

peripheral blood cells¹⁶⁻¹⁹. When *particulate* antigens are used with buffy coat cells, lymphocytes must be present for positive responses^{20,21}; in the mixed lymphocyte culture system production of an MIF capable of inhibiting buffy coat cell migration has been demonstrated²². Elaborate mechanisms have been proposed to demonstrate the presence of macrophages in positive soluble antigen buffy coat cell tests^{13, 23}. Nonetheless it remains an assumption, not critically proven, that *soluble* antigen induced buffy coat cell migration inhibition is mediated by MIF. The present experiments support this assumption by demonstrating the requirement for lymphocytes and for puromycin-sensitive protein synthesis.

Autologous serum and plasma clearly increase the inhibitory response of cells from skin-test positive donors, suggesting that immunoglobulin-antigen complexes can inhibit buffy coat cell migration as they can macrophage migration or that cytophilic antibody is measured in the migration test²⁴⁻²⁶. The failure of puromycin to block inhibition completely when AS is present is also consistent with at least a partial effect of immune complexes independent of protein synthesis. However findings that the AS-dependent response can be removed by dilution and that successive washes of leukocytes augment rather than diminish the positivity of the test indicate that serum antibody is not the sole determinant of the DMI response.

When certain conditions are met, the DMI test is reproducible, which is at variance with our earlier experience²⁷. We attribute the difference to changes in antigen dose, capillary size, and species of origin of sera in the media.

Résumé. La présence de lymphocytes et la synthèse de la protéine sont nécessaires à la production du MIF

(migration inhibition factor). Pour le test de migration des leucocytes du sang périphérique, nous avons pu montrer en utilisant un antigène soluble (PPD) et du sérum de cheval, qu'il n'y a point d'inhibition de migration en l'absence des lymphocytes ou en présence de puromycine. Quand le milieu contient du sérum de sujet testé, l'inhibition n'est que partiellement sensible à la puromycine.

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- ¹⁶ P. GLADE and K. HIRSCHHORN, *Am. J. Path.* 60, 483 (1970).
- ¹⁷ J. CLAUSEN, *Acta allergol.* 26, 56 (1971).
- ¹⁸ L. GOLDBERG, J. LOUIE and M. BAKER, *J. Immun.* 107, 906 (1971).
- ¹⁹ D. THOR and S. DRAY, *J. Immun.* 101, 51 (1968).
- ²⁰ J. ZABRISKIE and R. FALK, *Nature, Lond.* 226, 943 (1970).
- ²¹ M. SØBORG, *Acta med. scand.* 185, 221 (1969).
- ²² J. CLAUSEN, *J. Immun.* 108, 453 (1972).
- ²³ B. NORDQVIST and H. RORSMAN, *Acta derm.-vener Stock h* 50, 435 (1970).
- ²⁴ T. PACKALEN and J. WASSERMAN, *Int. Arch. Allerg. appl. Immun.* 41, 790 (1971).
- ²⁵ E. HEISE, S. HAN and R. WEISER, *J. Immun.* 101, 1004 (1968).
- ²⁶ H. AMOS, B. GURNER, R. OLDS and R. COOMBS, *Int. arch. Allerg. appl. Immun.* 32, 496 (1967).
- ²⁷ M. LOCKSHIN, *Proc. Soc. exp. Biol. Med.* 132, 928 (1969).
- ²⁸ Supported by a grant from the New York Chapter of the Arthritis Foundation.
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Preliminary Report on the Influence of the Plasma Upon the Effect of ADP on Arterial and Venous Platelets

In order to explain whether the phenomenon previously observed¹, i.e. that arterial platelets show less marked responsiveness to ADP than venous ones, we undertook experiments to check whether the different platelet behaviour in platelet-rich plasma (PRP) is inherent to platelets or to plasma.

Methods. Arterial blood was collected in open-chest animals from the left ventricle of artificially ventilated rats and venous blood from the right ventricle. PRP was obtained as previously described¹. ADP-responsiveness of washed platelets resuspended in different milieux was tested (see foreward).

The platelets were washed twice with a modified Tyrode's solution: 8 g NaCl, 0.2 g KCl, 1 g NaHCO₃, 1 g/l glucose, pH adjusted to 7.35 with NaOH. This solution does not contain calcium, magnesium or phosphate.

Washing was carried out as follows: PRP (3 ml) was diluted in a plastic centrifuge tube with 20 ml of modified Tyrode's solution and then centrifuged at 800 g for 8 min at room temperature. The supernatant was discharged and the pellet again resuspended and centrifuged.

After 2 washings: a) platelets were resuspended either in Tyrode's modified solution or in platelet-poor plasma (PPP) freshly obtained from arterial or venous blood; b) the samples were centrifuged at 100 g for 5 min in order to eliminate contaminating red blood cells and small aggregates. Platelet count was finally adjusted to a number of 700.000/μl; c) ADP-induced aggregation was estimated as previously described¹. The lapse of time between a) and c) was about 1 h.

Results. As can be seen (Figure 1a) twice washed 'venous' platelets resuspended in Tyrode's modified solution do not clump upon addition of ADP (6 experiments). Similar responses have been obtained from 'arterial' washed platelets resuspended in Tyrode's modified solution (8 experiments). When the platelets were resuspended in PPP freshly obtained, they recovered ADP-responsiveness, the extent of which was related to the type of plasma. There is evidence (Figure 1, b, c) that 'venous' washed platelets resuspended in PPP obtained from arterial blood (6 experiments) show a less-marked ADP-induced aggregation than 'arterial' washed platelets resuspended in PPP obtained from venous blood (9 experiments).

In 7 experiments we resuspended washed platelets in their own PPP; the ADP-responsiveness was quite similar to that of initial PRP.

Considering that the clumping power of twice washed platelets is lost in Tyrode's modified solution but restored by incubation in plasma, we studied the course of this recovering (8 experiments).

Figures 2 and 3 show the results obtained at different time intervals from the resuspension of platelets in plasma. It can be seen that ADP-responsiveness increases with the incubation time of 'arterial' or 'venous' platelets with the venous or arterial plasma, respectively.

Discussion. Experiments with washed platelets indicate that washing abolishes any clumping power, while resuspension in plasma restores it. So there should be a plas-

¹ D. BOTTECCHIA and M. G. DONI, *Experientia*, 29, 211 (1973).

matic component which allows platelets to aggregate. Its presence is necessary but not sufficient because platelets aggregation increases progressively with the incubation time in the plasma. So we may suppose that there is an interaction between some plasmatic component and platelets, although the mechanism of this interaction is a matter of speculation. The active substance in the plasma is probably fibrinogen²⁻⁶.

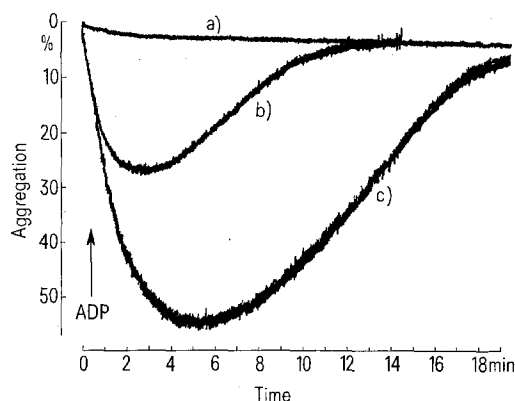


Fig. 1. ADP-induced changes in O.D. observed in twice washed 'venous' platelets resuspended in Tyrode's modified solution (a); 'venous' washed platelets resuspended in arterial PPP (b); and 'arterial' washed platelets resuspended in venous PPP (c). Platelet count 700,000/ μ l. Final concentration of ADP $9.2 \times 10^{-6}M$. Response quite similar to that of tracing (a) has been obtained from arterial washed platelets resuspended in modified Tyrode's solution.

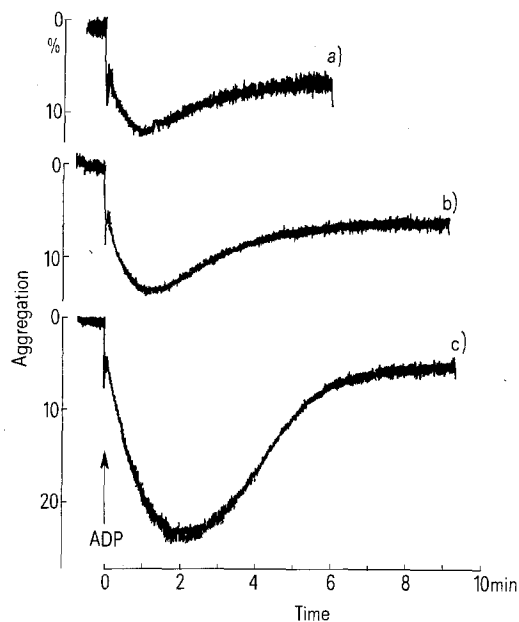


Fig. 2. ADP-induced changes in O.D. observed 7 (a), 15 (b), 48 (c) min after resuspension of 'venous' washed platelets in arterial PPP. Platelet count 700,000/ μ l. Final concentration of ADP $9.2 \times 10^{-6}M$.

The different influence of arterial and venous plasma on platelet adhesiveness suggests that the lungs also play an important role in this process. It could be supposed that the pulmonary contribution to the fibrinolytic activator activity of the plasma^{7,8} is involved in this phenomenon; but we can exclude this hypothesis because the fibrinolytic activator activity disappears a few minutes after storage at room temperature⁹. Besides the passage of the plasma through the lungs is without effect unless they are ventilated.

Present results point to the possibility that the greater 'venous' platelet adhesiveness compared to the 'arterial' one, play a role in thrombogenesis or in the development

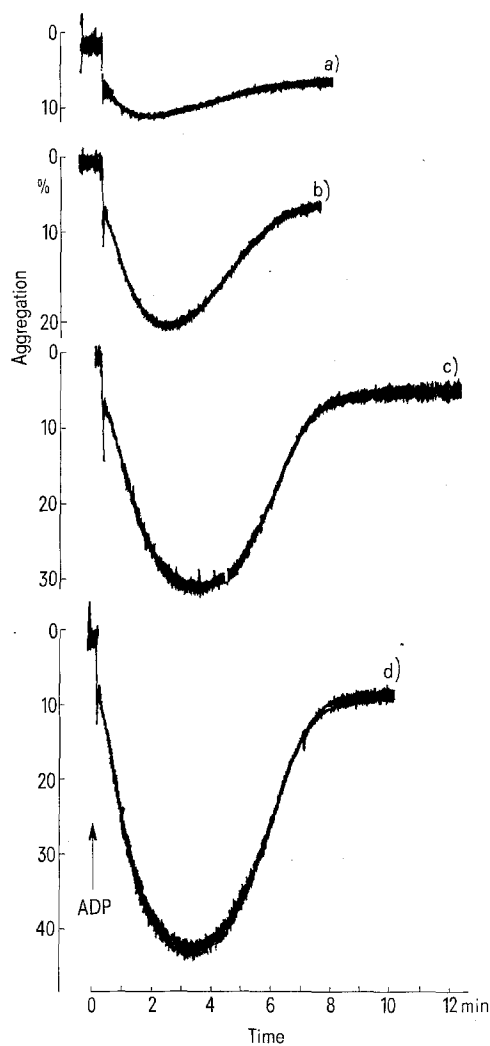


Fig. 3. ADP-induced changes in O.D. observed 7 (a), 30 (b), 47 (c), 75 (d) min after resuspension of 'arterial' washed platelets in venous PPP. Platelet count 700,000/ μ l. Final concentration of ADP $9.2 \times 10^{-6}M$.

² G. V. R. BORN and M. J. CROSS, *J. Physiol., Lond.* **170**, 397 (1964).

³ M. J. CROSS, *Thromb. Diath. haemorrh.* **72**, 524 (1964).

⁴ J. R. McLEAN, R. E. MAXWELL and D. HERTLER, *Nature, Lond.* **202**, 605 (1964).

⁵ N. O. SOLUM and H. STORMORKEN, *Scand. J. clin. Lab. Invest.* **17**, (Suppl. 84) 170 (1965).

⁶ R. G. MASON and M. S. READ, *Exp. molec. Path.* **6**, 370 (1967).

⁷ I. S. MENON, D. WEIGHTMAN and H. A. DEWAR, *Clin. Sci.* **36**, 427 (1969).

⁸ G. M. SMITH, 6th European Conference on Microcirculation (Ed. DITZEL-LEWIS, Karger, Basel 1971).

⁹ J. M. THOMSON, *A Practical Guide to Blood Coagulation and Haemostasis* (J. and A. Churchill, London 1970).

of venous thrombosis; they also provide evidence of the prominent role of plasma in regulating platelet adhesiveness.

Riassunto. È stato dimostrato mediante prove crociate e la osservazione del comportamento di piastrine lavate risospese in PPP venoso o arterioso che la causa della maggior risposta delle piastrine venose rispetto a quella delle piastrine arteriose all'ADP è nel plasma.

La responsività all'ADP aumenta col tempo di incubazione nel plasma delle piastrine lavate.

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Action des différentes formes actives de la vitamine A sur le mécanisme immunitaire chez le rat

On désigne généralement sous le nom de vitamine A le rétinol; vitamine A1; ou axérophthol, mais aussi dérivés physiologiquement actifs dans l'organisme animal. Plusieurs formes chimiques de vitamine A ont été mises en évidence, ainsi que leurs stéro-isomères. Ils ne diffèrent entre eux que par leur activité biologique, présente dans les tissus.

Depuis longtemps, cette vitamine est considérée surtout comme une vitamine qui lutte contre l'infection, d'où son appellation d'anti-infectieuse. Les travaux sur la vitamine A sont nombreux dans plusieurs domaines: son rôle dans le mécanisme photorécepteur de l'œil^{1,2}; son transport, son absorption, et son stockage^{3,4}, son effet sur la majeure partie des voies métaboliques et hormonales de l'organisme notamment dans le métabolisme glucidique⁵, lipidique⁶, et protidique⁷ ont été largement étudiés. Bien que les relations entre carence en vitamine A et infection soient étroitement liées et exercent ensemble un effet synergétique dans l'organisme hôte, l'action de cette vitamine sur la réactivité immunitaire n'est pas encore élucidée. La vitamine A, qui exerce un effet manifeste sur l'intégrité des membranes des organites cellulaires⁸, ainsi que sur les enzymes lysosomales⁹, et dont l'effet est semblable à celui des substances adjuvantes de l'immunité (toxines, antigènes cellulaires, produits carbohydratés, détergents non ioniques) pourrait-elle avoir une action sur le mécanisme immunitaire?

DRESSER¹⁰, en 1968, montra que le rôle de la vitamine A (rétinol) est celui d'une substance adjuvante de l'immunité à cause de sa destruction des membranes lysosomales ce qui entraîne une stimulation de la division cellulaire. En injectant à une souris des quantités différentes de *Bordetella pertussis* en présence de la globuline γ de bœuf (0-5 mg), la réponse immunitaire obtenue est fonction de la quantité d'adjuvant et d'antigène utilisée. DRESSER observa un effet similaire lorsqu'une même quantité de globuline γ de bœuf (0,5 mg) est injectée à une souris ayant déjà reçu différentes quantités de vitamine A

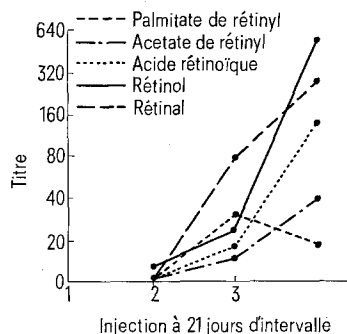
(rétinol) (1-10 mg). Cette possibilité de stimulation en présence de l'antigène dans la cellule conduit à une réponse immunitaire.

Dans ce travail sont exposés les résultats de l'adjuvantité des différentes formes actives de la vitamine A en présence d'un antigène qui est: la globuline γ de bœuf (BGG). L'expérience entreprise pour cette étude comprenait 48 Sprague Dawley rats mâles, âgés de 4-5 semaines et divisés en 6 groupes. Ces rats sont soumis à un régime normal. Le No 1 sert de témoin. Les 5 autres groupes reçoivent chacun une injection sous-cutanée de 16,500 UI (environ 5 mg) de l'une des formes actives de la vitamine A dissoute dans 0,5 ml d'huile de paraffine (substance non immunogène et non adjuvante en elle même). Les formes actives injectées de vitamine A sont: Le Rétinol (vitamine A alcool synthétique crist., K.L. Laboratories 5674); le rétinol (vitamin A aldehyde, Hoffman La Roche LTD., RO-01-6015/005); la vitamine A acetate crist. pure (K.L. Laboratories 5673t); la vitamine A palmitate (K.K. Laboratories Inc. 19862); et enfin l'acide rétinolique (Hoffman La Roche Lot. A. 263754).

Les 6 groupes de rats reçoivent après 24 h une injection i.p. de 1 mg de globuline γ de bœuf (BGG fraction II, Armour & Co) dans 0,1 ml. de NaCl 0,85/100. Le titre d'anticorps produit est déterminé par la technique de l'hémagglutination passive¹¹ au 21^e jour, après chaque injection de vitamine A et d'antigène (Figure).

En examinant ce graphique, on remarque la forte adjuvantité de la vitamine A (Rétinol) et (Rétinal) comparée aux effets adjuvantitaires donnés par les autres formes actives utilisées, c'est-à-dire l'acide rétinolique, la vitamine A acetate et palmitate. Il est à signaler aussi l'absence des anticorps provoqués par la globuline γ administrée uniquement aux rats témoins.

Dans ce travail, nous avons étudié le rôle des différentes formes actives de la vitamine A considérée comme substance adjuvante de la réactivité immunitaire chez le rat, et nous avons mis en évidence l'adjuvantité notable du rétinol et du rétinol par rapport aux autres dérivés actifs de cette vitamine en présence de la globuline γ de bœuf laquelle s'est montrée chez le rat témoin comme un antigène non immunogène en lui même. Bien que la vita-



Adjuvantité des différentes formes actives de la vitamine A en présence de la globuline γ de bœuf.

¹ G. WALD, Vitams Horm. 18, 417 (1960).

² K. D. FISCHER et C. G. CARR, Fedn. Proc. 205, 1605 (1970).

³ T. MOORE, Vitamin A P151 (Elsevier Amsterdam 1957).

⁴ J. GANGULY, Vitams Horm. 18, 387 (1960).

⁵ H. CHANDRA, G. VENKATACHALAM, B. V. BELAVDDY et P. REDDY, Indian J. Child Health 9, 589 (1960).

⁶ G. ARROYAVE, F. VITERI, M. BEHAR et N. S. SCRIMSHAW, Am. J. clin. Nutr. 7, 185 (1957).

⁷ G. A. PITT et R. A. MORTON, Rev. Biochem. 31, 491 (1962).

⁸ J. A. LUCY et J. T. DINGLE, in Metabolism and Significance of Lipids (DAWSON THODES., London, New York 1964).

⁹ J. T. DINGLE, Biochem. J. 79, 509 (1961).

¹⁰ D. W. DRESSER, Nature, Lond. 217, 527 (1968).

¹¹ A. B. J. STAVITSKY, Immunology 72, 360 (1954).