissue, and in the small vesicles. It had also passed into the vascular endothelial barriers, but any in the lumens had been washed away. These findings for the bladder should be regarded with some caution, because a 7% solution, as used in the bladder, was toxic to the heart.

These results show that Ferrous gluconate, with a mol. wt. of ~500, is a useful tracer. It passes through the endothelial barriers, and that of the epithelium of the bladder, via the junctions and the vesicles. Since, however, the vesicles only offer very slow transport relative to the junctions[1-3,6,8,12], we conclude that the junctional pathway is almost certainly the most important in the present instance. (Obviously the vesicles which open at the lumen will pick up some tracer, just as they pick up some of all the molecules in the plasma.) A priori, it would seem that any molecule smaller than 2000–5000 mol. wt. should pass mostly through the junctions[6], which almost certainly represent the pores of PAPPENHEIMER[15]. The zonulae occludentes usually do not stop flow completely, but supply the restraints necessary for molecular sieving[1,7,8,10].

Résumé. Le gluconate de fer a la propriété de suivre les molécules d'un poids moléculaire de ~500. Il traverse avec elles l'endothélium des vaisseaux sanguins et l'épithélium de la vessie en passant par les joints ou, pour une faible part, entre les vésicles.

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A Method for Studying the Ultrastructure of Intercellular Contacts in Leukocyte Cultures

To study the ultrastructure of the contacts between cells grown in tissue culture as well as the attachment of these cells to the substratum, it is often desirable to fix and embed the cells in situ in order to preserve the spatial relationship of these contacts. Previous investigators[1,2] have attempted to embed in epoxy resins cells cultured directly on glass coverslips, but, in contrast to results with the less satisfactory methacrylate embedding material[3-8], were unable to separate the coverslips from the epoxy-embedded cells unless the coverslips had been coated prior to culture with extraneous materials. We have briefly reported[9] a method to remove blocks of Epon-embedded leukocytes from uncoated glass coverslips, thereby making it unnecessary to complicate standard culturing procedures by exposing the cells to such extraneous coating substances. FLAXMAN et al.[10] also have recently reported a method by means of which a thin layer of Araldite, another epoxy resin, could be removed from an uncoated glass coverslip containing fibroblasts. In this paper we describe in detail our technique and its application to provide the first electron microscopic study of the unaltered, intercellular contacts between macrophages and lymphocytes grown on glass coverslips in human, peripheral blood leukocyte cultures stimulated to undergo blastogenesis.

Materials and methods. An individual's heparinized, peripheral, venous blood was centrifuged at 300*g* for 5 min. The resulting leukocyte-rich, supernatant plasma

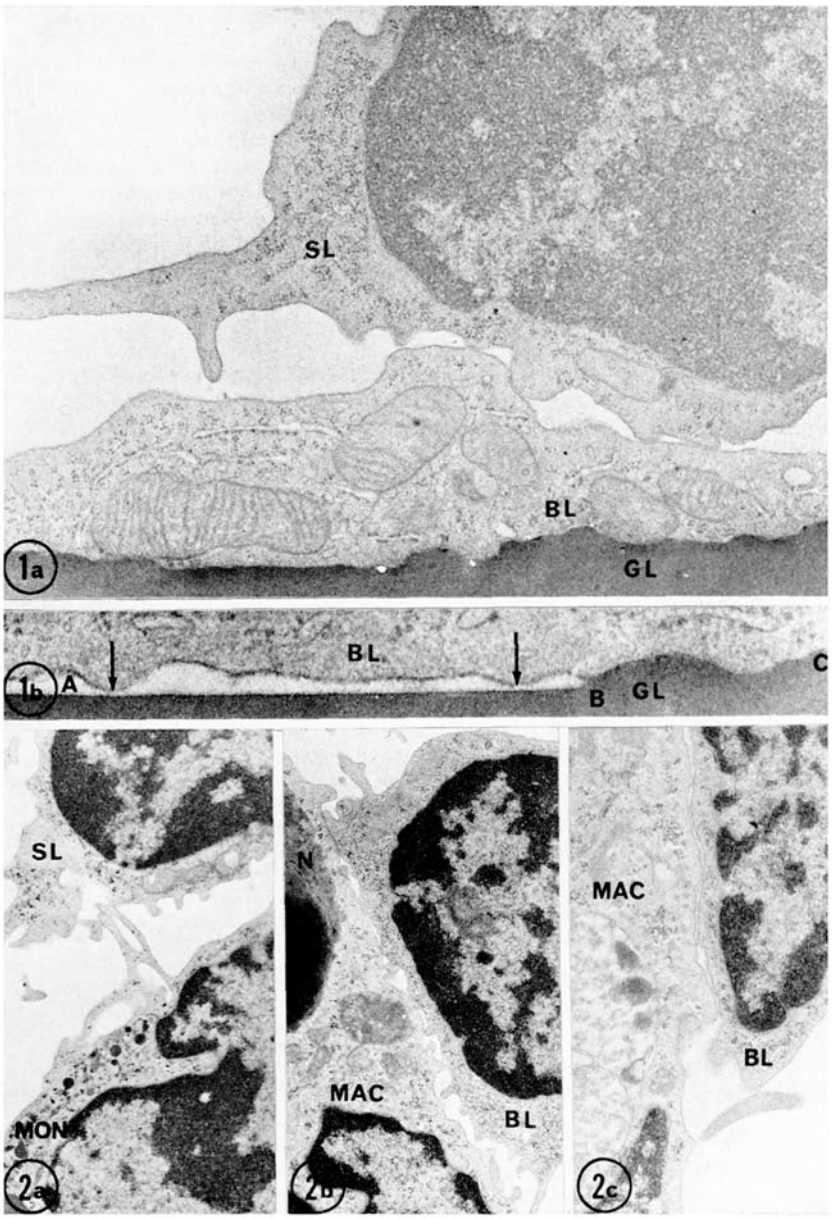

Fig. 1. Cells sectioned perpendicular to the surface of a coverslip on which a 9 h PHA culture was grown. a) A small lymphocyte (SL) is in contact with the upper surface of a blastoid lymphocyte (BL) through several cytoplasmic projections. The homogeneous, electron-dense material under the blastoid cell is an epoxy glue (GL) used to mount the specimen for perpendicular sectioning. × 13,000. b) Another section of the blastoid lymphocyte which illustrates that the Epon, which had permeated between the cells and the coverslip, may be separated cleanly from the removed coverslip as along A to B or removed with the coverslip as along B to C. The glue used to mount for sectioning permeates to the cleavage planes. Arrows indicate cell projections which had probably been in contact with the glass coverslip. × 39,000. Stained with uranyl acetate and lead citrate.

Fig. 2. Specimens sectioned parallel to the surface of the coverslip. a) A small lymphocyte (SL) in contact with microvillous projections of a monocyte (MON) in a 3 h PPD culture. b) and c) Blastoid lymphocytes (BL) in more extensive contact with macrophages (MAC) in 24 h cultures containing PPD and PHA, respectively. The macrophage in Figure 2, b contains an ingested neutrophil (N). a) × 5,000; b) × 7,900; c) × 12,400. Stained as in Figure 1.

was diluted with 4 parts of Medium 199 (Difco Laboratories) containing penicillin and streptomycin to give a culture fluid containing 10^6 leukocytes per ml. Aliquots of 0.4 ml of this culture fluid were incubated at 38 °C in stoppered, Leighton culture tubes containing 9 × 9 mm, No. 1 thickness, borosilicate glass coverslips (Bellco Glass, Inc.) cleaned in a silver-catalyzed, persulfate cleaning solution[11]. Each culture contained 1 µg of PHA (batch E-316A, Burroughs Wellcome and Co.) 0.5 µg of PPD (Parke, Davis and Co.), or no blastogenic factor.

Cells on coverslips were fixed for electron microscopy by decanting the warm culture fluid and replacing it with iced, 6% glutaraldehyde in pH 7.4, 0.1 *M* phosphate buffer. After fixation for 1 h at 0–4 °C, the coverslips were washed overnight in pH 7.4, 0.1 *M* phosphate-sucrose buffer, and dehydrated in graded alcohols. The cells were embedded by inverting a No. 3 gelatin capsule containing Epon 812[12] upon each coverslip. After curing at 60 °C for 48 h, the Epon-embedded cells were separated from the coverslips by differential thermal contraction and expansion induced by repeated transfers of the Epon block from boiling water to liquid nitrogen in each of which the block was held for 30 sec after each passage. Selected areas for thin sectioning

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were chosen under the light microscope and sectioned in an LKB microtome either perpendicular to, or parallel to, the plane defined by the surface of the coverslip on which the cells had grown.

Results and discussion. Perpendicularly sectioned specimens from a 9 h PHA culture are shown in Figure 1. In these sections the unit membranes of the upper and lower surfaces of the cells can be viewed. Figure 1, a shows a small lymphocyte on the upper surface of another cell which serial sections identified as belonging to a blastoid lymphocyte. The ultrastructure of the cells and their organelles has not been damaged by the extreme variations in temperature to which such embedded blocks had been subjected. It is apparent from the shape of the uppermost cell that there was no significant flattening or distortion during the preparative procedure.

Figure 1, b shows another section of the lowermost cell of Figure 1, a. The arrows indicate points at which the cell's membrane had been in contact with the glass coverslip. Along the straight surface between A and B the coverslip has been separated cleanly from the Epon. From B to C, however, the Epon which had permeated between the cell and the coverslip has been stripped away with the coverslip as a line of cleavage developed at the undersurface of the cell. The homogeneous, electron dense material (GL) under the cell is the epoxy glue used to mount the Epon block for sectioning.

In specimens cut in a plane parallel to the surface of the coverslip on which the cells were cultured, the membranes of the lateral surfaces of the cells can be viewed. As shown in Figure 2, a, microvilli of a monocyte are in contact with a small lymphocyte in a 3 h culture stimulated with PPD. Figures 2, b and c illustrate the fact that in later, 24 h cultures containing either PPD (Figure 2, b) or PHA (Figure 2, c) the apposition of cell membranes has become more extensive between the macrophages and the lymphocytes undergoing blastogenesis.

While contacts between lymphocytes and macrophages were more numerous in cultures stimulated with PHA than in those stimulated with PPD, the ultrastructure of the contacts appeared generally similar in both cases, consisting essentially of the apposition of unit membranes without any accumulation of cell organelles at these sites. Intercellular bridges such as those described connecting the cytoplasm of macrophages with that of lymphocytes in lymph nodes in vivo[13] were not found in any of our cultures. Such bridges, if they occurred, did so only fleetingly or extremely infrequently.

Résumé. Des globules blancs humains ont été cultivés sur des lamelles de verre puis fixées et inclus in situ pour l'examen au microscope électronique. Les lamelles furent enlevées des cellules incluses dans l'Epon par la méthode de contraction et d'expansion thermique induite par passage répété du bloc d'Epon de l'eau bouillante à l'azote liquide.

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Méthode de séparation des diverses classes d'ARN par électrophorèse sur gel de polyacrylamide en plaques

La séparation des différentes classes d'ARN[1] à partir d'un extrait brut est à la base de l'étude de cet acide nucléique et a fait l'objet de nombreux travaux de sorte que l'on dispose actuellement de plusieurs techniques éprouvées. Cependant, si les méthodes de séparation par centrifugation sur gradient de densité[2–5] ou par chromatographie sur colonne de MAK[6–8] sont universellement utilisées, celle d'électrophorèse prenant comme support un gel de polyacrylamide semble beaucoup moins répandue[9–11]. Un tel support devrait pourtant permettre l'obtention rapide d'un «profil» de la composition en ARN d'une cellule.

Lors de l'étude de cellules végétales, nous avons été amenés à mettre au point, pour l'ARN, une méthode d'électrophorèse sur gels de polyacrylamide-agarose dérivée de celle utilisée par URIEL[12] pour la séparation des protéines. (Des essais avec de l'ARN extrait de foie de rat ont donné des résultats analogues.)

Des régions apicales de racines sont mises à incuber dans une solution de Hoagland contenant des antibiotiques[3] et du phosphore 32 à l'activité spécifique désirée; après des temps d'incubation variables, les tissus sont lavés et broyés dans le milieu suivant: Tampon acétate de sodium: 0,05 *M*, pH 5,0; NaCl: 0,1 *M*; SLS: 1%; EDTA: 1 m*M*; bentonite: 0,5%.

L'ARN est extrait par les méthodes classiques de déprotéinisation au phénol à froid, puis, après plusieurs précipitations et lavages à l'alcool 70°, l'ARN est mis en solution dans des tampons différents selon son utilisation ultérieure; soit sur gradient de densité, soit dans des gels de polyacrylamide. Il est dosé en UV à 260 nm et la radioactivité comptée par effet Cerenkof[13] à l'aide d'un Pakard Tricarb.

Pour les contrôles, nous avons utilisé des gradients de sucrose 5 à 20% contenant du NaCl 0,1 *M* et de la bentonite. 0,2 ml d'ARN (600–1000 µg/ml) sont déposés sur ces gradients et centrifugés 5 h à 37000 × *g* dans une Spinco L, rotor SW 39; des fractions de 5 gouttes sont ensuite prélevées automatiquement diluées et dosées à 260 nm et leur radioactivité comptée.

Les gels de polyacrylamide sont bien connus comme support d'électrophorèse pour leur séparation extrêmement fine, supérieure à tout autre milieu. L'adjonction d'agarose accroit selon URIEL[12] la maniabilité de ces gels sans nuire à leurs propriétés.

La concentration en acrylamide est choisie en fonction de la masse moléculaire de la catégorie d'ARN à étudier. Nous voyons sur la Figure 1 (établie d'après diverses données de la littérature[14]) qu'un gel contenant 2,5 à 3% d'acrylamide laisse pénètrer toutes les classes d'ARN;