cinomas of the uterus[1] and malignant trophoblastic teratomas of the testis[14], but it is always absent from necrotic trophoblast[1,13]. In uterine trophoblast, HPF is restricted to the cytoplasm of the syncytiotrophoblast[1] but, in some malignant teratomas of the testis, it is present in the cytoplasm of occasional groups of cells identified as cytotrophoblast. Our finding of HPF in the cytotrophoblast of some cultured placental villi is therefore not surprising, and it possibly indicates a minor incoordination of differentiation in the organ cultures, as there is evidence that normal cytotrophoblast differentiates into syncytiotrophoblast[6,15].

The HPF antigen persists in organ cultures of human placenta for about 2 weeks although these cultures seemingly remain viable for at least 6 weeks. This is in marked contrast to the behaviour of an organ-specific microsomal antigen of human thyroid gland which persists in organ culture as long as the cells remain histologically viable[16]. It must be remembered, however, that the thyroid antigen is microsomal, whereas the placental antigen is a protein that is believed to be synthesized in and secreted by the syncytiotrophoblast in vivo[2]. If this is so, the depletion of the HPF antigen from the cytoplasm of the syncytiotrophoblast must result from an imbalance between the rates of synthesis in and loss, either by secretion or simple diffusion, from the organ-cultured tissue[17].

Zusammenfassung. Auswüchse menschlicher Placenten (12.–14. Woche Schwangerschaft) wurden in Organkultur gehalten. Obwohl sich die histologische Struktur langfristig erhalten lässt, bleibt ein dem menschlichen Wachstumshormon ähnliches Antigen, das in unkultiviertem Syncytiotrophoblast vorhanden ist, nur 10–14 Tage lang am Leben. Dasselbe erscheint vorübergehend in Cytotrophoblastzellen der Placentaauswüchse während der ersten 3 Monate.

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Study of the Lymphocyte Fractions in the Mixed Lymphocyte Culture in vitro

The culture of 2 lymphocyte populations from non-related individuals makes it possible to obtain the transformation of the lymphocytes into large blastic cells[1–4]. The % of these transformations is in proportion to the presence of incompatibility for some leucocyte antigens[5–7]. It is important to precise the nature of the substance which is responsible for these transformations. For this purpose the lymphocytes from one individual are disrupted and the different fractions are cultured with the living lymphocytes from another individual.

Our procedure is as follows: the lymphocytes are obtained from peripheric defibrinated blood after sedimentation of the red cells thanks to the addition of plasmagel (R. Bellon, Paris). The polynuclears are eliminated by sticking to the glass of Roux bottles while the lymphocytes remain in suspension. To disrupt the lymphocytes a hypotonic shock in distilled water for 30 min is used, which fragilizes the cells. Afterwards they are resuspended at a concentration of 3×10^6 lymphocytes/ml in a solution of $0.34 M$ saccharose and $0.0018 M$ CaCl. This suspension is treated by 4 successive freezing-thawings. During the whole procedure the temperature must never exceed 4 °C. The formation of aggregates is avoided by a permanent magnetic stir.

After the fourth freezing-thawing, a differential centrifugation at 600 *g* for 10 min, 17,000 *g* for 15 min and 105,000 *g* for 30 min produces 3 pellets: nuclei, mitochondria, and ribosomes; in addition the supernate remains. This procedure of fractionation is a modified version of that by RAPAPORT et al.[8]. The control under an electronic microscope shows the purity of our fractions.

The 3 pellets and the supernate are diluted in order to produce the equivalent of 1×10^6 cells/ml. All the fractions and the supernate are first tested in cultures with the autologous lymphocytes and then with the lymphocytes of one other individual. Four control cultures are carried out on the same day: (1) mixed lymphocyte culture (MLC) with the 2 living lymphocyte populations; (2) MLC with the killed lymphocytes of the donor of the fractions and the living lymphocytes of the other individual; (3) negative control culture (NCC) with the living lymphocytes of one of the individuals; (4) NCC with the living lymphocytes of the other individual.

The results, presented in the Table, show that only the pellet of the third centrifugation at 105,000 *g* is active. It produces the transformation of the isologous lymphocytes into blastic cells. Its activity is very similar to that observed when the killed lymphocytes are cultured in its place under the same conditions. Moreover, this demonstrates that the antigens responsible for the stimulation

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of the lymphocytes in the MLC are found in the third fraction. This fraction is composed by the ribosomes and the cell membranes.

These results confirm those of other authors who have found that transplantation antigens are in the ribosomal fraction[9]. The activity of the nuclear fraction is nul in our experiments. It is possible that it is due to the fact that our fraction is purer, which would explain the difference between our results and those of others[10].

The chemical nature of the substance responsible for the transformation of the lymphocytes in the MLC is not determined yet. It is however unlikely that the RNA is involved in this kind of reaction. Further work in this line is in progress[11].

Antigenic activity of the various lymphocyte fractions

3.1	A^a		B^a	2.5
1.6	A^b +	Pellet B 600 *g*	+ B	2
1.9	A^b +	Pellet B 17,000 *g*	+ B^b	2.2
6.5	A^b +	Pellet B 105,000 *g*	+ B^b	2.1
2.7	A^b +	Supernatant	+ B^b	2.5
		A + B^b: 12.5		
		A + B^c: 5.6		

A and B 2 lymphocyte populations; the fractions from B are tested against the living lymphocytes A. [a], 4×10^6 cells/culture; [b], 2×10^6 cells/culture; [c], killed cells. Numbers represent the percentage of transformed cells: mean value of 6 experiments.

Résumé. Les lymphocytes du sang périphérique d'un sujet ont été fractionnés. Trois fractions ont été obtenues. Chaque fraction a été cultivée avec les lymphocytes vivants d'un autre individu non apparenté au premier. Le pourcentage des cellules transformées a été évalué pour chaque culture. Seule la fraction ribosomale dans laquelle se trouvent également les membranes cellulaires est active et donne un pourcentage de transformations comparable à celui obtenu lorsqu'on met en culture dans les mêmes conditions les lymphocytes, entiers et tués par congélation-décongélation, qui ont servi pour la préparation des fractions.

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The Influence of Bacterial Endotoxin on the Formation of Antibody-Forming Spleen Cells in Mice Immunized with Sheep Red Blood Cells

In 1956 Johnson, Gaines and Landy[1] reported that purified lipopolysaccharide preparations from several gram-negative bacterial species are able to enhance antibody production to protein antigens in rabbits. This was repeatedly confirmed by other authors[2]. On the other hand, the same lipopolysaccharide preparations were found to be relatively ineffective in guinea-pigs and mice[1]. Finally, it has been reported that neither bacterial endotoxins nor complete Freund's adjuvants induce adjuvant effects with polysaccharide antigens[3]. That this cannot be considered as a general principle was shown recently[4] for when an accelerated and prolongated multiplication of antibody-forming spleen cells by *Bordetella pertussis* in mice immunized with sheep red blood cells (SRBC) was found. This prompted the authors to explore whether purified bacterial polysaccharides might also be able to increase the number of antibody-forming spleen cells to SRBC in mice under different experimental conditions. For the quantitative determination of plaque-forming spleen cells, the agar technique as described by Jerne et al.[5,6] was employed[4]. As complement guinea-pig serum was used diluted 1:3 (v/v) with physiological saline. For the experiments male albino mice of the inbred strain NMRI/Han. were used.

In a preliminary first investigation 60 mice were divided in 4 groups. The 15 mice of group I were immunized by an i.p. injection of 4×10^8 SRBC while the 15 mice of group II simultaneously with the SRBC received an i.v. injection of 50 μg of a commercially available endotoxin from *Serratia marcescens* (Difco). The immunization schedule of the animals of group III was the same, but each mouse was given an i.v. injection of 100 μg of endotoxin from *S. marcescens*. Finally, the 15 mice of group IV received only an i.v. injection of 100 μg of endotoxin from *S. marcescens*. Three mice out of each group, respectively, were sacrificed at various intervals after immunization and their spleens removed aseptically. The results presented in Table I show that endotoxin effects an accelerated formation of plaque-forming spleen cells. But the number of competent cells was not increased. On the other hand, the sharp decrease of plaque-forming spleen cells between the 7th and 14th day after immunization was delayed in the endotoxin-treated mice. Furthermore, it can be seen in Table I, too, that an endotoxin dose of 100 μg effected only a minute proliferation of hemolysin-forming spleen cells. In order to investigate the adjuvant effect of purified lipopolysaccharide from *S. marcescens* over a longer period of time and by use of greater numbers of mice, a second experiment was undertaken. A collective group of 100 male NMRI-mice was divided into 2 groups. The animals of group I received

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