ERYTHROCYTES FROM CANCER PATIENTS AND FROM BLOOD DONORS: HAEMAGGLUTINATION WITH MONOCLONAL ANTIBODY

Diagnosis	No. of samples	Haemagglutination result: Intact erythrocytes GM+ monoclonal Ab	Intact erythrocytes GM	Desialated erythrocytes GM+ monoclonal Ab	Desialated erythrocytes GM
Group I					
Leukaemia	25/25	+	−	+	−
Hodgkin's disease	4/4	+	−	+	−
Non-Hodgkin's lymphoma	2/2	−	−	+	−
Myeloma	1	+	−	+	−
Ewing's sarcoma	1	+	−	+	−
Wilms' tumour	1	−	−	+	−
Bronchus	3/3	+	−	+	−
Larynx	1	+	−	+	−
Lung	5/5	+	−	+	−
Ovary	1	+	−	+	−
Breast	5/5	+	−	+	−
Astrocytoma	1	+	−	+	−
Leukaemia*	1	−	−	+	−
Wilms' tumour*	1	−	−	+	−
Yolk sac carcinoma*	1	−	−	+	−
Ewing's sarcoma*	1	−	−	+	−
Hodgkin's disease*	1	−	−	+	−
Leukaemia, relapse	1	+	−	+	−
Group II					
Blood donors	2/50	+	−	+	−
	6/50	+/−	−	+	−
	42/50	−	−	+	−
Group III					
Random patients	4/32	+	−	+	−
	28/32	−	−	+	−

*Off treatment. GM = growth medium.

surface of their circulating erythrocytes. For these studies we first prepared a rat monoclonal antibody (an IgG) against desialated human erythrocytes. Antibody in the culture supernatant does not agglutinate normal erythrocytes but directly agglutinates desialated erythrocytes down to a titre of 1 in 100 (unpublished).[9] Preincubation of the monoclonal antibody with purified T antigen (kindly donated by Prof. G. Springer) inhibits this agglutination. Blood samples obtained from 50 tumour patients (group I) and from 50 blood donors (group II) were then tested for haemagglutination with the monoclonal antibody (unpublished). All samples were maintained at pH 7·4 and 4°C under sterile conditions and were tested within 24 h of sampling.

The results (see table) indicate that the T antigen is exposed on the erythrocytes of patients bearing a number of tumours, including an astrocytoma in 1 case. In this group 3 of the 5 samples were negative—2 from patients with non-Hodgkin's lymphoma and the third from a patient 5 weeks before cessation of treatment for a Wilms' tumour. Of the 50 blood donor samples 2 were positive and 6 showed slight agglutination; the remaining 42 were negative for T.

Besides the patients in group I, 5 out of 5 patients who had completed therapy for cancer and were off treatment were found to be negative for T on their erythrocytes. 1 patient, who had a relapse after 3 years off treatment for acute lymphoblastic leukaemia, was positive.

A third group (group III) consisted of samples obtained from patients at random: here the samples were tested before checking individual diagnoses. 4 out of 32 were positive, of which 1 turned out to be a sample from a patient on treatment for cancer. The other 3 were a 93-year-old with a poor diet and lung collapse, a patient with poor vitamin intake and recurrent stomatitis, and a patient with alcoholic liver failure. The remaining 28 were negative and included patients with anaemia, pernicious anaemia, diabetes mellitus, colitis, splenomegaly, pancreatitis, liver abscess, and arthritis.

The exposure of T antigen on the surface of erythrocytes of cancer patients was detectable at diagnosis (i.e., before the start of, and therefore unrelated to, chemotherapy). Control tests showed that:

(*a*) Treatment of erythrocytes from cancer patients or from normal individuals with irrelevant rat monoclonals did not cause haemagglutination.

(*b*) Those erythrocytes that did not agglutinate with "anti-T" monoclonal antibody subsequently agglutinated following desialation.

(*c*) Preincubation of the monoclonal antibody with purified T antigen abolished its ability to agglutinate intact erythrocytes from cancer patients.

(*d*) Those intact erythrocytes that could be agglutinated by the monoclonal antibody were not agglutinated by autologous plasma or by naturally occurring anti-T antibodies.

This last observation suggests that the epitope recognised by the monoclonal antibody is distinct from that which binds naturally occurring anti-T antibodies. Also, although the monoclonal antibody binds T antigen isolated from erythrocytes we have no direct evidence that it is binding to T on erythrocytes from cancer patients.

Studies are in progress to assess the value of this new monoclonal antibody in monitoring patients on treatment for leukaemia.

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Division of Virology,
Department of Pathology,
University of Cambridge,
Laboratories Block,
Addenbrooke's Hospital,
Cambridge CB2 2QQ

JO MILNER

Department of Surgery,
University of Cambridge,
Addenbrooke's Hospital

SU METCALFE

MESOTHELIOMA IN A BRAKE REPAIR WORKER

SIR,—Merewether's speculation in 1933[1] that asbestosis would manifest itself in industries, including the manufacture of asbestos brake linings, where it was then unknown, was soon realised. In the United States asbestosis was reported in a brake lining weaver in 1940[2] and the British Industrial Injuries Act of 1948 included asbestosis as a prescribed disease among "brake liners". Reports of lung cancer in asbestos-exposed workers closely followed those of fibrosis, but among "brake workers" such cases at first seemed to be confined to those who worked in friction product manufacture and fabrication. Subsequently there were occasional reports of lung cancer and mesothelioma among workers installing and repairing friction materials. However, asbestos-related cancer in brake repair workers has attracted little attention, in part because of the belief that fibre remained locked into the brake lining matrix or was much altered by heat generated by braking. Dust in brake drums was observed by light microscopy to be "asbestos-free". Furthermore, the capacity of chrysotile, the main form of asbestos used in brake linings, to cause mesothelioma may be less strong.

We describe here a diffuse pleural mesothelioma in a man whose sole exposure to asbestos was to the chrysotile form during brake maintenance and repair.

A 55-year-old man was admitted to hospital in October, 1980, with right-sided chest pain and soreness when he breathed deeply. Chest X-ray revealed a right-sided pleural effusion, and a cytological diagnosis of mesothelioma was confirmed by biopsy and examination of resected tumour. The patient died in September, 1981.

This man had for many years worked in used car, tyre, and car repair businesses. Since the age of 19 he had serviced automobiles, including the replacement of brake linings. He had no history of construction or shipyard work or any other occupational contact with asbestos and had never lived near an asbestos-fabricating plant.

1. Merewether ERA. A memorandum on asbestosis. *Tubercle* 1933; 15: 69–81, 109–18, 152–59.
2. Stone MJ. Clinical studies in asbestosis. *Am Rev Tuberculosis* 1940; 41: 12–21.

1

Fig. 1—Inorganic residues extracted from lung parenchyma.

Most chrysotile is present in fibril form: fibril A is 0·4 μm long, fibril B is 1·5 μm, fibril C is 12·0 μm (only 4·3 μm is shown). Additional short fibrils are scattered in matrix debris.

2

Fig. 2—Chrysotile fibre bundle in dust burden.

Selected area electron diffraction pattern (insert) shows chrysotile structure. Reciprocal a* axis is marked.

Necropsy revealed virtual obliteration of the right pleural space by a tumour that had extended through the diaphragm and metastasised to mediastinal lymph nodes, liver, and left lung. The histological appearance was in keeping with an epithelial type diffuse malignant mesothelioma. Slight interstitial fibrosis and anthracosis was noted in the pulmonary parenchyma. No typical asbestos bodies were observed but objects resembling such bodies were seen in a stained lung tissue block. After ashing only one typical asbestos body was found.

A tissue mass of 6310 mg was bulk digested.[3] Only 1·33 mg of inorganic debris was recovered (0·02%). The residue was immersed in distilled water, disrupted by sound waves (at 40 W for about 10 s), and analysed. 20 μl of suspension was put on a carbon-coated formvar support on a nickel grid and allowed to evaporate. Another coat of carbon was then placed over the dried residue.

Electron microscopy showed the residue to be full of exogenous particles, almost all non-fibrous. Magnification to 10 000 and more revealed chrysotile fibrils among these particles (fig. 1). Preliminary identification was based on morphology[3] but electron diffraction analysis confirmed the structure to be that of chrysotile (fig. 2 and insert). Fibrils less than 1 μm long and others longer than 5 μm were present. Long fibrils have been frequently encountered in lung tissues from occupationally exposed workers[4] but not in persons with only environmental exposure. 10% of the fibrils were longer than 10 μm.

No amphibole fibres were found, which was consistent with the occupational history. We estimate that about 1 μg chrysotile was present per 5 g wet lung parenchyma.

Although amphibole fibres (anthophyllite) were used experimentally for a brief time in the United States, to our knowledge only chrysotile has been used in brake pads since the 1940s.

Under certain test conditions of brake failure free chrysotile asbestos can be liberated, as shown, for example, by the work of Lynch,[5] Hatch,[6] Hickish and Knight,[7] Seshan,[8] and Rowson.[9] Some of these fibres are retained in drum dust[10,11] and tend to be submicroscopic. However, besides this submicroscopic chrysotile fibre in brake drum housing there is a more significant source of free, unaltered fibre in the bevelling, refurbishing, and refitting of brake pads.[10] There is thus ample opportunity, during brake maintenance and repair, for contact with chrysotile fibre both in drum debris (where it will usually be in a transformed state) and as long and predominantly unaltered fibres liberated by machining.

Controversy over the potential of chrysotile to cause mesothelioma has continued despite evidence from asbestos textile fabricators thought to have used only chrysotile,[12] from workers making brake pads,[13] from chrysotile miners and millers,[14] and from animal studies.[15]

3. Langer AM, Selikoff IJ, Sastre A. Chrysotile asbestos in the lungs of persons in New York City. *Arch Environ Health* 1971; **22**: 348–61.
4. Pooley FD, Oldham PD, Chang-Hyun UM, Wagner JC. The detection of asbestos in tissues. In: Shapiro HA, ed. Pneumoconiosis: Proceedings of 2nd International Conference (Johannesburg). London: Oxford University Press, 1970: 108–16.
5. Lynch J. Brake lining decomposition products. *J Air Pollut Control Assoc* 1968; **18**: 824–26.
6. Hatch D. Possible alternatives to asbestos as a friction material. *Ann Occup Hyg* 1970; **13**: 25–29.
7. Hickish DE, Knight KL. Exposure to asbestos during brake maintenance. *Ann Occup Hyg* 1970; **13**: 17–21.
8. Seshan K. On the utility of dark-field electron microscopy in the determination of the degree of deformation in chrysotile asbestos: an environmental research application. *Envir Res* 1978; **16**: 383–92.
9. Rowson DM. The chrysotile content of the wear debris of brake linings. *Wear* 1978; **47**: 315–21.
10. Rohl AN, Langer AM, Wolff MS, Weiman I. Asbestos exposure during brake lining maintenance and repair. *Envir Res* 1976; **12**: 110–28.
11. Rohl AN, Langer AM, Klimentidis R, Wolff MS, Selikoff IJ. Asbestos content of dust encountered in brake maintenance and repair. *Proc Roy Soc Med* 1977; **70**: 32–37.
12. Peto J. The hygiene standard for chrysotile asbestos. *Lancet* 1978; i: 484–89.
13. Mancuso TF, El Attar AA. Carcinogenic risk and duration of employment among asbestos workers. In: Biological effects of asbestos: Proceedings of Dresden International Conference. Dresden: Anspach, 1968: 161–66.
14. McDonald AD, Harper A, El Attar, McDonald JC. Epidemiology of a primary malignant mesothelial tumor in Canada. *Cancer* 1970; **28**: 914–19.
15. Wagner JC. Asbestos carcinogenesis. *Br J Cancer* 1975; **32**: 258–59.

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riction material. *Ann Occup Hyg* 1970;
during brake maintenance. *Ann Occup*
nicroscopy in the determination of the
in environmental research application.
debris of brake linings. *Wear* 1978; 47:
Asbestos exposure during brake lining
: 110–28.
S, Selikoff IJ. Asbestos content of dust
r. *Proc Roy Soc Med* 1977; 70: 32–37.
stos. *Lancet* 1978; i: 484–89.
and duration of employment among
f asbestos: Proceedings of Dresden
, 1968: 161–66.
ald JC. Epidemiology of a primary
ver 1970; 28: 914–19.
er 1975; 32: 258–59.

The pathological diagnosis of asbestos-related diseases in people exposed to chrysotile fibre is complicated by the fact that asbestos bodies do not form readily. Furthermore, in patients with mesothelioma asbestosis may not always be present. As in this case, analytical electron microscopy may be essential. Chrysotile in a biological environment is degraded chemically and physically so that light microscopy is a poor guide to chrysotile exposure.

The health and safety problem of brake maintenance and repair work has been outlined by Morgan.[16] In the United States there are about 1·7 million workers in the new and used car business, accessory stores, and service stations, and another 435 000 in repair shops and garages. The risk of malignant asbestos disease among these workers seems to be low[17] but mortality data have yet to be thoroughly evaluated. With the large numbers at risk, more cases may be seen in the future.

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Environmental Sciences Laboratory,
Mount Sinai School of Medicine,
New York, N.Y. 10029, U.S.A. — A. M. LANGER

Canadian Tumour Reference Centre,
Ottawa Civic Hospital, Ottawa, Canada — W. T. E. MCCAUGHEY

VENOUS ULCERATION IN HYPOGONADAL MEN

SIR,—Dr Howell and Dr Burton (Sept. 18, p. 630) suggest that venous stasis is related to hypogonadism. In trying to find an endocrine biochemical explanation for their findings they have underemphasised considerations of surgical pathology and powerful social factors.

The cause of venous ulceration is generally accepted to be incompetence of perforating veins, most commonly on the medial aspect of the ankle. Failure of the valves in these veins causes hypertension in the cutaneous venules and leads to ulceration.[1] Venous hypertension produces increased capillary leakage of fibrinogen and tissue deposition of fibrin, causing local hypoxia and ulceration.[2] There is no evidence that the blood in these veins is static or hypoxic, and the term stasis ulcer is outmoded.[3]

Incompetence of perforating veins normally follows deep vein thrombosis due to trauma such as that which occurs in women after childbirth and is, therefore, not necessarily related to androgen deficiency. The deep vein thrombosis resolves by recanalisation with damaged valves. The full hydrostatic pressure from the chest may, therefore, be transmitted to cutaneous venules; this effect will clearly be more severe in taller men.

Howell and Burton do not define hypogonadism, but testosterone assays by standard methods showed no evidence of reduced testicular androgen production, despite the fact that they were done on a selected subgroup of patients who were unmarried or infertile. The height and obesity of the patients cannot, therefore, be related to this. The infertility might be related to a reduced sperm count as happens in some generalised diseases (e.g., cystic fibrosis). Any metabolic abnormality causing a low sperm count might predispose to venous thrombosis or reduce fibrinolysis, but since sperm counts were not done this is only speculation, the two cases of Klinefelter's syndrome being clearly exceptional.

The men in the ulcer group were less likely to be married than were the controls—this, unlike fertility, cannot be directly related to sperm production. Howell and Burton discuss the fact that obesity might prevent men from marrying but ignore the possibility that this might also be true of men with venous ulceration or low socioeconomic class.

Department of Surgery,
St John's Hospital,
Chelmsford, Essex CM2 9BG — A. M. C. THOMAS

16. Morgan C. Trouble in the auto repair shop. *Job Safety Health* (OSHA, DOL) 1976 (Dec.): 21–28.
17. Nicholson WH. Brake maintenance investigation of health hazards in brake lining repair and maintenance workers occupationally exposed to asbestos: Final report to N.I.O.S.H. (in press).
1. Dodd H, Cockett FB. The pathology and surgery of the veins of the lower limb, 2nd ed. London: Churchill Livingstone, 1978: 246–66.
2. Burnand KG, Clemenson G, Whimster I, Browse NL. Extra vascular fibrin deposition in response to venous hypertension. *Br J Surg* 1976; 63: 660.
3. Browse NL, Burnand KG. The cause of venous ulceration. *Lancet* 1982; ii: 243–45.

VITAMIN A AND PAGET'S DISEASE

SIR,—The recent resurgence of clinical interest in vitamin A has come about primarily as a result of improved methodology, such as high-pressure liquid chromatography (HPLC), which has permitted the rapid separation and analysis of retinoids in small volumes of blood and tissue extracts.[1] In turn this is increasing our understanding of vitamin A metabolism and of the role played by the retinoids in disease—for example, the possible relation between serum retinol and the risk of cancer,[2] the development of hypercalcaemia in renal failure,[3] and the reduced fetal growth seen in immigrant Asians in Britain.[4]

The role of vitamin A in bone disease warrants closer examination. In vitamin A deficiency there is bulky overgrowth of bony tissue and failure of absorption of previously formed bone.[5] Both increased osteoblastic activity and reduced osteoclastic activity have been implicated in these changes, which are reversed in hypervitaminosis A. Retinol is a membranolytic agent and in organ culture directly causes destruction and resorption of bone. It also stimulates the release of parathyroid hormone both in man in vivo and in isolated bovine parathyroid glands.[6]

While studying the response of patients with previously untreated Paget's disease to the injection of a single dose of calcitonin we noted that the plasma calcium usually fell while circulating parathyroid hormone (PTH) concentrations (measured by a radioimmunoassay directed mainly at the amino-terminal of the peptide) increased. However, the magnitude of these changes and the time of peak response varied considerably from patient to patient. In view of the above reported effects of vitamin A, we measured (by HPLC) plasma retinol on the basal plasma samples obtained from the patients to see whether vitamin A might be a factor in this variability.

So far sixteen patients have been studied. Four showed no response to calcitonin (i.e., plasma calcium and PTH values did not change). One patient showed a continuous increase in plasma PTH over the 24 h when samples were taken and was thought to have hyperparathyroidism. We excluded this patient from our calculations. The relations between the plasma retinol and changes in plasma calcium (ΔCa = maximum fall of calcium occurring at a time, T_{min} Ca, after injection) and PTH (ΔPTH = maximum rise of PTH occurring at a time T_{max} PTH, after injection) were as follows:

	Correlation (r)
ΔCa*	0·519 ($p<0·05$)
ΔPTH*	0·417 ($p>0·1$)
T_{min} Ca	−0·606 ($p<0·05$)
T_{max} PTH	−0·853 ($p<0·001$)

*Including the 4 non-responders.

T_{max} PTH varied from 5 to 12 h in the eleven patients who responded to calcitonin (figure).

These results support the view that vitamin A has a role in bone metabolism and PTH release. Our interpretation would be that inasmuch as plasma retinol is a measure of vitamin A status and/or availability of vitamin A for metabolism, the reduced interaction of vitamin A with the parathyroid glands results in a delayed response in the release of PTH after the hypocalcaemia induced by calcitonin. This hypocalcaemic response to calcitonin is characteristic of patients with increased osteoclastic activity.[7] Patients with a reduced availability of vitamin A may have less

1. De Ruyter MGM, De Leenheer AP. Simultaneous determination of retinol and retinyl esters in serum or plasma by reversed phase high performance liquid chromatography. *Clin Chem* 1978; 24: 1920–23.
2. Kark JD, Smith AH, Hames CG. Serum retinol and the inverse relationship between serum cholesterol and cancer. *Br Med J* 1982; 284: 152–54.
3. Farrington K, Miller P, Varghese Z, Baillod RA, Moorhead JF. Vitamin A toxicity and hypercalcaemia in chronic renal failure. *Br Med J* 1982; 282: 1999–2002.
4. Maxwell JD, Ang L, Brooke OG, Brown IRF. Vitamin D supplements enhance weight gain and nutritional status in pregnant Asians. *Br J Obstet Gynaecol* 1981; 88: 987–91.
5. Navia JM, Harris SS. Vitamin A influence on calcium metabolism and calcification. *Ann N Y Acad Sci* 1980; 355: 45–56.
6. Chestow BS, Williams GA, Norris RM, Baker GR, Hargis GK. Vitamin A stimulation of parathyroid hormone: Interaction with calcium, hydrocortisone and vitamin E in bovine parathyroid tissues and effects of vitamin A in man. *Eur J Clin Invest* 1977; 7: 307–14.
7. Burckhardt PM, Singer FR, Potts JT. Parathyroid function in patients with Paget's disease treated with salmon calcitonin. *Clin Endocrinol* 1973; 2: 15–22.