(a result of inadequate Australian-U.K. communications by the investigator) this was unlikely to have been a major source of bias, as the comparisons between areas with known and unknown response rates in the U.K. showed both sections of data to be comparable with each other. With regard to the problem of chest illness causing absence from school during the week of the survey, this likelihood was substantially lessened by circulating the questionnaires during the summer months when the prevalence of both bronchitis and asthma were at their lowest levels. Significant bias could have occurred through smoking by some of the children, but this hardly begins in children before the age of 11 years (Todd, 1969), and the U.K.-Australian differences in the prevalence of bronchitis were most prominent before this age. The accurate differentiation between bronchitis and asthma is frequently difficult when using only a questionnaire as the method of study. Fortunately for the purposes of this report both disorders showed very similar relations with climatic factors.

These results suggest that children develop or receive some protection from any disadvantages that may be inherent in the more temperate zones of rural areas. Perhaps mothers in the warmer climates do not provide sufficient clothing for their children during the cold weather. Another potential disadvantage of warm climates is the dust (or pollens) in farming areas during the summer months, particularly in the wind-blown areas in the north of Australia. The finding that children had fewer respiratory symptoms when from areas with higher summer rainfalls could be related to the better summer pastures and consequently less dust in the environment.

Despite the higher respiratory death rates during childhood in England and Wales compared with the Netherlands and Scandinavia (Reid, 1969), the data suggest that rural people should hesitate before migrating to warmer climates—for example, Australia—in the hope of benefiting the chest illnesses in their children.

In the absence of any major bias incurred by the methods of study, the present report supports the hypothesis that the predominant factors in chronic respiratory disease are environmental and preventable, rather than climatic ones from which there can be inadequate protection. Although climate could be important when associated with other respiratory hazards, the reasons for geographical differences in respiratory disease should usually be found in the factors already incriminated, such as smoking, dust, micro-organisms, pollution, and social conditions (Colley and Reid, 1970; Colley, 1971).

This study was undertaken through the Royal Colleges of General Practitioners in the United Kingdom, Australia, and New Zealand. The following members participated. England: Drs. G. I. Watson, I. C. Fuller, C. Ward, D. M. Wilkinson, J. McA. Williams, R. H. Parrott, W. T. Mills, A. C. Rumsey, L. A. C. Wood, and M. Wigram. Scotland: Drs. D. Hendry, J. Hepburn, G. W. Morrison, and A. Marquis; Queensland: Drs. P. Bridgman and H. C. Fox. New South Wales: Dr. R. E. Coolican. Victoria: Dr. W. Fabb. Tasmania: Dr. N. Rutledge. South Australia: Drs. W. J. Pryor, C. Leeson, W. J. Sleeman, C. P. Mattner, B. W. Thompson and J. Linn. Western Australia: Drs. A. Jacobs, E. D. Cullen, K. J. Cullen, J. Lubich, and A. L. Walsh. New Zealand: Dr. C. M. Hockin.

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Postoperative Depression of Tumour-directed Cell-mediated Immunity in Patients with Malignant Disease

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Summary

Leucocytes from 46 melanoma patients, 45 breast carcinoma patients, and 95 control donors were tested by the leucocyte migration test against the supernatants of homogenates of malignant melanomas, breast carcinomas, simple breast tumours, and breasts showing simple cystic disease. By comparison with controls inhibition of migration occurred significantly more frequently when tumour patients' leucocytes were exposed to extracts of histogenetically similar tumours.

Cell-mediated immunity to tumour-associated antigens was measured in 12 patients with breast carcinoma and 12 with malignant melanoma immediately before surgical operation and in the postoperative period. All patients tested before operation showed significant inhibition of migration on contact with extracts of histogenetically similar tumours. Postoperatively the degree of leucocyte migration inhibition was reduced in all patients with melanoma and breast carcinoma. Significant inhibition of leucocyte migration returned in most patients 6-22 days after operation.

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Introduction

Metastasis formation remains a major problem in the management of patients with malignant disease. Despite the high frequency of spread from the primary site only a minority of the malignant cells which reach the blood stream or lymphatics give rise to secondary tumours (Madden and Karpas, 1967). There

are reports of a general diminution in immunological activity after surgical operations, especially in patients with cancer or heart disease (Riddle and Berenbaum, 1967; Park *et al.*, 1971; Han, 1972). We have investigated this situation by examining cell-mediated immunity to tumour-associated antigens preoperatively and during the postoperative period by the leucocyte migration technique (Bendixen and Søborg, 1969).

We chose to use the leucocyte migration inhibition technique, which is a modification of the macrophage migration inhibition technique (George and Vaughan, 1962), because much evidence suggests that this technique is a sensitive measure in vitro of delayed hypersensitivity in vivo (Ferraresi *et al.*, 1969). In addition the leucocyte migration inhibition technique has been used successfully to study cell-mediated immunity to human tumour-associated antigens (Andersen *et al.*, 1970; Cochran *et al.*, 1972).

Patients and Methods

The patients studied were admitted for surgical treatment of clinically suspected malignant melanoma or breast carcinoma. The clinical diagnosis was confirmed in every case by histological examination of tumour tissue.

LEUCOCYTE MIGRATION TECHNIQUE

This was modified from the technique of Bendixen and Søborg (1969). A 25-ml sample of venous blood was drawn into a syringe containing 1 ml of heparin (5,000 IU/ml). The syringe was then stood vertically with the needle pointing upwards for two hours at 37°C. The white cell rich component was then expressed from the syringe and washed twice in saline free from calcium and magnesium ions, buffered with phosphate to a pH of 7·4, and, finally, with Eagle's minimal essential medium with 10% fetal calf serum, penicillin, and streptomycin. The resulting cell pellet was then resuspended in 10 times its own volume of Eagle's medium with fetal calf serum. Then 200-μl samples of this suspension were placed in 10 × 65-mm glass test-tubes, the appropriate amount of antigen was added, and the tubes were stood in air for one hour at 37°C. Control tubes were prepared in the same way but contained only cells and medium.

The test-tubes were then agitated and four aliquots drawn into capillary tubes and the tubes sealed with inert clay at one end. These were then centrifuged at 600 *g* for four minutes and the tubes cut with a diamond at the cell-fluid interface. The cell buttons were mounted in a spot of silicone grease in disposable tissue culture plates, and the Eagle's medium with fetal calf serum was added to fill the plate, which was then closed with a coverslip held in place by silicone grease.

The complete plates were incubated in air at 37°C for 18-24 hours and the areas of cell migration drawn by means of a drawing tube attached to a light microscope. The areas were measured by planimetry. The migration area of cells pre-exposed to antigen in Eagle's medium with fetal calf serum was compared with that of the same cells exposed only to Eagle's medium with fetal calf serum. This ratio is the migration index. Results obtained with the same cells in the presence and absence of antigen were compared by the Mann-Whitney-Wilcoxon U test of ranking. Significance was assessed at the 5% level.

ANTIGENIC EXTRACTS

Tumour samples were received at operation and placed in a sterile container. Fat and fibrous tissues were dissected away and the specimen was finely chopped with a scalpel blade in Eagles minimal essential medium containing penicillin and streptomycin. The resulting material was homogenized in a blade homogenizer and then spun for 30 minutes at 1,500 *g* at 4°C. The supernatant from these tubes was separated and its protein concentration estimated by spectrophotometry. The optimum antigen concentration was established in each case by determining the concentration which maximally inhibited leucocyte migration. For the antigenic preparations used in this study optimum concentrations lay in the range 12·5-100 μg/ml.

Results

MAIN GROUP

The patients reported on in detail in this paper are part of a continuing study of tumour-directed cellular immunity in malignant melanoma and breast carcinoma. The detailed results of this study will be published separately. The following information is intended to indicate the specificity of the reactions reported.

Malignant Melanoma

Homogenates were prepared from 10 malignant melanomas (Table I). Leucocytes from 46 patients with histologically-proved malignant melanoma were tested individually against up to six of these extracts. Significant inhibition of leucocyte migration on contact with at least one antigen was observed with cells from 37 patients (80%). Most of these tests involved allogeneic combinations of leucocytes and antigen, but seven autologous tests were performed and inhibition occurred in four of these (57%). In an attempt to exclude the possibility that keratinocyte antigens were involved homogenates were prepared from secondary melanomas remote from the skin. While the proportion of patients reacting with secondary-derived homogenates (41%) was lower than that reacting with primary-derived material (59%), both were significantly greater ($P < 0{\cdot}002$, both cases by χ^2 test) than the figure observed with leucocytes from control donors (16%). Leucocytes from eight melanoma patients were tested against 16 breast carcinoma homogenates and weak inhibition was observed in only one test.

TABLE I—*Frequency of Inhibition of Migration of Leucocytes from Patients with Breast Carcinoma or Malignant Melanoma and from Control Donors on Contact with Tumour Homogenates (Number showing Positive Reaction with at least One Antigen/Total Number Tested)*

Source of Antigen	Leucocytes from:		
	Melanoma Patients	Breast Carcinoma Patients	Control Donors*
Malignant melanoma (all)	37/46	1/9	5/31
Primary malignant melanoma ..	27/46	—	5/31
Secondary malignant melanoma ..	19/26	—	1/31
Autologous tumour	4/7	6/8	—
Primary breast carcinoma	1/8	28/45	18/64

* Normal donors and patients with other malignancies or non-malignant diseases.

Breast Carcinoma

Homogenates were prepared from 16 breast carcinomas (Table I). Leucocytes from 45 patients with histologically-proved breast carcinoma were tested against up to six of these extracts. Significant inhibition of leucocyte migration on contact with at least one antigen occurred with cells from 28 patients (62%). While most tests involved allogeneic combinations the leucocytes of six out of eight patients tested against homogenates of their own tumours showed significant inhibition in this situation (75%). Control studies were performed by testing leucocytes from normal subjects, patients with fibroadenomas of breast, non-neoplastic breast disease, other malignancies (including malignant melanoma), and other non-neoplastic diseases against breast carcinoma antigens. Leucocytes from a total of 64 donors

were tested and inhibition on contact with at least one antigen occurred with the cells of 18 (28%). The difference between the frequency of inhibition of migration of the leucocytes of breast carcinoma patients and controls is highly significant ($P < 0.01$ by χ^2 test).

Leucocytes from nine patients with breast disease (five cancers, four non-tumourous breast disease) were tested against eight melanoma homogenates. Significant migration inhibition occurred with the leucocytes of only one patient (simple cystic disease) on contact with one melanoma preparation. Leucocytes from 11 breast carcinoma patients were tested against three homogenates of non-cancerous breast conditions (two simple cystic disease, one fibroadenoma). Inhibition occurred with the cells of one patient on contact with one of the homogenates of a breast showing simple cystic disease.

PATIENTS STUDIED OVER OPERATING PERIOD

Malignant Melanoma.—Peripheral blood leucocytes from 12 patients with malignant melanoma were studied on at least two and up to seven occasions (Table II). All were tested against from one to six extracts of allogeneic or autochthonous malignant melanomas. A preoperative assessment of reactivity

TABLE II—*Frequency of Significant Inhibition of Leucocyte Migration in Patients with Malignant Melanoma Before and After Operation (Positive Reaction/Antigens Tested)*

Case No.	Pre-operative	Postoperative (Days) 2	4	6	8	10	12	14	15	22
1	2/2		0/2		0/2			2/2		
2	—		0/1		0/1	0/1		1/1		
3	1/1	0/2		0/2			2/2		0/1	1/2
4	1/1		0/1	0/1		1/1				
5	2/2	0/2	0/2				1/2			
6	1/2	0/2								
7	3/3	0/3	0/3			1/2				
8	6/6		0/6		2/6		3/3			
9	5/6	0/6		2/4						
10	—	0/4		0/4				0/4		3/4
11	2/3		0/3		1/3					
12	2/2		0/2		0/2	1/2				

was available in 10 cases, all of which showed significant inhibition of leucocyte migration on contact with at least one antigen. In all 10 there was complete loss of this inhibition during the four to five days after operation. One patient (Case 2), who was not tested preoperatively, showed no reactivity on postoperative days 4, 8, and 10 but a definite inhibition on day 14. Another patient (Case 10), who also did not have a preoperative test, showed no return of reactivity until day 22. Tests run on days 6-22 after operation showed a gradual return of migration inhibition in all 11 patients examined in the period. Actual migration indices from several representative experiments are shown in Table III.

Breast Carcinoma.—Twelve patients with breast carcinoma were studied (Table IV). All had tumour clinically localized to the primary site. The migration of peripheral leucocytes was assessed on up to four occasions against the homogenates of allogeneic or autochthonous breast carcinomas. In 10 patients tests were run preoperatively and all showed significant leucocyte migration inhibition on contact with at least one antigen. None of these patients showed significant migration inhibition in tests between one and six days after operation. Migration

TABLE IV—*Frequency of Significant Inhibition of Leucocyte Migration in Patients with Breast Carcinoma Before and After Operation. (Positive Reaction/Antigens Tested)*

Case No.	Pre-operative	Postoperative (Days) 1	2	3	4	5	6	7	8	10
1	2/2			0/3				1/1		
2				0/3					3/3	
3							0/3			2/3
4	3/3						0/3			
5	3/3				0/3					
6	2/3	0/4						0/3		
7	1/3		0/3							
8	2/2				0/2					1/1
9	2/2			0/2			0/2			1/1
10	1/1						0/1			0/1
11	2/2				0/3					1/1
12	1/1				0/1			0/1		0/1

inhibition of about the degree noted before operation was seen in tests of the leucocytes of six out of nine patients examined after the sixth postoperative day (Table IV). Of the patients who did not show a return of reactivity two were tested only in the early postoperative period, one was negative up to day 7 and two up to day 10 after operation. Two patients not tested preoperatively showed complete lack of reactivity in the first postoperative week. Repeat testing eight and 10 days after operation showed significant reactivity of one patient's leucocytes against all three antigens and of the other patients' cells against two of the three antigens used. All the patients had operations of similar type (simple mastectomy) and about similar duration. The premedication and anaesthetic agents were similar in all cases.

Discussion

The large group of patients and controls included in Table I are not discussed in any detail here and will be the subject of a separate report. We believe, however, that our results to date suggest that inhibition of leucocyte migration occurs selectively in tumour-bearing patients on exposure of their cells to homogenates of histogenetically similar tumours. The occurrence of inhibition in autologous tests appears to exclude transplantation antigens as the cause of the inhibition. The low frequency of reactions against homogenates of histogenetically dissimilar tumours and (in the case of breast cancer) preparations from non-cancerous breasts make it unlikely that the tissue preparations were toxic. Wolberg (1971) reported that toxicity of tissue preparations or the production of toxic materials by tumour extract and leucocyte interaction may be a cause of inhibition of leucocyte migration. Nevertheless he did not indicate the protein concentration of the extracts used in his study. The disparity in the frequency of inhibition of our tumour patients' leucocytes and control donors' leucocytes seems to exclude toxicity as the mechanism of inhibition (at least in most of the tests). It should be emphasized that the present tests were conducted in the presence of total added homogenate protein concentrations of 25-100 μg. At concentrations above 200 μg we found frequent inhibition of migration of both patients' leucocytes and controls' leucocytes. These inhibitions we regard as due to non-specific toxicity.

Immune reactivity, as evidenced by the ability of lymphoid cells to undergo blast transformation on exposure to mitogens, is noticeably reduced in the postoperative period (Riddle and

TABLE III—*Representative Experiments Showing Variations in Migration Index over Operative and Immediate Postoperative Period*

Patient	Tumour	Homogenate	Migration Index: Pre-operatively	On Postoperative Day 2	4	6	8	10	12	14	16	18	20	22
A	Melanoma	Melanoma	0·77			1·03			0·64					0·64
B	Melanoma	Melanoma	0·71	1·17		0·88		0·54						
C	Breast carcinoma	Breast carcinoma	0·67		1·03		0·77							
D	Breast carcinoma	Breast carcinoma	0·72	0·93		0·88		0·68						

Berenbaum, 1967; Park *et al.*, 1971; Han, 1972). This reactivity is, of course, immunologically non-specific, but an appreciable diminution in mitogen responsiveness seems likely to be indicative of a high degree of immune depression (Rubin, 1970). While it might be predicted that specific immune reactivity would be depressed as part of the general process, no evidence on this point is currently available.

We were unable to show significant inhibition of migration of the peripheral leucocytes of 24 patients with either malignant melanoma or breast carcinoma in the immediate postoperative period in the presence of the appropriate antigens. All patients who had been tested preoperatively (20) had had striking reactivity to one or several tumour extracts immediately before operation. The minimum period of loss of reactivity was six days, and migration inhibition was restored in 17 out of 20 patients tested between six and 22 days.

The mechanism of the immunological depression is not known. Since the effect appeared to occur in all patients examined it could not be related to duration of operation, though anaesthetic agents do interfere reversibly with lymphocyte function (Nunn *et al.*, 1970), and this is therefore a potentially relevant factor. Postoperative variations in adrenal cortical activity are well known, and the role of corticosteroids as proved immunosuppressive agents is worthy of consideration. Studies of the mechanism of this phenomenon are in progress. Regardless of its mechanism the deficiency of specific anti-tumour immune defence seems real and may well be relevant to the establishment of immediately aggressive metastases or slow growing latent metastases.

These preliminary observations clearly require confirmation and extension. If confirmed, the timing of operative procedures in cancer patients may require reappraisal. This seems especially the case with sequential procedures such as excision of a primary tumour followed after an interval of 10-14 days by removal of clinically involved nodes.

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"Ischaemic" Colitis in Young Adults

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Summary

Four cases of transient haemorrhagic colitis seen in young adults resemble 10 others in the literature. The 14 cases are distinguished from the classical transient ischaemic colitis syndrome by their youth, the low incidence of stricture formation, and the prevalence of right-sided lesions.

Introduction

Since the first description of ulcerative colitis (Wilks and Moxon, 1875) two further types of colitis of unknown aetiology have been defined. Crohn's disease (Crohn, Ginzburg, and Oppenheimer, 1932) was soon shown to affect the colon as well (Colp, 1934) and could occur there in the absence of small-bowel lesions (Pugh, 1945). Analysis of cases described as segmental colitis (Crohn, Garlock, and Yarnis, 1947; Neuman, Bargen, and Judd, 1954) suggests that most showed the features of Crohn's disease. The third type of non-specific colitis has been labelled pseudomembranous or nectorizing (Pettet, Baggenstoss, Dearing, and Judd 1954). It should be distinguished from staphylococcal enteritis, which it closely resembles, and from ischaemic colitis (Goulston and McGovern, 1965).

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Ischaemic colitis was first described by Boley, Schwartz, Lash, and Sternhill (1963). Marston, Pheils, Thomas, and Morson (1966) classified the syndrome depending on the degree of ischaemia, which produced either infarction of the whole bowel wall or severe haemorrhagic colitis leading eventually to stricture formation or transient bloody diarrhoea with complete recovery. We report four cases of acute haemorrhagic colitis in young adults with complete resolution in three and progression to stricture in the fourth. The features are similar to those of 10 cases reported by others. The whole group differ appreciably from the classical ischaemic colitis syndrome in age and distribution of lesion. Furthermore the incidence of stricture formation appears to be very low.

Case Reports

Case 1.—A 21-year-old minicab driver had been investigated for transient attacks of abdonimal pain and vomiting, each lasting about one day. He had had six attacks in two years. No cause had been found. In May 1971 he was admitted to hospital with similar but more severe pain and vomiting. He had had diarrhoea since the onset of the pain and the stools contained a small amount of bright blood. On examination he was afebrile with upper abdominal tenderness. Sigmoidoscopy showed normal mucosa to 20 cm. Barium-enema examination showed sawtooth irregularity (Fig. 1). Laparotomy showed oedematous thickening of the colon from the appendix to the upper part of the descending colon. The vessels appeared normal. The appendix was removed and showed a non-specific chronic inflammatory infiltration. Postoperatively he recovered rapidly, and a further barium-enema film two months later showed the colon to have returned to normal. He remained symptom free.

Case 2.—A 26-year-old West Indian man was admitted to hospital in August 1971. He had been fit until the day before admission, when he developed colicky periumbilical pain. The pain soon became more generalized and severe, accompanied by