response to cellular antigens by peripheral lymphocytes from normal and lung cancer patients in serum free medium in vitro (Aaskov and Anthony, Biomedicine, 1973, 19, 369). In common with results published for the one-stage MMI test, occasional stimulation of migration has been noted in this assay. Because of the problem of assaying migration inhibition factor (MIF) in the presence of stimulatory material, attempts have been made to identify the materials responsible for this stimulation.

Sephadex chromatography yielded an MIF (mol. wt 20–30,000) and a chemotactic factor (protein, mol. wt approximately 12,500) from supernates of antigenically stimulated lymphocytes. The chemotactic material had no stimulatory effect on macrophage migration. Stimulation of migration was observed in fractions from 2 peaks (mol. wt >150,000 and mol. wt 60–70,000). Assay after isoelectric focusing has shown this to be due to Hb and IgG. The stimulatory effect of these substances at certain concentrations has been confirmed.

**Spleen Size in Patients with Breast Cancer.** J. G. Roberts, M. Wisbey, K. G. Leach and M. Baum, Departments of Surgery and Medical Physics, Welsh National School of Medicine and the University Hospital of Wales.

Gamma camera images of the spleen obtained following the intravenous injection of heat damaged, autologous, $^{99m}$Tc labelled red cells provide a means of estimating spleen size in patients.

A new method of calculating spleen size has been validated in 10 patients. The computed weight correlated well with the exsanguinated weight of the excised spleens ($r = 0.9894$) with 95% confidence limits of less than 50 g. This technique has been applied to 40 patients with breast cancer and 5 controls. The cancer patients had significantly larger spleens ($P < 0.05$) and spleen size varied with clinicopathological stage. In 10 patients spleen size has been estimated at primary treatment and some 6 months later. All patients except one showed a reduction in spleen size ($P < 0.01$).

These results suggest that in man, as in experimental animals, splenomegaly occurs as a component of the host response to cancer.

**Immunity in Patients with Breast Cancer.** A. J. Cochran, R. M. Mackie, C. E. Ross, R. M. Grant and D. E. Hoyle, University Department of Pathology, Western Infirmary, Glasgow.

We have examined tumour directed immunity and nonspecific immunological activities in 200 patients with breast carcinoma. Techniques employed included skin testing with recall antigens, the leucocyte migration technique (LMT), mitogen induced transformation, an immune adherence technique, immunoglobulin and complement assays and T and B cell quantification.

The main findings are: (1) A majority of breast cancer patients (54%) showed evidence of sensitization to tumour derived materials; (2) sensitization is rare in control donors (15%). This is true of normal donors, patients with simple breast disease and those with other malignancies; (3) inhibition of migration was similarly frequent with homologous (53%) and autologous (56%) combinations of antigens and leucocytes; (4) sensitization is less commonly demonstrable in patients with advanced disease (27%); (5) patients with local recurrences were as frequently reactive as those with primary tumours only (60%); (6) extracts of mastopathic breasts only rarely inhibited the migration of leucocytes whether from cancer patients (21%) or controls (17%); (7) there is some evidence that non-cancerous tissues in cancerous breasts may possess tumour-like antigens. The significance of these results, obtained mainly with the LMT, can be assessed by relating them to results obtained by the other techniques.

**The Relationship between Prognosis and Lymphocyte Response to PHA in Breast Cancer.** P. M. Bolton, R. H. Whitehead, R. G. Newcombe, S. L. James and L. E. Hughes, Departments of Surgery and Medical Statistics, Welsh National School of Medicine, Cardiff.

Studies of lymphocyte response to PHA in cancer patients have shown variable results, probably due to methodological differences. We have used a microtest method with 3 PHA dose levels (0.3 μg/ml, 0.8 μg/ml and 4.0 μg/ml) to study the lymphocyte response in 119 patients with breast disease. These patients were classi-
fied into 6 groups dependent on pathology, tumour stage and prognosis, without knowledge of results of PHA testing.

Control and early cancer patients responded best to 0·8 μg PHA/ml but also had a good response to 0·3 μg/ml. Patients with advanced disease responded maximally to 4·0 μg/ml and poorly to 0·3 μg/ml. The highest dose (4·0 μg/ml) did not discriminate as well between the groups, as did the other 2 doses.

A dose-response curve of PHA response provides more meaningful information than estimations performed at one dose. Results correlate with the expected prognosis in breast cancer.

**DETECTION OF DISEASE OF THE BREAST IN WOMEN ATTENDING A FAMILY PLANNING ASSOCIATION CLINIC.** T. Hamilton, R. J. Prescott and N. B. Loudon, Department of Clinical Surgery, and Medical Computing Group, University of Edinburgh and Family Planning Association.

A long-term project has been established to determine the influence of contraceptive habit on the breast. A total of 13,451 women attending a Family Planning Association Clinic in Edinburgh over a period of 5 years were offered annual clinical examination of the breast. In this time 233 women (1 in 58) attending were referred for surgical opinion; 115 were examined and reassured; 118 (1 in 114) were submitted to biopsy; benign lesions were present in 106 and carcinoma detected in 12 women. Those with carcinoma were all included in the 17% of the screened population aged over 35 years (1 in 195). It is concluded that screening, certainly for carcinoma, might reasonably be restricted to women over 35 years, and that below this age, examination of the breast is probably unnecessary for those attending for contraceptive advice.

**HUMAN TUMOUR CELLS IN SHORT TERM MONOLAYER CULTURE—CELL CYCLE KINETICS AND EFFECT OF HORMONAL STIMULATION.** M. E. Lloyd and P. P. Dendy, Neurology Research Laboratory, The London Hospital.

Cell cycle parameters of human tumours in primary monolayer culture were established using a method of continuous exposure to 3H-TdR. The effect of prednisolone on kinetics was also studied.

A remarkable similarity in durations of cell cycle parameters was found between specimens, S phases averaging 6 h and TEs 35 h. However, the wide range in values of maximum Labelling Index (24 → 95%) indicated considerable differences in the percentage of cells cycling for different specimens. For some specimens prednisolone increased the % cycling cells with no effect on phase durations of cells already cycling. The durations of S phase are low, compared with those found by other workers. This may be due to the short period of growth in culture but the implications of these results in the study of S phase of tumour cells in vivo is uncertain.

Prednisolone studies indicate that some of the cells unlabelled during long exposure to 3H-TdR have not necessarily left the cell cycle permanently.

**A PILOT STUDY OF THE VARIABILITY OF NUCLEAR FORM AND DNA CONTENT IN SMEARS OF HUMAN URO-EPITHELIAL BLADDER TUMOURS.** R. C. L. Feneley and J. M. N. Boss, Bristol United Hospitals and Department of Physiology, University of Bristol.

From 19 patients, subsequently observed for 2–2½ years, 27 smears (20 Feulgen and 7 haematoxylin stained) of transurethrally resected material were made, with independent histological grading as “low”, “average”, or “high”. “Pleomorphic” smears had nuclei > 20 μm × > 10 μm with indentations > 2 μm deep. “Variable” smears had standard deviation > 0·60 × mean for microdensitometry of 25 nuclei each. The 3 sites without tumours and 7 patients treated and clear on repeated review, yielded neither “pleomorphic” nor “variable” smears. Of the 4 “pleomorphic” smears 3 were from patients since dead, and the 4 “variable” from patients now dead (3) or still with recurrence (1). In the clinically heterogenous “average” grade are 6 living (none “pleomorphic”; 1, with recurrence, “variable”) and 4 dead (3 “pleomorphic”, none variable, but 3 with no Feulgen stain). (The work was assisted by an M.R.C. grant to J. M. N. Boss.)