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LYMPHOCYTE TRANSFORMATION BY PHYTOHAEMAGGLUTININ (PHA) AND PURIFIED PROTEIN DERIVATIVE (PPD) IN PRIMARY BILIARY CIRRHOSIS

EVIDENCE OF SERUM INHIBITORY FACTORS

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SUMMARY

Factors inhibitory to lymphocyte transformation *in vitro* by PHA and PPD have been demonstrated in the majority of sera from patients with primary biliary cirrhosis. These factors inhibited transformation in response to PHA both of autologous lymphocytes, and of 'reference' lymphocytes which were obtained from a single donor. 'Reference' lymphocyte transformation in response to PPD was significantly less when cultured in sera from both primary biliary cirrhosis patients and control subjects than when cultured in AB serum.

INTRODUCTION

In a previous communication (MacSween *et al.*, 1973) we have shown that when cultured in the presence of phytohaemagglutinin (PHA), the blood lymphocytes of patients with primary biliary cirrhosis show a sub-normal transformation response, particularly with low doses of PHA. Insofar as lymphocyte transformation induced by PHA is considered to be a property primarily of thymus-derived 'T' lymphocytes, these results provide good evidence of T cell abnormality in this disease.

In this communication we report on the occurrence in primary biliary cirrhosis of serum factors inhibitory to lymphocyte transformation *in vitro* by PHA and purified protein derivative (PPD).

MATERIALS AND METHODS

A diagnosis of primary biliary cirrhosis had been established in the twenty patients studied on the basis of clinical, biochemical and serological findings (Goudie, MacSween & Goldberg, 1966; Sherlock, 1971), and liver biopsy material, available from seventeen patients, showed appearances consistent with this diagnosis (Goudie *et al.*, 1966; Scheuer, 1967).

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PHA studies were completed on eleven of the patients, and PPD studies were completed on all. The seven control subjects studied were composed of four healthy members of the laboratory staff, and three hospital patients, two of whom had had a cerebral infarct and one a myocardial infarct. The ages and sex distribution of patients and controls are summarized in the Tables of results.

Lymphocyte transformation studies

A 50-ml blood sample was collected from each subject and 20 ml was used to provide serum. Thirty millilitres were mixed in a universal container with 300 units of phenol-free heparin (Weddell Pharmaceuticals) and 1·7 ml of a 5% aqueous solution of 500,000 mol. wt dextran. The red cells were allowed to settle for 40–50 min at 37°C and the leucocyte-rich plasma was withdrawn and treated with a 3 μm particle size carbonyl iron suspension in order to obtain a higher lymphocyte concentration (Yeung Laiwah, 1971). The lymphocytes were washed three times in Eagle's medium, and then resuspended in sufficient Eagle's medium containing 20% of appropriate serum to give a cell suspension of 6×10^6 ml. Lymphocyte cultures were set up in triplicate. Control cultures without added mitogen were also set up in triplicate. To each culture tube was added 0·3 ml (2×10^6 cells) of the lymphocyte suspension, 1·2 ml of Eagle's medium containing 20% serum, together with 100 units of penicillin and 100 μg of streptomycin per ml, and 0·15 ml of the mitogen solution (0·15 ml saline in the case of the control cultures). All cultures were incubated for 120 hr at 37°C. Eighteen hours before termination of culture 0·1 μCi of [^{14}C]thymidine (Radiochemical Centre, Amersham) was added to each culture tube. Following incubation the culture tubes were centrifuged and the cell button washed twice with normal saline, twice with 10% TCA, and once with a 9:1 methanol–ether mixture. The DNA-incorporated isotope in the acid precipitated material was determined in a Packard Tri-Carb liquid scintillation counter. The mean results for the three culture tubes were calculated, and are expressed as counts per minute (cpm) per 2×10^6 lymphocytes.

The mitogens used were as follows: (a) freeze-dried reagent grade PHA (Wellcome Reagents) reconstituted to 5 ml and then diluted 1 in 10 in saline—0·15 ml used in each culture tube; (b) PPD (Evans Medical Ltd), reconstituted to give a solution containing an equivalent of 2000 units of old tuberculin per ml and used after dialysis for 24 hr against phosphate-buffered saline, pH 7·2—0·15 ml used in each culture tube.

Design of experiments

'Reference' lymphocytes, isolated as above, were obtained for each experiment from the same subject, a healthy medical member of the laboratory staff known to be strongly Mantoux positive. 'Test' lymphocytes (i.e. lymphocytes from primary biliary cirrhosis patients or control subjects) were cultured, with and without mitogens, both in medium containing autologous (test) serum and in pooled group AB serum, these cultures being set up simultaneously using lymphocytes from the same isolate. Parallel experiments were set up in which 'reference' lymphocytes were cultured in the same media with and without mitogens as the test lymphocytes, i.e. both in medium containing serum derived from the primary biliary cirrhosis patients or controls (test serum) and in medium containing AB serum. Similar cross-over experiments were carried out for both PHA and PPD. In comparing the results statistical analysis was performed using the paired *t*-test.

TABLE 1. PHA (phytohaemagglutinin)-induced lymphocyte transformation in a medium containing serum from primary biliary cirrhosis patients or control subjects and in a medium containing AB serum. Results are expressed as counts per minute (cpm)—mean ± standard error

	Number tested	Sex	Mean age ± SD	Test lymphocytes		'Reference' lymphocytes	
				Test (autologous) serum (range)	AB serum (range)	Test serum (range)	AB serum (range)
Primary biliary cirrhosis patients	11	9F	57 ± 13	2881 ± 583 (687–7,109)	5423 ± 1090 (1,683–15,268)	2753 ± 640 (653–7,776)	3859 ± 603 (1,524–7,138)
Control subjects	6	5F	44 ± 17	8612 ± 1132 (4,128–11,542)	7159 ± 1608 (3,132–14,380)	5888 ± 1341 (924–10,449)	5847 ± 1940 (1,774–15,094)

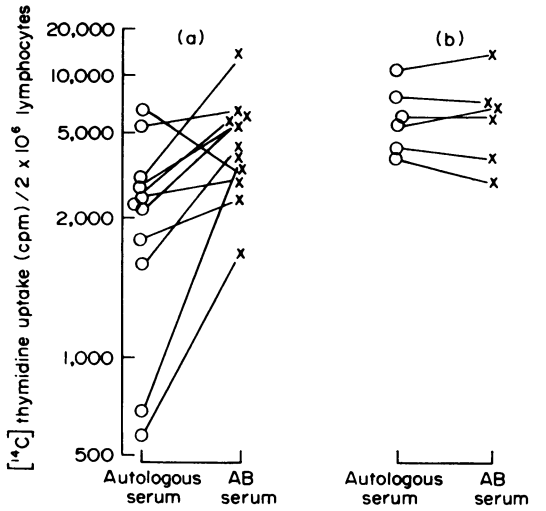

FIG. 1. PHA-induced lymphocyte transformation in autologous serum and in AB serum. (a) Primary biliary cirrhosis patients' lymphocytes. (b) Control subjects' lymphocytes.

RESULTS

PHA-induced lymphocyte transformation. (Table 1 and Figs 1 and 2)

In ten of the eleven primary biliary cirrhosis patients, lymphocyte responsiveness to PHA was greater in the presence of AB serum than with autologous serum ($t = 2{\cdot}386$; $P > 0{\cdot}05$). In the similar cross-over experiments with control subjects' lymphocytes, two showed greater and four lesser responsiveness in AB serum, and the overall results are not signifi-

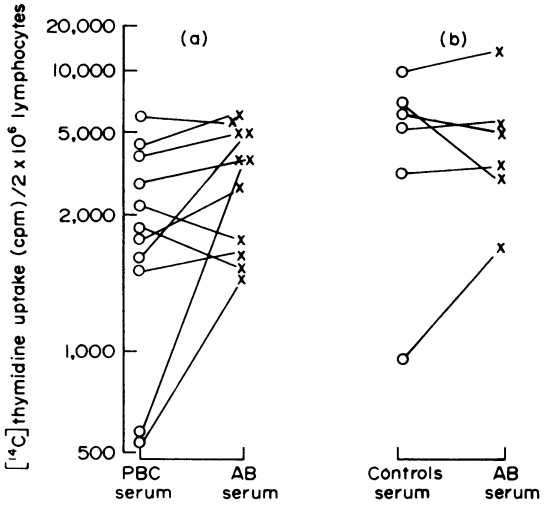

FIG. 2. PHA-induced lymphocyte transformation. 'Reference' lymphocytes in AB serum and (a) in serum from primary biliary cirrhosis patients and (b) in serum from control subjects.

TABLE 2. PPD (purified protein derivative)-induced lymphocyte transformation in a medium containing serum from primary biliary cirrhosis patients ('responders') or control subjects and a medium containing AB serum. Results are expressed as counts per minute (cpm) – mean ± standard error

	Number tested	Sex	Mean age ± SD	Test lymphocytes		'Reference' lymphocytes	
				Test (autologous) serum (range)	AB serum (range)	Test serum (range)	AB serum (range)
Primary biliary cirrhosis patients	11	10F	59 ± 10	2086 ± 610 (399–5,455)	5495 ± 1907 (220–15,128)	3942 ± 1101 (566–14,087)	16,004 ± 2153 (4,372–31,034)
Control subjects	6	4F	39 ± 18	4262 ± 1299 (1,025–10,296)	6092 ± 2862 (622–22,408)	6908 ± 1151 (3,631–9,691)	12,389 ± 2073 (9,293–19,531)

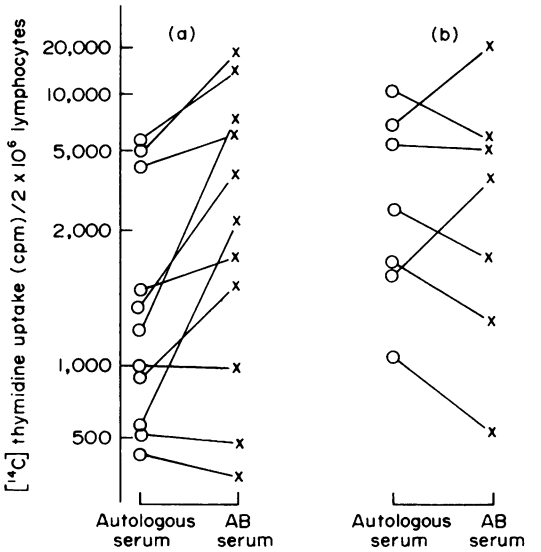

FIG. 3. PPD-induced lymphocyte transformation in AB serum. (a) Primary biliary cirrhosis patients' ('responders') lymphocytes. (b) Control subjects' lymphocytes.

cant ($t = 0{\cdot}876$; $P<0{\cdot}40$). The 'reference' lymphocytes were less responsive to PHA in the presence of eight of the eleven primary biliary cirrhosis sera than in AB serum ($t = 2{\cdot}038$; $P>0{\cdot}10$). Responsiveness of the 'reference' lymphocytes in control subjects' sera was not significantly different from that in AB serum ($t = 0{\cdot}75$; $P<0{\cdot}40$). A high degree of correlation was observed in the depressing effect of the primary biliary cirrhosis sera on autologous and 'reference' lymphocytes ($r = 0{\cdot}7149$; $t = 3{\cdot}07$; $P>0{\cdot}02$).

PPD-induced lymphocyte transformation

A greater than fourfold increase in cpm/2×10^6 lymphocytes in stimulated as compared with unstimulated controls was accepted as indicating responsiveness to PPD. Using this index nine of the twenty primary biliary cirrhosis patients and one of eight controls exhibited lymphocyte unresponsiveness to PPD. Results are thus presented for 'responder' and 'non-responder' sub-groups.

TABLE 3. PPD-(purified protein derivative) induced lymphocyte transformation in a medium containing serum from primary biliary cirrhosis patients ('non-responders') or control subjects and in a medium containing AB serum. Results are expressed as counts per minute (cpm)—mean ± standard error

	Number tested	Sex	Mean age ± SD	'Reference' lymphocytes	
				Test serum	AB serum
Primary biliary cirrhosis patients	9	8F	56 ± 12	3773 ± 555 (1,651–5,144)	14,835 ± 2501 (5,481–25,780)
Control subject	1	F	58	1,032	9,074

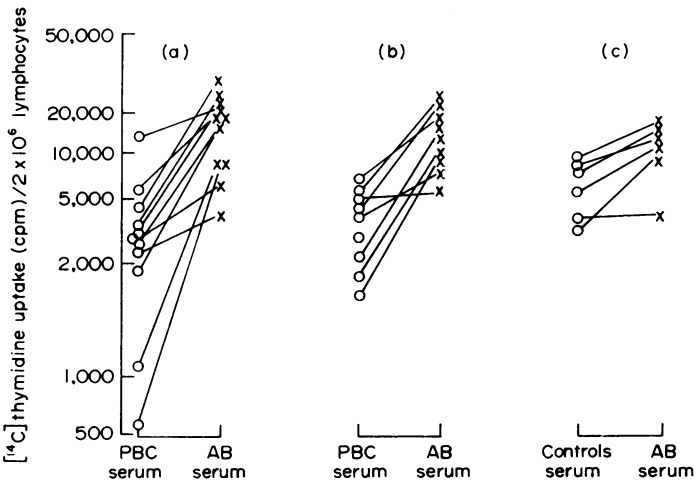

FIG. 4. PPD-induced lymphocyte transformation. 'Reference' lymphocytes in AB serum and (a) in serum from primary biliary cirrhosis patients ('responders'), (b) in serum from primary biliary cirrhosis patients ('non-responders'), (c) in serum from control subjects ('responders').

In the 'responder' sub-group (Table 2 and Figs 3 and 4) in eight of eleven primary biliary cirrhosis patients, lymphocyte stimulation by PPD was greater in AB serum than in autologous serum ($t = 2{\cdot}434$; $P > 0{\cdot}05$); a corresponding increase was found in two of seven control subjects, but the results are not statistically significant ($t = 0{\cdot}75$; $P < 0{\cdot}40$). In all experiments 'reference' lymphocytes cultured in AB serum showed significantly greater responsiveness to PPD than when cultured in primary biliary cirrhosis sera ($t = 5{\cdot}155$; $P > 0{\cdot}001$) or in control subjects' sera ($t = 4{\cdot}351$; $P > 0{\cdot}005$).

In the 'non-responder' sub-group (Table 3 and Fig. 4) greater 'reference' lymphocyte responsiveness to PPD was found using AB serum than with serum from nine primary biliary cirrhosis patients ($t = 4{\cdot}627$; $P > 0{\cdot}005$). A similar increase was found in the one control subject studied.

DISCUSSION

Serum factors which inhibit lymphocyte transformation *in vitro* in response to non-specific mitogens and/or specific antigens have been described in a number of conditions including tuberculosis (Heilman & McFarland, 1966), histoplasmosis (Newberry *et al.*, 1968), leprosy (Bullock & Fasal, 1971; Mehra *et al.*, 1972), syphilis (Levene *et al.*, 1969), chronic mucocutaneous candidiasis (Canales *et al.*, 1969), ataxia telangiectasia (Oppenheim *et al.*, 1966; McFarlin & Oppenheim, 1969), renal failure (Silk, 1967; Newberry & Sanford, 1971), idiopathic steatorrhoea (Winter *et al.*, 1967; Blecher *et al.*, 1969), multiple sclerosis (Knowles *et al.*, 1968; Burns *et al.*, 1971), and malignant disease (Trubowitz, Masek & Del Rosario, 1966; Gatti, Garrioch & Good, 1970; Whittaker, Rees & Clark, 1971; Burns *et al.*, 1971; Scheurlen, Schneider & Pappas, 1971).

Attempts have been made to demonstrate similar inhibitory factors in liver diseases, and inhibition of PHA responsiveness has been reported in acute hepatitis (Mella & Lang, 1967; Willems, Melnick & Rawls, 1969; Mella & Taswell, 1970), drug-induced hepatitis (Paro-

netto & Popper, 1970), alcoholic cirrhosis (Winter *et al.*, 1967; Hsu & Leevy, 1971) and in primary biliary cirrhosis (Fox *et al.*, 1973). In their study on PHA responses in renal failure Newberry & Sanford (1971) found no evidence of serum inhibitory factors in one anicteric cirrhotic patient whom they used as a control. More recently, however, these same workers (Newberry *et al.*, 1973) examined sera from patients with a variety of liver abnormalities and demonstrated depression of lymphocyte reactivity in acute viral hepatitis (three patients), chronic Australia antigenaemia (two patients), common bile duct obstruction (four patients), primary biliary cirrhosis (one patient), halothane hepatitis (one patient), and alcoholic cirrhosis (one patient). Furthermore, in two of the patients with common bile duct obstruction, surgical relief of the obstruction resulted in correction within 1 week of the inhibition of PHA-induced transformation, although at this time there was still a significant degree of bilirubinaemia.

In the present study we have shown that serum inhibitory factors to PHA-induced lymphocyte transformation were present in ten of eleven patients with primary biliary cirrhosis. Resuspension of lymphocytes from these patients in AB serum resulted in an increase in responsiveness but to a level below that found in normal control subjects. This is in agreement with our previous report (MacSween *et al.*, 1973) in which lymphocytes from primary biliary cirrhosis patients were found to have impaired responsiveness to PHA in AB serum as compared with a control population. In the present study we have also shown reduction of PHA-induced transformation of 'reference' lymphocytes by eight of eleven sera from primary biliary cirrhosis patients. Taken in conjunction, our investigations suggest that in primary biliary cirrhosis there is an intrinsic impairment of lymphocyte responsiveness to PHA, and that, in addition, extrinsic serum factors inhibitory of PHA transformation are present. The possibility that both of these observations result from a factor which is adherent to lymphocytes despite three washings in medium cannot be entirely excluded. We have not observed any correlation of these findings with either the severity of the disease, or any of the abnormal biochemical or serological indices in the patients. Similarly Newberry *et al.* (1973) could find no correlation between the reduction of PHA responses and serum indices of abnormal liver function, and in addition showed that bile acids were unlikely to be of significance in this context.

Fox *et al.* (1973) in their study found evidence of serum inhibitory factors in the plasma of four of twelve patients with primary biliary cirrhosis, when lymphocytes from normal subjects were cultured in the patients' sera. In experiments in which lymphocytes from patients were cultured in autologous plasma and in normal homologous plasma, increase in responsiveness to PHA was noted in all five patients who had impaired transformation in their own plasma, but in only one of the five did the transformation return to within the normal range.

In the PPD experiments we found that the lymphocytes of eleven of twenty patients (55%) with primary biliary cirrhosis were unresponsive, whereas Fox *et al.* (1969) noted that twenty-seven of forty-two (64%) of their primary biliary cirrhosis patients were Mantoux negative.

In eight of our eleven patients PPD-induced lymphocyte transformation was impaired in autologous serum as compared with AB serum, and the overall reduction in the group was statistically significant. A similar reduction was noted in two of the seven control subjects, but the overall results in this group were not significant. Serum inhibitory factors of PPD-induced lymphocyte transformation have been reported in tuberculosis (Heilman & McFar-

land, 1966), leprosy (Bullock & Fasal, 1971), malignant disease (Scheurlen *et al.*, 1971) and in pregnancy (Smith, Caspary & Field, 1972).

Our results with the 'reference' lymphocytes in the PPD studies were entirely unexpected and are of considerable interest. Responses were impaired by serum from both 'responders' and 'non-responders' with primary biliary cirrhosis, and also by control subjects' sera, the percentage reduction as compared with the responses in AB serum being respectively 75·4, 74·7 and 44·2%. We have no ready explanation for these observations. It may be that enhancing factors were present in the AB serum. A more likely possibility, however, would seem to be that in the other sera there were factors which blocked specific antigen receptors on the lymphocytes. Greaves, Torrigiani & Roitt (1969) were able to suppress the responses of sensitized lymphocytes to PPD *in vitro* when the cells were cultured in the presence of the Fab component of anti-light chain antibody. We have found rheumatoid factor–like substances in the serum of 64% of our primary biliary cirrhosis patients (MacSween *et al.*, 1973) and the relationship of these factors to the present observations are now being investigated. Preliminary experiments have shown that the inhibitory factor is not heat-labile.

Attempts to establish the nature of serum inhibitory factors to non-specific and specific lymphocyte mitogens have not succeeded. Newberry & Sanford (1971) showed that the serum inhibitory factor in renal failure was dialysable and did not depend for its action in interference with the initial PHA/cell membrane interaction. Serum α_2-globulins have been shown to depress lymphocyte responses to PHA (Cooperband *et al.*, 1968) and there has been continued speculation on whether these protein fractions contain the serum inhibitors. Serum α_2-macroglobulins are present in significantly raised amounts in primary biliary cirrhosis (MacSween *et al.*, 1972). Furthermore, we were able to show an increased prevalence of a little investigated serum α_2-macroglobulin (pregnancy–associated globulin) in the sera of primary biliary cirrhosis patients (Horne *et al.*, 1973). However, although this protein has been suspected of having immunosuppressive properties, we could not demonstrate any correlation between its concentration in some of the sera examined in the present study and their ability to depress PHA- and PPD-induced lymphocyte transformation.

Gatti (1971) did not detect any correlation between α_2-globulin levels and serum inhibition of PHA responsiveness in malignant disease, nor was he able to show any correlation of inhibitory effect with RNase levels. Scheurlen *et al.* (1971) in gel filtration experiments showed that the inhibitory factors they demonstrated in serum from patients with malignant disease were not contained in the α_2-macroglobulin fraction, but were probably (poly-) peptides.

The demonstration of these *in vitro* serum inhibitory factors continues to be of considerable interest, but whether they have an important role in the homeostasis of immune responses is by no means clear. At present their possible biological significance is obscure. We have shown them to be present in the majority of sera from patients with primary biliary cirrhosis, a disease in which there is substantial evidence of disturbed immune mechanisms. The studies of Newberry *et al.* (1973), albeit in a small number of patients, suggests they may occur in a number of liver diseases, and this clearly merits further study.

REFERENCES

BLECHER, T.E., BRZECHWA-AJDUKIEWICZ, A., MCCARTHY, C.F. & READ, A.E. (1969) Serum immunoglobulins and lymphocyte transformation studies in coeliac disease. *Gut*, **10**, 57.

BULLOCK, W.E. & FASAL, P. (1971) Studies of immune mechanisms in leprosy. III. The role of cellular and humoral factors in impairment of the *in vitro* immune response. *J. Immunol.* **106**, 888.

BURNS, C.P., ARMENTROUT, S.A., GROSS, S., VAN DEN NOORT, S. & STJERNHOLM, R.L. (1971) The effect of a lymphotoxic factor in the sera of patients with multiple sclerosis and acute leukemia on the metabolism of normal and leukemic lymphocytes. *J. Lab. clin. Med.* **78,** 508.

CANALES, L., MIDDLEMAS, R.O., LOURO, J.M. & SOUTH, M.A. (1969) Immunological observations in chronic mucocutaneous candidiasis. *Lancet*, **ii,** 567.

COOPERBAND, S.R., BONDEVIK, H., SCHMID, K. & MANNICK, J.A. (1968) Transformation of human lymphocytes: inhibition by homologous alpha-globulin. *Science*, **159,** 1243.

FOX, R.A., DUDLEY, F.J., SAMUELS, M., MILLIGAN, J. & SHERLOCK, S. (1973) Lymphocyte transformation in response to phytohaemagglutinin in primary biliary cirrhosis: the search for a plasma inhibitory factor. *Gut*, **14,** 89.

FOX, R.A., JAMES, D.G., SCHEUER, P.J., SHARMA, O. & SHERLOCK, S. (1969) Impaired delayed hypersensitivity in primary biliary cirrhosis. *Lancet*, **i,** 959.

GATTI, R.A. (1971) Serum inhibitors of lymphocyte responses. *Lancet*, **i,** 1351.

GATTI, R.A., GARRIOCH, D.B. & GOOD, R.A. (1970) Depressed PHA responses in patients with non-lymphoid malignancies. *Proceedings of the Fifth Leucocyte Conference* (Ed. by J. E. Harris), p. 339–357. Academic Press, New York, U.S.A.

GOUDIE, R.B., MACSWEEN, R.N.M. & GOLDBERG, D.M. (1966) Serological and histological diagnosis of primary biliary cirrhosis. *J. clin. Path.* **19,** 527.

GREAVES, M.F., TORRIGIANI, G. & ROITT, I.M. (1969) Blocking of the lymphocyte receptor site for cell mediated hypersensitivity and transplantation reactions by anti-light chain sera. *Nature (Lond.)*, **222,** 885.

HEILMAN, D.H. & MCFARLAND, W. (1966) Inhibition of tuberculin-induced mitogenesis in cultures of lymphocytes from tuberculous donors. *Int. Arch. Allergy*, **30,** 58.

HORNE, C.H.W., MCLAY, A.L.C., TAVADIA, H.B., CARMICHAEL, I., MALLINSON, A.C., YEUNG LAIWAH, A.A.C., THOMAS, M.A. & MACSWEEN, R.N.M. (1973) Studies on pregnancy-associated globulin. *Clin. exp. Immunol.* **13,** 603.

HSU, C.C.S. & LEEVY, C.M. (1971) Inhibition of PHA-stimulated lymphocyte transformation by plasma from patients with advanced alcoholic cirrhosis. *Clin. exp. Immunol.* **8,** 749.

KNOWLES, M., HUGHES, D., CASPARY, E.A. & FIELD, E.J. (1968) Lymphocyte transformation in multiple sclerosis. Inhibition of unstimulated thymidine uptake by a serum factor. *Lancet*, **ii,** 1207.

LEVENE, G.M., TURK, J.L., WRIGHT, D.J.M. & GRIMBLE, A.G.S. (1969) Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. *Lancet*, **ii,** 246.

MCFARLIN, D.E. & OPPENHEIM, J.J. (1969) Impaired lymphocyte transformation in ataxia-telangiectasia in part due to a plasma inhibitory factor. *J. Immunol.* **103,** 1212.

MACSWEEN, R.N.M., GALBRAITH, I., THOMAS, M.A., WATKINSON, G. & LUDLAM, G.B. (1973) Phytohaem-agglutinin–(PHA) induced lymphocyte transformation and *Toxoplasma gondii* antibody in primary biliary cirrhosis. Evidence of impaired cell-mediated immunity. *Clin. exp. Immunol.* (In press.)

MACSWEEN, R.N.M., HORNE, C.H.W., MOFFAT, A.J., & HUGHES, H.M. (1972) Serum protein levels in primary biliary cirrhosis. *J. clin. Path.* **25,** 789.

MACSWEEN, R.N.M., ROSS, S.K., WILLIAMSON, J. & WATKINSON, G. (1973) Primary biliary cirrhosis. Clinical and immunological associations. (In press.)

MEHRA, V.L., TALWAR, G.P., BALAKRISHNAN, K. & BHUTANI, L.K. (1972) Influence of chemotherapy and serum factors on the mitogenic response of peripheral leucocytes of leprosy patients to phytohaemag-glutinin. *Clin. exp. Immunol.* **12,** 205.

MELLA, B. & LANG, D.J. (1967) Leukocyte mitosis: suppression *in vitro* associated with acute infectious hepatitis. *Science*, **155,** 80.

MELLA, B.A. & TASWELL, H.F. (1970) Suppression of leukocytic mitosis by sera of hepatitis-implicated donors. *Amer. J. clin. Path.* **53,** 141.

NEWBERRY, W.M., CHANDLER, J.W., CHIN, T.D.Y. & KIRKPATRICK, C.H. (1968) Immunology of the mycoses. I. Depressed lymphocyte transformation in chronic histoplasmosis. *J. Immunol.* **100,** 436.

NEWBERRY, W.M. & SANFORD, J.P. (1971) Defective cellular immunity in renal failure: depression of reactivity of lymphocytes to phytohaemagglutinin by renal failure serum. *J. clin. Invest.* **50,** 1262.

NEWBERRY, W.M., SHOREY, J.W., SANFORD, J.P. & COMBES, B. (1973) Depression of lymphocyte reactivity to phytohaemagglutinin by serum from patients with liver disease. *Cell. Immunol.* **6,** 87.

OPPENHEIM, J.J., BARLOW, M., WALDMANN, T.A. & BLOCK, J.B. (1966) Impaired *in vitro* lymphocyte transformation in patients with ataxia-telangiectasis. *Brit. med. J.* **ii,** 330.

PARONETTO, F. & POPPER, H. (1970) Lymphocyte stimulation induced by halothane in patients with hepatitis following exposure to halothane. *New Engl. J. Med.* **283,** 277.

SCHEUER, P.J. (1967) Primary biliary cirrhosis. *Proc. roy. Soc. Med.* **60,** 1257.

SCHEURLEN, P.G., SCHNEIDER, W. & PAPPAS, A. (1971) Inhibition of transformation of normal lymphocytes by plasma factor from patients with Hodgkin's disease and cancer. *Lancet,* **ii,** 1265.

SHERLOCK, S. (1971) *Diseases of the Liver and Biliary System.* Fourth Edn, revised third printing, p. 305. Blackwell Scientific Publications, Oxford.

SILK, M.R. (1967) The effect of uremic plasma on lymphocyte transformation. *Invest. Urol.* **5,** 195.

SMITH, J.K., CASPARY, E.A. & FIELD, E.J. (1972) Immune responses in pregnancy. *Lancet,* **i,** 96.

TRUBOWITZ, S., MASEK, B. & DEL ROSARIO, A. (1966) Lymphocyte response to phytohemagglutinin in Hodgkin's disease, lymphatic leukemia and lymphosarcoma. *Cancer (Philad.),* **19,** 2019.

WHITTAKER, M.G., REES, K. & CLARK, C.G. (1971) Reduced lymphocyte transformation in breast cancer. *Lancet,* **i,** 892.

WILLEMS, F.TH.C., MELNICK, J.L. & RAWLS, W.E. (1969) Viral inhibition of the phytohaemagglutinin response of human lymphocytes and application to viral hepatitis. *Proc. Soc. exp. Biol. (N.Y.),* **130,** 652.

WINTER, G.C.B., MCCARTHY, C.F., READ, A.E. & YOFFEY, J.M. (1967) Development of macrophages in phytohaemagglutinin cultures of blood from patients with idiopathic steatorrhoea and with cirrhosis. *Brit. J. exp. Path.* **48,** 66.

YEUNG LAIWAH, A.A.C. (1971) Lymphocyte transformation by Australia antigen. *Lancet,* **ii,** 470.