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Interactions of chrysotile asbestos fibres with the complement system

J.-M. R. SAINT-REMY & P. COLE *Host Defence Unit, Department of Medicine, Cardiothoracic Institute, Brompton Hospital, London*

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Summary. Type A chrysotile fibres (white asbestos) were tested *in vitro* for activation of the complement system. Fibres were incubated in normal human serum (NHS), factor B-depleted human serum, and normal and C4-deficient guinea-pig sera; the supernates were assayed for the remaining complement activity. Activation of the alternative pathway (AP) was shown in three ways. First, quantitative measurement of factor B; second, kinetic analysis of rabbit red blood cell lysis in whole alternative pathway (AP) and factor B lytic assays; third, qualitative measurement of C3 and factor B conversion by crossed immunoelectrophoresis. No C3 convertase activity could be demonstrated on the fibres but other possible mechanisms of AP activation are discussed. Magnesium itself is not responsible for this activation because acid-treated fibres retain this property. The early classical pathway is not involved as shown by normal whole complement activity of a factor B-depleted human serum and the absence of decrease of C4 functional activity. Knowing that complement proteins are present in pulmonary alveoli, mainly provided by cell synthesis, we suggest that complement activation *in vivo* may be relevant to the genesis of the chronic inflammation and fibrosis in the lung.

Correspondence: Dr Peter Cole, Host Defence Unit, Department of Medicine, Cardiothoracic Institute, Fulham Road, London SW3 6HP.

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INTRODUCTION

Occupational and environmental exposure to asbestos fibres has been known to be a health hazard for several decades. The mechanism by which asbestos dust induces lung fibrosis and mesothelioma is still poorly understood but an extensive review of *in vitro* biological properties of asbestos fibres has been published recently (Harington, Allison & Badami, 1975).

Experimental approaches to this question have followed two lines. First, *in vitro* studies have shown that chrysotile asbestos fibres are highly cytotoxic to macrophages by interaction with the cell membrane (Allison, 1970), that this effect is largely inhibited by the presence of serum and is dependent on the presence of the fibre's magnesium outer sheet (Beck, Holt & Nasrallah, 1971; Light & Wei, 1977). Hydrolytic enzymes are known to be released by reaction of the fibre within the lysosome after phagocytosis by mononuclear phagocytes (Davies, Allison, Ackerman, Butterfield & Williams, 1974), but controversy exists about the secretion of a fibrogenetic factor from the macrophages (Heppleston & Styles, 1967; Harington, Ritchie, King & Miller, 1973). Chrysotile is also a potent haemolytic agent (Harington, Miller & MacNab, 1971), this property being a function of the magnesium content of the fibre.

Second, a number of *in vivo* models have been described mainly using animals exposed to asbestos in inhalation chambers (Wagner, Berry, Skidmore & Timbrell, 1974) or by intrapleural or intraperitoneal injection of asbestos. Lung fibrosis, mesotheliomata

and other tumours have been produced (Davis, 1972; Wagner, Berry & Timbrell, 1973).

However, it is difficult to extrapolate *in vitro* results to *in vivo* mechanisms because of physical (fragmentation) and chemical (leaching) modification of the fibres, coating with bronchial secretions, increase of surfactant production and changes in the free alveolar cell population (Tetley, Hext, Richard & McDermott, 1976). At present it is thought that complex cell interactions occur wherein macrophages play a central role. Humoral mechanisms are likely to be involved as well (Turner-Warwick & Parkes, 1970). Kagan and colleagues (Kagan, Solomon, Cochrane, Beissner, Gluckman, Rocks & Webster, 1977a; Kagan, Solomon, Cochrane, Kuba, Rocks & Webster, 1977b) found significant cutaneous anergy and T-cell depletion, diminished lymphocyte response to phytohaemagglutinin and serum inhibitors of mitogen-induced lymphocyte transformation in a group of patients with asbestosis. Moreover, they found high levels of IgA (salivary, secretory and serum), IgG, IgM and IgE, a high frequency of autoantibodies without organ specificity and cold-reactive lymphocytotoxins.

Very little information about interactions of asbestos fibres and the complement system is found in the literature. However, it is known that polymorphonuclear cells and mononuclear phagocytes are able to synthesize proteins of the complement system (Lai A Fat & Van Furth, 1975; Einstein, Hansen, Ballow, Davis, Davis, Alper, Rosen & Colten, 1977; Ferluga, Schorlemmer, Baptista & Allison, 1978; Bentley, Fries & Brade, 1978). Moreover, some of those proteins have been recovered from lung lavage fluids (Robertson, Caldwell, Castle & Waldman, 1976). On the other hand the role of complement in chemotaxis and cellular metabolism is well known. For instance, factor B of the alternative pathway of complement activation has been shown *in vitro* to influence macrophage spreading (Bianco, Eden & Cohn, 1976; Gotze, Bianco & Cohn, 1979). For these reasons and because of the availability of sensitive methods to assay alternative pathway (AP) activation, a series of experiments was carried out on the interactions of chrysotile asbestos fibres with the complement system.

MATERIALS AND METHODS

Asbestos fibres

Type A chrysotile fibres (UICC) were obtained from Dr V. Timbrell as a kind gift. An aliquot was washed extensively and suspended at 12 mg/ml in sterile PBS for 7 days at pH 7·2. A second aliquot was suspended in 1 M HCl for 7 days, washed twice in PBS pH 7·2 and finally resuspended in the same buffer before use. A sample of PBS and of acid-treated fibres was autoclaved at 120° for 2 h.

Sera

Normal human serum (NHS) was obtained by venepuncture and used within 2 h. Factor B-depleted NHS was made by heating fresh NHS at 50° for 20 min (Lachmann & Hobart, 1978), the loss of C2 was estimated and a correction factor applied to the results. Guinea-pig serum from normal and C4-deficient animals was withdrawn by cardiac puncture.

Buffers

The buffers used were phosphate-buffered saline (PBS), pH 7·2 0·15 M, veronal-buffered saline (VBS) containing calcium and magnesium (CFD from Oxoid Ltd, London), PBS containing 0·01 M/l ethylene glycol tetraacetic acid (EGTA) and 0·008 M/l magnesium.

Incubation

Serial dilutions of the fibres were made in serum and incubated at 37° for 10–120 min with continuous slow inversion. The samples were then rapidly chilled in ice and centrifuged at 9000 ***g*** for 20 min. The supernates were tested immediately for their remaining complement activity. Control experiments included serum incubated without asbestos, asbestos without serum, asbestos and serum EDTA buffer and buffer alone.

Complement assay

Whole complement haemolytic activity was tested by the Mayer technique (Mayer, 1961). Sheep erythrocytes were sensitized with rabbit antibody (rabbit haemolytic serum, Wellcome Reagents Ltd, Beckenham). Serial dilutions of the supernates were tested for the ability to lyse the sensitized red cells (EA) in VBS.

C4 was assayed by an haemolytic agarose plate method (Lachmann & Hobart, 1978). Samples were loaded in wells cut in an agarose gel containing EA and C4-deficient guinea-pig serum; lysed areas were proportional to the amount of C4 in the samples.

Activation of AP was detected by three different techniques. (i) A quantitative measurement of factor B activity in an agarose plate containing guinea-pig erythrocytes and factor B-depleted NHS in EGTA buffer (Martin, Lachmann, Halbwachs & Hobart, 1976). (ii) A kinetic analysis of whole AP haemolytic activity (Platts-Mills & Ishizaka, 1974; Polhill, Pruitt & Johnston, 1978). Briefly, rabbit red cells (known activators of the AP) were mixed at 37° with the supernates in EGTA buffer and the lysis was followed in a spectrophotometer at 700 nm optical density. A modification of this method (which will be described elsewhere) has been introduced for factor B alone. (iii) A qualitative measurement of factor B and C3 conversion using crossed immunoelectrophoresis (Laurell, 1965) in EGTA buffer. Anti-C3 (β_1A/β_1C) was purchased from Meloy Laboratories and anti-factor B from Behring Institute, Marburg, West Germany. Samples were also analysed by rocket electrophoresis against human β_1H (factor H) sheep antiserum. (Kindly provided by Dr M. Hobart).

C3-cleaving enzyme

0·5 ml from a serial dilution of asbestos fibres was mixed with 0·5 ml of NHS and incubated at 27° for 10 min (Burger, Bitter-Suermann, Loos & Hadding, 1977). The fibres were then washed six times in ice-cold VBS at half ionic strength, resuspended and incubated at 37° for 30 min with a solution of purified C3. After centrifugation the extent of C3 conversion in the supernate was determined by crossed immunoelectrophoresis. C3 was purified according to the method of Lachmann & Hobart (1978). Briefly, this method involves a sequential precipitation of serum with Na_2SO_4 (20 g and 11 g/100 ml), a DEAE-Sephacel ion exchange and hydroxylapatite adsorption chromatography.

RESULTS

Alternative pathway

Figure 1 summarizes the results of AP kinetic assay. There was an actual increase in the time required to lyse 50% of the rabbit red cells ($t_{\frac{1}{2}}$) even with as little as 0·5 μg/ml asbestos fibres. A plateau was reached at 50 μg/ml. No differences could be seen between the PBS- and acid-treated fibres, except at very low concentrations where there was a *decrease* in $t_{\frac{1}{2}}$ for the PBS-treated fibres. By varying incubation times, it was found that maximal inactivation of the serum was obtained in about 30 min. The same experiments were performed with normal and C4-deficient guinea-pig serum and similar results were obtained (data not shown). Control experiments included asbestos fibres without serum in order to exclude direct lysis of rabbit red cells by fibres still present in the supernate.

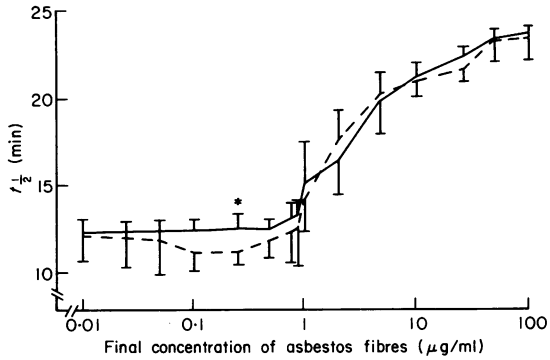

Figure 1. Whole alternative pathway lytic activity on rabbit red blood cells. The ordinate shows the time required to lyse 50% ($t_{\frac{1}{2}}$) of cells at 37° in EGTA buffer. The abscissa shows the final concentration of chrysotile fibres incubated with normal human secrum in EGTA buffer for 60 min at 37°. Asbestos was incubated for 7 days in PBS or 1 M HCl. Each point represents the mean of five experiments. Bar represents 1 SD. *$0{\cdot}02 > P > 0{\cdot}05$ (t test). (——) HCl treated; (---) PBS EGTA.

Factor B

Quantitative assessment of factor B activity on

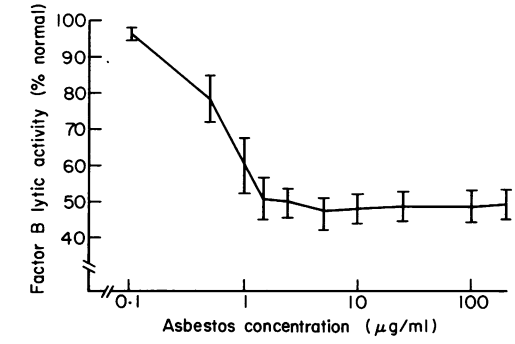

Figure 2. Haemolytic assay for factor B in an agarose plate after incubation of normal human serum with chrysotile asbestos. Incubation for 60 min at 37° in EGTA buffer. Each point is the mean of eight determinations. A sample without asbestos serves as control. Bar represents 1 SD.

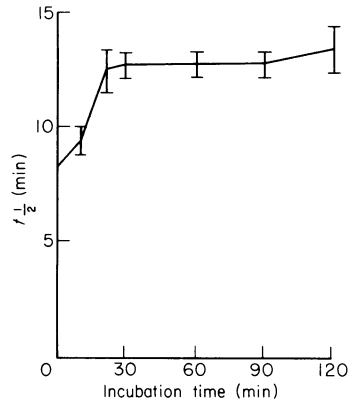

Figure 3. Kinetic assay of factor B activity. The time required to lyse 50% ($t_{\frac{1}{2}}$) rabbit red cells at 37° in EGTA buffer is recorded. The abscissa shows incubation time of 10 μg/ml chrysotile fibres in normal human serum and EGTA buffer. Similar results were obtained with 1·5 μg/ml chrysotile fibres. Bar represents 1 SD.

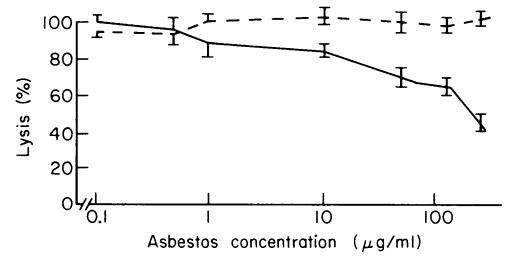

Figure 4. Whole complement lytic activity of the supernatant from incubation of normal human serum and factor B-depleted human serum with asbestos. Results are expressed as percentage of a control serum incubated without asbestos. Each point represents the mean of four determinations. The abscissa shows the final concentration of chrysotile incubated for 60 min at 37° in VBS. Bar represents 1 SD. (——) Supernatant from NHS; (– – –) supernatant from factor B-depleted NHS.

haemolytic agarose plates (Fig. 2) shows a decrease of about 50% with similar amounts of fibres. Here again a plateau was reached so that increasing the concentration of asbestos even one hundred-fold did not produce any further decrease in factor B activity. A kinetic analysis of factor B (Fig. 3) shows that this activity was almost completely lost in 20 min. Conversion of factor B and C3 on crossed immunoelectrophoresis were demonstrated over a similar range of fibre concentration (Table 1).

β_1H (factor H)

No variation in the amount of β_1H in the supernates could be shown by rocket electrophoresis.

Classical pathway

Whole complement haemolytic activity was slightly decreased in proportion to the amount of asbestos added to the solution (Fig. 4). On repeating this experiment with a factor B-depleted NHS, no effect whatsoever could be demonstrated. Quantitative assessment of C4 activity on haemolytic agarose plates revealed no diminution even with higher doses of asbestos fibres.

Table 1. Crossed immunoelectrophoresis of C3 and factor B after incubation of normal human serum with chrysotile asbestos

Final concentration of chrysotile fibres (μg/ml)	C3 Conversion in supernatant	Factor B Conversion in supernatant	Residual C4 (% of control) in supernatant*
250	++	++	100
125	++	++	96
50	++	++	102
10	++	++	100
1	++	+	ND
0·5	+	−	ND
0.1	±	−	ND
0	−	−	100

(++) 25–50% conversion, (+) 10–25%, (±) less than 10%, (−) no conversion, ND; not done.

* After incubation with asbestos in VBS for 69 min at 30°.

C3-cleaving enzyme

No C3 convertase activity could be demonstrated on the fibres after incubation with serum in the presence of either VBS- or PBS-EGTA buffer (data not shown).

Autoclaved fibres

No difference in results was obtained when using a sample of asbestos fibres autoclaved to remove endotoxin.

DISCUSSION

This study was initiated to find out whether chrysotile asbestos fibres could trigger AP activation and, ultimately, whether this could be one mechanism generating chronic inflammatory infiltrates in the lung.

Using a sensitive method for assaying AP activation, we have found that *in vitro* activation does occur. Five pieces of evidence suggest that this activation occurs via the AP. First, experiments were done in the presence of EGTA which chelates Ca^{2+} but not Mg^{2+} (Fine, Marney, Colley, Sergent & Des Prez, 1972) thus avoiding activation of C1. Second, no loss of functional C4 activity could be detected. Third, similar results were obtained with C4-deficient guinea-pig serum. Fourth, conversion products of C3 (C3c of β_1A mobility) and factor B (Ba of γ mobility) were detected by crossed immunoelectrophoresis of the supernates in EGTA buffer. Fifth, the loss of haemolytic activity in the whole complement assay was shown to be due to the involvement of factor B as seen by the abrogation of this reduction when factor B-depleted NHS was used.

Chrysotile fibres (white asbestos) were chosen because they are the most widely used in the world, causing more than 90% of industrial exposure in the United States and Western Europe. These fibres have a cylindrical shape consisting of a core of silicate and an outer sheet of $Mg(OH)_2$. They are usually long and very flexible (Harington *et al.*, 1975). Their behaviour in the lung is still controversial. Because of their small diameter, fibres are aspirated into small airways and alveoli where they come into contact with epithelial cells and macrophages. They have a tendency to fragment and rapidly lose their Mg^{2+} content. Smaller fibres can perforate the alveolar wall and reach the pleura.

As early as 1971, Harington, MacNab, Miller & King (1971b) showed that addition of fresh serum increased the haemolytic properties of amosite and crocidolite but not of chrysotile fibres. However, in their study the added fresh serum was diluted several times more than in our experiments, which is a critical aspect when assaying AP activation. Moreover, haemoglobin release from red cells is a rather crude estimate for haemolysis especially when fresh serum-induced lysis is superimposed on a marked direct lytic effect. We believe our method to be much more sensitive in detecting subtle changes in complement activity. Tiny amounts of fibres (less than 1 μg/ml) were shown to be sufficient to trigger the AP of complement. A study by Wilson, Gaumer & Salvaggio (1977) showed activation of AP with chrysotile fibres at a final concentration of 250 μg/ml. The observed 34·3 $\pm$ 13·2% lysis of glutathione-sensitized human erythrocytes (100% lysis being obtained with 250 μg/ml inulin) is comparable to the 45% seen in this study but unfortunately no dose–response curve for chrysotile fibres was presented. There were differences in the techniques used. In Wilson's study the red cells were mixed with the serum and the fibres and results were then calculated as difference between lysis obtained with fresh serum and heat-inactivated serum. In our study there was no contact between rabbit red cells and the fibres and no haemolysis was seen when supernate from a serum-free sample was mixed with the red cell. Very recently, Hasselbacher (1979) demonstrated chrysotile A-induced C3 and factor B conversion in EGTA buffer (20% and 6% respectively) as well as classical pathway activation. However this study is not comparable to ours in several respects. Mainly, a single concentration of 2·3 mg fibre/ml was used. Also electrostatic adsorption of IgG upon unleached fibres is likely to induce some complement activation as pointed out by the author. When chrysotile has lost its soluble Mg^{2+}, its biological activity is markedly changed. As the surface charge decreases with release of Mg^{2+}, protein adsorption upon the fibre remains almost unchanged until more than 30% Mg^{2+} is lost. Further leaching rapidly diminishes the adsorption capacity (Morgan, Davies, Wagner, Berry & Holmes, 1977) and direct haemolytic activity is also reduced. It is known that leaching occurs *in vitro* (Pelfrene, 1977). Moreover, fibres isolated from human lungs have been shown to have lost up to 50% of their Mg^{2+} (Pooley, 1972). On the other hand, Mg^{2+} itself is an activator of AP (Lew,

Yikiyama, Waks & Osler, 1975). For these reasons we felt that a more realistic approach would be to leach the fibres before use in our *in vitro* assay. From Fig. 1 it can be seen that acid treatment of the fibres for 7 days (ie. not more than 80% Mg^{2+} leached; Morgan *et al.*, 1977) does not alter the capacity for AP activation. After incubation in saline a very small amount of fibres seem to decrease the $t_{\frac{1}{2}}$ (left hand part of the curve). This somewhat intriguing result may be explained by the presence of trace amounts of Mg^{2+} upon the fibres, but enough to increase the speed of AP activation via rabbit red cells. The right hand part of the curve indicates that the fibre activates the AP independently of its Mg^{2+} content. Because of the well recognized action of endotoxin upon the AP of complement (Fine, 1974), a series of samples were autoclaved at 120° for 2 h. Comparable results were obtained, therefore it seems unlikely that endotoxins are the causal factor.

The mechanism by which chrysotile fibres activate the AP is puzzling. We were unable to demonstrate any C3-cleaving enzyme on the fibre, either via the classical ($\overline{C42}$) or the alternative ($\overline{C3bB}$) pathway of activation. This is known to be the case with zymosan activation of the AP, for instance (Brade, Lee, Nicholson, Shin & Mayer, 1973; Medicus, Schreiber, Gotze & Müller-Eberhard, 1976). Another possibility for AP activation lies in adsorption and/or inhibition of regulatory proteins (β_1H, C3bINA or C3bINA cofactor). It has recently been shown (Bitter-Suermann, 1978) that particulate polyanions are able to adsorb one of these proteins. We have no evidence from this study to confirm or refute this possibility. Rocket electrophoresis on β_1H excludes adsorption of this protein on the fibre. Nevertheless, it is worth noting that in our assay we were unable to activate the AP further by increasing the amount of asbestos by as much as one hundredfold. This is in favour of interaction with a serum factor present in limited amount in the test tube.

Mechanisms involved in pulmonary fibrogenesis due to asbestos exposure are far from clear (Bateman, Emerson & Cole, 1980). Much is known about physical configuration, chemistry and biological effects *in vitro*, but the way in which the fibres behave in the lung is controversial. In particular, chemical and physical modifications *in vivo* almost certainly influence cellular interactions and humoral responses. We suggest that the capability of chrysotile fibres to activate the AP of complement may be one of the mechanisms through which chronic inflammatory infiltrates are generated in the lung (Schorlemmer, Bitter-Suermann & Allison, 1977). Proteins from the complement pathways are present in alveoli due to *in situ* cell synthesis and transudation from the plasma. Knowing the effects of C3b (Schorlemmer & Allison, 1976; Schorlemmer, Davies & Allison, 1976) and factor B (Gotze *et al.*, 1979) upon cellular regulation, it is also tempting to incriminate continuous complement activation *in vivo* in the genesis of fibrotic changes in the lung.

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REFERENCES

ALLISON A.C. (1970) Effects of silica and asbestos on cells in culture. *Inhaled Part. Vap.* **3**, 437.

BATEMAN E., EMERSON R. & COLE P.J. (1980) Mechanisms of fibrogenesis. In: *Occupational Diseases: Research Approaches and Methods* (Ed. by M. Turner-Warwick and H. Weill). In: *Lung Biology in Health and Disease.* (Exec. Ed. Claude Lenfant) Marcel Dekker Inc, New York. (In press).

BECK E.G., HOLT P.F. & NASRALLAH E.T. (1971) Effects of chrysotile and acid-treated chrysotile on macrophage cultures. *Br. J. Indust. Med.* **28**, 179.

BENTLEY C., FRIES W. & BRADE V. (1978) Synthesis of factors, D, B and P of the alternative pathway of complement activation, as well as C3, by guinea-pig peritoneal macrophages *in vitro*. *Immunology*, **35**, 971.

BIANCO C., EDEN A. & COHN Z.A. (1976) The induction of macrophage spreading: role of coagulation factors and the complement system. *J. exp. Med.* **144**, 1531.

BITTER-SUERMANN D. (1978) Polyanionic blockade of regulatory proteins: an efficient fluid phase C3b amplification model (abstract). *Fourth European Complement Workshop*, Cambridge, England.

BRADE V., LEE G.D., NICHOLSON A., SHIN H.S. & MAYER M.M. (1973) The reaction of zymosan with the properdin system in normal and C4 deficient guinea-pig serum. *J. Immunol.* **111**, 1389.

BURGER R., BITTER-SUERMANN D., LOOS M. & HADDING U. (1977) Insoluble polyanions as activators of both pathways of complement. *Immunology*, **33**, 827.

DAVIES P., ALLISON A.C., ACKERMAN J., BUTTERFIELD A. & WILLIAMS S. (1974) Asbestos induces selective release of lysosomal enzymes from mononuclear phagocytes. *Nature (Lond.)*, **251**, 423.

DAVIS J.M.G. (1972) The fibrogenic effects of mineral dusts

injected into the pleural cavity of mice. *Br. J. exp. Path.* **53,** 190.

EINSTEIN L.P., HANSEN P.J., BALLOW M., DAVIS A.E., DAVIS J.S., ALPER C.A., ROSEN F.S. & COLTEN H.R. (1977) Biosynthesis of the third component of complement (C3) *in vitro* by monocytes from both normal and homozygous C3 deficient humans. *J. clin. Invest.* **60,** 963.

FERLUGA J., SCHORLEMMER H.U., BAPTISTA L.D. & ALLISON A.C. (1978) Production of the complement cleavage product, C3a, by activated macrophages and its tumorolytic effects. *Clin. exp. Immunol.* **31,** 512.

FINE D.P. (1974) Activation of the classical and alternate complement pathways by endotoxin. *J. Immunol.* **112,** 763.

FINE D.P., MARNEY S.R. JR, COLLEY D.G., SERGENT J.S. & DES PREZ R.M. (1972) C3 shunt activation in human serum chelated with EGTA. *J. Immunol.* **109,** 807.

GOTZE O., BIANCO C. & COHN Z.A. (1979) The induction of macrophage spreading by factor B of the properdin system. *J. exp. Med.* **149,** 372.

HARINGTON J.S., MILLER K. & MACNAB G. (1971a) Hemolysis by asbestos. *Envir. Res.* **4,** 95.

HARINGTON J.S., MACNAB G.M., MILLER K. & KING P.C. (1971b) Enhancement of haemolytic activity of asbestos by heat-labile factors in fresh serum. *Med. Lavoro,* **62,** 171.

HARINGTON J.S., RITCHIE M., KING P.C. & MILLER K. (1973) The *in vitro* effects of silica-treated hamster macrophages on collagen production by hamster fibroblasts. *J. Path.* **109,** 21.

HARINGTON J.S., ALLISON A.C. & BADAMI D.V. (1975) Mineral fibres: chemical, physicochemical and biological properties. *Adv. Pharmacol. Chemother.* **12,** 323.

HASSELBACHER P. (1979) Binding of immunoglobulin and activation of complement by asbestos fibres. *J. Allergy clin. Immunol.* **64,** 294.

HEPPLESTON A.G. & STYLES J.A. (1967) Activity of a macrophage factor in collagen formation by silica. *Nature (Lond.),* **214,** 521.

KAGAN E., SOLOMON A., COCHRANE J.C., BEISSNER I.E., GLUCKMAN J., ROCKS P.H. & WEBSTER I. (1977a) Immunological studies of patients with asbestosis. I. Studies of cell-mediated immunity. *Clin. exp. Immunol.* **28,** 261.

KAGAN E., SOLOMON A., COCHRANE J.C., KUBA P., ROCKS P. & WEBSTER I. (1977b) Immunological studies of patients with asbestosis. II. Studies of circulating lymphoid cell numbers and humoral immunity. *Clin. exp. Immunol.* **28,** 268.

LACHMANN P.J. & HOBART M.J. (1978) Complement technology. In: *Handbook of Experimental Immunology,* Vol. 1, 3rd edn (Ed. by D.M. Weir). 5A. 14–15. Blackwell Scientific Publications, Oxford.

LAI A FAT R.F.M. & VAN FURTH F. (1975) *In vitro* synthesis of some complement componenets (Clq, C3 and C4) by lymphoid tissues and circulating leucocytes in man. *Immunology,* **28,** 359.

LAURELL C.B. (1965) Antigen-antibody crossed electrophoresis. *Anal. Biochem.* **10,** 358.

LEW F.T., YUKIYAMA Y., WAKS H.S. & OSLER A.G. (1975) Activation of the alternative (properdin) pathway by divalent cations. *J. Immunol.* **115,** 884.

LIGHT W.G. & WEI E.T. (1977) Surface charge and asbestos toxicity. *Nature (Lond.),* **265,** 537.

MARTIN A., LACHMANN P.J., HALBWACHS L. & HOBART M.J. (1976) Haemolytic diffusion plate assays for factors B and D of the alternative pathway of complement activation. *Immunochemistry,* **13,** 317.

MAYER M.M. (1961) Complement and complement fixation. In: *Experimental Immunochemistry,* 2nd edn (ed. by E.A. Kabat and M.M. Mayer), p. 133. Charles C. Thomas, Springfield, Illinois.

MEDICUS R.G., SCHREIBER R.D., GOTZE O. & MÜLLER-EBERHARD H.J. (1976) A molecular concept of the properdin pathway. *Proc. natn. Acad. Sci. (U.S.A.),* **73,** 612.

MORGAN A., DAVIES P., WAGNER J.D., BERRY G. & HOLMES A. (1977) The biological effects of magnesium-leached chrysotile asbestos. *Br. J. exp. Path.* **58,** 465.

PELFRENE A.F. (1977) Magnesium and cytotoxic effects of asbestos fibres. *Med. Lavoro,* **68,** 349.

PLATTS-MILLS T.A. & ISHIZAKA K. (1974) Activation of the alternate pathway of human complement by rabbit red cells. *J. Immunol.* **113,** 348.

POLHILL R.B., Jr., PRUITT K.M. & JOHNSTON R.B. Jr. (1978) Kinetic assessment of alternative complement pathway activity in a hemolytic system. *J. Immunol.* **121,** 363.

POOLEY F.D. (1972) electron microscope characteristics of inhaled chrysotile asbestos. *Br. J. indust. Med.* **29,** 146.

ROBERTSON J., CALDWELL J.R., CASTLE J.R. & WALDMAN R.H. (1976) Evidence for the presence of components of the alternative (properdin) pathway of complement activation in respiratory secretions. *J. Immunol.* **117,** 900.

SCHORLEMMER U. & ALLISON A.C. (1976) Effects of activated complement components on enzyme secretion by macrophages. *Immunology,* **31,** 781.

SCHORLEMMER U., DAVIES P. & ALLISON A.C. (1976) Ability of activated complement components to induce lysosomal enzyme release from macrophages. *Nature (Lond.),* **261,** 48.

SCHORLEMMER U., BITTER-SUERMANN D. & ALLISON A.C. (1977) Complement activation by the alternative pathway and macrophage enzyme secretion in the pathogenesis of chronic inflammation. *Immunology,* **32,** 929.

TETLEY T.D., HEXT P.M., RICHARDS R.J. & MCDERMOTT M. (1976) Chrysotile-induced asbestosis: changes in the free cell population, pulmonary surfactant and whole lung tissue of rats. *Br. J. exp. Path.* **57,** 505.

TURNER-WARWICK M. & PARKES W. (1970) Circulating rheumatoid and antinuclear factors in asbestos workers. *Brit. med. J.* **3,** 492.

TURNER-WARWICK M. (1973) Immunological mechanisms in occupational disorders. *Pro. R. Soc. Med.* **66,** 927.

WAGNER J.C., BERRY G. & TIMBRELL V. (1973) Mesotheliomata in rats after inoculation with asbestos and other materials. *Br. J. Cancer,* **28,** 123.

WAGNER J.C., BERRY G., SKIDMORE J.W. & TIMBRELL V. (1974) The effects of the inhalation of asbestos in rats. *Br. J. Cancer,* **29,** 252.

WILSON M.R., GAUMER H.R. & SALVAGGIO J.E. (1977) Activation of the alternative complement pathway and generation of chemotactic factors by asbestos. *J. Allergy clin. Immunol.* **60,** 218.