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Effect of the PAF antagonist UK-74 505 on experimental lung injury in mice

JM MIOTLA, TJ WILLIAMS, PK JEFFERY, PG HELLEWELL *Departments of Lung Pathology and Applied Pharmacology, National Heart and Lung Institute, London* We have shown that intravenous lipopolysaccharide (LPS) and zymosan treatment induces plasma protein accumulation in the lung, which is dependent upon neutrophil accumulation but not their emigration into alveolar interstitium (Miotla *et al. Am Rev Respir Dis* **147**: A69). We have now investigated the role of PAF in the induction of neutrophil sequestration and vascular permeability changes. BALB/c mice (20 g) received i.v. LPS (3 mg/kg) two hours prior to i.v. zymosan (10 mg/kg). The PAF antagonist, UK-74 505 (0·5 mg/kg) was injected i.v. 15 minutes prior to LPS. Accumulation of ^{125}I-human serum albumin (^{125}I-HSA), co-injected with zymosan, was assessed in lung tissue after 30 minutes. ^{131}I-HSA injected i.v. five minutes before sacrifice was used as a marker of intravascular volume. The activities of ^{125}I-HSA and ^{131}I-HSA in lung tissue were assessed simultaneously in a gamma counter and compared with that in plasma. Albumin accumulation in the lung was expressed as µl of plasma retained extravascularly. Accumulation of plasma increased from 1·3 (0·4) µl (n = 4) in the saline-treated group to 10·7 (1·9) µl (n = 7, $p < 0.001$) after combined LPS and zymosan. Pretreatment with UK-74 505 reduced plasma accumulation to 2·9 (0·8) µl (n = 5, $p < 0.001$). Combined LPS and zymosan treatment resulted in a marked diffuse accumulation of neutrophils and focal aggregates as seen by light microscopy. UK-74 505 had no noticeable effect on the diffuse accumulation of neutrophils nor on the intensity and frequency of the aggregates. In conclusion, blockade of PAF receptors reduces lung plasma protein extravasation induced by LPS and zymosan but does not modify neutrophil accumulation. The attenuation of plasma protein accumulation by UK-74 505 may be by blockade of PAF receptors located on vascular endothelial cells or on the intravascular neutrophils.

Prevalence of asthma and probable "asthma" in Blackburn

P MYERS, LP ORMEROD *Chest Clinic, Blackburn Royal Infirmary, Blackburn, Lancashire* A respiratory questionnaire administered by health visitor and translator was completed by 1783 persons, 96·6% of all the Asian patients in three local practices. The age breakdown of the survey was similar to that for the Asian district population in the 1991 census. 181 (10·2%) were known asthmatics. Only 121 (6·8%) were current, and 28 (1·6%) ex-smokers. There was no correlation between any symptoms/treatment and social class or the Jarman index of the patients' addresses. Those born in the UK were more likely to describe respiratory symptoms, but there were increasing rates of symptoms and medication use with increasing time in the UK. The prevalence of diagnosed asthma was 9–16% in the under 15s, fell to 4·5–9% between the ages of 20–49, but rose to 12–17% in those aged over 50. Positive responses to the questions: night time cough (n = 157), morning tightness (n = 127), wheeze after exercise (n = 152), cough with change of air temperature (n = 170), and a history of wheezy breathing (n = 231) all suggested under diagnosis of asthma. If those non-smokers with positive responses to one or more of the above were not known to be asthmatic they were regarded as having "probable" asthma. The total prevalence of asthma (diagnosed and probable) rises to 17–22% in children, 9–19% between ages 20–49, and to 22–34% in the over 50s. Patients born in the UK were more likely to be diagnosed as asthmatic than those born abroad; those born abroad had a similar percentage with symptoms and were more likely to have undiagnosed "probable" asthma. The survey shows a high prevalence of asthma in Asian patients of all ages in the UK.

Effects of geography and gender on the prevalence of asthma symptoms: results of a postal survey

G DEVEREUX, SMT AYATOLLAHI, K FISHWICK, SJ BOURKE, DJ HENDRICK *Chest Unit and Regional Unit for Occupational Lung Disease, Newcastle General Hospital, University of Newcastle Upon Tyne* We have used the postal questionnaire of the EC Study of Asthma Prevalence and Risk Factors as the initial phase of a comparable investigation within the Northern Health Region. We hope to evaluate the background prevalence of asthma (and airway responsiveness) in young adults of working age (20–44 years) and to assess its dependence on area of residence (west *v* east coast) and gender in order to provide control data for industrial populations in our region at risk of developing occupational asthma. The questionnaire was mailed to random samples of West Cumbrian (WC) men, Newcastle men, and Newcastle women identified from FHSA registers. Investigation of a sample of non-responders indicated that most had moved from the addresses provided, leaving participation rates for those presumed to have received the questionnaire (and up to two reminders) of 85·1% from WC men, 83·9% from Newcastle men, and 85·4% from Newcastle women. There were no significant differences in the age distribution between the three groups. The results are shown in the table. We conclude that (1) the only evidence of a geographic effect was to the question "have you ever wheezed" (more frequent in Newcastle), and (2) the only evidence of a gender effect was to the question "have you woken coughing in the last year" (more frequent in women).

Symptom	*WC men (n = 1802)*	*Newcastle men (n = 1368)*	*Newcastle women (n = 1679)*
Ever wheezed	*39·9%	*44·0%	41·7%
Waking with tight chest in last year	17·1%	18·8%	20·6%
Waking breathless in last year	8·6%	9·8%	10·2%
Waking coughing in last year	23·0%	**24·7%	**36·1%
Asthma attack in last year	5·2%	4·7%	6·1%
Currently taking asthma medication	7·3%	6·9%	7·9%

*p = 0·019; **p = 0·017 (χ^2 test)

Association of age, sex and smoking with total IgE and specific IgE in young adults living in East Anglia

D JARVIS, P BURNEY, S CHINN, C LUCZYNSKA, E LAI, R HALL, B HARRISON, J STARK *Department of Public Health Medicine, UMDS; Department of Respiratory Medicine, The Ipswich Hospital; Department of Respiratory Medicine, West Norwich Hospital; Department of Respiratory Medicine, Addenbrookes Hospital, Cambridge* As part of the British arm of The European Community Respiratory Health Survey blood samples for determination of total IgE and specific IgE to *D pteronyssinus*, cat and timothy grass were taken from a random sample of 854 young adults living in three areas in East Anglia. Smoking was recorded as non-smoker (NS), ex-smoker (EXS), and current smoker (CS) (table 1). In a multiple regression model with adjustment for age, sex and area the overall effect of smoking on total IgE was significant ($p < 0.05$), the lowest total IgE values being in EXS. In a logistic regression model, excluding subjects who had experienced childhood asthma attacks, and with adjustment for age, sex, area and total IgE, the odds ratios for a positive RAST (> 0·35 kU/l) to the three allergens were as shown in table 2. The relation between smoking and specific IgE after adjustment for total IgE is not the

Table 1 Geometric mean total IgE (kU/l)

	Men		*Women*	
Age	*Mean*	*(95% CI)*	*Mean*	*(95% CI)*
20–24	40·3	(26·7–60·6)	24·3	(14·7–40·1)
25–29	47·1	(30·7–72·4)	21·0	(15·6–28·5)
30–34	29·4	(17·7–48·8)	32·2	(23·1–44·8)
35–39	33·7	(24·4–46·6)	22·8	(16·9–30·7)
40–44	34·3	(24·0–49·0)	19·6	(17·3–32·5)

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Table 2 Odds ratios for positive RAST to allergens

	D pteronyssinus	*Cat*	*Grass*
NS	1·00	1·00	1·00
EXS	1·26 (0·75–2·08)	0·78 (0·37–1·60)	0·64 (0·39–1·05)
CS	1·60 (1·02–2·48)	0·47 (0·22–1·00)	0·45 (0·28–0·71)

same for all allergens. [This work was supported by the National Asthma Campaign.]

What is an asthma death? Results of a cohort study of patients discharged following inpatient treatment for asthma

H GUITE, A FITZGERALD, P BURNEY *Department of Public Health Medicine, St Thomas' Hospitals, UMDS, London* To quantify the risk of death following inpatient treatment for asthma, vital status was ascertained on 2291/2382 (96%) of patients aged 15–64 years discharged from hospitals in the South East Thames Region with a primary diagnosis of asthma. In the first year of follow up all cause mortality rate was 18·5/1000 person years (95% CI 13–23/1000 person years), six times greater than would have been expected in a similar age and sex matched group of the general population. This excess mortality occurred in all age groups and in both sexes but was expecially marked in women over the age of 45 years. Of the excess deaths 80% were accounted for, approximately equally, by deaths from asthma, chronic obstructive pulmonary disease and circulatory disorders. The strongest risk marker for death was age. Other risk markers (adjusted for age and sex) were length of stay $\geqslant$ six days (RR 2·3 (95% CI 1·4–3·5)) and a second recorded diagnosis (RR 2·2 (95% CI 1·4–3·4)). Studies of asthma mortality should include deaths from all causes in patients with asthma. [This work was supported by the National Asthma Campaign and Glaxo Group Research Limited.]

East Anglian confidential enquiry into asthma deaths 1992

BDW HARRISON, G MOHAN, S MILDENHALL, RM BADMINTON, NJ WAREHAM FOR EAST ANGLIAN CHEST PHYSICIANS AND DIRECTORS OF PUBLIC HEALTH *Department of Respiratory Medicine, West Norwich Hospital* Seventeen deaths were notified. Thirteen patients under the age of 65 died of asthma. The asthma was thought to be potentially reversible in 12. In the year before death, asthma had been severe in eight, moderate in one, mild in three, and severity unknown in one. Two patients had been discharged from hospital within the previous six weeks. In nine of 12 patients there were significant social factors that might have contributed to death (living alone; alcoholism; single parenthood; self employed or low socioeconomic status; stress with close relatives or at work; smoking or passive smoking). In seven of 10 patients there were felt to be psychological characteristics which might have contributed to death (denial in six; depression in two). Six of 12 patients were felt to have good understanding of asthma, but only four appeared willing to cope with their disease. Ten of 13 had received appropriate advice and education but interviews with next of kin suggested that they would frequently have liked more information and education about asthma. None of the patients had a written management plan and peak flow (PF) monitoring of asthma was only appropriate in five. In seven of 10 patients the final attack developed against a background of under-controlled asthma; six were not making PF measurements appropriately in the days and weeks before death. Approximately half the patients did not respond to their worsening symptoms appropriately and half their relatives failed to respond appropriately. The overall management in the days or weeks leading up to the final attack was felt to be inappropriate in seven of 13 patients. Factors included premature discharge from hospital; inadequate medical monitoring in the days and weeks following discharge; not following GP's advice; not attending outpatients and GP's surgery with no subsequent attempt made to contact the patient; failure to monitor PF at home; over using β agonists; failure of relatives to respond appropriately; reluctance to call the GP. This is the first of the multiregional ongoing enquiries, coordinated by the Mortality Working Group of the UK Asthma Task Force, to be established. A list of recommendations which were sent to all general practitioners and consultant physicians in the region as a result of this first year's enquiry will be shown.

Community treatment of worsening asthma symptoms in patients requiring hospital admission

BJ MCDONOUGH, SW WATKIN, CRK HIND *Royal Liverpool University Hospital, Liverpool* As part of a continuing programme of asthma audit at this hospital (*Thorax* 1992;47:884P) a questionnaire survey was prospectively performed on 41 consecutive admissions with acute severe asthma. The objective was to compare actual community management with recommended guidelines for the management of asthma. Patient characteristics were: 30 females of mean age 38 (range 18–67); seven current smokers. Seventeen (44%) had had asthma for less than 10 years. Thirty nine of 41 (95%) had consulted their GP with asthma symptoms in the past year (mean 10 visits; range 1–52). Twenty one of 41 (51%) were using a home peak flow meter (mean 2·0, range 0–4 times per day), in 14 for over six months. Night waking had occurred on each of the seven nights prior to admission in 23/41 (56%). Medication prior to the index attack had included: beta-2 agonists – inhaled (37), oral (6), nebulised (9); anticholinergics (14); theophyllines (14); and steroids – inhaled (32, with spacer 16), oral (4). During the index asthma attack changes had then been made prior to admission in the majority (31/41 (76%)) either by the GP (27) or by the patient (4). Changes included: increased anti-inflammatory treatment with or without other measure (16/31 (52%)); increased bronchodilator treatment alone (4/31 (13%)); or antibiotics alone (7/31 (23%)). These results suggest that the majority of patients requiring hospital admission for acute severe asthma have a prolonged time course of worsening symptoms. Despite this apparent window of opportunity, appropriate changes in asthma management did not take place in all patients. Such changes might have prevented the need for admission to hospital.

High risk asthmatic patients: indications for emergency listing

H MOUDGIL, AG LEITCH, GJR MCHARDY *Respiratory Medical Unit, University of Edinburgh and City Hospital, Edinburgh* Approximately 1–2% of patients with severe asthma die as a result of their disease and so much attention is focused on investigating the nature of disease in fatal asthma and in attempting to identify individuals at high risk. Delay in receiving specialist care doubtless contributes to morality and to this end, 25 years ago, an Edinburgh group introduced a relatively unique self referral system for pre-identified asthmatics (*BMJ* 1975;4:680–2); such a system depends on being able to identify patients with potentially fatal asthma and the impact of this system has previously been described by the original group (*BMJ* 1979;2:1199–201). The City Hospital in Edinburgh has adopted the Greencard system for asthmatics over the past 15 years and this retrospective review reports on the current cohort of asthmatics (Register Jan 93) outlining patient characteristics, identifying the indications for inclusion on the emergency list, describing the nature of concurrent disease, and reviews current prescribed medications as indicators of disease severity. Clinical notes for 83/104 (~80%) of the patients were reviewed identifying a group with a mean age of 39·6 y (range 16–85) with 23 males (mean age 42·4y, range 18–78) and 60 females (mean age 38·5y, range 16–85). Forty one were non-smokers, 18 ex-smokers, and 24 current smokers. Fifty one with mean (SD) age at onset of 6 (5·1)y had documented onset of disease during childhood (< 18 years), and 32 with mean age at onset 41·2 (13·8)y had onset in adult life (> 18 years). Mean duration of disease for the group was 20.0 (11.9), range 1–54y and period of surveillance 5·5 (3·8)y (range 1–15), although the majority of the childhood group had been followed up elsewhere previously. Forty seven of 83 (56·6%) of patients had experienced at least one life threatening event, of whom 12 (14·5%) had been ventilated, 11 (13·3%) had experienced a respiratory arrest but not ventilated, and 24 (28·9%) had documented hypercapnia alone. The nature of disease where discriminated was described as acute deterioration to status asthmaticus in 18 (21·7%), persistently unstable disease in 26 (31·3%), predominantly nocturnal disease with fluctuant PEFR in 14 (16·9%), and asphyxial anaphylaxis in six (7·2%). Excluding chronic airflow limitation due to asthma or chronic bronchitis, concurrent disease was noted in 60% of patients, with, as a percentage of the full group, 22 (26·5%) describing pulmonary disease, 17 (20·5%) cardiac, and 24 (28·9%) psychosocial problems. All patients were prescribed β_2 agonists (36 (43·4%) by nebuliser), and inhaled steroids (15 (18·1%) by nebuliser); 56·6% were also maintained on parenteral steroids with 41 (49·4%) on oral prednisolone and six (7·2%) on intramuscular triamcinolone. Additionally 31 (37·3%) were prescribed a theophylline, 20 (24·1%) salmeterol, and 12 (14·5%) an oral long acting β_2 agonist. One patient was prescribed a sedative, and two patients opiates regularly. This review concurs with previous reports that the high risk population are predominantly middle aged women, usually have an identifiable previous life threatening event, have severe asthma reflected by the number of asthma medications including maintenance on parenteral steroids, possibly have concurrent disease of either respiratory or cardiac nature, and may have psychosocial problems (*Aust NZ J Med* 1991;21:681–5).

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Use of peak flow meters in acute asthma by patients and doctors: BTS theory and practice

G DEVEREUX, G PILKINGTON, K FISHWICK, SJ BOURKE, AND DJ HENDRICK *Chest Unit and Regional Unit for Occupational Lung Disease, Newcastle General Hospital, University of Newcastle Upon Tyne* The British Thoracic Society (BTS) asthma guidelines stress the need for measurement of peak flow (PF) as an index of asthma severity. We therefore studied the current use of PF meters in acute asthma by patients, GPs, and hospital doctors. For a six month period all patients admitted to our hospital with acute asthma were invited to participate in an audit of asthma management. Of 44 eligible local subjects, 40 (53 admissions) participated. Thirty six had an established diagnosis of asthma; 22/36 (61%) had a PF meter, 11/22 used it daily, and 22/22 claimed to use it when they felt their asthma was deteriorating. In 15/22 (68%) a falling PF played a significant role in the recognition of deterioration, the remaining 7/22 (32%) relied on deteriorating symptoms. 20/22 (91%) said that the PF meter was useful, 2/22 found the meter useless. GP records showed 16/36 (44%) had PF records before the first study admission, ranging from the day of admission to 31 months beforehand (mean 4·5 months). GPs saw 31/40 patients immediately before the admission and used PF to assess asthma severity in 13 (42%). All patients had a PF recorded in their hospital admission notes and had regular inpatient PF recordings. PF was recorded on the day of discharge for 51 of the 53 episodes. The BTS recommendation that PF should be >75% predicted or best by discharge was not met for 14/51 (27·5%) discharges. Additionally the BTS recommendation that PF variability should be <25% was not met for 13 of the 40 episodes (32·5%) where calculable. None of these episodes were readmitted within a month. On discharge 32/40 patients had PF meters, including all newly diagnosed asthmatics. This study provides a broad experience of current usage of PF meters in acute asthma in our area. Although PF meters are widely (though not universally) used in accordance with BTS guidelines, some patients and GPs rely more on other clinical features in assessing asthma severity. BTS discharge criteria proved to be unobtainable or impractical in 30% of patients. PF meters are not considered appropriate for all patients and some patients feel that they do not benefit from a PF meter.

PF	*Admission*				*Discharge*
	<50%	*<40%*	*<30%*	*Mean*	*Mean*
% Best (n=49)	37 (76%)	28 (57%)	20 (41%)	38·8%	84·8%
%Predicted (n=20)	17 (85%)	14 (70%)	10 (50%)	31·3%	80·6%

Assessment of asthma morbidity in the first four weeks after discharge from hospital following admission for acute severe asthma

BJ MCDONOUGH, SW WATKIN, CRK HIND *Royal Liverpool University Hospital, Liverpool* To evaluate the continuing care of patients recently admitted with acute asthma we studied 41 consecutive hospital asthma admissions by questionnaire both at the time of admission and one month later. The follow up data were obtained by postal survey but telephone contact was made if necessary. Data were obtained on all patients. Patient characteristics were: 30 female; mean age 38 (range 18–67); seven current smokers; 17 (44%) had asthma for less than 10 years. We found that 30/41 (73%) had received an outpatient follow up appointment, but of these only 50% were within the month since discharge. During this period 22/41 (54%) had consulted the GP about their asthma, and four patients had required a further admission to hospital. Treatment at one month compared with treatment before the index attack occurred was as follows: systemic steroid 11 *v* 4; high dose inhaled steroid 29 *v* 19; low dose inhaled steroid 10 *v* 13; no inhaled steroid 2 *v* 9; regular use of inhaled beta-2 agonist 20 *v* 8; long-acting beta-2 agonist 10 *v* 8; use of spacer device 24 *v* 16. In the month after discharge 11 patients (27%) had been seen by an asthma nurse (eight GP based, three hospital). Peak flow meters were used by 21 patients before the attack and by 37 at one month. Whilst these data suggest that frequent treatment step-ups do take place as a result of acute admission, early follow up remains a problem and 10% of patients were readmitted within a month.

Long term outcome following an acute attack of asthma treated in the A&E department

JM MEIGHAN, VHF MAK, NT BATEMAN *Department of Thoracic Medicine, St Thomas' Hospital, London* Patients who have suffered an acute attack of asthma bad enough to require treatment in an A&E department constitute an "at risk" group who should receive careful monitoring and adjustment of their treatment. However, patients do not always follow the advice given to them. We have followed patients who were discharged from the A&E department for a six month period to examine the pattern of attendance to their GP or A&E department. The practice records of 58 patients discharged from A&E after emergency treatment were analysed at three weeks, three and six months to assess whether they attended the GP or an A&E department. We also looked to see if non-attenders were contacted by the practice to encourage them to attend. Forty two A&E discharge letters were received by the GPs. Only 18 of the 58 patients attended regular review over all of the six month period, and only two of these patients required A&E treatment, one on two occasions. Of six patients who made no contact with their GP at all, one was attending the hospital chest clinic and only one was sent a reminder to attend. None of these non-attenders required further A&E treatment during the six month follow up. Twenty patients did not attend within the first three weeks following discharge from A&E. Of these 20, 13 visited their GP at least once within three months and a further one by six months. Of the 14 who attended by six months, five may have attended because of deteriorating asthma and five because they were sent reminders. Twenty one out of 22 patients (95%) whose GPs held asthma clinics were sent reminders compared with only 11 of 36 patients (31%) of GPs without asthma clinics. There were no reported deaths amongst the 58 patients followed. Over half the patients who attended A&E for acute asthma visited their GP for further advice. Subsequent GP visits seem to be determined by deteriorating asthma or being sent a reminder, and this is dependent upon whether the practice runs an asthma clinic.

Follow up of young adults after acute severe asthma: who attends?

I RYLAND, J JACKSON, MG PEARSON *Aintree Chest Centre, Liverpool* The Asthma Guidelines recommend that all patients with acute severe asthma should be followed up in the hospital outpatients (OPD). The BTS/RCP/NAC audit showed that young adults were particularly likely to default (*Thorax* 1992;47:209P). Does this matter? Are GPs picking these patients up? We prospectively identified 50 consecutive patients (aged <40 years) admitted with acute severe asthma, recorded basic data on admission, obtained data on follow up from their case notes, and then 6–9 months later obtained details of current treatment and attendance at their general practitioner. The median age was 27 (16–39) years and 54% were female. Forty were given an OPD appointment (33/34 by chest physicians, 7/16 by other physicians, $p<0·01$). Two were readmitted before the OPD. We compared the 21 who attended with the 27 who were not seen again at hospital. Age (28 *v* 27 y), history of previous admissions (65% *v* 63%), previous treatment (β agonists 90% *v* 93%, inhaled steroids 76% *v* 59%, oral steroids 28% *v* 21%) and physician type were similar. Those not followed up had had worse PEF on admission (median 200 *v* 260 l/min, $p<0·05$) and included disproportionately more males (59% *v* 29%, $p<0·05$). Care in hospital was similar and all but four (none from chest physicians) went home on inhaled steroids; 76% also had oral steroids. Some 6–9 months later those who were not seen at hospital OPD were less likely to be seeing their GP regularly (22% saw GP most months and 4% attended an asthma clinic compared with 70% and 25% respectively, $p<0·01$). The hospital non-attenders were less likely still to be prescribed inhaled steroids (78% *v* 95%) and less likely to be collecting prescriptions regularly (55% *v* 70%, $p<0·05$). Young adults not seen at the hospital OPD are not being picked up in the community in spite of having had a more severe acute episode. Hospital physicians may be missing those who most need help.

Successful avoidance of hospital readmission in acute asthma

LM OSMAN, JAR FRIEND, JS LEGGE, JG DOUGLAS *Centre for Medical Education, University of Edinburgh; Thoracic Medicine Unit, City Hospital, Aberdeen* In 1987 92 patients with acute asthma were admitted to a Grampian hospital for the first time. By 1991 30 of these had had further admissions, three had died, and 59 had successfully avoided readmission. Of these 59, 43 were interviewed in 1991. Present (1991) medical management and patient behaviour in this group (Singles) was compared with that of 70 patients admitted to hospital with acute asthma for the first time in 1991 (Firsts). The Singles were more likely than the Firsts to be currently prescribed an inhaled steroid (84% *v* 61%, $p<0·05$), for this to be at 800+μg per day (74% *v* 35%, $p<0·001$), and to report they took their inhaled

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steroid daily (83% *v* 64%, $p < 0.07$). Although the Singles last episode was not severe, they were as likely to have seen a GP (45% *v* 46%) during it as the Firsts in the month before admission and more likely to have used oral steroids (47% *v* 23%, $p < 0.05$). Singles were also more likely to use oral steroids at early signs of exacerbation (60% *v* 30%, $p < 0.05$). The Singles were significantly more likely to have been referred to specialist care over the past four years (46% *v* 24%, $p < 0.05$). Referral to specialist care over this time was significantly associated with prescription of inhaled steroid ($p < 0.001$), inhaled steroid at 800+μg ($p < 0.001$), and daily use of inhaled steroid ($p < 0.001$). Those referred to specialist care had used oral steroids slightly more often in their last episode (38% *v* 27%, NS). Most first admissions will not be readmitted. These results suggest that this is because both medical management and patient behaviour change after a first admission for asthma. These changes are apparent even four years after the admission, and they are most likely to occur if the patient is referred to specialist care.

Association of positive skin prick tests with work related chest symptoms and bronchial hyperresponsiveness in a study of allergy to acid anhydrides

RD BARKER, J HARRIS, M VAN TONGEREN, KM VENABLES, AJ NEWMAN TAYLOR *Department of Occupational and Environmental Medicine, National Heart and Lung Institute, London; Institute of Occupational Medicine Birmingham* Clinical studies have shown that acid anhydrides (AA) can cause asthma that is associated with bronchial hyperresponsiveness (BHR) and the presence of IgE directed against AA-human serum albumin (HSA) conjugates. Few epidemiological studies have investigated these relationships. In spite of this, it has been suggested that clinical evaluation plus antibody studies should be an accurate method for the diagnosis of TMA asthma (*J Allergy Clin Immunol* 1981;**188**:188–93). We have examined the relationship between sensitisation to AA and asthma symptoms, asthma treatment and BHR in an epidemiological survey. The cohort was defined to study exposure–response relationships in asthma caused by acid anhydrides. Participants completed questionnaires on respiratory symptoms, had skin prick tests (SPT) with three AA-HSA conjugates, and a measurement of bronchial responsiveness. A total of 622 workers were identified; 449 (73%) workers did all the tests (436 men; age range 18–78, median 43 years). Of these, 57 (13%) had BHR ($PD_{20} \leqslant 8$ μmol histamine), 41 (9%) had new work related chest symptoms (NWRCS), 114 (28%) had had or were having treatment for their chest, and 14 (3%) had a positive SPT to an AA-HSA conjugate. Workers with a positive AA SPT were more likely to have NWRCS, to have been treated for chest disease, and to have BHR (NWRCS 11/14 *v* 30/435, RR 11·4, CI 7·33–17·7, $p < 0.0001$; treated 12/14 *v* 102/435, RR 3·7, CI 2·78–4·8, $p < 0.0001$; BHR 5/14 *v* 52/435, RR 2·99, CI 1·41–6·31, $p = 0.023$). These data give epidemiological evidence that sensitisation to AA is associated with asthma.

Determinants of bronchial responsiveness in an occupational cohort study

RD BARKER, J HARRIS, M VAN TONGEREN, KM VENABLES, AJ NEWMAN TAYLOR *Department of Occupational and Environmental Medicine, National Heart and Lung Institute, London; Institute of Occupational Medicine Birmingham* A cohort was defined to study exposure–response relationships in asthma caused by acid anhydrides. It included all people with at least one month's work at four industrial sites in the UK. Entry began in 1960 at two sites and in 1979 at two. Participants completed questionnaires on respiratory symptoms, smoking, and work experience, and had skin prick tests (SPT). Bronchial responsiveness was measured by the method of Yan *et al* (*Thorax* 1983;**38**:760–5). Bronchial hyperresponsiveness (BHR) was defined as a $PD_{20} \leqslant 8$ μmol histamine. A total of 622 workers were identified and 470 (76%) had a histamine challenge (455 men; age range 18–78 median 42 years). Of these, 62 (13%) had BHR, 151 (33%) were atopic on SPT with common aeroallergens, 169 (37%) were current smokers, and 14 (3%) had a positive SPT to an acid anhydride. BHR was associated with atopy (OR = 1·7, $p = 0.049$) and a positive SPT to acid anhydrides (OR = 4·0, $p = 0.024$), but not significantly with current smoking (OR = 1·5, $p = 0.13$). The distribution of BHR was "U" shaped across five age quintiles with the nadir in the fourth decade. The prevalence of atopy fell with age. The proportion of the population who were smokers was similar in each age group. In the youngest age quintile 7/7 (100%) of those with BHR were atopic and this proportion fell to 7/23 (30%) in the oldest quintile. In contrast, the proportion of BHR associated with current cigarette smoking increased with age: 2/8 (25%) in quintile 1 compared with 10/24 (42%) in quintile 5. Further analysis suggested that atopy was associated with BHR at all ages whereas current cigarette smoking was only associated with BHR in those over 40 years old. Apart from the influence of atopy on BHR in the elderly, these results are very similar to those found by Burney *et al* (*Thorax* 1987;**42**:38–44) in a community survey of the epidemiology of bronchial responsiveness.

Effects of air pollution in wintertime on peak expiratory flow measurements in subjects with asthma or chronic bronchitis

SCO TAGGART, BG HIGGINS, HC FRANCIS, AM FLETCHER, CJ YATES, CAC PICKERING, AA WOODCOCK *Wythenshawe Hospital, Manchester* We have previously reported an association between peak expiratory flow (PEF) measurements and environmental pollution at normal British summertime levels in subjects with asthma or chronic bronchitis (*Thorax* 1993;**48**:417). Since pollution levels change seasonally and because differences in temperature may alter the effects of pollution, we have repeated these measurements during winter months (Jan–Feb 1992). Subjects from our earlier study in Widnes and Runcorn were asked to record PEF at two-hourly intervals during waking hours for a period of four weeks. A bronchial challenge test was performed using methacholine administered by the method of Yan: 21 subjects with a $PD_{20} < 12{\cdot}25$ μmol were identified and classified as reactors. Pollution levels were monitored continuously using absorption spectroscopy. The relations between pollution levels and PEF variability (expressed as amplitude % mean), mean and minimum PEF levels were determined using multiple linear regression. Of the original group of 76 subjects 55 (29 male, ages 20–82) were able to keep a satisfactory PEF record during the winter period. Mean 24-hour pollution levels were below WHO guide levels but maximum 24-hour levels were higher: SO_2 168·8 μg/m³ (WHO level 125 μg/m³); NO_2 150·5 μg/m³ (WHO level 150 μg/m³). Ozone levels were negligible. In the reactors a significant association was found between increasing 24-hour lag SO_2 and increasing amplitude % mean ($p = 0.035$ in the reactors; $p = 0.08$ in the whole subject group) and there was an association of borderline significance between 24-hour lag SO_2 and mean PEF ($p = 0.06$). There were no significant associations between NO_2 and any PEF index.

Consequences of a diagnosis of occupational asthma: a controlled study

J CANNON, P CULLINAN, AJ NEWMAN TAYLOR *Department of Occupational and Environmental Medicine, National Heart and Lung Institute, London* In order to examine the socioeconomic consequences of a diagnosis of occupational asthma (OA) we have surveyed all patients referred to our specialist clinic for investigation of the disease over the past five years. 178 (89%) patients completed a postal questionnaire enquiring into changes in occupation, earnings and social activities; the responses from those with asthma induced by exposures at work (OA, n = 87) were compared with those whose asthma was exacerbated by work (WRA, 26) and those in whom there was no work relationship (NWRA, 65). Patients with OA or WRA (89%) were more likely than those with NWRA (54%) to have changed jobs and to report that the diagnosis had made employment more difficult to find; those with OA had suffered a greater loss of income and one third (compared with 15% with WRA and none with NWRA) reported that their preferred career was no longer feasible. There were no consistent differences between the proportions in each group reporting difficulties with various social activities. This controlled study highlights the important professional consequences of a diagnosis of OA.

Pneumoconiosis associated with lobar or segmental lung collapse

J CONGLETON, MD PEAKE *Chest Unit, Pontefract General Infirmary, West Yorkshire* Coal worker's pneumoconiosis (CWP) is the description given to a collection of respiratory conditions caused by inhaling large volumes of coal dust over a length of time. We describe lobar or segmental lung collapse which we feel is a newly described manifestation of CWP. We present eight men, all of whom had previously worked underground as coal miners for many years. None had a history of previous tuberculosis. Their chest radiographs showed varying degrees of simple pneumoconiosis. One man also had a lesion consistent with progressive massive fibrosis. In addition they all showed evidence of a degree of lobar or segmental collapse (six

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right middle lobe (RML) only, one left upper lobe (LUL) only, one RML and RUL). Fibreoptic bronchoscopy was performed. In all cases there was circumferential narrowing by fibrotic looking tissue of the lobe corresponding to the area of loss of volume on chest radiography. Biopsies or brush cytology were taken in six cases and these showed no evidence of malignancy. In four cases all there was, in addition, was generalised narrowing of the peripheral airways. CT scans of the chest have to date been performed in three of these patients and have shown no evidence of a mass lesion to explain the areas of collapse. All have shown a degree of subcarinal lymphadenopathy with more generalised lymphadenopathy in one, (lymphadenopathy is recognised to be a feature of CWP). On follow up for several years none of these patients has developed bronchial carcinoma and the area of collapsed lung has persisted. We propose that CWP is a cause of collapse or partial collapse of a lobe or segment, particularly the RML. We are not aware of this being reported previously. The mechanism could either be by a focus of coal dust causing a dense local fibrotic reaction, or possibly by a "middle lobe syndrome" similar to that seen in tuberculosis. The latter explanation seems less likely on the basis of the bronchoscopic appearances.

Clinicopathological correlation in end stage primary pulmonary hypertension

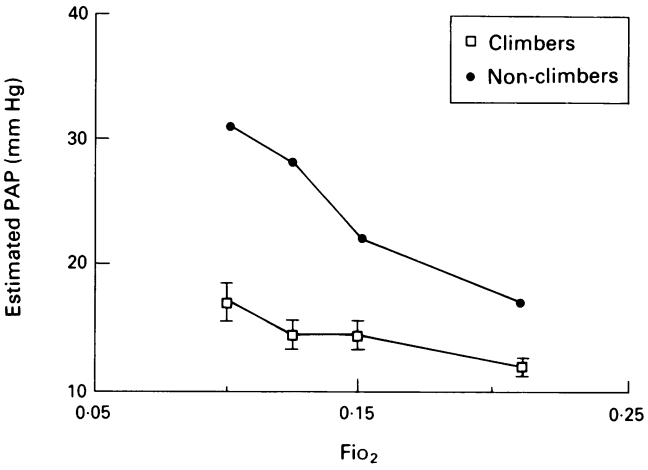
BP MADDEN, JR GOSNEY *Royal Brompton National Heart and Lung Hospital, London; Royal Liverpool University Hospital, Liverpool* A clinicopathological correlation was performed on 20 consecutive patients with primary pulmonary hypertension (PPH) who underwent heart–lung transplantation; 14 females, six males, mean age 26 (range 2–51) years. From a review of their preoperative notes, all pertinent clinical data were retrieved. Two patients had Raynaud's phenomenon. The mean pulmonary artery pressure for the group was 77 mm Hg, mean pulmonary vascular resistance 29 Wood units, and mean cardiac output 2·4 l/min. Five patients received a vasodilator challenge with prostacyclin but none demonstrated a therapeutic response. The histological features of PPH were present in all subjects and active migration of myofibroblasts was noted in five. Numbers of pulmonary endocrine cells immunoreactive for gastrin releasing peptide (GRP) and calcitonin correlated significantly ($p < 0.001$) with activity of myofibroblasts. There was a statistically significant inverse relationship between mean pulmonary artery pressure and numbers of pulmonary endocrine cells immunoreactive for both GRP and calcitonin ($p < 0.025$). Using multivariate regression analysis no clinical feature was strongly predictive of any histological finding. Although the patients who received prostacyclin had contracted small muscular pulmonary arteries, intimal proliferation was present in each case. Whereas the prognosis of PPH cannot be defined by other than its clinical manifestations, intimal proliferation as well as vasoconstriction may be important in its pathogenesis. The release of GRP from pulmonary endocrine cells may possibly be involved in this process.

Effect of intravenous prostacyclin on the pulmonary circulation in systemic sclerosis

N MENON, L MCALPINE, R MADHOK, A PEACOCK *Department of Rheumatology, Gartnavel General Hospital; Pulmonary Vascular Unit, Western Infirmary, Glasgow* Pulmonary vascular disease is a major cause of morbidity and mortality in patients with systemic sclerosis (SS). It is unknown whether the pulmonary vessels in patients with SS will respond to vasodilators although there is evidence that hypoxic pulmonary vasoconstriction may contribute to the elevated pulmonary vascular resistance (Morgan JM *et al. Chest* 1991;**99**:551–6). Prostacyclin has been used as the gold standard to determine the vasodilatory response in patients with primary pulmonary hypertension (Rubin *et al. Ann Intern Med* 1990;**112**:485–9). We therefore studied the haemodynamic effect of 100% oxygen and prostacyclin (PGI_2) in a group of patients with SS and pulmonary hypertension. We studied six patients (aged 41–68; all female). All patients were breathless on mild exertion; mean Pao_2 9·2 kPa; mean TLC 83% pred; mean Kco 52% pred. All had echo Doppler evidence of pulmonary hypertension but normal V/Q scans. Each patient underwent right heart catheterisation with measurements of pulmonary artery pressure (mean PAP 39 mm Hg), pulmonary artery occlusion pressure, and cardiac output by thermodilution. Baseline measurements were made and then the haemodynamic responses to 100% oxygen and PGI_2 were determined. PGI_2 was infused via a peripheral vein starting at 2 ng/kg/min and increased by 2 ng/kg/min every 10 minutes until a plateau response was observed. 100% oxygen had no effect. Five patients responded to PGI_2 with a rise in cardiac output (1·04 (0·2) l/min; $p < 0.05$) and a fall in pulmonary vascular resistance (33 (4)%; $p < 0.05$). We conclude that in our patients 100% oxygen had no effect but prostacyclin did cause an improvement in pulmonary haemodynamics. Further studies are needed, but our results suggest that vasodilators should be considered in patients with systemic sclerosis and pulmonary hypertension.

Estimation of the pulmonary haemodynamic response to hypoxia using Doppler echocardiography in subjects travelling to extreme altitude: results from the British 40th anniversary Everest expedition

AJ PEACOCK, T MCDONAGH, H DARGIE, R NAEIJE *Departments of Respiratory Medicine and Cardiology, Western Infirmary Glasgow; Intensive Care, Erasme Hospital, Brussels* Performance at high altitude may be related to the pulmonary vascular response to hypoxia – a vigorous response is believed to increase the risk of high altitude pulmonary oedema (HAPE). We have previously shown that this response can be assessed in normal subjects using echo Doppler (Peacock *et al. Respir Med* 1990;**84**:335–7). We studied nine elite climbers (aged 32–53; 8F, 1M) three months before they travelled to Mt Everest. All had previously climbed above 4000 m and four above 6000 m without difficulty. We measured the velocity of the tricuspid regurgitation jet (TR) while the subjects breathed air (Fio_2 0·21) or a hypoxic mixture containing Fio_2 of 0·1, 0·125 or 0·15. The TR wave velocity (v) provides an estimate of pulmonary artery pressure (PAP) using the Bernouilli theorem where PAP = 4.v + right atrial pressure (which was assumed to be 5 mm Hg). The mean (SD) results for the nine climbers are compared with the previous results from non-climbing subjects in the figure. None of the climbers suffered from HAPE



despite climbing to very high altitudes (all > 6350 m; four > 8000 m, and one to the summit at 8848 m). The climbers had a less vigorous pulmonary haemodynamic response to hypoxia than the non-climbers ($p < 0.05$), supporting the hypothesis that a blunted vascular response to hypoxia is an advantage for high altitude climbing. [AJP was supported by the Wellington Foundation.]

Method for assessing the dynamic nature of pulmonary blood flow reduction during lobar hypoxia in man

NW MORRELL, KS NIJRAN, T BIGGS, WA SEED *Departments of Medicine and Nuclear Medicine, Charing Cross and Westminster Medical School, London* We have previously shown (*Thorax* 1993; **48**: 1077P) that acute hypoxic pulmonary vasoconstriction (AHPV) at the lobar level in man is a stronger reflex than previously reported in entire lungs. In this study we have estimated the time course as well as the magnitude of the response with the use of an intravenous infusion of krypton-81 m (^{81m}Kr) as a flow tracer. During fibreoptic bronchoscopy lobar hypoxia was induced by occluding left upper lobe bronchi with balloon-tipped catheters in eight normal volunteers. The PAo_2 and $PAco_2$ inside the occluded lobes were monitored intermittently with a mass spectrometer. In three subjects lobes were made hyperoxic prior to occlusion by breathing 100% oxygen. Prior to and during an occlusion a solution of ^{81m}Kr was infused at a constant rate into the right antecubital vein with the subject lying over a gamma camera. Dynamic images of ^{81m}Kr delivery were obtained by each 10 second period of acquisition. Images were analysed by drawing a region of interest over the left upper lobe (anatomy defined by introducing ^{81m}Kr gas directly into the occluded lobe) and generating a graph of activity *v* time. PAo_2 and $PAco_2$ within hypoxic occluded lobes rapidly

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approached those of mixed venous blood: 38 (SE 1·5) mm Hg and 39·9 (0·9) mm Hg, respectively. In three hyperoxic lobes the mean gas tensions measured just prior to balloon deflation were P_{AO_2} 127·8 (31·5) mm Hg and P_{ACO_2} 40·2 (1·3) mm Hg. Following occlusion there was an initial rise in the ^{81m}Kr activity because of reduction in the ventilatory removal of isotope to zero. In hypoxic lobes the activity then fell in a curvilinear fashion that approximated a single exponential, reaching a mean of 61·2 (2·6)% of the initial peak level after 4·5 minutes of occlusion. In hyperoxic lobes the activity did not change significantly after the initial rise. This technique has allowed us to characterise both the time course and magnitude of AHPV in man during short periods of lobar hypoxia.

Atrial natriuretic peptide modulates pulmonary haemodynamics in hypoxic COPD

TK ROGERS, W SHEEDY, J WATERHOUSE, P HOWARD, AH MORICE *Department of Medicine and Pharmacology, University of Sheffield, Royal Hallamshire Hospital, Sheffield* Pulmonary artery pressure (PPA) is elevated in advanced chronic obstructive pulmonary disease (COPD). Atrial natriuretic peptide (ANP) release is increased in pulmonary hypertension and this hormone may both selectively vasodilate pulmonary vessels and inhibit pulmonary vascular remodelling. We have hypothesised that ANP has a physiological role in protection of the pulmonary circulation from pressure overload, and may be beneficial in COPD at pathophysiological doses. Ten patients with hypoxic COPD were infused for 30 minute periods with saline, then 0·4, 2, and 10 pmol/kg/min of ANP respectively, via a pulmonary artery catheter, monitoring haemodynamics and oxygenation. Immunoreactive ANP levels were increased significantly from a mean (SD) of 23(15) to a maximum of 94(41) pmol/l ($p < 0·0005$) – i.e. to levels seen in severe pulmonary hypertension. Neither systemic blood pressure, cardiac output, nor total systemic vascular resistance showed any correlation with irANP levels. There were significant negative correlations between ANP levels and falls in mean PPA (28·7 to 25·9 mm Hg), pulmonary artery wedge pressure (6·5 to 4·2 mm Hg), total pulmonary vascular resistance (489 to 428 dynes s cm^{-5}) and P_{ACO_2} (6·2 to 5·9 kPa). Venous admixture and oxygen delivery both increased non-significantly. At these pathophysiological concentrations there was evidence that ANP had a selective effect on pulmonary compared with the systemic circulation. These data support the hypotheses that ANP may regulate the pulmonary circulation in humans, and that increased plasma levels may be beneficial in hypoxic COPD.

L-Arginine selectively increases renal blood flow in normal subjects but not in patients with cor pulmonale

TQ HOWES, CR DEANE, SV BAUDOUIN, J MOXHAM *Department of Thoracic Medicine and Medical Engineering and Physics, King's College Hospital, London* The pathogenesis of oedema in cor pulmonale (CP) remains elusive. Renal blood flow has long been known to be reduced but the mechanism of its reduction is unknown (Richens *et al. Clin Sci* 1982;**62**:255–9). We have shown that oxygen is a potent vasodilator in hypoxaemic normocapnic patients with chronic airflow limitation (CAL) (Baudouin *et al. Thorax* 1992;**47**:550–4)). This response is not present in normal subjects or those with advanced CAL and hypercapnia (Howes *et al. Am Rev Respir Dis* **147**:A343) which may be due to a failure of an oxygen dependent vasodilator. Nitric oxide (NO) is known to be produced from L-arginine by NO synthase in the presence of oxygen (Russe *et al. Circulation* 1993;**87**:V18–25) and to be a potent vasodilator. We have infused L-arginine (20 g/30 min) intravenously in 10 normal volunteers and five patients with CP. Using colour flow Doppler, previously validated (Howes *et al*), we measured changes in renal and aortic blood flow. Blood pressure and pulse were monitored. Normal subjects (group A) were recruited from hospital staff (mean age 31 years). Group B had CAL with hypoxaemia (Pao_2, mean (SE) 7·8 (1·2) kPa; $Paco_2$, 4·9 (0·5); FEV_1, 1·25 (0·3) l). In group A renal blood velocity increased by 18% (range 8–36%) with L-arginine ($p < 0·001$). There was no change in pulse rate, blood pressure or aortic blood velocities. In group B the same dose of L-arginine had no effect on any of the parameters and in particular renal blood flow was not increased ($p > 0·05$). These results suggest that the nitric oxide system may play a part in the pathogenesis of CP.

Comparison between nebulisers and spacers for home bronchodilator treatment in COPD

MB ALLEN, MF MUERS *Respiratory Unit, Regional Cardiothoracic Unit, Killingbeck Hospital, Leeds* Nebulisers for long term high dose bronchodilators improve symptoms and home peak flow (PF) measurements in patients with COPD (*Respir Med* 1992;**86**:33–8), although they are inconvenient to use and expensive. Some patients prefer smaller doses of bronchodilators given through a large volume spacing device. We have now compared the changes in home peak flow and treatment preferences betwen spacer and nebulised bronchodilators in a group of COPD patients still symptomatic on other treatment. A total of 120 patients with COPD, mean (SD) FEV_1 39 (17)% predicted and FEV_1/FVC ratio 49·1 (14·8)% undertook twice daily home PF recordings while receiving salbutamol (S) or ipratropium bromide (IB) four times daily for one week in the regimens and order listed below. The mean (SD) PF for the period and the significance of any improvements over the first regimen by paired *t* tests are shown in the table. A response, defined as a 15% increase in PF over regimen 1 (*Eur Respir J* 1992;**5**:424–9), was found in 10 (2) patients with regimen 2, 30 (9) with regimen 3, and 46 (34) with nebulised bronchodilators; the preference for that treatment is shown in parentheses. Forty six (38%) of the 120 patients showed a response to nebulised treatment with 30 (25%) responding to the same drugs in lower dosages through a spacer. Although most responding patients prefer a nebuliser, the MDI + spacer regimen is cheaper and, we suggest, should be a routine component of nebuliser assessments in COPD.

		PF		
Regimen	*Drugs + dose*	*Mean*	*SD*	*p*
1 MDI + spacer	S 2p	198	69	
2 MDI + spacer	S 4p	205	72	<0·001
3 MDI + spacer	S 4p + IB 4p	210	75	<0·001
4 Nebuliser	S 5 mg + IB 500 µg	227	79	<0·001

Corticosteroid trial reproducibility in COPD

DPS SPENCE, L RIMINGTON, M NISAR, JE EARIS, PMA CALVERLEY, MG PEARSON *Aintree Chest Centre, Fazakerley Hospital, Liverpool* Oral corticosteroids are given in COPD to distinguish those with steroid response from those with "fixed" disease. While such steroid trials select patients with a better prognosis (Rimington, *Thorax* 1993;**48**:419P) and may be used to identify subjects who may benefit from inhaled steroids, few data are available on the reproducibility of such trials in COPD. We studied a group of 54 COPD patients (ATS criteria) (age (SD) 59 (8) years, FEV_1 1·03 (0·53) l) who form a representative cohort of survivors from a group we have previously studied (Nisar, *Thorax* 1990). Patients received 30 mg prednisolone daily for two weeks, spirometry being measured before and after. Spirometric reversibility to 5 mg nebulised salbutamol, smoking history, and IgE were all repeated five years apart. Forty two patients had had a negative steroid trial at the outset and, of these, 29 were still unresponsive at five years whilst 13 increased their FEV_1 significantly (>0·2 l) (range 0·2–0·8 l) when retested. Among the 12 initial steroid responsive patients seven still showed an improvement in FEV_1 after steroids. In two the baseline FEV_1 increased by >1 litre over the five years and they showed no further increase with oral steroids. In the remaining three patients the FEV_1 was unchanged after the second trial of oral steroids (initial increase in FEV_1 0·29 (0·08) l). These data suggest that a clear steroid response may not exclude subsequent reversibility which may be substantial.

Steroid response	*Baseline FEV_1*	*Δ FEV_1 (steroid trial 1)*	*Δ FEV_1 (steroid trial 2)*	*Δ FEV_1 (salbutamol trial)*	*IgE*	*Age*
Always (n=9)	1·86 (0·45)	0·78 (0·43)	0·31 (0·36)	0·54 (0·33)	141 (136)	56 (5)
Once (n=16)	0·93 (0·51)	0·02 (0·2)	0·29 (0·19)	0·21 (0·15)	155 (219)	59 (8)
Never (n=29)	0·83 (0·27)	0 (0·12)	0·03 (0·1)	0·2 (0·17)	110 (197)	59 (8)

Effect of withdrawal of inhaled steroids in patients with chronic obstructive pulmonary disease (COPD)

N AL JARAD, NM FASEY, NC BARNES, RM RUDD, JA WEDZICHA ON BEHALF OF THE ISOLDE GROUP *The London Chest Hospital, London* Many patients with irreversible COPD are treated with inhaled steroids though the benefits are controversial. In addition, the effect of withdrawal of inhaled steroids in those patients is unknown. We have investigated the effect of withdrawal of inhaled steroids in patients with COPD. Patients were included in the study if post-bronchodila-

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tor FEV_1 was >799 ml and <65% of predicted, FEV_1/FVC ratio <65%, and the reversibility of FEV_1 to 400 µg salbutamol was less than 10% of their predicted FEV_1. We investigated 60 patients (18 female and 42 male), mean age 64 years (range 47–75), mean FEV_1 43% predicted (SD 13%) during an eight week run-in period of the ISOLDE study. They were all current or previous smokers. In the year prior to study 23 (38%) patients received regular inhaled steroids and intermittent courses of prednisolone on exacerbations, 30 (50%) received inhaled steroids only, and seven (12%) received neither. An exacerbation was defined as increased shortness of breath with or without sputum production requiring oral steroids and/or antibiotics. Of all patients 22 (36%) had at least one exacerbation and 16 (28%) were steroid dependent and were withdrawn from the study. Patients who had two exacerbations had received a greater dose of inhaled steroid (beclomethasone/budesonide) in the year prior to the study than the rest of the patients (median 412 mg compared with 191 mg, p=0·0025). The group who had at least one exacerbation (n=22) received greater dose of inhaled steroid (median 351 mg) than the non-exacerbators (n=38) (median 193 mg; p=0·0077). Even when we excluded those who had not received any oral or inhaled steroid prior to the study, patients who were steroid dependent received a greater dose of inhaled steroid (median 412 mg) than the non-dependent group (234 mg; p=0·044). There was no difference between the study groups with respect to the total dose of oral prednisolone taken by patients in the year prior to the study, baseline lung function (FEV_1, PEFR, TLCO and KCO), age, sex distribution, pack years of cigarette smoking, eosinophil counts, plasma IgE, and duration of illness. Over a third of patients with irreversible COPD suffer exacerbations within eight weeks of withdrawal of their inhaled steroids. The risk of exacerbation was only related to the dose of inhaled steroid received in the year prior to study.

Effect of nebulised morphine and morphine 6-glucuronide on exercise endurance in patients with chronic obstructive pulmonary disease

CL DAVIS, C HODDER, S LOVE, R SHAH, M SLEVIN, J WEDZICHA *ICRF Department of Medical Oncology and Pharmacy, St Bartholomew's Hospital, London; Department of Thoracic Medicine, The London Chest Hospital, London; ICRF Department of Medical Statistics, Lincoln's Inn Fields, London* Nebulised morphine may be efficaceous in the treatment of breathlessness: the mechanism of action is unknown. We have conducted a randomised, double blind, placebo controlled, crossover comparison of the effects of equianalgesic, single doses of nebulised morphine (M) and morphine 6-glucuronide (M6-G; the major active metabolite of M). Eighteen patients (median age 66) with clinically stable COPD (mean FEV_1 0·93; mean FVC 2·53; mean Po_2 9·5 kPa; mean Pco_2 5·8 kPa) whose exercise tolerance was severely limited by breathlessness were studied before and after the administration of three nebulised solutions; M 12·5 mg, M6-G 4 mg, and normal saline (P). Objective measurements included spirometry, ear lobe blood gases, six minute walking tests and endurance bicycle exercise tests at 60% $\dot{V}o_2max$. No toxicity was experienced by any patient during the study. Significant changes were found in bicycle endurance time, $\dot{V}o_2max$ and $\dot{V}Emax$. There was no significant change in resting spirometry, six minute walking distance or blood gases at rest and peak exercise. Nebulised M6-G is more effective at increasing exercise endurance in patients with COPD than nebulised morphine and placebo. This may reflect differences in potency between M and M6-G, or these two compounds may have different actions on ventilatory control.

		% *increase in*	
	Bicycle endurance	$\dot{V}o_2$	$\dot{V}Emax$
M *v* P	1·9 (0·7)	6·6 (0·2)	4·0 (0·3)
M6-G *v* P	21·7 (0·004)	13·1 (0·01)	8·3 (0·03)
M6-G *v* M	19·8 (0·01)	6·5 (0·2)	4·3 (0·3)

Values in parentheses are p values.

Effect of cognitive and behavioural therapy on dyspnoea in the "pink puffer" syndrome

N EISER, S EVANS, C WEST *Lewisham and Guy's Hospitals, London* Anxiety is common in the "pink puffer" syndrome. The degree of anxiety correlates well with perceived dyspnoea (Gift *et al. Heart Lung* 1986;**15**:544). This study examines the effect of group psychotherapy on anxiety, dyspnoea, and quality of life. Eleven patients with severe stable chronic airflow obstruction (mean FEV_1 = 1·151) had six weekly 90-minute sessions of cognitive and behavioural psychotherapy. Patients completed Hospital Anxiety-Depression (HAD), MRC Dyspnoea + St George's (StG) Respiratory Questionnaires one week before and after therapy. They also performed spirometry, six-minute walks, and had blood gas analysis. Eight control patients with similar FEV_1 values attended for lung function and walks for six consecutive weeks but had no psychotherapy. Mean results are shown in the table. Baseline HAD score was significantly higher in the psychotherapy group but other parameters were very similar between groups. Walking distance increased significantly (by a mean of 20%) after psychotherapy but no change occurred in the control patients. Neither questionnaire scores, FEV_1, nor blood gases altered significantly in either group during the study. In conclusion, six sessions of cognitive and behavioural psychotherapy improved exercise tolerance in our "pink puffer" patients. Future studies of more prolonged, intensive psychotherapy would establish whether better symptom and quality of life scores might accompany further improvements in exercise tolerance in "pink puffers".

Groups	*Distance walked (m)*	Po_2 *(kPa)*	Pco_2 *(kPa)*	*Questionnaire score*		
				HAD	*MRC*	*StG*
Treatment						
Baseline	351	8·6	5·3	12	3	45
After treatment	423	8·4	5·3	11	3	49
Control						
Baseline	370	8·9	5·3	7	3	61
At 6 weeks	378	8·6	5·3	7	3	64

Preterminal disease and dying in adult cystic fibrosis

AJ MOORCROFT, AK WEBB, ME DODD *Monsall Hospital, Newton Heath, Manchester* Survival in cystic fibrosis is increasing although the majority of patients still die in early adulthood. There are few published data characterising adult patients at time of dying. Deaths over the last 10 years at a regional centre were reviewed for age of death, cause of death, sputum colonisation, sex, diabetes and the requirement for IV antibiotics. The best FEV_1, FVC, and body mass index (BMI) were recorded for the three months prior to death and at two years before death. Fifty patients from a population of 225 referrals died over the decade; 44 from respiratory failure, one from septicaemia, and five following heart/lung transplantation. The mean age of death was 22·4 years. In those deaths from respiratory failure the mean FEV_1 three months prior to death was 24·7% (predicted) and the mean FVC 40% (predicted). These were significantly (p<0·001) lower than two years previously when the mean FEV_1 was 38·6% and FVC 58·9%. The BMI was very low both at death (16·7) and two years previously. Comparing deaths from respiratory failure in the first half of the decade with those in the second, there was no significant difference in mean age of death, spirometry, or BMI. There was a trend towards higher antibiotic requirement in the second half of the decade. Similarly, no significant difference in BMI, spirometry or age of death was found when patients were split according to sex, *P cepacia* colonisation or diabetes. There was a tendency to higher antibiotic requirement in those colonised by *P cepacia*. In conclusion, irrespective of adverse features such as *P cepacia*, diabetes and female sex during two consecutive five year periods, patients with cystic fibrosis dying of respiratory failure had similar clinical characteristics.

Effect of antibiotic therapy on lung function in cystic fibrosis patients with *Pseudomonas cepacia* colonisation compared with patients with *Pseudomonas aeruginosa* colonisation

N HIRANI, D PECKHAM, AJ KNOX *Respiratory Medicine Unit, City Hospital, Nottingham* *Pseudomonas cepacia* (PC) is usually reported in vitro to be resistant to many of the antibiotics commonly used to treat infective exacerbations of cystic fibrosis (CF). Our clinical impression, however, is that patients with PC do tend to improve with antibiotic therapy regardless. We therefore retrospectively compared lung function before and after antibiotic therapy in 13 patients (aged 18–24) with established PC colonisation, with 13 patients (aged 19–24) with established *Pseudomonas aeruginosa* (PA) colonisation who had never grown PC in their sputum. Where PC was cultured, the organism was invariably found to be resistant to ciprofloxacin, gentamicin, azlocillin, and tobramycin. In three cases sensitivity to ceftazidime was reported. PA sensitivities were variable, but the organism was never pan-resistant. The mean FEV_1 and FVC in the PC and PA groups prior to starting therapy were 1·46 l and 2·09 l *v* 7·35 l and 2·11 l respectively. The mean weight in the PC and PA

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groups prior to therapy was 51·6 kg and 52·0 kg respectively. Antibiotic therapy significantly improved FEV_1, FVC and weight (all $p < 0.05$). Changes in mean FEV_1, FVC and weight in the PC group were +0·21 l, +0·27 l, and +0·9 kg respectively, and in the PA group, +0·13 l, +0·18 l, and +1·0 kg respectively. There was no significant difference between PC and PA groups in the change in any of these variables. This suggests that patients with multiresistant PC colonisation and those with PA colonisation respond similarly to antibiotic therapy.

Preliminary report on in vivo reduction of sputum viscoelasticity in cystic fibrosis patients treated with aerosolised recombinant human DNase

PL SHAH, S SCOTT, A LANSLEY, G MARTIN, C MARRIOTT, ME HODSON *Royal Brompton National Heart and Lung Hospital, London; Department of Pharmacy, King's College London* Recombinant human DNase I (rhDNase) has been shown to depolymerise extracellular DNA and thereby reduce viscosity of cystic fibrosis (CF) sputum in vitro. A phase II double blind placebo controlled study demonstrated that rhDNase improves pulmonary function in CF patients (Ranasinha *et al. Lancet* 1993;**342**:199). Patients were treated with either placebo or rhDNase 2·5 mg twice daily for 10 days. Fresh sputum samples were collected in sterile containers from the participating patients prior to treatment and on day 10 of treatment with study drug, and stored at −20°C. Preliminary rheological analysis was conducted on sputum samples of 10 patients from each treatment group. Dynamic oscillatory analysis with a parallel plate was used on a CSL 100 rheometer (Carrie-Med, UK). The mean dynamic storage modulus (G′), a measure of elasticity, and the mean dynamic loss modulus (G″), which is related to viscosity, at 9·5 Hz are reported. Pre-treatment: $G' = 51{\cdot}83$ (SE 13·29) Nm^{-2}; $G'' = 13{\cdot}99$ (2·96) Nm^{-2} for placebo, and $G' = 59{\cdot}53$ (20·51) Nm^{-2}; $G'' = 15{\cdot}15$ (3·97) Nm^{-2} for rhDNase groups. Post treatment: $G' = 54{\cdot}67$ (12·09) Nm^{-2} and $G'' = 15{\cdot}16$ (2·51) Nm^{-2} for placebo, and $G' = 21{\cdot}38$ (9·0) Nm^{-2} and $G'' = 6{\cdot}33$ (2·24) Nm^{-2} for rhDNase groups. Treatment with rhDNase significantly reduced G′ ($p < 0.0025$) and G″ ($p < 0.0025$) compared with placebo (significance was calculated with a Mann–Whitney U test on the difference between pre- and post-treatment samples). These preliminary data confirm that rhDNase reduces viscoelasticity of CF sputum in vivo.

Preliminary report on using aerosolised recombinant human DNase I in the treatment of patients with stable stage cystic fibrosis for six months

PL SHAH, S SCOTT, D GEDDES, H FUCHS, ME HODSON *Royal Brompton National Heart and Lung Hospital, London; Genentech Inc., San Francisco, USA* A phase II multicentre double blind placebo controlled study demonstrated that short term treatment with recombinant human DNase I (rhDNase) improves pulmonary function in cystic fibrosis (CF) patients with stable stage lung disease (Ranasinha *et al. Lancet* 1993;**342**:199). We conducted a six month open label treatment study in these patients to assess the medium term effects of rhDNase. Patients who completed the phase II study and were stable for 15 days prior to treatment were eligible. Fifty nine patients (31M,28F) age range 16–55 years were recruited. Mean baseline values for FEV_1 and FVC were 41·5% and 72·4% of predicted, respectively. The mean increase in FEV_1 over the first month of treatment was 13·1% (range 12–14·1%) and then stabilised at 6·2% (4·6–7·8%) for the subsequent five months. FVC was similarly improved. Administration of rhDNase improved the severity of dyspnoea, CF related symptoms, and the Taussig/NIH score. All patients apart from two who died completed the study. These two deaths were considered to be due to progression of their pulmonary disease from CF and unrelated to treatment with rhDNase. The adverse events and intercurrent illnesses were no different from those expected in a CF population. Pharyngitis was the only possible drug related adverse event which occurred at least once in 10% of patients during the six month period. Administratiion of rhDNase for six months was safe, well tolerated and improved pulmonary function in CF patients.

Correction of the ion transport defect in the airways of the insertional cystic fibrosis mouse model by liposome mediated gene therapy

EWFW ALTON, PG MIDDLETON, NJ CAPLEN, SN SMITH, DM STEEL, FM MUNKONGE, PK JEFFERY, DM GEDDES, SL HART, R WILLIAMSON, KI FASOLD, AD MILLER, P DICKINSON, BJ STEVENSON, G MCLACHLAN, JR DORIN, DJ PORTEOUS *Ion Transport Laboratory and Department of Lung Pathology, National Heart and Lung Institute, London; Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School, London; Department of Chemistry, Imperial College, London; MRC Human Genetics Unit, Edinburgh* We have assessed the possibility of using liposome mediated gene therapy to correct the electrophysiological defect characteristic of cystic fibrosis (CF) in the insertional mouse model for this disease (*Nature* 1992;**359**:211). The human CFTR gene complexed with the cationic liposome DC Chol:DOPE was nebulised into the airways of these mutant mice (n = 8). Human CFTR mRNA was detected from the trachea of all tested animals, but not from control mutant mice (n = 7) treated with the reporter gene β-galactosidase, nor in untreated mutant animals (n = 12). Expression was variable within and between animals. Both in vivo and in vitro measurements of ion transport showed that gene therapy produced restoration of approximately 50% of the deficit in cAMP mediated chloride secretion between mutant and wild type mice. Some animals showed complete correction whilst others demonstrated little improvement. Calcium mediated chloride secretion does not differ between mutant and wild type animals and was not altered following treatment. We conclude that complete correction of the characteristic electrophysiological defect of CF can be achieved by non-invasive gene delivery. However, some of the difficulties encountered in this study may be relevant to recently started trials of gene therapy in CF patients.

Chloride impermeability in the airways of males with congenital bilateral absence of the vas deferens

PG MIDDLETON, LR OSBORNE, GK SANTIS, DM GEDDES, EWFW ALTON *Ion Transport Laboratory and Department of Cystic Fibrosis, National Heart and Lung Institute, London* Congenital bilateral absence of the vas deferens (CBAVD), found in almost all males with cystic fibrosis (CF), accounts for approximately 20% of infertility in non-CF males. It has been proposed that CBAVD may represent a mild form of CF, with no other phenotypic features of the disease. Recently Dumar *et al.* (*Lancet* 1990;**336**:512) found an increased incidence of CF mutations in a group of males with CBAVD, including some with previously undiagnosed CF (compound heterozygotes). We report the nasal bioelectric properties, sweat tests, and genotypes of a group of nine subjects with CBAVD: two had elevated sweat electrolytes and nasal potential difference (NPD), fulfilling the diagnostic criteria of CF; one (genotype ΔF508/R347H) had a history of recurrent chest infections, whilst the other (ΔF508/unknown) was asymptomatic. The other seven subjects included five who were heterozygous for CF mutations (one R553X/-, four ΔF508/-) and two who had no CF mutations. Baseline measurements of NPD in these seven subjects were compared with those following superfusion with amiloride (1 mM) in Krebs Henseleit solution and a low chloride (5·5 mM) equivalent. Mean (SE) results (as absolutes) were compared with controls (n = 9), CF subjects (CF/CF n = 9), and CF heterozygotes (+/CF n = 5) (table). We conclude that this group of patients appears to have isolated airway chloride impermeability, yet normal sodium transport, differentiating them from subjects with CF.

	Controls	*CF/CF*	*CBAVD*	*+/CF*
Baseline	14·8 (1·2)	38·2 (1·3)*†	17·4 (1·4)	14·6 (1·7)
Δ Amiloride	−6·2 (0·4)	−21·7 (1·6)*†	−5·4 (1·2)	−6·1 (0·4)
Δ Low Cl^-	9·3 (1·4)†	−1·7 (0·6)*†	1·7 (1·0)*	7·3 (0·8)†

*$p < 0.05$ *v* controls; †$p < 0.05$ *v* CBAVD.

BTS study of cryptogenic fibrosing alveolitis (CFA): clinical features at presentation

IDA JOHNSTON, RM RUDD on behalf of the BTS Research Committee *University Hospital, Nottingham and London Chest Hospital, London* Mortality ascribed to CFA is increasing, but little is known about the current presentation and natural history of the disease. All BTS consultant members in England, Wales and Scotland were invited to participate in the present study in which new patients presenting with CFA between December 1990 and November 1992 would be entered and followed up for up to six years. In all, 320 physicians agreed, and 150 actually entered patients. A total of 601 patients with CFA, defined on standard clinical criteria, were entered over the two years. Mean (SD) age was 67·4 (10·2) years (range 27–100); 381 (63%) were male and 582 (97%) were of white ethnic group; 75% of patients were primary referrals from General Practice. Physicians considered the diagnosis definite at presentation in 55%,

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but an alternative diagnosis has emerged in only 11 additional patients entered but excluded from current analysis. Mean length of symptoms before presentation was 15 months, with an acute onset in 19% and gradual in 76%. Presenting symptoms were: breathlessness 89% (35% were breathless on minimal exertion or at rest), cough (75%), arthralgia (12%), and 5% were asymptomatic; 20% were current and 57% ex-smokers. Ninety (15%) were noted to have had some asbestos exposure (in 70 this was graded as light). Finger clubbing was present in 49%. This is the largest group of CFA patients reported. Patients were much older than in earlier surveys, but otherwise clinical features at presentation were broadly similar to previous reports.

BTS study of cryptogenic fibrosing alveolitis (CFA): investigations, lung function and treatment at presentation

IDA JOHNSTON, RM RUDD on behalf of the BTS Research Committee *University Hospital, Nottingham and London Chest Hospital, London* Between December 1990 and November 1992 601 patients with CFA, defined on standard clinical criteria, were entered into the BTS study of CFA. Clinical details at presentation are presented in an accompanying abstract. Mean (SD) haemoglobin was 14·2 (1·6) g/dl, WCC 9·9 (8·9) $\times 10^9$/l, and ESR 32 (23) mm/h. Rheumatoid factor was positive in 93/494 (19%) patients and antinuclear factor in 124/491 (25%). Chest radiography at entry was described as normal (3%), fine nodular/short linear (50%), ground glass (5%), patchy/confluent (26%), honeycomb (15%). Lung function as mean percent predicted (SD) was: FEV (n=564), 79·4 (24·7); FVC (n=564), 79·0 (25·3); TLC (n=416), 72·5 (21·0); TLCO (n=441), 57·5 (41·6); KCO (n=427), 67·8 (40·0). Transbronchial biopsy had been performed at patient entry or was planned in 165 (28%), open lung biopsy in 75 (12%), and BAL in 68 (11%). In 364 (61%) patients no biopsy procedure or BAL had been performed at entry, and were not planned. Initial management plan was observation in 293 (49%), initiation of treatment in 266 (44%), and change in existing treatment in 42 (7%). Lung volumes were relatively well preserved at presentation but gas transfer was substantially reduced. Participating physicians considered histological information unnecessary or inappropriate in the majority of cases, however, and were prepared to observe up to half the patients at entry.

Pulmonary function tests (PFT) and thin section computed tomography (CT) in lone cryptogenic fibrosing alveolitis (CFA): a functional morphological correlation

AU WELLS, AD KING, MB RUBENS, RM DU BOIS, DM HANSELL *Royal Brompton National Heart and Lung Hospital, London* In lone CFA, functional indices are a more accurate reflection of the underlying disease process than are symptoms or chest radiographic findings. However, it is unclear which lung function parameter is most indicative of disease severity. CT provides a reproducible method of quantifying the morphological extent of fibrosing alveolitis. The aim of this study was to examine the relationships between extent of disease and a number of functional indices which are used in clinical practice. Seventy six patients with CFA underwent concurrent CT scans and PFT, including measures of lung volume, gas exchange at rest, and exercise desaturation. Twelve patients with emphysema on CT were excluded. CT sections (3 mm/10 mm) were acquired throughout the lungs and scored by two radiologists. Visual estimates were made of the percentage of abnormal lung (to the nearest 5%) at five levels defined by anatomical landmarks; these figures were combined to give the overall percentage of abnormal lung. There were significant correlations between the overall extent of disease and all lung function indices. The percentage predicted TLCO was more closely related to extent of disease on CT ($r=0·69$, $p<0·005$) than other functional parameters (r values: $FEV_1=0·28$; FVC=0·39; TLC=0·43; $FEV_1/FVC=0·38$; KCO=0·38; $Pao_2=0·40$; alveolar-arterial oxygen gradient=0·42; desaturation on exercise=0·63). We conclude that the carbon monoxide diffusing capacity is the functional parameter most indicative of disease extent in CFA: measurement of the TLCO should be part of routine assessment in fibrosing alveolitis.

Functional impairment in fibrosing alveolitis after matching for CT findings, demographic factors and smoking: a comparison between cryptogenic fibrosing alveolitis and the fibrosing alveolitis of systemic sclerosis

AU WELLS, AD KING, MB RUBENS, CM BLACK, DM HANSELL, RM DU BOIS *Royal Brompton National Heart and Lung Hospital, London* Lone cryptogenic fibrosing alveolitis (CFA) and fibrosing alveolitis associated with systemic sclerosis (FASS) are indistinguishable, histologically and on lung CT. However, survival analyses indicate that there are differences in the biological behaviour of the two entities. CT provides a method to match morphological abnormalities in the two diseases and compare functional impairment. Patients with emphysema on CT or primary pulmonary vascular disease were excluded. 123 patients (CFA, n=64; FASS, n=59) underwent CT concurrently with full lung function tests, including blood gases; 82/123 completed formal exercise testing. Three mm CT sections were scored by two observers for disease extent (percentage of abnormal lung) and pattern (a ground glass pattern predominates, mixed appearances, a reticular pattern predominates). A multivariate linear regression model was used to identify whether age, sex, smoking history, disease extent on CT, CT pattern, and the type of fibrosing alveolitis were independent determinants of the level of functional impairment. After matching for other factors, gas exchange was more impaired in CFA than in FASS (TLCO, $p=0·032$; resting Pao_2, $p<0·001$; alveolar-arterial O_2 gradient, $p<0·001$; desaturation on exercise, $p=0·013$). There was no significant difference in lung volumes between the two diseases. In conclusion, there are marked differences in indices of gas exchange between CFA and FASS indicative of a fundamental pathophysiological difference between the diseases.

Endogenous nitric oxide is increased in the exhaled air of asthmatic patients

S KHARITONOV, D YATES, R ROBBINS, E SHINEBOURNE, P BARNES *Department of Thoracic Medicine, National Heart and Lung Institute, London* Nitric oxide (NO) is a gas produced by a variety of cells within the lower respiratory tract, including inflammatory and epithelial cells, and has been previously detected in the exhaled air of humans. Asthma is a chronic inflammatory disease of unknown aetiology. We hypothesised that exhaled NO levels would be higher in asthmatics compared with normal controls. NO was measured reproducibly using a slow vital capacity manoeuvre and a specially adapted chemiluminescence analyser in 64 normal subjects and 58 asthmatics. NO was detectable in all control subjects (mean peak expired NO concentration 80(4) ppb). Exhaled NO was significantly reduced by inhalation of a specific NO synthase inhibitor. Asthmatic subjects not receiving corticosteroids had significantly higher peak expired NO concentrations than controls (mean 300(27) ppb, n=22, $p<0·001$). However, asthmatic patients receiving inhaled corticosteroids did not differ significantly from normal control subjects (mean 104(11) ppb, n=36). Elevated exhaled NO concentrations in asthmatic patients may reflect induction of NO synthase which is known to be inhibited by steroids. Measurement of exhaled NO concentrations may have a clinical use in the detection and management of inflammatory lung disorders.

Activation of the renin-angiotensin system by nebulised β_2 agonists

EA MILLAR, RM ANGUS, NC THOMSON *Department of Respiratory Medicine, Western Infirmary, Glasgow* We have previously demonstrated activation of the renin-angiotensin system (RAS) in acute severe asthma. The stimulus to this is unclear but high dose β_2 agonists used in the treatment of asthma could be responsible, since β adrenoceptors are known to be present on juxtaglomerular cells. In a randomised, double blind study, we have examined the effect of nebulised fenoterol, salbutamol and placebo on the activity of the RAS in a group of eight mild asthmatics, mean (SD) age 38 (8·4) years, FEV_1 82 (15·2)% predicted, and eight normal subjects, mean age 34 (7·6) years. Following a 20 minute rest, a baseline blood sample was withdrawn for estimation of plasma renin, angiotensin II (AII) and electrolytes. Patients then received either fenoterol (2·5 mg), salbutamol (5 mg), or placebo by nebuliser and remained supine for the remainder of the study. The above measurements were repeated at intervals of 15, 30, 60 and 120 mins after nebulisation. There was no significant difference between baseline values of plasma renin and AII on either study day or between the asthmatic patients and normal subjects. In the asthmatic patients, mean (SE) baseline renin was 24·2 (4·4), 31·5 (8·4) and 30·4 (7·5) μU/ml, and AII 19·1 (8·3), 8·6 (1·1) and 8·1 (3·5) pg/ml prior to inhalation of fenoterol, salbutamol and placebo respectively. In the normal subjects mean (SE) baseline renin was 32·8 (6·1), 29·2 (6·3) and 26·5 (6·7) μU/ml, and AII 11·0 (2·6), 11·5 (3·3) and 13·1 (3·9) prior to fenoterol, salbutamol and placebo respectively. Plasma renin levels were significantly elevated in the asthmatic patients after both salbutamol and fenoterol at 120 mins

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when compared with placebo (mean (SE) absolute change in plasma renin of 21·2 (12·6) and 34·6 (16) mU/ml respectively; $p<0.05$ *v* placebo). In the asthmatic group fenoterol caused a significant elevation of AII after 15 mins (mean (SE) absolute change in AII of 13·8 (19·1) pg/ml; $p<0.05$ *v* placebo). In the normal subjects AII levels were significantly elevated at 30 and 60 minutes after salbutamol but not fenoterol (mean (SE) absolute change in AII of 12·7 (10·5) and 12·6 (10·3) pg/ml; $p<0.05$ *v* placebo). We conclude that nebulised β_2 agonists activate the RAS in asthmatic patients and normal subjects. [EM was supported by the National Asthma Campaign.]

Bronchodilator subsensitivity after chronic dosing with eformoterol in patients with asthma

DM NEWNHAM, DG MCDEVITT, BJ LIPWORTH *Department of Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, Dundee* In vivo and in vitro β_2 adrenoceptor responsiveness was evaluated after chronic inhaled therapy with the long acting β_2 agonist eformoterol or placebo in patients with stable asthma. After a two week run in period without β_2 agonist therapy, seven patients age 34 (5) years, FEV_1 58 (5)% predicted (1·90 (0·27) l), were randomised to receive regular treatment with eformoterol (E) 24 µg twice daily or placebo (P) twice daily for four weeks. Dose–response curves (DRC) to eformoterol in cumulative doses of 6 µg, 30 µg (6 µg + 24 µg), 78 µg (30 µg + 48 µg), and 126 µg (78 µg + 48 µg) were constructed at the end of each treatment period with increments given every 50 minutes. Measurements of airways and systemic β_2 responses were performed at baseline, 30 minutes after each dose, and for six hours after the last dose. In addition, in vitro parameters of lymphocyte β_2 receptor function were evaluated prior to the DRC after each treatment period. Values are shown as means and 95% CI for the difference between E and P (as change from baseline). Baseline values were not significantly different between the two treatment periods except for finger tremor. The peak bronchodilator response from the DRC and the response six hours after the last dose were both significantly attenuated after chronic dosing with E compared with P. The values for FEV_1 were: peak 0·26 (0·09, 0·43) l, $p<0.05$; and six hours 0·39 (0·20, 0·58) l, $p<0.005$. Systemic β_2 responses and in vitro parameters of lymphocyte β_2 receptor function were also significantly blunted after E compared with P. In conclusion, chronic therapy with eformoterol produces (i) tachyphylaxis to its bronchodilator response both in terms of peak response and response six hours after the last dose; (ii) tachyphylaxis of extrapulmonary β_2 mediated responses; and (iii) subsensitivity of in vitro lymphocyte β_2 adrenoceptor function.

Withdrawing chronic theophylline causes immunomodulation in the blood of asthmatic patients

JC KIDNEY, M DOMINGUEZ, M ROSE, S AIKMAN, KF CHUNG, PJ BARNES *Department of Thoracic Medicine, National Heart and Lung Institute, London; Department of Immunology, Harefield Hospital, Uxbridge* Theophylline may have immune modulating properties. Recently Ward *et al* (*Am Rev Respir Dis* 1993) showed that chronic treatment attenuates the late asthmatic response to allergen and the associated T cell changes in the blood. The aim of our study was to see if there were immunological changes in the blood after withdrawing theophylline and to determine the effect of drug levels. The current plasma levels recommended >5 mg/l. In a double blind study we withdrew chronic theophylline therapy (>6 months). Subjects were seen every two weeks for six weeks. After a run in of two weeks theophylline was substituted by placebo in a double blind manner during either of the remaining two two-week periods. A diary card recorded PEF three times daily, symptoms, and agonist use. FEV_1 was measured at each visit. Blood was taken for determination of theophylline levels and inflammatory indices by flow cytometry CD4, CD8 (dual stained HLA-DR and CD25), and EG2. Twenty five asthmatic (16 atopic) subjects (age 46·8 years, range 25–70) completed the study. All were taking maintenance inhaled steroids (mean 1548 µg daily), three were taking maintenance oral steroids (5, 7·5 and 10 mg). Nocturnal symptoms and diurnal variation of PEF increased on placebo. The FEV_1 was 2·2 (0·18) l which fell to 2·01 (0·15) l ($p<0.01$) after placebo. Levels of activated T cells were high in all patients on theophylline: CD4/DR was 6·59 (0·8)%, CD4/CD25 was 18·3 (1·45)%, CD8/DR was 12·06 (2·04)%, CD8/CD25 was 3·75 (0·63)%. On placebo CD8/DR fell to 10·09 (1·53)%, ($p<0.05$), CD4/CD25 fell 15·8% where level of theophylline was >5 mg/l ($p<0.01$, $n=19$). The monocytes fell from 7·79 (0·6)% to 7·04 (0·43)% ($p<0.05$) in the whole group. When chronic theophylline treatment is withdrawn there is a deterioration in asthma and an alteration in inflammatory cells in the blood.

Withdrawing chronic theophylline treatment increases airway lymphocytes in asthma

JC KIDNEY, M DOMINGUEZ, P TAYLOR, M ROSE, S AIKMAN, KF CHUNG, P BARNES *Department of Thoracic Medicine, National Heart and Lung Institute, London; Department of Immunology, Harefield Hospital, Uxbridge* We have recently shown that when chronic theophylline treatment is withdrawn there are increased airway symptoms, a fall in FEV_1, and modulation of activated inflammatory cells in the blood (Kidney *et al. BTS* December 1993). The pattern of inflammatory cell change suggested recruitment of lymphocytes into the airway. Our hypothesis was that theophylline affects airway lymphocytes. The aim of this study was to determine if withdrawal of chronic theophylline treatment increased airway lymphocytes. Subjects who had taken part in our previous study were enrolled into a single blind placebo substitution of chronic theophylline treatment. At the end of each two week treatment period subjects underwent fibreoptic bronchoscopy and bronchial biopsy. In the first two weeks chronic theophylline treatment was continued, in the second two weeks placebo was substituted. Airway biopsies were stained for T lymphocytes (CD3), helper/inducer T cells (CD4), cytotoxic/suppressor (CD8), monocyte/macrophage series (CD14), IL-2 receptor (CD25) and EG2, a marker of cleaved ECP. Eight subjects were enrolled into the study. All subjects were taking inhaled corticosteroids (mean (SE) dose 1537 (134·9)). The mean FEV_1 was 2·16 (0·13) l which fell to 1·77 (0·1) l ($p<0.01$) after placebo. The mean theophylline level was 7·69 (0·8) before and undetectable after placebo. The number of CD8 cells per 0·25 mm of submucosa was 5·0 (1·25) on theophylline and 8·88 (2·3) after placebo ($p<0.05$). The number of CD4 cells was 10·38 (3·1) on theophylline and 17·25 (5·2) after placebo ($p>0.05$). In the airway epithelium the number of CD4 cells per 100 epithelial cells was 1·5 (0·5) on theophylline and 4·25 (1·0) on placebo ($p<0.05$), whereas the number of CD8 cells was 3·1 (1·2) on theophylline and 4·3 (0·9) on placebo ($p>0.05$). There were no significant changes in CD14 and CD25. When chronic theophylline treatment is withdrawn there is a deterioration in asthma and a significant increase in T lymphocytes in the airway.

Cytokine generation by blood peripheral monocytes from patients with corticosteroid sensitive (CS) and resistant (CR) chronic bronchial asthma

SP MATUSIEWICZ, M GORDON, AP GREENING *Respiratory Unit, Western General Hospital, Edinburgh* Cytokine release by monocytes from patients with chronic severe asthma, spontaneously and with lipopolysaccharide (LPS) stimulation, was studied together with the effects of corticosteroids (dexamethasone (Dex)). We obtained blood from 30 asthmatics with chronic symptoms despite >800 µg inhaled steroid/day; 11 CR (as defined by failure of FEV_1 or mean peak flow to improve by >15% following two weeks' prednisolone despite >15% responses to β agonist (*Am Rev Respir Dis* 1991;**144**:1016)), 19 CS, and 20 normal controls. Monocytes (Mo) were purified by density centrifugation and adherence. Cells were cultured in RPMI 1640 plus 5% fetal calf serum for 20 h ± LPS (100 ng/ml) ± Dex 10^{-7} M. Cell free supernatants were stored at −80°C until batch assay of TNFα, GM-CSF, IL-1β and IL-6 by ELISA, expressed as mean (SE) values, ng/10^6 Mo. Group mean data were analysed by ANOVA, Bonferroni and *t* tests (table). The results showed that CS monocytes spontaneously release less TNFα and IL-1β than normal subjects, but no differences were seen for GM-CSF or IL-6. Responses to dexamethasone were not the same for each cytokine.

	Normal	*Asthma (CS)*	*Asthma (CR)*
TNFα			
LPS 0	7·7 (1·3)	4·4 (0·6)*	4·1 (0·6)
LPS 0 + Dex	4·7 (0·8)	2·3 (0·5)	2·8 (0·6)
LPS 100	22 (3·4)	21 (4)	14 (3·3)
LPS 100 + Dex	14 (2)	11 (2·2)	6·6 (1·6)
IL-1β			
LPS 0	9·6 (2·3)	3·9 (0·8)*	5·2 (1·2)
LPS 0 + Dex	6·6 (1·6)	2·8 (1·6)	5·4 (1·2)
LPS 100	39·5 (7)	24·9(6·2)	29 (7·1)
LPS 100 + Dex	38·5 (6·3)	27·1 (7·3)	18·4 (3·9)

*$p<0.05$ *v* normal

Acute lung injury in fulminant hepatic failure following paracetamol poisoning

SV BAUDOUIN, P HOWDLE, JG O'GRADY, NR WEBSTER *Intensive Care and Liver Units, St James' University Hospital, Leeds* There is little information on the incidence of acute lung injury or changes in the

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pulmonary circulation in fulminant hepatic failure. We have retrospectively assessed the incidence of acute lung injury in fulminant hepatic failure caused by paracetamol poisoning and documented associated pulmonary circulatory changes. The degree of lung injury was assessed by a standard scoring system (Murray *Am Rev Respir Dis* 1988;**138**:720–3) on all patients with paracetamol induced fulminant hepatic failure, admitted to the intensive care unit over a one year period. Twenty four patients with paracetamol induced liver failure were admitted and nine (33%) developed lung injury of whom eight had severe injury (Murray score >2·5). Patients with lung injury had higher mean encephalopathy grades (3·4 *v* 2·5 in the non-injured group) and APACHE II scores (30 *v* 20). Circulatory failure, requiring vasoconstrictor support, occurred in all patients with lung injury but in only 40% of those without. Cerebral oedema, as detected by abnormal rises in intracranial pressure, also occurred in all patients with lung injury but in only 27% of the non-injured patients. The incidence of renal failure requiring renal replacement therapy was similar in both groups (67% and 54%). Pulmonary artery occlusion pressures were normal in the lung injury group. Cardiac output was high (mean 12·8 l/min), systemic vascular resistance low (mean 471 dynes/s/cm^{-5}), and pulmonary vascular resistance low (mean 68 dynes/s/cm^{-5}). Mortality was much higher in the lung injury group than in the non-injured group (89% *v* 13%). Acute lung injury is common in patients with paracetamol induced fulminant hepatic failure and is associated with systemic circulatory failure and cerebral oedema. The development of acute lung injury was associated with high mortality.

Altered levels of soluble L-selectin adhesion receptor in plasma is correlated with development of the adult respiratory distress syndrome in at risk patient groups

SC DONNELLY, C HASLETT, I DRANSFIELD, CE ROBERTSON, DC CARTER, JA ROSS, IS GRANT, TF TEDDER *Respiratory Medicine Unit, University of Edinburgh; Department of Accident and Emergency, Royal Infirmary, Edinburgh; Department of Surgery, Royal Infirmary, Edinburgh; Department of Intensive Therapy Unit, Western General Hospital, Edinburgh; Division of Tumor Immunology, Harvard Medical School, Boston, Massachusetts, USA* The adult respiratory distress syndrome (ARDS) represents a catastrophic form of acute lung injury, the early pathogenesis of which is characterised by inflammatory cell accumulation and activation in the lung. Soluble L-selectin (sL-selectin) is a functional circulating adhesion receptor shed from the cell surface of circulating leucocytes following activation. Since sL-selectin serves an important role in the control of neutrophil/endothelial interactions, we investigated whether plasma sL-selectin levels in at risk patients were associated with subsequent ARDS development. Eighty two patients at risk of ARDS were enrolled from three well defined groups (multiple trauma, pancreatitis, perforated bowel). Plasma samples were obtained on hospital presentation and sL-selectin was quantified using a sandwich ELISA. Fourteen patients subsequently developed ARDS. Initial plasma sL-selectin levels were significantly lower in those patients who progressed to ARDS than in those who did not ($p=0·0001$). In addition, a significant correlation was found between low values of sL-selectin and indices of subsequent lung injury including requirement for ventilation ($p=0·0001$) and lowest documented Pao_2/Fio_2 ratio ($p=0·004$). A significant correlation was also found between low values of sL-selectin and patient mortality ($p=0·001$). Measurement of circulating sL-selectin in patients at risk for ARDS on initial hospital presentation is of prognostic significance, with low levels being closely associated with the development of ARDS and mortality. These results also further our understanding of neutrophil/endothelial interactions in the early stages of ARDS pathogenesis.

Pulmonary endothelial permeability in patients with severe lung injury

DG SINCLAIR, S BRAUDE, PL HASLAM, TW EVANS *AICU, Royal Brompton National Heart and Lung Hospital, London* The pathophysiology of the adult respiratory distress (ARDS) syndrome remains unclear. We studied 11 patients meeting accepted diagnostic criteria for ARDS to further examine this process. Pulmonary capillary permeability was measured on up to three occasions throughout the clinical course. A double isotope technique (*Nucl Med Commun* 1990;**11**:879–84) was used which allows the lung extravascular protein accumulation index (PAI) to be calculated. On each occasion that PAI was measured the lung injury score (LIS) was calculated (*Am Rev Respir Dis* 1988;**138**:720–3). Bronchoalveolar lavage (BAL) was performed once with three 60 ml aliquots of buffered normal saline following a PAI measurement. Cell counts were undertaken, neutrophil content being expressed as a percentage of the total cells recovered. The mean (SE) PAI (normal range $0–1 \times 10^{-3}$) was 2·84 (0·44), 3·14 (0·57), and 2·95 (1·05) $\times 10^{-3}$. Corresponding LIS values were 2·12 (0·27), 2·54 (0·15), and 2·08 (0·3). BAL neutrophil content was 53·4 (10·5)%. The correlation between various parameters is as follows: PAI and LIS, $r=0·71$, $p<0·001$; PAI and BAL neutrophils, $r=0·81$, $p<0·01$. These data suggest that increased pulmonary vascular permeability persists throughout the course of ARDS and is related to a clinical score of lung injury severity and BAL neutrophil content. [This work was supported by the British Lung Foundation.]

Role of TNF in the increased airspace epithelial permeability in acute lung inflammation

XY LI, K DONALDSON, W MACNEE *Respiratory Medicine Unit, University of Edinburgh; Napier University, Edinburgh* Lung inflammation is characterised by an increase in the permeability of the airspace epithelial barrier. We studied the mechanisms of the increased epithelial permeability in an acute neutrophil alveolitis induced by intratracheal (IT) instillation of the heat killed bacterium *C parvum* (CP) in rat lungs. Whereas the peak neutrophil influx into the airspaces occurred 16 hours after CP (control $0·06 \times 10^6$, CP-16 h $34·40 \times 10^6$), the maximum permeability occurred at 16 hours (1·74 times control values) measured by the clearance of IT ^{125}I-BSA. Reducing both the neutrophil and macrophage influx into the airspaces prevented the increase in epithelial permeability produced by CP. Reinstillation of neutrophil and macrophage enriched cell populations showed that macrophages, rather than neutrophils, accounted for the increased epithelial permeability. There was no evidence of a protease mediated mechanism for the increased epithelial permeability in this inflammatory model, as shown by the similarity of airspace leucocytes from 8 and 16 hour CP treated rats, to degrade a fibronectin matrix in vitro and the high functional antiprotease activity in bronchoalveolar lavage at both time points. Furthermore, reducing lung GSH, a major antioxidant, to 52% of control values with buthionine sulphoximine did not enhance the increased epithelial permeability induced by CP. However, a role for TNF was supported by (1) increased TNF in the airspaces eight hours but not 16 hours after CP; (2) the greatest TNF release by alveolar macrophages eight hours after CP; (3) the inhibition of epithelial permeability in vivo by TNF-antibody; (4) the induction of permeability of an airspace epithelial cell monolayer in vitro by TNF. [Supported by the Norman Salvesen Emphysema Research Trust.]

Interleukin 5 and interleukin 8 gene expression: key cytokines in fibrosing alveolotis

AM SOUTHCOTT, CM GELDER, P PANTELIDIS, M PETREK, JFJ MORRISON, RM DU BOIS *Royal Brompton Hospital, London; Leeds General Infirmary, Leeds* Fibrosing alveolitis (FA) is characterised by the accumulation of inflammatory cells within the lower respiratory tract. The mechanisms by which these cells migrate to the lungs include complex interactions between cytokines and expression of cellular adhesion molecules. We aimed to investigate the profile of cytokines relevant to inflammatory cell traffic in FA. We assessed cytokine expression by lavage (BAL) cells using reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from BAL cells in nine patients with FA (five cryptogenic, four systemic sclerosis) and from six normal subjects. Oligonucleotide primer pairs specific for cytokines in three functional groups were used. Group I = cytokines released during the initial response to injury/endotoxin; initiate cytokine cascade and enhance adhesion molecule expression (interleukins 1 and 6 (IL-1, IL-6) and tumour necrosis factor α (TNF-α)). Group II = granulocyte chemotaxins (interleukins 5 (IL-5) (eosinophils) and 8 (IL-8) (neutrophils)). Group III = β chemokines; chemotaxins for T lymphocytes [RANTES (R)] and monocytes/macrophages (macrophage inflammatory proteins 1α and 1β (M1α and M1β), monocyte chemoattractant protein 1 (MCP-1)). Thus group I and group III cytokines are present in normal and diseased lung reflecting the important immune surveillance role of the lung. The striking difference between the normal and FA groups was the expression of the granulocyte chemotaxins in FA. We therefore conclude that IL-5 and IL-8 are critical determinants of the development of fibrosing lung disease, presumably through mechanisms involving granulocyte induced injury.

	Number expressing PCR product								
	IL-1	*IL-6*	*TNF-α*	*IL-5*	*IL-8*	*R*	*M1α*	*M1β*	*MCP*
Normal (n=6)	5	2	6	2	0	6	6	5	5
FA (n=9)	4	2	8	6	4	9	8	7	9

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Inability to produce IgG_4 in the lungs in sarcoidosis is modified by treatment

HJ MILBURN, GM COCHRANE, M KEMENY *Departments of Thoracic Medicine and Allergy, Guy's Hospital, London* IgG_4 is locally produced in normal lungs but its function in the lower respiratory tract is unclear. It appears to be involved in the normal response to chronic antigen stimulation and may have a blocking rather than a sensitising role. A deficiency of IgG_4 may therefore be a factor in the pathogenesis of disorders associated with uncontrolled inflammation. In this study we have measured levels of the four subclasses of IgG by radial immunodiffusion in serum and bronchoalveolar lavage fluid (BAL) from 15 patients with untreated cryptogenic fibrosing alveolitis (CFA), 14 patients with untreated sarcoidosis, and five normal non-smoking volunteers. Measurements were repeated in six sarcoid patients after 6–12 weeks' treatment with oral corticosteroids. Albumin was used to correct for serum leakage. Results are given as mean (SE) corrected BAL/serum ratios. All normals showed significant local production of IgG_4 (76·8 (19·2)) and some production of IgG_1 (3.1 (2)) in the lungs. IgG_2 could be detected in BAL but there was no evidence for local production and IgG_3 could not be detected in normal BAL. In CFA and sarcoid IgG_1 and IgG_2 levels were similar to normals. IgG_4 could be detected in only 7/15 patients with CFA (8·3 (5), $p<0·01$) and 5/14 patients with sarcoid before treatment (0·12 (0·07), $p<0·001$) but in all six of those on steroids (3·16 (2·5)). Although still significantly lower than in normals, this shows an increase over pretreatment levels ($p<0·01$). Furthermore, IgG_3 could be detected in BAL of 12 untreated patients (CFA 1·9 (0·9), sarcoid 0·58 (0·19)), but only in one patient after starting treatment (0·13). This study demonstrates disordered local immunity in the lungs of patients with untreated interstitial lung disease and some improvement on treatment. Reduced local production of IgG_4 may be a factor in the uncontrolled inflammatory response found in these disorders.

Effect of weal diameter on definition of atopy

JF MILES, RM CAYTON, JG AYRES *Chest Research Institute, Birmingham Heartlands Hospital, Birmingham* Definitions of atopy involve the presence of at least one positive skin test to an identified allergen. However, the definition of "positivity" remains contentious with weal diameters 1–4 mm greater than that of a negative control, in the presence of a positive weal to histamine solution, being cited as "diagnostic". We have assessed the effects of such differing definitions on the categorisation of atopy in 29 pairs of well characterised brittle and non-brittle asthmatics (18F, mean age 45·8 (range 17–62) years). Each patient was tested to 19 common allergens, the mean two diameters at right angles being measured 15 minutes after prick testing. No differences were seen between the two groups between the numbers classed as atopic, but there was significant within group variation in patients with non-brittle asthma (table). The extent or severity of atopy as measured by numbers of positive skin tests, however, significantly varied between brittle and non-brittle asthma, at atopy definitions of 2 mm and below. These results highlight the importance for adhesion to a unified definition or classification of atopy in order to allow meaningful comparisons between studies. [JM was a West Midlands RHA Sheldon Research Fellow.]

	Diameter for definition of "positivity"			
	4 mm	*3 mm*	*2 mm*	*1 mm*
Skin tests				
Brittle	4·07	6·69	9·48	11·76
Non-brittle	2·10	3·62	6·00	8·76
Wilcoxon Z score	−1·9	−1·8	−2·1	−2·4
p value	0·07	0·07	0·03	0·02
No. of atopic patients				
Brittle ($\chi^2=5·52$; $p=0·14$)	21	24	26	27
Non-brittle ($\chi^2=7·54$; $p=0·05$)	19	22	26	26

Age and exercise induced bronchospasm in asthmatic children

A CUSTOVIC, N ARIFHODZIC, SCO TAGGART, A WOODCOCK *University Childrens Hospital, Sarajevo, Bosnia and Herzegovina; North-West Lung Centre, Wythenshawe Hospital, Manchester* Pulmonary function was assessed before and five minutes following six minutes of free running exercise in a group of 48 normal and 70 children with mild/moderate atopic asthma who were divided into five age groups (I: 6–7 years, II: 8–9 years, III: 10–11 years, IV: 12–13 years, and V: 14–15 years). Exercise induced bronchospasm (EIB) (defined as normal group mean − 2SD) occurred with >10% fall in forced expiratory volume in one second (FEV_1), >17·5% fall in peak expiratory flow rate (PEFR), >26% in mid expiratory flow rate (FEF_{25-75}), and >40% in forced expiratory flow at 25% of VC (FEF_{25}). Sixty three of 70 patients had EIB by at least one of the definitions, and combination of FEV_1 and FEF_{25-75} criteria enabled detection of all subjects with EIB. There was no difference in baseline lung functions between different age groups, and the fall in lung functions after exercise (expressed as a percentage of baseline values) was as shown in the table. There was no difference in EIB in boys compared with girls, but EIB decreased significantly with age. EIB should be defined by more than one flow-volume curve parameter (FEV_1 and FEF_{25-75}).

Group	*No.*	*FEV_1*	*PEFR*	*FEF_{25-75}*	*FEF_{25}*	*% of responders*
I	13	68·9*	66·2	47·0	48·1	100
II	15	67·8†	71·8	46·1	42·1	100
III	15	69·5*	64·5	48·5	45·8	100
IV	15	77·0	75·8	60·1	62·1	80
V	12	82·2*†	84·4	72·4	68·3	66·7

* $p<0·05$; † $p=0·02$.

Prevention of exercise induced bronchoconstriction by inhaled frusemide in children

PF MUNYARD, KF CHUNG, A BUSH *Royal Brompton National Heart and Lung Hospital, London* Nebulised frusemide has been shown to prevent bronchoconstriction induced by exercise in asthmatic adults. To determine if inhaled frusemide (40 mg) from a metered dose inhaler (MDI) and spacer had a similar effect in children we conducted a randomised double blind placebo controlled crossover trial in 12 children (aged 7–14) years (8M, 4F), with standardised exercise testing. Patients had long standing asthma and complained of exercise induced symptoms. All were on inhaled prophylactic therapy (one cromoglycate, eleven steroids). Cromoglycate was withheld for 24 hours, oral β_2 agonists for 12 hours, inhaled β_2 agonists for six hours, inhaled steroids were continued; no patient was receiving oral steroids or salmeterol. All were clinically stable and free from symptoms of respiratory infection for one month. Exercise testing was performed three times (control, placebo, and frusemide in random order) at the same time of day within a seven day period on a treadmill for eight minutes until 85% of age predicted maximum heart rate was reached. A portable Vitalograph was used to record lung function at baseline, during exercise, and at 0, 2, 4, 6, 8, 10, 15, 20, and 30 minutes after exercise. Baseline lung function was the same on all study days. The mean (95% CI) maximal % falls in FEV_1 were: control 15·4 (7·5–23·3); placebo 14·4 (7·7–21·0); frusemide, 5·7 (2·3–9·0). There was a significant reduction with frusemide (paired *t* test, $p<0·01$). There were no adverse effects. Frusemide via MDI merits further evaluation in children with exercise induced asthma.

Effect of inhaled beclomethasone dipropionate on overnight urinary cortisol in prepubertal mildly asthmatic children

IJM DOULL, P WOOD, NJ FREEZER, ST HOLGATE *University Medicine, Centre Block, Southampton General Hospital, Southampton* We investigated the effect of inhaled corticosteroids on linear growth and overnight urinary cortisol, a sensitive marker of adrenal function, in 104 prepubertal children aged 7–9 years with mild to moderate asthma and not using either oral or inhaled corticosteroids. The children were randomised to receive in a double blind manner either beclomethasone dipropionate (BDP) 200 µg twice daily or placebo via Diskhaler. After a run in period of at least two weeks children received treatment for seven months followed by a washout period of five months. Overnight urine samples were collected prior to randomisation and after three and six months of treatment. Urinary cortisol was measured by radioimmunoassay and urinary creatinine by conventional methods. Overnight urinary cortisol production was expressed as rate of cortisol production (nmol/hour) and urinary cortisol/creatinine (COR/CR) ratio (nmol/mmol creatinine) and log transformed to normalise distribution. Eighty four children completed the study. We have previously shown that growth during the treatment period in the BDP group was significantly less than the placebo group (0·112 mm/day *v* 0·163 mm/day, $p<0·0001$) (*Am Rev Respir Dis* 1993;**147**:A265). There were no significant differences in baseline demographic data between the BDP and placebo groups. There were no significant differences between the BDP and placebo groups in cortisol production at any of the three time points (baseline 80·5 *v*

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88·9 nmol/hour, three months 82·6 *v* 74 nmol/hour, six months 67·7 *v* 77·1 nmol/hour), or in COR/CR at any of the three time points (baseline 10·3 *v* 10·5 nmol/mmol, three months 9·58 *v* 9·36 nmol/mmol, six months 9·24 *v* 9·82 nmol/mmol). In this group of children there was decreased growth on inhaled corticosteroids without biochemical evidence of adrenal suppression. The effects on growth of inhaled corticosteroids are unlikely to be mediated via the pituitary-adrenal axis.

Height velocity standard deviation scores in 162 prepubertal children receiving beclomethasone dipropionate, budesonide, or sodium cromoglycate

GJJ HUNT, ATE EDMUNDS, CJH KELNAR *Department of Child Life and Health, University of Edinburgh, Edinburgh* A study was undertaken to determine whether any difference in height velocity exists in prepubertal asthmatic children on inhaled steroids or cromoglycate and whether the delivery system has any influence on this. The study was carried out in a large asthma clinic in a children's hospital serving a population of approximately one million as a tertiary centre. One hundred and sixty two prepubertal children, having no other significant medical condition, on no other steroid preparation at the time of observation such as nasal sprays, not on regular oral steroids though some, rarely, had short courses to deal with exacerbations, took part. Height velocity was measured over at least nine months and as near to one year as possible by a single trained observer using a wall mounted stadiometer; the reported dose of steroid was taken at each appointment and averaged over the period of observation. Mean height velocity was reduced for beclomethasone ($p < 0.05$) and this effect showed increasing growth suppression with increase in dose. Growth suppression was more marked with dry powder administration than with aerosol. No significant effects were seen with the other two drugs. Beclomethasone appears to have a dose dependent negative effect on height velocity, accentuated by dry powder administration.

Audit of home nebuliser use in childhood asthma

P MADGE, JY PATON, J MCCOLL, JO BEATTIE *Departments of Child Health and Statistics, University of Glasgow; Stirling Royal Infirmary NHS Trust* Nebulised therapy is an effective and widely used method of delivering inhaled therapy both to children with severe asthma and those too young to use simpler devices. For children with severe asthma, having a nebuliser at home may reduce the need for hospital attendance or admission by allowing parents to give high dose bronchodilator therapy early and avert an asthma attack. The British Thoracic Society guidelines on the management of asthma in children (*BMJ* 1993;**306**:776–82) recommend that nebulised therapy should only be initiated after careful consideration and such patients should already be taking adequate prophylactic therapy. Families should be made aware of how and when to use the nebuliser, how to perform and interpret peak flow monitoring, and when to seek medical help, and should have ongoing supervision including regular compressor servicing. We have performed an audit by postal questionnaire looking at home nebuliser use in two large health boards in Central Scotland (Greater Glasgow and Stirling and Forth Valley). Parents of 297 children with asthma responded. We found that 29% (86/297) used the nebuliser for bronchodilator therapy only and were on no prophylactic therapy. Many children had evidence of chronic symptoms such as sleep disturbance (59%, 174/297) and exercise limitation in the previous two weeks (42%, 125/297); 52% (87/168) had missed school due to increased asthma symptoms. Only 60% (102/168) of school age children had a peak flow meter to measure the response to nebulised treatment. Supply and servicing arrangements were poorly organised; only 11% of compressors had ever been serviced. Adherence to National Guidelines continues to be poor. Better organisation of home nebuliser use is required if recognised standards of care are to be met.

Acid air measurements in East Birmingham

GT ARCHER, RM HARRISON, JG AYRES, S WALTERS *Institute of Public and Environmental Health, University of Birmingham and Chest Research Institute, Heartlands Hospital, Birmingham* Levels of aerosol strong acid have been shown to correlate to symptoms of cough and sputum in the Six Cities Study. To explore this in the UK setting the daily mean concentration of air pollutants, including aerosol strong and gaseous acids and ammonia, were measured for two month periods during summer and winter 1993 in Birmingham. The measurements were conducted as part of a wider study examining the relationship between air quality and respiratory health in a panel of asthma patients. Levels of aerosol acid during the winter ranged between -102 nmol m^{-3} and 170 nmol m^{-3} (the negative value corresponding to a basic sample), and on days where there was net acidity averaged 43 nmol m^{-3}. These are lower than those experienced in the only previous UK study, and those in the eastern USA (typically 0–600 nmol m^{-3}) as a result of the higher levels of ambient ammonia observed in this study. The correlations between the aerosol acid concentration and sulphur dioxide and sulphate aerosol ($r = 0.15$ and 0·13 respectively) suggest these are poor surrogates, consistent with other published works. Levels of SO_4^{2-} correlated better with those of PM_{10} ($r = 0.57$) which we have found to be most closely associated with a reduction in PEF within a panel of brittle asthmatics (Walters *et al*, Summer BTS, 1993). Although overall levels of aerosol acid were low, changes in levels may be as important as absolute values in terms of health effects. This is currently being investigated. [The work has been funded by a BLF/Fisons Bursary.]

Variations in gas volumes inspired over one hour: implications for exposure systems used in the study of the health effects of air pollutants

WS TUNNICLIFFE, RJ ATKINS, JG AYRES, PS BURGE *Chest Research Institute, Birmingham Heartlands Hospital, Birmingham; Vitalograph Limited, Co. Clare, Ireland* As part of a study of the effects of nitrogen dioxide (NO_2) exposure on house dust mite challenge in mild asthmatics, we assessed intra- and inter-individual variations in ventilation rates during gas challenges in six subjects. On three occasions separated by at least seven days subjects breathed a gas mixture for one hour. Exposures were via a mouth piece attached to a Rudolph valve. Gases were inspired from a flaccid Douglas bag via an in-line Fleisch pneumotachograph through rigid, wide bore nylon tubing. The gas mixtures were air, 100 ppb NO_2 and 400 ppb NO_2 in air. Exposures were performed in a double blind, random order with the subjects seated at rest, with nose clips in place. Pneumotachograph readings were relayed to a personal computer using Vitalograph Spirotrach III system and collated using specially adapted software. The volume, duration and start time of each breath were recorded allowing the volume of gas per minute and per hour to be calculated. The mean maximal intra-individual variation for three exposures (%Max) was 18·85%, range 34·48–3·23%. There was no order effect. The mean total volume inspired for all exposures (1) was 501·67, range 373·1–781·0. Relative hyperventilation occurred during the first five minutes (14·33% > hourly mean, range -6.53% to 27·70%) and last five minutes (5·81% > hourly mean, range -5.97% to 21·22%) of the hour. From the 10th to the 50th minutes dose was proportional to duration of exposure. Significant inter- and intra-individual variation occurs in the volumes of gas individuals breathe over an hour at rest. Defining "an exposure" to a pollutant in terms of time, when performing repeated exposures, may not be valid, and measured volume may be more appropriate. If time is to be used, we would recommend that a run in period of at least 10 minutes be employed before true gas exposure is begun.

	Total volume (l) inspired for each exposure		
Subject	*Maximum*	*Intermed (%Max)*	*Minimum (%Max)*
1	443·61	433·24 (97·66)	416·10 (93·80)
2	781·00	605·31 (77·50)	521·66 (66·79)
3	623·74	573·35 (91·92)	530·29 (85·02)
4	414·99	406·05 (97·85)	401·59 (96·77)
5	569·47	500·54 (87·90)	373·10 (65·52)
6	537·23	474·30 (88·29)	424·53 (79·02)

Asthma in prawn workers: serum antibody isotopes to prawn-derived tropomyosin identified by Western blotting

C MCSHARRY, MJ COLLOFF, IC MCKAY, C LEWIS, DM KEMENY, RB WILSON, K ANDERSON *Departments of Immunology and Respiratory Medicine, Western Infirmary SPDL, Stobhill Hospital, Glasgow; Department of Medicine, Guy's Hospital, London* Workers in a seafood processing factory developed wheeze while operating water jets directed into the carapace of Norway prawns to remove the meat. As part of the assessment of these workers, serum was obtained for laboratory investigation. Raised levels of IgE antibodies to prawn antigens were found in 15 of 26 workers with respiratory symptoms and in one of 26 case matched asymptomatic controls ($p < 0.001$). Raised IgG antibody titres against the same antigens were found in 18 subjects in each group. The median titres of IgG antibody did not differ significantly, and within the IgG subclasses only IgG_4 antibody

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activity was significantly greater in the symptomatic subjects. Western blotting, using fresh prawn extract, demonstrated that tropomyosin was the major antigen, with other minor antigenic proteins of 70, 54, 45, 23, and 16 kDa. These antigens were recognised by both IgE and IgG in most positive sera from the factory workers. Serum from a different group of patients with a history of reaction to ingested prawn tended to have higher specific IgE antibody titres but a more restricted immunoblotting pattern recognising mainly tropomyosin, possibly reflecting its heat stability. Our findings suggest that tropomyosin is the predominant protein causing sensitisation following exposure by inhalation or ingestion of prawn antigens.

Upper and lower respiratory symptoms in two mushroom farms

JCG SIMPSON, LA OLDHAM, CAC PICKERING, AM FLETCHER, HC FRANCIS, RMCL NIVEN *Department of Occupational and Environmental Medicine, Wythenshawe Hospital, Manchester* Mushroom workers are known to suffer from extrinsic allergic alveolitis and asthma. A variety of microbial elements may be implicated. As part of a study of respiratory disease in a mixed cohort of workers exposed to organic dusts, 122 workers at two mushroom farms were investigated. An interviewer administered, MRC adapted, questionnaire identified the following work related symptoms: chronic bronchitis 1·6%, persistent cough 3·3%, chest tightness 6·6%, wheeze 3·3%, and shortness of breath 9·8%. Work related nasal symptoms were most prevalent (25·4%) and eye symptoms (13·9%). There was no difference in the age or sex distributions of symptomatic and asymptomatic workers (either upper or lower respiratory symptoms). Lower respiratory symptoms were not associated with smoking, but work related eye and nasal symptoms were more common in current smokers ($p < 0·05$). A trend of higher dust exposure was identified in the group with work related lower respiratory symptoms (0·74 mg/m^3 CI 0·65–0·87) *v* asymptomatics (0·68 mg/m^3 CI 0·65–0·72). No significant relationship existed between dust exposures in those workers with and without work related eye or nasal symptoms (0·70 mg/m^3 CI 0·64–0·76 *v* 0·69 mg/m^3 CI 0·65–0·73). This study identifies high levels of work related eye and nasal symptoms in mushroom workers. Current smoking is associated with an increased prevalence of symptoms. Respiratory work related symptoms, as identified by questionnaire technique, was associated with higher dust exposures.

Occupational asthma in workers exposed to cyanoacrylates

RMCL NIVEN, D SHACKLETON, CAC PICKERING, CJ WARBURTON, AM FLETCHER *Department of Occupational and Environmental Medicine, Wythenshawe Hospital, Manchester* Methyl cyanoacrylate (MCA) is a known cause of both occupational asthma and dermatitis. Cyanoacrylates are widely used in contact adhesives including "Super-glue". Three suspected cases of occupational asthma to cyanoacrylates have been identified. Two worked at the same factory making lamp shades with ethyl cyanoacrylate (ECA), not previously identified as a sensitiser. Neither had respiratory disease before exposure but developed symptoms within one month of first exposure. Symptoms of breathlessness, wheeze and cough improved at weekends and on holidays. Unfortunately the factory closed before reliable occupational peak flow recordings were made. Both workers underwent double blind placebo controlled challenge with ECA. In each case small early and late asthmatic responses were produced. Both had significant increases in bronchial reactivity. Case 1: $PC_{20} > 4·0$ mg/ml pre-challenge, PC_{20} 1·8 mg/ml post-challenge; case 2: 4·8 mg/ml and 0·7 mg/ml respectively. The entire work force of the factory were interviewed ($n = 26$), of whom the above two had evidence of occupational asthma; all the others had at least one upper or lower respiratory symptom suggestive of an irritant response. Case 3 was exposed to MCA (treating circuit boards) and developed symptoms two months after first exposure. Peak expiratory flow recordings demonstrated a typical pattern of occupational asthma. Cases 1 and 2 confirm ethyl cyanoacrylate as a likely respiratory sensitiser, in addition to methyl cyanoacrylate.

Asthma in roof bolting coal miners

SJ BOURKE, P SANDHU, SC STENTON, M MOHAN, DJ HENDRICK *Chest Unit and Regional Unit for Occupational Lung Disease, Newcastle General Hospital, Medical Boarding Centre and University of Newcastle upon Tyne* Roof bolting is increasingly being used in British coal mines to replace prop supports. In this process the roof of the mine is secured to the strata above using bolts anchored in place by a binding resin whose components include polyester resin in styrene with benzoyl peroxide catalyst. Mixing of the resin and catalyst occurs when a bolt is drilled through the twin component capsule placed in a bolt hole. Eight miners (mean age 42·6 years) who worked as roof bolters were recently referred for evaluation of suspected occupational asthma. The diagnosis of asthma was confirmed in six. All had developed asthma de novo after exposure to roof bolting with a mean latent period of 2·5 years (six months to five years) between first exposure and onset of symptoms. Five described symptoms of late asthmatic reactions after roof bolting with a clear pattern of improvement away from work and deterioration on re-exposure. Two also had immediate asthmatic reactions, two had nasal symptoms, but none had dermatitis. Three had never smoked, two were ex-smokers, and one was a current smoker. Four had airway hyperresponsiveness to methacholine (PD_{20} 12·5, 25, 131, 1400 µg) and two had reversible airway obstruction (FEV_1/VC 1·9/2·8 to 2·6/3·5; 1·8/2·9 to 3·6/4·0 l) with FEV_1 too low to permit methacholine challenge. Chest radiographs and transfer factor for carbon monoxide were normal. Skin tests for atopy were negative. Five had left the industry (two on medical advice) a mean 6·6 months previously. Although their symptoms had improved they were still troublesome, requiring ongoing treatment, and all still had airway hyperresponsiveness or reversible obstruction. The development of asthma de novo after a latent period of exposure to roof bolting suggests a specific hypersensitivity reaction. Inhalation challenge studies are needed to confirm the suspicion of occupational asthma and to identify which component of the work environment may be the sensitising antigen.

Use of nebulised antimicrobial agents and precautions taken to avoid occupational exposure of health care workers in the West Midlands

JR BEACH, SC STENTON, M CAMPBELL, TC AW *Institute of Occupational Health, University of Birmingham; Chest Unit, Newcastle General Hospital, University of Newcastle upon Tyne* Nebulised drugs are increasingly being used both in asthma and in other chest diseases in an attempt to target drugs at the lungs. Although a number of antimicrobial agents are used in this way there is relatively little information available about the prevalence and method of their use. Often nebuliser type, flow rate, and precautions used to prevent exposure of health care staff to the waste aerosol produced may not be specified by drug manufacturers, although these factors are important in ensuring adequate drug delivery to patients and proper protection of staff. To investigate this we sent a questionnaire to all consultant physicians, paediatricians, and geriatricians employed by West Midlands Regional Health Authority asking for details of nebulised antimicrobial agents used in the past year, whether the nebuliser type and flow rate was specified, and whether any exhaust system was used to reduce exposure of staff to waste aerosol. In all, 295 consultants in the appropriate categories were sent questionnaires and 226 (76%) were returned. Of those who replied, 20 of 129 (16%) physicians, 15 of 64 (23%) paediatricians, and one of 33 (3%) geriatricians had used nebulised antimicrobial agents in the past year. Colomycin, pentamidine, amoxycillin, and gentamicin had been used for both inpatients and outpatients, ribavirin and netilmycin had been used for inpatients, and carbenicillin and tobramycin had been used for outpatients. The commonest agent used was colomycin which had been used by 18 consultants on inpatients and by 21 on outpatients. Ribavirin was the next most commonly prescribed to inpatients (seven consultants), although used exclusively by paediatricians. When prescribing these agents most consultants specified a nebuliser type (79%), and most either specified a flow rate or used a fixed rate compressor (85%). A smaller proportion specified the use of an exhaust device whether for inpatient use (50%) or outpatient use (62%). All the drugs given in this way for inpatients were occasionally given without an exhaust device (six of seven consultants who used it). Colomycin was also used relatively frequently without an exhaust device (six of 18 consultants), while in contrast only one of six consultants used pentamidine without an exhaust device. Nebulised antimicrobial agents are therefore mostly being given by a specified and effective method, but adequate precautions to protect staff from waste aerosol are not always in use.

Increased airway responsiveness among shipyard workers exposed to welding fume

JR BEACH, SC STENTON, AJ AVERY, DJ HENDRICK *Chest Unit and Regional Unit for Occupational Lung Disease, Newcastle General Hospital, University of Newcastle upon Tyne* 682 shipyard workers

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and 254 school leaver controls (prospective employees) took part in a cross sectional study of the effects of welding fume. Subjects were categorised according to their trade into three categories representing high (welders and caulkers), ambient (other shopfloor trades), and negligible (office workers) exposure to welding fume. All subjects completed a respiratory questionnaire and underwent skin prick tests for atopy, spirometry, and airway responsiveness (AR) measurements ($PD_{20}FEV_1$ to methacholine). Participation represented 97% of all eligible subjects. Among the workers there were no significant differences in symptoms and lung function between exposure subgroups, but the proportion with quantifiable AR ($PD_{20} \leqslant 6400\ \mu g$) was greater in the high exposure subgroup. There were no subgroup differences in the distribution of quantifiable AR among the school leavers – high 44% (33/75), ambient 41% (51/125), low 44% (24/54). The high exposure subgroups (both workers and school leavers) together contained higher proportions of smokers than the ambient and negligible subgroups (43% *v* 23% *v*18%), and fewer females (3% *v* 4% *v* 14%). Regression analyses suggest that these do not fully account for the differences in PD_{20}. The data suggest that welding fume exposure might cause increased AR and asthma. [Supported by grants from the MRC and HSE.]

	High	*Ambient*	*Negligible*	*p*
n	284	219	179	
Wheeze	19%	23%	20%	0·3
Any symptom	22%	29%	23%	0·2
FEV_1%	108·4%	109·3%	110·2%	0·4
FEV_1/FVC	87·2%	87·2%	87·5%	0·9
$PD_{20} \leqslant 6400\ \mu g$	49%	44%	36%	<0·05

Diurnal peak expiratory flow variability and a respiratory symptoms questionnaire in laboratory animal and flour workers

K MCKINLAY, D LOWSON, K VENABLES, AJ NEWMAN TAYLOR *Department of Occupational and Environmental Medicine, National Heart and Lung Institute, London* The development and validation of a nine item asthma questionnaire by this department (*Thorax* 1993;**48**:214–9) prompted us to study its usefulness in conjunction with serial peak expiratory flow rate measurement in two occupational groups. 297 laboratory animal workers (mean age 28, 64%F) and 157 flour workers (mean age 34, 27%F) completed the questionnaire, recorded seven days of PEF readings (4 × daily, >21 readings) and had a histamine challenge test. Mean daily PEF variability was expressed as: Amp % mean = (max − min)/mean(%); Amplitude = max − min (l/min). PEF variability was greater in those reporting three or more symptoms (positive responses) in the questionnaire (table). In addition, we confirmed our previous findings that three or more symptoms were good indices of bronchial hyperresponsiveness and of a history of asthma.

Symptoms (no.)	*Laboratory animal*			*Flour*		
	n	*Amp % mean GM*(gsd) (%)*	*Amplitude GM (gsd) (l/min)*	*n*	*Amp % mean GM*(gsd) (%)*	*Amplitude GM (gsd) (l/min)*
0	100	5·0 (1·7)	26·5 (1·6)	43	5·8 (2·0)	28·7 (2·0)
1–2	162	5·7 (1·8)	29·0 (1·8)	98	5·9 (2·1)	30·8 (1·9)
⩾3	35	7·1 (2·3)	33·3 (2·2)	16	9·3 (1·9)	42·8 (1·8)

* $p < 0.05$ ANOVA

Respiratory symptoms and pulmonary function in airport workers

SP O'HICKEY, W TUNNICLIFFE, T FLETCHER, JF MILES, PS BURGE, JG AYRES *Chest Research Institute, Birmingham Heartlands Hospital, Birmingham* To explore the effect of aircraft fuel exposure on respiratory symptoms and asthma, we studied a workforce at Birmingham International Airport. Subjects were assigned to either high, medium, or low exposure groups according to their occupation and a randomised sample proportional to size were invited to attend on one of three study days for completion of a respiratory questionnaire, and to measure spirometry, exhaled carbon monoxide and skin prick test reactivity. 222 subjects/317 (70%) agreed to be studied (high = 86; medium = 83; low = 53). Mean time per day exposed to the aircraft taxi-ing area was 6.03 hours, 1.46 hours, and 0.62 hours for high, medium and low groups respectively. The high exposure group were significantly younger (mean age 36·7 years) and all male compared with the medium (mean age 43·2 years M:F ratio 1·96:1) and low (mean age 44·8, M:F ratio 0·59:1) exposure groups. There were no differences in FEV_1 or FVC between the groups. Smoking was more prevalent in the high and medium exposure groups than the low exposure group (34%, 36%, 17%, respectively ($p = 0.007$)). The prevalence of atopy was similar in each group (58%, 52%, 48%). Symptoms of cough were more common amongst the high exposure group (60%) compared to the medium (36%) and low (43%) exposure groups, with similar differences for productive cough (36%, 16%, and 21%). Differences were significant between the high and medium exposure groups (cough odds ratio 2·69 (95% CI 1·24 to 5·88) and cough with sputum odds ratio 4·50 (95% CI 1·49 to 13·88)) which was only partially explained when allowing for cigarette smoking. These results suggest an increase in respiratory symptoms in workers heavily exposed to aircraft fuel.

Peak flow based asthma self-management: a randomised controlled study in general practice

KP JONES, MA MULLEE *Faculty of Medicine, University of Southampton, on behalf of the British Thoracic Society* Asthma self-management based on home peak flow monitoring has become a widespread component of disease care protocols, but its effectiveness has only been investigated in small hospital based studies and single general practices. Under the auspices of the British Thoracic Society, 127 asthmatic subjects aged 15–40 years on inhaled steroids from 25 general practices in the Wessex region were randomised to receive either an asthma self-management programme based on home peak flow monitoring with surgery review by their GP or practice nurse (SM), or to a programme of planned visits only (PV) completed the study protocol, but diary card data for at least three months were available on a total of 84 (39 SM, 45 PV). Home peak flow monitoring was felt to be useful by both doctors and patients in 28 (85%) and 27 (82%) cases respectively in the SM group. However, there were no significant between group differences during the study period in terms of lung function, symptoms, quality of life, and prescribing costs (excluding the peak flow meters). Some within group improvements were noted in the SM group only in terms of disturbance of daily activities and quality of life. Teaching self-management at visit 2 took significantly longer (medians 14·2 minutes SM, 10·0 minutes PV, $p < 0.01$). Possible explanations for these negative results include: small numbers of subjects, the mild nature of their asthma (mean initial peak flow rate 87% predicted) and poor understanding of SM by subjects (inhaled steroid dose was not doubled in 278/534 (52%) instances when this was indicated). Rigid adherence to long term daily peak flow measurement is clearly not beneficial in the management of mild asthma in general practice. Self-management and the use of prescribed peak flow meters need to be tailored to individual circumstances.

Increased atopy in brittle compared with non-brittle asthma

JF MILES, RM CAYTON, JG AYRES *Chest Research Institute, Birmingham Heartlands Hospital, Birmingham* To test the hypothesis that patients with brittle asthma (diurnal variation in peak flow >40% for >50% of days over six months despite high dose inhaled steroids) are more atopic than non-brittle, age, sex and inhaled steroid matched controls, 29 patients with brittle asthma (18 F, mean age (range) 45·8 (17–62) years) underwent skin prick testing to 19 common inhaled or ingested allergens. Weal diameters were measured in two planes at right angles to each other and the absolute value subtracted from the mean negative control diameter. All patients showed a positive reaction to 10% histamine. Overall atopy score (the sum of all corrected weal diameters) was significantly greater in the brittle group than the controls (44·35 mm *v* 23·72 mm, Z score 2·06; $p = 0.039$). Responses to *Alternaria*, suggested as a risk factor for asthma deaths, were rare. These findings support the hypothesis that allergic sensitisation is more marked in brittle asthma and that atopy may be a risk factor for severity in asthma. [JM was a West Midlands RHA Sheldon Research Fellow.]

Selected allergen	*Mean weal diameter (mm)*		*Wilcoxon rank*	
	Brittle	*Non-brittle*	*Z score*	*p*
Der P1	7·52	4·60	−1·45	0·147
Cat	4·86	3·09	−1·70	0·089
Dog	2·78	0·71	−3·04	0·002
Horse	6·28	2·64	−2·34	0·018
Grass pollen	4·64	2·17	−2·56	0·011
Feathers	2·96	1·52	−2·72	0·007
Wheat	1·48	0·66	−3·34	0·001
Chocolate	1·09	0·41	−2·00	0·045
Alternaria	0·90	0·64	−1·54	0·123

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Salbutamol inhaler using a non-chlorinated propellant, GR106642X: systemic pharmacodynamic activity in healthy volunteers

SM KIRBY, J SMITH, GP VENTRESCA *Clinical Pharmacology Division and Department of Medical Statistics, Glaxo Group Research Ltd, Greenford, Middlesex* Metered dose inhalers, currently the most common delivery system for asthma therapy, use as propellants chlorofluorocarbons. These face an international ban on production from 1995–6 due to their potential depleting effect on the ozone layer. Efforts are being made to formulate antiasthma drugs with non-chlorinated propellants that do not have this potential. Salbutamol (SAL), a selective short acting β_2 agonist, has been reformulated using GR106642X, tetrafluoroethane (also known as HFA134a), as propellant. The safety, tolerability, and systemic pharmacodynamic activity of the SAL/GR106642X inhaler, the current SAL inhaler and placebo (GR106642X) were compared in 12 healthy male volunteers. For the SAL/GR106642X inhaler two actuators were tested, one identical to the current SAL inhaler. The study design was a double blind, randomised crossover on four different days separated by a washout of at least 36 hours. Dosing was cumulative, with doses of 200, 200, 400, 800, and 1600 µg SAL given at 35 min intervals. The cumulative total after each dose was 200, 400, 800, 1600, and 3200 µg. Measurements of pulse rate, blood pressure, QT_c interval, tremor, plasma potassium, and glucose were made at 15 and 30 minutes after each dose. The systemic pharmacodynamic response to SAL/GR106642X was related to the dose over the range used (200–3200 µg). No significant differences on any of the parameters measured were seen between the SAL/GR106642X inhaler with the current actuator and the current SAL inhaler. The mean difference in pulse rate after the last dose was 7 bpm (95% CI 0 to 14, p=0·058) and in plasma potassium was −0·09 mmol/l (95% CI −0·23 to 0·05, p=0·204). The SAL/GR106642X inhaler with the different actuator gave changes significantly different from the current SAL inhaler for most of the parameters measured. The SAL/GR106642X inhaler with the current actuator is at least as safe and well tolerated as the current SAL inhaler, and has a similar systemic pharmacodynamic activity.

Salmeterol inhaler using a non-chlorinated propellant, GR106642X: systemic pharmacodynamic activity in healthy volunteers

SM KIRBY, J SMITH, GP VENTRESCA *Clinical Pharmacology Division and Department of Medical Statistics, Glaxo Group Research Ltd, Greenford, Middlesex* Metered dose inhalers, currently the most common delivery system for asthma therapy, use as propellants chlorofluorocarbons. These face an international ban on production from 1995–6 due to their potential depleting effect on the ozone layer. Efforts are being made to formulate antiasthma drugs with non-chlorinated propellants that do not have this potential. Salmeterol (SLG), a potent selective long acting β_2 agonist, has been reformulated using GR106642X, tetrafluoroethane (also known as HFA134a), as propellant. The safety, tolerability and systemic pharmacodynamic activity of the SLG/GR106642X inhaler, the current SLG inhaler and placebo (GR106642X) were compared in 12 healthy male volunteers. The study design was a double blind, randomised crossover on three different days separated by a washout of at least one week. Dosing was cumulative, with doses of 50, 50, 100, and 200 µg SLG given at 60 min intervals. The cumulative total after each dose was 50, 100, 200, and 400 µg. Measurements of pulse rate, blood pressure, QT_c interval, tremor, plasma potassium and glucose were made at 30 and 55 minutes after each dose. The systemic pharmacodynamic response to SLG/GR106642X was related to the dose over the range used (50–400 µg). No clinically significant differences were seen between the SLG/GR106642X inhaler and the current SLG inhaler on any of the parameters measured. Between the two SLG inhalers the mean difference in pulse rate after the last dose was −1 bpm (95% CI −6 to 4, p=0·718) and in plasma potassium was 0·12 mmol/l (95% CI 0·02 to 0·22, p=0·025). For both parameters the observed effects were less with the SLG/GR106642X inhaler. The SLG/GR106642X inhaler is at least as safe and well tolerated as the current SLG inhaler, and has a similar systemic pharmacodynamic activity.

Poor output of salbutamol from a spacer device: effect of spacer static charge and multiple actuations

PW BARRY, C O'CALLAGHAN *Department of Child Health, University of Leicester, Leicester* The output of drugs from spacers is affected by static charge (C O'Callaghan *et al. Thorax* 1993;**48**:603–6). The field strength arising from the static charge on the inside of a previously unused Volumatic spacer (VS), measured using an electrostatic meter, was more than five times (2800 arbitrary units in the new VS, 550 units in the old). The field strength of the new spacer was reduced to 90 units by wiping with an antistatic cloth. A multistage liquid impinger was used to measure the amount of salbutamol available for inhalation from the new and used VS, and to determine the effect of more than one actuation into the VS before sampling. The table shows the amount of salbutamol recovered in particles smaller than 5 µm per 100 µg actuation. The output of salbutamol from the VS is reduced by multiple actuations into the spacer and by high static charge carried on the spacer wall. This charge may be reduced by wiping the VS with an antistatic cloth. This should be done when the VS is first used and if the VS is only used occasionally. In the emergency treatment of acute severe asthma in adults, two actuations of a metered dose inhaler into a large volume spacer, such as the VS, are recommended (*Thorax* 1993;**48**:S1–S24). If this is done, drug delivery will be lower than expected which may contribute to therapeutic failure.

Spacer type	*Field strength*	*No delay*	*20 second delay*	*2 actuations*	*5 actuations*
New	2800	19·3 µg	3·3 µg	—	—
Used	500	54·2 µg	40·8 µg	42·4 µg	20·7 µg

Pulmonary deposition and effect of terbutaline administered by metered dose inhaler or Turbohaler

E DEROM, E WÅHLIN-BOLL, L BORGSTRÖM, R PAUWELS *University Hospital, Department of Respiratory Diseases, De Pintelaan 185, B-9000 Ghent; Astra Draco, Clin R&D, Box 34, S-221 00 Lund, Sweden* The development of new inhalation devices raises the question regarding comparative efficacy for administering drugs by inhalation. The present study compared both pulmonary deposition and lung function following single inhalations of 0·25 and 0·5 mg terbutaline via a pressurised metered dose inhaler (pMDI) and via Turbohaler. The study was randomised, double blind, double dummy, and the four treatments were administered on four separate days. Thirteen asthmatics (FEV_1, 59% pred; 34% reversibility) completed the study. Pulmonary deposition was evaluated with the charcoal block method (Borgström, Nilsson. *Pharm Res* 1990;7:1068–70); FEV_1, FVC, FEF_{25} FEF_{50}, FEF_{75}, PEF, and sGaw were measured in a body plethysmograph before and up to six hours after inhalation. Pulmonary deposition was 8·1% and 8·3% of the nominal dose for the pMDI days, and 19·0% and 22·0% for the Turbohaler days. Thus, the degree of pulmonary deposition was at least twice as high from Turbohaler as from pMDI; the deposition values are in accordance with previously published budesonide data (Thorsson, Edsbäcker. *Thorax*, 1993;**48**:434). The increase in FEV_1 after 0·25 mg terbutaline via pMDI was significantly smaller than the increase after all other treatments. Inhalation of 0·25 mg terbutaline via Turbohaler resulted in a similar increase in FEV_1 as 0·5 mg via pMDI. An increase in Turbohaler dose from 0·25 to 0·5 mg only slightly increased the response further. Other lung function variables exhibited a similar behaviour. We conclude that: (1) the dose of terbutaline deposited in the lungs is dependent on inhalation device; (2) Turbohaler delivers about twice the amount of drug to the lungs as pMDI; (3) the observd difference in deposition is also reflected in the pulmonary effects.

Acute bronchoconstriction following the use of metered dose inhalers in adult asthmatics

JG AYRES, C BENINCASA *Chest Research Institute, Birmingham Heartlands Hospital, Birmingham; Allen and Hanburys Limited, UK* Acute bronchoconstriction following metered dose inhaler (MDI) use is a recognised adverse reaction, the cause of which remains unknown. It was decided to investigate and compare the incidence of MDI-associated bronchoconstriction in an adult asthmatic population following the use of three different MDIs. Two different placebo MDI preparations containing the same chlorofluorocarbons and differing in the dispersant chemicals – one containing oleic acid (MDI-OA), the other containing lecithin NF (MDI-L) – were compared with an MDI containing salmeterol xinafoate (25 µg) and lecithin NF (MDI-S). In a double blind, parallel group design, 11 850 asthmatic patients were randomly assigned to receive two puffs from one of the inhalers, MDI-S (n=3948), MDI-L (n=3942),

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or MDI-OA (n = 3960). Peak expiratory flow (PEF) was measured before and five minutes after inhalation. A drop of at least 20% in PEF was defined as clinically significant bronchoconstriction. Overall, 180 (1·5%) patients had a decrease of 20% or more in PEF, 43 (1·1%) with MDI-S, 67 (1·7%) with MDI-L, and 70 (1·8%) with MDI-OA. There was a significantly lower incidence of bronchoconstriction seen with MDI-S than with either MDI-L ($p = 0·021$) or MDI-OA ($p = 0·011$). The risk of acute bronchoconstriction increased with both age ($p = 0·005$) and decreasing baseline PEF ($p < 0·001$), and these were independent risk factors. This study showed that bronchoconstriction following the use of MDIs is uncommon. The causative factor is unlikely to be the active medication (in this study, salmeterol xinafoate) but rather one of the other constituents present in the MDI.

Nebuliser performance, systemic aborption, and efficacy of salbutamol delivered via a novel nebuliser system (Ventstream)

DM NEWNHAM, BJ LIPWORTH *Department of Clinical Pharmacology, University of Dundee, Ninewells Hospital and Medical School, Dundee* An initial in vitro comparison of 10 Ventstream (V) and 10 Hudson (H) nebulisers showed a significantly higher percentage of particles $< 5\,\mu m$ in diameter using V compared with H (mean and 95% CI for difference: 25·4 (22·4 to 28·3)%, $p < 0·005$), and also a higher aerosol output: 0·08 (0·05 to 0·11) g/min, $p < 0·005$. Eight asthmatic patients of mean (SE) age 41 (5) years, FEV_1% predicted (litres) 55 (2)%, (2·01 (0·22) l) were then randomised to receive salbutamol via V or H on separate days. On each study day cumulative doses of inhaled salbutamol were given: 1·25 mg, 2·5 mg (1·25 + 1·25) and 5·0 mg (2·5 + 2·5). Airways, systemic responses, and plasma salbutamol concentrations were measured at each dose and for up to 240 minutes after the final dose. At the 5·0 mg dose V produced an approximately twofold greater concentration of plasma salbutamol compared with H (AUC0-240, mean and 95% CI for difference: 392·1 (240·6 to 543·6) ng/min/ml, $p < 0·001$). There was a significantly higher AUC0-240 for PEF when using V compared with H (mean and 95% CI for difference: 74·16 (39·50, 108·82) $l \times 10^2$, $p < 0·001$). For FEV_1 and FEF_{25-75} there was a significant ($p < 0·05$) difference in the peak response between the 5·0 mg and 2·5 mg doses with V, but not with H. Extrapulmonary β_2 responses (serum potassium, heart rate, finger tremor and T wave amplitude) were significantly ($p < 0·05$) greater with V than with H at 2·5 mg and 5·0 mg doses, although differences were not clinically relevant. In conclusion, V produced a twofold increase in the delivery of salbutamol to the lung compared with H; and there was an associated significant increase in systemic β_2 effects and an improvement in some parameters of bronchodilator efficacy.

A placebo controlled comparison of the effect of oral bambuterol and inhaled terbutaline against exercise induced asthma (EIA)

F STAFFORD, O MCCALLION, A MCLEAN, Y GNOSSPELIUS, CF STANFORD *Royal Victoria Hospital, Belfast; Astra Clinical Research Unit, Edinburgh; Astra Draco, Sweden* The most effective drugs for preventing EIA are inhaled β_2 agonists. Even though the same drugs when given orally can induce significant bronchodilatation, they are less effective at preventing EIA. The aim was to evaluate the effect of oral bambuterol (a prodrug of terbutaline) on EIA compared with inhaled terbutaline and placebo. The study was of a double blind, crossover design which comprised three treatment periods. Each treatment period lasted seven days. Fourteen patients were randomised to receive either bambuterol 20 mg mane, terbutaline Turbohaler 500 µg tid, or placebo. At each clinic visit patients underwent exercise testing for eight minutes on a treadmill. Bambuterol was taken two hours and terbutaline one hour prior to the test. FEV_1 was measured before and immediately after and at intervals up to 20 minutes post-exercise. A fall of 15% or more in FEV_1 post-exercise was clinically defined as EIA. A diary card was completed daily in which patients recorded PEF, β_2 agonist use, and asthma symptom score. The baseline FEV_1 was 85% predicted at study entry. Twelve patients took inhaled steroids before inclusion and continued use during the treatment period. The mean maximum % fall in FEV_1 after exercise was 11·2% for terbutaline, 12·2% for bambuterol, and 19·5% for placebo. Both bambuterol and terbutaline were significantly different from placebo. Night-time asthma scores were significantly reduced with terbutaline when compared with placebo ($p = 0·035$). There were no other significant treatment differences. Bambuterol and terbutaline both attenuated the asthmatic response to exercise. There was no significant difference between the oral and the inhaled method of drug delivery.

Comparison of addition of salmeterol with increase in inhaled steroids in adult asthmatics symptomatic on 200 µg twice daily (bd) inhaled steroids

AP GREENING, PW IND, ON BEHALF OF AN ALLEN AND HANBURYS UK STUDY GROUP *Western General Hospital, Edinburgh; Hammersmith Hospital, London* In a double blind study of general practice asthmatic patients symptomatic on inhaled beclomethasone dipropionate (BDP) 200 µg bd, the effects of adding in salmeterol have been compared with more than doubling the dose of inhaled steroid. 426 patients were randomised to (a) salmeterol 50 µg bd by Diskhaler plus BDP 200 µg bd by MDI (SM; n = 220), or (b) BDP 500 µg bd by MDI (↑BDP; n = 206), for six months. Relief medication was inhaled salbutamol (salb). Primary outcome measures were peak expiratory flow (PEF) and exacerbation rates. Mean (SD) baseline morning PEF (l/min) was 349 (109) for the SM group and 339 (99) for the (↑BDP group (NS). Mean morning PEF improved in both groups but the improvement was greater at all time points for the SM group ($p < 0·01$). At week 1 the treatment difference in PEF improvement was 17 l/min (95% CI: 10 to 24) and at week 21 was 21 l/min (95% CI: 5 to 34). Mean evening PEF and diurnal variation were also significantly better at all time points in the SM group. Exacerbations were categorised as mild (increased salb), moderate (short courses of oral steroids), or severe (hospitalisation). Exacerbations were experienced by a minority of patients, with mean 28-day exacerbations rates of 0·21 and 0·29 for the SM and ↑BDP groups respectively (NS). Secondary outcome variables, including daytime symptoms, nocturnal wakening, salb use, all favoured the SM group (statistically significant at some time points only). Both treatment regimens were well tolerated with similar overall incidence and nature of adverse events. In this defined patient group the addition of salmeterol 50 µg bd was more effective than increasing the inhaled steroid dose to BDP 500 µg bd.

Effects of fluticasone propionate 1 mg and 2 mg daily and budesonide 1·6 mg daily in severe asthmatics

JG AYRES, TAJ HARRIS, ED BATEMAN, B LUNDBACK ON BEHALF OF AN INTERNATIONAL STUDY TEAM *East Birmingham Hospital, UK; Respiratory Department, Glaxo Group Research Ltd, UK; Groote Schuur Hospital, South Africa; Central Hospital, Boden, Sweden* A double blind, parallel group study was conducted in 671 severe asthmatic patients (already taking 0·8–2·0 mg daily of inhaled steroid) to compare the safety and efficacy of six weeks' treatment with inhaled fluticasone propionate (FP) 1 mg daily with FP 2 mg daily and budesonide (BUD) 1·6 mg daily. Daily peak expiratory flow (PEF) measurements, clinic lung function, asthma symptoms, rescue medication usage, and morning serum cortisol levels were assessed. The change in mean morning PEF from baseline for weeks 1–6 of the treatment period for patients receiving FP 2 mg daily was + 24 l/min (FP 2 mg *v* BUD $p < 0·001$), for FP 1 mg daily was + 21 l/min (FP 1 mg *v* BUD $p < 0·02$), and for BUD 1·6 mg daily was + 13 l/min. A similar rank order was seen for daily evening PEF, clinic lung function parameters, reduction of asthma exacerbations, symptom scores, and requirements for rescue bronchodilators and oral corticosteroids. Analysis of serum cortisols (ANOVA) showed a change in the rank order. The geometric mean cortisol ratio (treatment/baseline ratio for cortisol levels after six weeks' treatment) for FP 1 mg daily was 1·04 (FP 1 mg *v* FP 2 mg $p < 0·001$), for BUD 1·6 mg daily was 0·97 (BUD *v* FP 2 mg $p < 0·01$), and for FP 2 mg daily was 0·88, although mean serum cortisol levels remained within the normal range for all groups. Both doses of FP produced statistically significant improvements in lung function compared with BUD. Suppression of serum cortisol levels in the FP 1 mg daily group was less than in the BUD 1·6 mg daily group.

Comparison of budesonide and beclomethasone dipropionate on indices of bone turnover in COAD

D MORRISON, R RICHARDS, N ALI, S CAPEWELL, K SKWARSKI, M SANDERSON, P ROUTLEDGE, W MACNEE *Unit of Respiratory Medicine, Department of Medicine (RIE), City Hospital, Edinburgh* Previous studies have suggested a difference between the effects of budesonide (BUD) and beclomethasone dipropionate (BDP) on bone turnover. Biochemical markers of bone turnover were studied in 10 patients with

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chronic obstructive airways disease (COAD) (mean (SE) age 64 (3) years, FEV_1 1·5 (0·2) l) in a placebo controlled crossover design. Active treatments (BUD or BDP both 2000 µg daily) were given for four weeks each with a two week run-in period and two week washout periods after each treatment. All patients were clinically stable and none had received systemic steroids within six weeks. Venous blood and a two hour urine sample were taken between 9 and 10 am after an overnight fast. Neither the mean urinary hydroxyproline creatinine ratio nor the urinary calcium creatinine ratio changed following BUD or BDP. There was a significant reduction in urinary pyridinoline creatinine ratio (PYR) and deoxypyridinoline creatinine ratio (DPYR) [BUD: PYR 60·0 (12·1) to 48·2 (7·4), $p < 0·05$; DPYR 12·8 (2·1) to 10·1 (1·4), $p < 0·05$; BDP: PYR 61·7 (15·5) to 44·2 (5·8), $p < 0·01$; DPYR 10·4 (1·4) to 9·1 (1·1), $p < 0·05$]. All are indicators of bone resorption. There was no difference between the two treatments. Serum alkaline phosphatase, a marker of bone formation, did not change following BUD or BDP. Serum osteocalcin (OC) showed a significant treatment period interaction ($p < 0·01$). Analysis of the first period only ($n = 5$) showed a 26% reduction following BUD (10·8 (1·5) to 8·0 (1·3) ng/ml, $p < 0·05$) and an 18% reduction following BDP (4·5 (0·6) to 3·7 (0·7), $p = 0·08$). However the baseline OC levels were significantly different ($p < 0·05$) and may account for the difference. In conclusion, both BUD and BDP 200 µg daily for four weeks reduced bone resorption and tended to reduce bone formation.

Easy bruising and adrenocortical function in patients taking inhaled corticosteroids

R MELCHOR, VHF MAK, SG SPIRO *University College Hospital, London* The use of inhaled corticosteroids (ICS) in the control of asthma has the advantage of reduced systemic side effects in comparison with oral steroids. However, at doses over 1500 µg beclomethasone/day, adrenocortical suppression may occur. We have recently reported that easy bruising (defined as bruising resulting from a slight knock or without apparent cause) occurred in over 40% of patients taking ICS but not taking oral steroids (Mak *et al. Eur Respir J* 1992;5:1068–74). The prevalence of easy bruising was dose related, so there may be an association between easy bruising and adrenocortical suppression. We have therefore studied patients who have noticed easy bruising since commencing ICS for evidence of impaired adrenocortical function. A prospective open study was performed in 32 asthmatic patients not taking oral corticosteroids, who had reported easy bruising *after* commencing inhaled corticosteroids. Platelet count, clotting screen, bleeding time, 9 am basal plasma cortisol and plasma cortisol 30 minutes following 250 µg intramuscular tetracosactrin were measured (short Synacthen test). A normal result was taken to be an increment from basal levels of >200 nmol/l or a rise to a level >500 nmol/l. Patients were considered to have suppressed adrenocortical function if they had abnormal basal levels and an impaired Synacthen test. The mean (SD) dose of inhaled corticosteroid was 1590 (651) µg/day beclomethasone dipropionate and the mean duration of use was 46·4 (34·6) months. All had platelet counts and bleeding times within the normal range. Only two of 32 patients fulfilled the criteria for adrenocortical suppression. Three other patients had normal basal cortisol levels but had an increment of less than 200 nmol/l or did not reach 500 nmol/l following tetracosactrin. We conclude that the prevalence of impaired adrenocortical function in patients taking high dose inhaled corticosteroids who report easy bruising is no higher than in an unselected population of asthmatic patients taking similar doses of inhaled corticosteroids (Brown *et al. Respir Med* 1991;**85**:501–10).

Patient information and bronchoscopy: an audit of clinical practice

E LOUGHNEY, D FISHWICK, L BRADSHAW, GJ GIBSON, PA CORRIS *Respiratory Division, Department of Medicine, Freeman Hospital and University of Newcastle upon Tyne* Recent studies have explored methods of improving the tolerance to fibreoptic bronchoscopy (FB). We wished to assess the impact of written patient information on the attitudes of patients to FB. Thirty three consecutive patients, seen as new referrals to the respiratory medicine unit, were assessed. An information leaflet was given to 16 consecutive patients. This contained information regarding the practical details and possible side effects of FB. A further 17 consecutive patients were given only a verbal explanation of the procedure. These patients were similar in age and sex. All 33 patients had spirometry and the results were similar in both groups (mean FEV_1 1·78, 95% CI 1·50 to 2·02). On attendance for FB the patients were asked by the nursing staff to complete a questionnaire. A visual category scale was used to assess anxiety levels (1 = very relaxed, 10 = very anxious). Premedication with intramuscular atropine was given to all patients. Sedation with intravenous midazolam was given at patient or operator request. Topical anaesthesia was obtained using lignocaine to the oropharynx and via the bronchoscope. After recovery, patients were asked to complete a questionnaire related to the presence of minor side effects and anxiety levels and asked whether they would prefer sedation in the event of a further bronchoscopy. Anxiety levels prior to FB were lower in patients who had read the leaflet (Wilcoxon rank sum test, $p < 0·05$). This was reflected by significantly fewer requests for further sedation (Fisher's exact test, $p = 0·0002$). There were no differences in the percentage of patients with pain (19% without leaflet *v* 7% with leaflet, NS) or nausea (7% *v* 3%, NS) after bronchoscopy. As a result of this audit the information leaflet is now distributed to all patients prior to bronchoscopy.

Percutaneous intrathoracic biopsy using the Biopty gun: five year experience in a district hospital

HP LAUBENSTEIN, DR MORGAN, MS PHILLIPS, DYH WAI *Scarborough District Hospital, Scarborough, North Yorkshire* Between July 1988 and June 1993 105 patients (75 men, age range 32–88 years) were investigated by percutaneous intrathoracic biopsy using the Biopty gun. All biopsies were performed by one operator (DYHW) using ultrasound guidance in 61 and radiography in 44. Radiological presentations were: intrapulmonary mass lesions (single (78) multiple (3)); consolidation (4); pleural thickening (9) or mass lesion (10), and one anterior mediastinal mass. Sixty two (79%) of the solitary intrapulmonary mass lesions were malignant (primary bronchial carcinoma 55, secondary carcinoma 5, sarcoma 2). Sixteen were benign, non-specific inflammatory change or fibrosis (13) (one false negative for carcinoma) cryptogenic organising pneumonitis (1) hamartoma (1), and Blesovsky's syndrome (1). The multiple lesions were malignant (1), Wegener's granulomatosis (1) and non-specific (false negative for carcinoma) (1). Consolidation was due to cryptogenic organising pneumonitis (2), Wegener's granulomatosis (1), and acute alveolitis (1). Pleural thickening was benign fibrous thickening in six patients (one false negative for mesothelioma) and malignant in three (two carcinoma, one mesothelioma). There were seven malignant pleural mass lesions (five carcinoma, two mesothelioma) and three benign (one false negative for mesothelioma). The anterior mediastinal mass was a thymoma. There were 21 pneumothoraces (20%), two requiring intercostal tube drainage, and two haemoptyses. Examination of more tissue at surgery or post mortem enabled three of the false negatives to be identified and the diagnosis to be confirmed in 25 other patients, malignancy in 24, with concordant cell type in 22 and one hamartoma. This technique has proved a satisfactory alternative to fine needle aspiration biopsy.

Treatment of superior vena cava obstruction (SVCO) with expandable metal stents

SW WATKIN, JAH LITTLER, PC ROWLANDS, RD EDWARDS, CRK HIND *Royal Liverpool University Hospital, Liverpool; Clatterbridge Centre for Oncology, Liverpool* SVCO carries a significant morbidity and mortality and requires urgent palliation. Radiotherapy is often effective but there may be a delay in response and some patients develop the complication after previous radiotherapy. Between January and August 1993 we have treated eight patients with expandable metal stents and other transvenous procedures. There were five men and three women, mean age 63 years (range 52–79). All had classical features of SVCO for four weeks or less in seven patients and 30 weeks in one. The underlying diagnosis was non-small cell lung cancer in seven (stage T_1 N_3 in five) and previously untreated sclerosing B cell lymphoma in one. Two of the lung cancer patients had received prior radical hyperfractionated radiotherapy and the remaining five had received palliative radiotherapy, only one for SVCO. Either Gianturco stents or Wallstents were placed across radiologically evident stenoses. Three patients received thrombolytic therapy and heparin, and two of these were also treated with an Amplatz thrombectomy device. Five patients showed complete clinical resolution and one had a partial response but was left with evidence of unilateral subclavian vein thrombosis. The remaining two patients died of advanced carcinoma before formal re-evaluation. Five patients required only one treatment session and three returned for venography and further treatment. There were no serious complications, but one patient developed phlebitis in the antecubital fossa and extravasation of contrast from the brachiocephalic vein was seen in another during stent insertion. We conclude that these techniques can have a dramatic effect in palliation of SVCO and provide a means of treatment when further radiotherapy cannot be given, although appropriate radiological expertise is required.

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Percutaneous dilational tracheostomy in the intensive care unit: a review of 50 cases

SV BAUDOUIN, AE BODENHAM *The Intensive Care Unit, Leeds General Infirmary, Leeds* Percutaneous dilational tracheostomy is a new technique which can be performed as a bedside procedure in the Intensive Care Unit (Bodenham. *Anaesthesia* 1993;**48**:101–2). There is little information on outcome or complications of this procedure compared with traditional surgical methods. We have therefore retrospectively reviewed our experience with 50 consecutive cases. Percutaneous tracheostomies were performed on 42 men and eight women with a mean age of 49 years (range 18–81). Major admitting diagnoses were: multiple trauma (12); head injury (9); surgical sepsis (8); COAD (5); pancreatitis (5); post neurosurgery (4); pneumonia (2), and miscellaneous problems (5). Patients were intubated for a mean (SD) of 5 (4) days before tracheostomy and were mechanically ventilated for a further 6 (4) days. Indications for tracheostomy were as a weaning aid in 36, due to depressed conscious level in 13, and due to upper airways obstruction in one. Thirty two patients survived their hospital admission. The technique was successful in all cases and there were no major complications. A moderate bleed occurred during the procedure in one patient and minor subcutaneous emphysema occurred in two patients. Percutaneous dilational tracheostomy is a safe and effective method that can be used as a bedside technique in the ICU.

Video assisted thoracoscopic pleurectomy in the management of persistent pleural effusion

DA WALLER, GN MORRITT, J FORTY *Freeman Hospital, Newcastle upon Tyne* The optimal method of obtaining pleurodesis in patients with persistent pleural effusion remains undetermined. Open pleurectomy is the most effective but has been associated with high morbidity and mortality (Martin *et al. Cancer* 1975;**35**:734–8). Video assisted thoracoscopic (VAT) pleurectomy is a potentially safer approach. We report our initial experience with this technique in 14 patients (median age 63 years, range 52–84) presenting with persistent pleural effusion. Surgery was indicated for symptomatic relief and diagnosis. All patients represented a high anaesthetic risk due to impaired lung function (median FEV_1 43% predicted; median FVC 62% predicted). VAT parietal pleurectomy was performed via two 2 cm incisions with a median operating time of 35 min (range 15–50). Operating blood loss was not excessive with a median 24 hour fall in haemoglobin of 0·95 g/dl and postoperative pain was minimal (median 12 hour morphine requirement 6 mg). All patients were extubated on table without the need for reventilation. In nine patients no preoperative diagnosis had been obtained but postoperatively all but two were found to have malignant effusions: malignant mesothelioma in eight patients and metastatic adenocarcinoma in four. All patients were successfully discharged with a median postoperative stay of five days (range 3–20 days). At current median follow up of 4·5 months (range 1–11 months) two patients have died but in the remainder recurrent effusion has not been observed. VAT pleurectomy is a safe, effective method of obtaining a diagnosis and achieving pleurodesis in the management of persistent pleural effusion.

Video assisted thoracoscopic surgery for secondary spontaneous pneumothorax in high risk patients

DA WALLER, J FORTY, AK SONI, ID CONACHER, GN MORRITT *Freeman Hospital, Newcastle upon Tyne* Video assisted thoracoscopy (VAT) is potentially preferable to thoracotomy for surgical treatment of secondary spontaneous pneumothorax (SSP) in patients with poor lung function, but its use has not been validated in this condition. We report our experience with VAT surgery in 25 patients (median age 71 years, range 46–92) who all had SSP and emphysema (median FEV_1 48% predicted and FVC 64% predicted) and were at high anaesthetic risk (ASA grade 3 or 4). The median interval to surgical referral was 14 days (range 6–40). VAT pleurectomy ± bullectomy was performed via two 2 cm incisions under general anaesthesia with a median overall operating time of 50 minutes (range 24–90). General anaesthesia was used in each case. Single lung ventilation was found to be superior to high frequency jet ventilation and was tolerated in all but five patients. Postoperative pain was minimal (median 12 hour morphine requirement 12 mg, range 1–44 mg) and no patient required reventilation. Initial treatment was successful in 21 cases (84%) but revisional thoracotomy for air leak was required in four patients, one of whom subsequently died in respiratory failure. The median postoperative stay was seven days (range 3–26). At current median follow up of 10·3 months (range 1·1–17·7) there has been no recurrent pneumothorax. VAT surgery is an effective alternative surgical treatment for SSP in elderly patients at high risk from thoracotomy.

Surgical resection for bronchiectasis: functional and computed tomographic follow up

MJ TOLAN, MF FINK, CD FLOWERS, FC WELLS *Cardiothoracic Surgical Unit, Papworth Hospital, Cambridge* In the decade 1980–90 13 patients aged 36–60 years have had lung resections for bronchiectasis at Papworth Hospital. Preoperatively all patients had long standing symptoms requiring repeated hospitalisation and continuous antibiotic therapy. Eleven patients had unilateral and two bilateral disease. Surgical clearance of all radiologically demonstrated disease was achieved in seven patients. There was zero mortality and morbidity. All 13 patients have had major symptomatic improvement. No patient required further hospitalisation nor more than two courses of antibiotics per year. Pulmonary function testing demonstrates maintained improvement in FEV_1 and VC in nine patients. Computed tomography (CT) has demonstrated no further disease in those seven patients in whom surgical clearance of disease was achieved. Of the remaining six patients there is CT evidence of progression of disease in previously affected segments in five and two patients had newly affected segments. Using a CT scoring system (Bhalla *et al. Radiology* 1991;**179**:783–8) a high correlation between FEV_1 and CT score was demonstrated. It is concluded that surgical resection in advanced bronchiectasis is low in morbidity and highly efficacious even if all disease is not resected. Thin section CT scanning is the optimum method of follow up.

Referral for lung transplantation: experience of an adult cystic fibrosis centre

PJ RYAN, L GUMERY, C STACK, DE STABLEFORTH *Adult Cystic Fibrosis Clinic, Birmingham Heartlands Hospital, West Midlands* Over a six and a half year period commencing February 1987 we have referred 42 patients with cystic fibrosis for lung/heart transplantation to four centres in England. Of these 42 patients four are awaiting assessment. The mean time from referral to assessment was 6·5 months (range 1–18 months). One patient was refused a transplant because of a coexisting aspergilloma and another because of multiple resistant *Staphylococcus aureus*. Initially 18 patients were put on the "non-active" list though half of these subsequently joined the active list. One patient died on the non-active list. Twenty seven patients joined the active transplantation waiting list and 10 patients (37%) died whilst awaiting a transplant. For the patients who died the mean duration on the active list was 6·8 months (range 2 weeks to 25 months) and time from referral to death 13 months. Five patients remain on the active list (average wait of 10·6 months) and 12 patients have been transplanted. Of the 12 patients who have received transplants nine survive with the longest living over five years (average 2 years 9 months). Two transplantation deaths were due to *Pseudomonas cepacia* septicaemia in the postoperative period and the third died of progressive bronchiectasis and severe *Pseudomonas aeruginosa* infection after 10 months. These figures illustrate the difficulties faced by patients with cystic fibrosis when being referred for heart/lung transplantation. Over one third of the patients on the active waiting list died and this situation is likely to deteriorate unless the problems regarding the lack of donor organs improves. We have been worried by the small number of transplantations that have been carried out between 1989 and 1992 and wonder whether being a non-transplant centre is disadvantageous to our patients.

Cytomegalovirus (CMV) carriage in the lung after pulmonary transplantation

M HEALY, S HAMILTON, R SCOTT, C TAYLOR, KF GOULD, JH DARK, PA CORRIS *Department of Cardiopulmonary Transplantation, Cardiothoracic Centre, Freeman Hospital, Newcastle* CMV is a well recognised cause of pneumonia in patients following lung transplantation. Some patients, however, have evidence of persistent CMV carriage in the lungs without pneumonia, as demonstrated by recovery of this organism from BAL fluid after surveillance bronchoscopy. Little is known of the significance of this finding. As part of an ongoing study of the local immune response to CMV in the lung we have prospectively studied the clinical significance in the first six months following transplantation of CMV carriage in the lungs in 46 patients. Three patients developed CMV pneumonia and required treatment with

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Ganciclovir. A further 11 transplant recipients (six single lung (SL), five sequential lung (SSL)) regularly had CMV isolated from BAL without pneumonia (excretors). The 32 remaining patients had no evidence of CMV in their lungs (non-excretors). Mean (SD) frequency of rejection and infection in excretors and non-excretors were 1·1 (0·4) *v* 0·9 (0·6), and 1·9 (1·3) *v* 1·6 (1·4) respectively (both not significant). Similarly there were no significant differences between FEV_1 % predicted or TLCO % predicted at six months between excretors or non-excretors in patients following SLT or SSLT. We conclude that asymptomatic CMV recovery from BAL fluid is not associated with clinically significant complications in the short term. [Supported by the British Lung Foundation.]

Spectrum of *Aspergillus* disease following lung transplantation

JJ EGAN, N YONAN, AN RAHMAN, AK DEIRANIYA, TN STANBRIDGE, KB CARROLL, A WOODCOCK *North West Lung Centre, Department of Cardiothoracic Surgery, Department of Microbiology, Wythenshawe Hospital, Manchester* Transplanted lungs are prone to infection by opportunistic organisms. Of 39 patients (26 heart/lung, nine single lung, four sequential lung) who received lung allografts, five (13%) became culture positive for *Aspergillus* (*fumigatus*) following transplantation. Aspergillus infection contributed directly to death in three patients (8%). None of the five patients were culture positive for aspergillus prior to transplantation. Two patients developed aspergillus infection localised to the suture line at six and 15 days following transplant and were treated with intravenous liposomal amphotericin, oral itraconazole, and nebulised amphotericin. Despite treatment one case progressed to aspergillus pneumonia and died. Two cases were sputum culture positive in the absence of invasive disease and sputum clearance was achieved with liposomal amphotericin, itraconazole and nebulised amphotericin. However, one of these patients had recurrent infection while on maintenance itraconazole and died from aspergillus pneumonia and respiratory failure 18 months following transplant. A fifth patient became sputum positive for aspergillus in association with post transplant lymphoproliferative disease (PTLD) and died as a result of progressive lymphoma and aspergillus pneumonia. Renal function deteriorated in four of the five patients (two developed acute renal failure) when liposomal amphotericin and itraconazole were administered in standard dosage concurrently with reduced doses of cyclosporin. Of the remaining patients 12 were tested for aspergillus precipitins before transplantation with four being positive. One of these and three others were sputum culture positive for aspergillus. Aspergillus infection carries significant morbidity and mortality following lung transplantation, particularly in the presence of invasive disease.

Bronchiolitis obliterans syndrome after heart/lung transplantation (HLT)

TW HIGENBOTTAM, C DENNIS, K MCNEIL, G CREMONA, N CARY, S STEWART, F WELLS, S LARGE, J WALLWORK *Respiratory Physiology Department, Papworth Hospital, Papworth Everard, Cambridge* Long term survival after lung transplantation is limited by the frequent development of disabling severe irreversible airflow obstruction. This clinical state is associated with histological evidence on specimens of lung at post mortem or lung biopsy of an obliterative bronchiolitis. The prevalence and severity of this complication remains unknown in the lung transplant population. Recently an international classification system based on disturbance of lung function was agreed. This sets four grades of bronchiolitis obliterans syndrome (BOS); FEV_1 >80% predicted, FEV_1 <79> 65%, FEV <64%> 50%, and FEV_1 <50%. We have reviewed 72 surviving patients from HLT in terms of grade. The numbers of patients with severe grade 3 BOS after HLT increased with time after surgery. Only 48% of patients had normal lung function at 5 years.

Grade	*Time after surgery (months)*						
	6	*12*	*18*	*24*	*36*	*48*	*60*
0	87	81	70	65	53	51	48
1	9	3	6	17	17	12	32
2	1	7	8	8	9	16	5
3	3	9	16	19	21	21	25

Neutrophil sequestration and activation in the pulmonary vasculature in acute alveolitis

DM BROWN, GM BROWN, JH WILLIAMS, K DONALDSON, W MACNEE *Institute of Occupational Medicine and Unit of Respiratory Medicine, University of Edinburgh, Napier University, Edinburgh; Pulmonary and Critical Care Medicine, UC Irvine Medical Centre, Orange, California, USA* Neutrophil (PMN) sequestration in the pulmonary microvasculature is a prerequisite for cell migration. Intratracheal instillation of *Corynebacterium parvum* (CP) produces a PMN alveolitis in the rat lung with increased sequestration of harvested and radiolabelled ^{51}Cr-PMN (*Am Rev Respir Dis* 1991;**143**:A230). In this study we confirmed PMN sequestration in this model as an excess of unlabelled PMN in pulmonary vascular lavage (PVL). PVL PMN tended to be less deformable and showed increased expression of CD18/11b integrins compared with circulating cells. Circulating and PVL PMN in CP treated animals also had increased TNF secretion compared with control cells. Superoxide anion (O_2) release from circulating and PVL PMN of control rats were similar. In contrast, PVL PMN from CP rats had higher spontaneous O_2 production than control PVL PMN (table). These data suggest that sequestered PMN in this model are activated with the potential to produce tissue injury. [Supported by The Wellcome Trust and the Norman Salvesen Emphysema Research Trust.]

	Untriggered	*PMA*
Peripheral blood		
Control	1·132 (0·251)	6·028 (1·246)
C parvum	0·646 (0·174)	16·702 (2·245)
Microvascular		
Control	0·834 (0·649)	9·569 (0·470)
C parvum	3·906 (1·673)	21·620 (1·230)

Data expressed as nmoles O_2/2·5 × 10 cells).

Expression of circulating membrane bound receptors (CD11b/18, CD14, CD15, L-selectin) from patients at risk of the adult respiratory distress syndrome (ARDS) does not predict subsequent lung injury and ARDS

SC DONNELLY, LJ BRUCE, I DRANSFIELD, D STEEDMAN, DC CARTER, I GRANT, C HASLETT *Respiratory Medicine Unit, City Hospital; Department of Accident/Emergency, Royal Infirmary; Department of Surgery, Royal Infirmary; Intensive Therapy Unit, Western General Hospital, Edinburgh* There is considerable evidence implicating the neutrophil in the pathogenesis of ARDS. Specific neutrophil receptors are known to be involved in the neutrophil/endothelial adhesion which appears to be critical for injury to occur. These include CD11b/18, CD14, CD15, and L-selectin. We investigated whether the expression of these receptors on circulating neutrophils, obtained from ARDS at-risk patient groups on initial hospital presentation, was related to subsequent lung injury and ARDS. Receptor expression on circulating neutrophils was quantified by flow cytometric analysis of fluorescent MoAbs directed against these adhesive molecules at the neutrophil surface. Forty two patients at risk of ARDS were enrolled (multiple trauma (n=37), pancreatitis (n=3), perforated bowel (n=2)), of whom six subsequently progressed to ARDS. Mean (SE) values for receptor expression are summarised in the table. No significant association was found between receptor expression and subsequent ARDS development. CD14 expression was significantly associated with mortality (p=0·03), days requiring assisted ventilation (p=0·045), severity of organ failure (p=0·005), and supplemented oxygen requirements (p=0·009). We conclude that the expression of selected membrane bound neutrophil receptors from ARDS at-risk patients on hospital presentation does not have a predictive role for subsequent ARDS development. However, the expression of these molecules on peripheral blood PMN may not provide a true reflection of events in pulmonary microvessels. Nevertheless a low expression of membrane bound CD14 was of prognostic significance as significant correlations were found with severity of subsequent organ failure and with mortality.

	Non-ARDS (n=36)	*ARDS (n=6)*
CD11b/18	6·46 (1·12)	6·40 (2·50)
CD14	1·37 (0·07)	1·31 (0·08)
CD15	2·69 (0·34)	2·55 (0·62)
L-selectin	6·05 (0·78)	8·27 (3·45)

Bronchoalveolar lavage and pulmonary capillary permeability after cardiopulmonary bypass

DG SINCLAIR, R LANEY, PL HASLAM, JR PEPPER, TW EVANS *AICU, Royal Brompton National Heart and Lung Hospital, London* Lung injury following cardiopulmonary bypass (CPB) is not uncommon. We studied 20 patients undergoing routine CPB surgery to further

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examine this phenomenon. Bronchoalveolar lavage (BAL) was performed with three 60 ml aliquots of buffered normal saline immediately pre- and postoperatively. Cell counts were undertaken on each sample, neutrophil content being expressed as a % of the total cells recovered. Pulmonary capillary permeability was measured once postoperatively by a double isotope technique (*Nucl Med Commun* 1990;**11**:879–84) which allows the lung extravascular protein accumulation index (PAI) to be calculated. The mean (SE) neutrophil content of the BAL fluid preoperatively was 20·34 (5·7)% rising to 48·1 (5·3)% postoperatively ($p < 0.001$). The mean (SE) PAI was 1·57 (0·29) $\times 10^{-3}$ (range 0–44, normal range $< 1 \times 10^{-3}$). The correlation between various parameters is as shown in the table. These data suggest that the increase in pulmonary capillary permeability following CPB is attributable to an inflammatory response which may be generated by the contact of blood with non-physiological surfaces. [Work supported by the British Lung Foundation.]

	r	*p*
Postop % neutrophils and PAI	0·51	<0·05
PAI and bypass time	0·64	<0·01

Time course of permeability increase induced by hyperoxia in cultured endothelial cell monolayers

AM WOODS, DJ GODDEN, PW JOHNSTON, JAS ROSS, HM WALLACE *Departments of Medicine and Therapeutics, Biomedical Sciences, Pathology and Environmental and Occupational Medicine, University of Aberdeen, Aberdeen* An increase in lung interstitial albumin, suggesting increased endothelial permeability, has been observed in rats following exposure to 100 kPa oxygen for 24 hours (O'Gorman *et al. Thorax* 1993;**48**:1071P). To study the mechanism of this permeability increase it would be desirable to establish a model in which cellular function could be examined. The aim of this study was therefore to investigate the effect of oxygen exposure on the permeability of a confluent human endothelial cell monolayer in culture. Human umbilical vein endothelial cells at second pass were seeded at a density of 2×10^4 cells/cm^2 on microporous membranes in a two compartment system (Transwell, Costar, Cambridge) and cultured for 120 hours. This time period had been determined previously to permit the formation of a functionally confluent cell monolayer which was a barrier to passage between the two compartments. [^{14}C]-methyl albumin was then added to the top compartment and its rate of passage across the cell monolayer was determined following exposure to 100 kPa oxygen (hyperoxia) in a specially adapted chamber. Control cells, prepared identically, were exposed to 21 kPa oxygen. The passage of [^{14}C]-methyl albumin was determined after 6, 12, 16, 20, 24, and 28 hours of exposure. Six replicates were performed at each time point, and albumin clearance rate from the upper chamber was calculated as the slope of the clearance/time relationship determined by least squares linear regression. Clearance rate was significantly greater in the cells exposed to 100 kPa oxygen (mean 1·38 µl/min, 95% CI 1 to 1·8) compared with those exposed to 21 kPa oxygen (mean 0·66 µl/min, 95% CI 0·25 to 1) ($t = 3.35$, dF 8, $p = 0.01$). We conclude that permeability to albumin of this human endothelial cell monolayer is increased during the first 28 hours of exposure to hyperoxia. This system provides a useful model to examine mechanisms of endothelial injury and permeability change during hyperoxia.

Respiratory epithelial permeability to plasma proteins following exposure to 100% oxygen in rats

AJW HENDERSON, MC MAIN, PW JOHNSTON, JAS ROSS, PJ HELMS, DJ GODDEN *Departments of Child Health, Medicine and Therapeutics, Environmental Medicine and Pathology, University of Aberdeen, Aberdeen* Oxygen exposure has been shown to increase pulmonary epithelial permeability to ^{99m}Tc-DTPA in rats (Royston *et al. J Appl Physiol* 1990;**69**:1532–7). We have examined pulmonary epithelial permeability in oxygen exposed rats by measuring concentrations of albumin and transferrin in bronchoalveolar lavage fluid (BALF). Twenty four adult, male Sprague Dawley rats (wt 250 (34) g) were studied. Eight animals each were exposed to 100% O_2 for 48 hours and 60 hours and eight control animals received no supplemental O_2. Whole lung lavage was performed with five $\times$ 2 ml aliquots warmed 0·9% saline. Concentrations of albumin and transferrin were measured in the centrifuged BALF supernatant and in serum from a contemporaneously taken blood sample using rocket immunoelectrophoresis. Mean (SD) results are expressed as a percentage of normal rat serum concentrations (table). We have shown that exposure of rats to 100% O_2 leads to a significant increase in albumin and transferrin concentration in BALF. At 48 hours the serum:BALF albumin ratio is increased but transferrin ratio does not rise significantly until 60 hours' exposure. These findings indicate progressive loss of selectivity of permeability to plasma proteins with increasing duration of exposure to hyperoxia. The site and mechanism of these changes remains to be defined.

	Control	*48 hours O_2*	*60 hours O_2*
[Alb] BALF	8·89 (3·96)	16·75 (10·80)	30·00 (7·64)**
[Trans] BALF	12·14 (4·61)	15·83 (9·39)	30·78 (10·2)**
[Alb] BALF/[Alb] serum	0·119 (0·05)	0·254 (0·15)*	0·375 (0·06)**
[Trans] BALF/[Trans] serum	0·143 (0·06)	0·159 (0·08)	0·321 (0·19)*

* $p < 0.05$; ** $p < 0.01$ *v* controls.

Effects of hyperoxia on endothelial cells: evidence for early biochemical damage

AM WOODS, DJ GODDEN, PW JOHNSTON, JAS ROSS, HM WALLACE *Departments of Medicine and Therapeutics, Biomedical Sciences, Pathology and Environmental and Occupational Medicine, University of Aberdeen, Aberdeen* Injury to endothelial cells is an important feature of pulmonary oxygen toxicity (Crapo. *Am Rev Physiol* 1986;**48**:721–31). We have recently shown that pulmonary interstitial albumin in rats increases within 24 hours of hyperoxic exposure (O'Gorman *et al. Thorax* 1993;**48**:1071P). This suggests that endothelial damage is occurring but the mechanism is unknown. The aim of this study was to examine the time course of biochemical changes in endothelial cell cultures exposed to hyperoxia. Human umbilical vein endothelial cells at second pass were exposed to 100 kPa oxygen at atmospheric pressure (hyperoxia) for 24 hours in a specially adapted chamber. Control cells were exposed to 21 kPa oxygen. Measurements of [^{3}H] thymidine incorporation ($n = 6$), cellular ATP content ($n = 6$), cell number ($n = 3$), and cellular protein content ($n = 6$) were made after 6, 12, 16, 20, and 24 hours' exposure. Inhibition of [^{3}H] thymidine incorporation and ATP depletion were observed after six hours of exposure to hyperoxia. Cell number was unchanged up to 20 hours but was significantly reduced after 24 hours. Cellular protein content, expressed as mg/plate, was unchanged. Mean values for cells exposed to hyperoxia, expressed as % control, are shown in the table. We conclude that injury to biochemical functions of endothelial cells in culture occurs within six hours of exposure to hyperoxia. Loss of cell number is a later event.

	Duration of exposure (hours)				
	6	*12*	*16*	*20*	*24*
[^{3}H] thymidine incorporation	*66	*65	*18	*24	*37
ATP content	*58	*41	*47	80	*59
Cell number	100	87	101	99	*71
Protein content	118	107	107	96	106

* $p < 0.05$ *v* control.

Agalactosyl IgG antibody levels are elevated in patients with sarcoidosis and their spouses

HM FIDLER, CM O'CONNOR, C QUIGLEY, MX FITZGERALD, C ABOU-ZEID, GAW ROOK *Department of Medical Microbiology, UCL Medical School, London* Recent studies have given rise to renewed speculation on the possible involvement of mycobacterial infection in sarcoidosis (Fidler *et al. BMJ* 1993;**306**:546–9). The percentage of serum IgG molecules that lack terminal galactose from their F_c heavy chains (G_0) is elevated in tuberculosis, erythema nodosum leprosum, and a subgroup of patients with sarcoidosis (O'Connor *et al. Sarcoidosis* 1992;**9**:453–4). We therefore assessed %G_0 levels in patients with sarcoidosis and their spouses ($n = 47$ each) using an ELISA. %G_0 levels were higher in the sarcoid group and their spouses than in the control group ($p < 0.001$). The detection of raised serum %G_0 in spouses of sarcoid patients lends support to the suggestion that a transmissible/environmental agent is involved in this disease.

Subject	*No of patients*	*%G_0 level**
Sarcoidosis	47	6·09 (0·88)
Spouses	47	3·32 (0·75)
Controls	38	0·34 (0·99)

* Mean (SD) optical density expressed as a % of a positive control area.

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Circulating antibodies to lung antigen(s) in patients with cryptogenic fibrosing alveolitis (CFA)

WAH WALLACE, H CALDWELL, SN ROBERTS, E THORNTON, AP GREENING, D LAMB, SEM HOWIE *Department of Pathology, Edinburgh University Medical School; Department of Respiratory Medicine, Western General Hosptial, Edinburgh* It is recognised that patients with CFA may show disregulation of the humoral immune system. However attempts to demonstrate autoantibodies to lung antigens have been inconclusive. Plasma samples from 20 patients with CFA, 22 patients with sarcoidosis, and 17 healthy controls were screened for the presence of autoantibodies to lung antigen(s) in CFA, sarcoid and control lung tissue as well as four normal non-pulmonary tissues using Western blotting. Eighteen of 20 CFA patients had reactive IgG to lung antigen(s) in the 70–90 kD range compared with five of 18 sarcoid patients and one of 17 healthy controls. Plasma from CFA patients recognised antigen(s) of similar molecular weight in control and sarcoid lung tissue, but not non-pulmonary tissues. Patients with CFA have a high frequency of circulating IgG which recognises antigen(s) within lung tissue. The significance of these antibodies to the aetiology and pathogenesis is as yet unclear.

Tissue location and distribution of a pulmonary autoantigen in patients with cryptogenic fibrosing alveolitis (CFA)

WAH WALLACE, D LAMB, SEM HOWIE *Department of Pathology, Edinburgh University Medical School, Edinburgh* Patients with CFA show evidence of disregulation of the humoral immune system. We have recently shown a high incidence of circulating IgG autoantibodies to a 70–90 kDa lung fraction (*Thorax* 1993;**48**:463). Immunohistochemical staining of CFA biopsy material with IgG purified from patient plasma suggests the antigen(s) is associated with pulmonary epithelial cells. To confirm this and study further the distribution of the antigen(s) we immunised a rabbit with this 70–90 kDa extract from human CFA lung biopsy material. The extract was given subcutaneously at four weekly intervals following a baseline serum sample (R_0). A post immunisation serum sample was obtained four weeks after the third dose (R_3). Western blotting revealed that the R_3 sample, but not the R_0 sample, recognised 70–90 kDa antigen(s) on Western blots of human CFA lung biopsy material. Immunohistochemical staining of cryostat sections of CFA lung biopsies using the rabbit IgG showed staining of the type II epithelial cells. Cytospin preparations of the type II cell line A549 were also positive and the staining pattern appeared cytoplasmic in these cells. This was confirmed by flow cytometry. No positivity with the rabbit IgG was seen on similar preparations of peripheral blood leucocytes, human T cell lines or primary cultures of chondrocytes or pre-osteoblasts. The 70–90 kDa autoantigen(s) we have previously demonstrated are found in association with pulmonary epithelial cells and appear to be cytoplasmic.

Alveolar macrophages from patients with fibrosing alveolitis secrete more TNFα than patients with sarcoidosis and normal individuals

P PANTELIDIS, AM SOUTHCOTT, RM DU BOIS *Royal Brompton Hospital, London* TNFα plays a central role in fibrogenesis. In sarcoidosis (Sarc) and fibrosing alveolitis (FA), two conditions with quite distinct fibrotic responses, the control of TNFα secretion by mononuclear phagocytes at disease sites may be subject to influences within the local milieu and dependent on the degree of mononuclear phagocyte differentiation. To examine this concept we employed the reverse haemolytic plaque assay (RHPA) to evaluate the secretion of TNFα by autologous peripheral blood (PBM) and lavage (BAL) mononuclear cells, at the single cell level and under conditions that minimise paracrine influences, from patients with Sarc and FA and normal individuals (N). PBM and BAL cells were isolated from six N, seven Sarc, and 11 FA and cultured in monolayers with protein A-coated sheep erythrocytes (sRBC) in the presence of polyclonal anti-TNFα antibody with or without lipopolysaccharide (LPS). Secretion of TNFα by individual cells was visualised following complement lysis of the cytokine/anticytokine antibody complex bound sRBC and the areas of lysis were analysed using image analysis following immunocytochemical staining. In paired studies: (a) FA BAL cells spontaneously secreted more TNFα than Sarc ($p < 0.02$) and N ($p < 0.05$); (b) no differences were found using PBM; (c) in all samples there was variability in TNFα secretion; (d) LPS increased the number of TNFα secreting cells in all groups. We conclude that FA BAL cells spontaneously secrete significantly more TNFα than Sarc and N; and that there is heterogeneity within the mononuclear phagocytic population with respect to TNFα secretion.

Lymphocyte chemokine RANTES is expressed in patients with fibrosing lung disease and also in normal lung

M PETREK, AM SOUTHCOTT, P PANTELIDIS, RM DU BOIS *Royal Brompton Hospital, London* CD45RO+T cells ("antigen-primed") accumulate in the lungs of patients with fibrosing alveolitis (FA) but the mechanism of recruitment is not known. RANTES, an 8 kD chemokine, is chemoattractant to CD45RO+ lymphocytes in vitro. To explore whether RANTES plays a role in T cell recruitment in man, we (1) investigated RANTES mRNA expression in lung cells in health and in disease; and (2) correlated RANTES mRNA expression to lavage lymphocyte numbers. RNA was extracted from cells obtained by lung lavage (BAL) from seven normal individuals, four patients with fibrosing alveolitis (FA), 13 with systemic sclerosis and fibrosing alveolitis (SSc-FA), and four with systemic sclerosis without lung disease (SSc). Following reverse transcription, polymerase chain reaction (PCR) with RANTES specific primers was performed; PCR products were visualised by ethidium bromide staining after gel electrophoresis. As controls, mRNA for the constitutively expressed β-actin gene was identified using similar methodology. For quantification, ratios of the optical densities of RANTES and β-actin were calculated. Mean (SE) RANTES/β-actin ratios were: N, 0·24 (0·08); FA, 0·20 (0·05); SSc-FA, 0·22 (0·04); SSc, 0·14 (0·03). In all subjects there was a striking correlation ($p < 0.001$) between RANTES/β-actin ratio and relative ($r_s = 0.56$) as well as total ($r_s = 0.57$) BAL lymphocyte counts. We conclude that (1) RANTES mRNA is expressed in lung cells in health and in disease; and (2) RANTES mRNA expression correlates with lymphocyte numbers implicating RANTES in lymphocyte traffic to the lung. [M Petrek is an EC research fellow.]

Gene therapy for cystic fibrosis: safety of the cationic liposome

PG MIDDLETON, NJ CAPLEN, X GAO, L HUANG, H GAYA, DM GEDDES, EWFW ALTON *Ion Transport Laboratory, National Heart and Lung Institute; Department of Biochemistry and Molecular Genetics, St Mary's Hospital Medical School; Laboratory of Drug Targeting, Department of Pharmacology, University of Pittsburgh School of Medicine; Department of Microbiology, Royal Brompton Hospital, London* We and others have recently demonstrated that in vivo liposome mediated gene delivery of plasmid DNA containing the human cystic fibrosis transmembrane conductance regulator (CFTR) gene can correct the cystic fibrosis (CF) bioelectric defect found in mouse models of CF (*Nature Genetics*, in press; *Nature* 1993;**262**:250). Before cationic liposome-DNA complexes are introduced into the airways of human subjects with CF, we sought confirmation that the liposome itself is safe and does not alter the bioelectric properties of the human airway epithelium. Nasal potential difference (PD) is both a sensitive marker of epithelial integrity and reflects the chloride impermeability characteristic of CF. We therefore assessed whether nasal application of the cationic liposome DC-Chol:DOPE altered nasal ion transport. Lung function was also used as a further measure of safety. As the airways of the subjects with cystic fibrosis are chronically infected, we also studied whether DC-Chol:DOPE complexes altered the bacterial growth or sputum sensitivities of CF subjects. The mean (SE) results are given in the table. Sputum culture showed no change in growth or sensitivities following the addition of cationic liposome. Thus it is likely that DC-Chol:DOPE is appropriate for use in human trials of gene therapy for CF.

	Nasal PD		*Lung function*	
	Baseline	*ΔCl*	*FEV_1*	*FVC*
Before	14·0 (1·3)	9·9 (1·0)	4·1 (0·2)	5·3 (0·2)
After 1 hour			4·1 (0·2)	5·0 (0·3)
After 1 day	16·5 (1·4)	11·8 (1·8)	4·0 (0·3)	4·8 (0·3)
After 5 days	15·4 (1·1)	9·8 (1·8)	4·0 (0·2)	5·1 (0·2)

Assessment of an organ culture model incorporating an air interface for the study of bacterial interactions with human respiratory mucosa

AD JACKSON, CFJ RAYNER, PJ COLE, R WILSON *Host Defence Unit, Department of Thoracic Medicine, Royal Brompton National Heart and Lung Institute, London* Adherence to human respiratory mucosa

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is thought to be a critical event in bacterial colonisation. Organ cultures immersed in cell culture medium have been used to study adherence of pathogenic bacteria to the mucosa, but this is unphysiological and bacteria replicating in the medium can reinfect the tissue and cause damage by toxin production. Also bacterial adherence is affected by the composition of the medium. We have developed a new nasopharyngeal organ culture incorporating an air interface. The function and structure of inferior nasal turbinate tissue within the model was assessed by light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) for 10 days. Ciliary beat frequency (CBF) remained constant at 11–12 Hz over five days. At 10 days CBF fell significantly to 7·9 (0·8) Hz ($p = 0·0004$). By SEM mucus cover varied from 29% to 84% but there was no significant time related trend. The percentage tissue cover of ciliated cells, non-ciliated cells, and tissue damage did not change significantly over 10 days. By TEM levels of heterochromatin in the nuclei were reduced at five days and virtually absent at 10 days. Nucleoli were also much less frequently observed. Mitochondria appeared normal at four days, slightly damaged at five days, and severely damaged at 10 days. Tight junctions, cell projection, cytoplasmic blebbing, and cell vacuolation remained normal over 10 days. Nasal turbinate tissue in this novel organ culture model with an air interface remained normal for five days. The model is more physiological than total immersion models for the study of microbial interactions with the mucosa, and its longevity will permit studies of viral/bacterial interactions and pharmacological studies.

Ciliary disorientation in patients with chronic upper respiratory tract (URT) inflammation

CFJ RAYNER, A RUTMAN, PJ COLE, R WILSON *Host Defence Unit, Department of Thoracic Medicine, Royal Brompton National Heart and Lung Institute, London* Random ciliary orientation (CO) has been described as a possible variant of primary ciliary dyskinesia (PCD); cilia have normal ultrastructure and nearly normal beat frequency (CBF) but lack efficacy because the direction of their beat is random. We have investigated the nasal cilia of patients with URT inflammation and two patients with clinical PCD who have random CO (index group). Bronchiectatics (Bx) had positive nasal cultures: *Pseudomonas aeruginosa* (PA) 5, *Streptococcus pneumoniae* 3, *Haemophilus influenzae* 4. All cystic fibrosis (CF) patients were taking antibiotics, two had positive cultures for PA, seven were sterile. Other cultures were negative. Cilia ultrastructure was normal in all cases. Disorientation of the central microtubules and basal feet (expressed as the mean standard deviation of ciliary angles) was significantly increased in both the index and Bx groups. Disorientation was greater in the Bx patients with PA (22·03 *v* 15·07, $p < 0·05$) and in the two CF patients with PA at 15·8 ($p < 0·02$). Comparison of NMCC and CO demonstrated a positive correlation ($r = 0·9$). Antibiotic treatment resulted in a reduction in disorientation in one patient with Bx and PA from 23·41 to 10·25 ($p < 0·05$). These findings show that disorientation contributes to abnormal mucociliary clearance and suggest that disorientation may occur secondary to bacterial infection and inflammation.

Patient group	*n*	*NMCC (min)*	*CBF (Hz)*	*Mean (SD)*
Control	10	12·6	12·2 (1·2)	10·47 (0·53)
Index	2	>60	13·8 (2·8)	22·09 (0·25)*
Bronchiectasis	12	38·83	12·4 (0·9)	17·72 (4·15)**
Cystic fibrosis	9	18·11	11·4 (1·4)	12·3 (2·17)

* $p < 0·05$, ** $p < 0·001$.

Effect of salmeterol on cyclic AMP mediated slowing of human ciliary beat in vitro by the *Pseudomonas aeruginosa* toxin pyocyanin

K KANTHAKUMAR, M JOHNSON, PJ WILLS, GW TAYLOR, PJ COLE, R WILSON *Host Defence Unit, Royal Brompton National Heart and Lung Institute, London; Department of Cardiovascular and Respiratory Pharmacology, Glaxo Group Research Ltd, Ware; Department of Clinical Pharmacology, Royal Postgraduate Medical School, London* Pyocyanin (Pyo) is a phenazine pigment produced by *P aeruginosa* which is present in the sputum of colonised patients, reduces human ciliary beat frequency (CBF) in vitro, and slows mucociliary transport in vivo in the guinea pig. Pyo-induced ciliary slowing is associated with a substantial fall in intracellular cyclic AMP, and agents which raise cyclic AMP prevent ciliary slowing. Isoprenaline (2×10^{-7} M) inhibited pyo (20 μg/ml) induced slowing of CBF by 39%. We have now investigated the effects of salmeterol, a long acting β_2 agonist, on human CBF in vitro ($n = 6$). Salmeterol ($1–4 \times 10^{-7}$ M) inhibited the effects of pyo in a concentration-dependent manner. Salmeterol (2×10^{-7} M) was found to reduce pyo (20 μg/ml)-induced slowing of CBF by 53% and fall in intracellular cAMP by 26%. Thirty minutes' exposure to salmeterol was required to maximally inhibit pyo action and the effects were retained after washing the cells. Propranolol (10^{-7} M) and the β_2-specific antagonist, ICI118551 (10^{-6} M) blocked the protective effects of salmeterol completely, but atenolol (10^{-6} M) was less effective. These results suggest that the effects of salmeterol were mediated primarily via the stimulation of β_2 adrenoreceptors. Mucociliary clearance is an important defence mechanism of the airways against bacterial infection. Salmeterol may benefit patients colonised by *P aeruginosa*, not only by its bronchodilator action, but also by protecting epithelial cells from pyo-induced slowing of CBF.

Damage to bronchial wall examined in an experimental model of bronchiectasis due to persistence of bacteria

D GUERREIRO, R FLORIO, H TODD, MN SHEPPARD, PJ COLE *Host Defence Unit and Department of Lung Pathology, Royal Brompton National Heart and Lung Institute, London* A "vicious circle" of events enables persistent *Pseudomonas aeruginosa* (Pa), *Haemophilus influenzae* (Hi) and *Staphylococcus aureus* (St) to colonise the respiratory tree of patients leading to severe alteration of the bronchial wall components resulting in chronic bronchial sepsis (CBS) and progressive bronchiectasis (Bx). The histopathology has been described but no quantitation of the relative damage imparted to these components by different bacteria has been reported. We have produced an in vivo model of progressive Bx using isolates from sputum of patients with CBS and Bx and quantitated bronchial wall components. The rat apical bronchus was ligated (LIG) and 10^9 (cfu) of the individual bacterial isolates were injected into the distal bronchial lumen. Three groups of five rats were prepared with either Pa (PaL + LIG), Hi (HiL + LIG) or St (StL + LIG), and 12 control groups (normal (N); sham operated; LIG alone; each of the three viable bacteria alone; each of the three bacteria killed: with and without LIG). Rats were sacrificed from each group at one, two, and three months after surgery, lungs inflated, apical lobes orientated and sectioned uniformly for staining with monoclonal antibody to smooth muscle actin, Millers' elastin stain to differentiate both bronchial wall collagen and elastin stainable tissue. Axial bronchial wall, blood vessels and their muscle, collagen and elastin tissue were assessed blind by a modified point counting technique, no less than 10^3 points being counted (× 400) and tested using Kruskal–Wallis nonparametric analysis of variance. Severity of bronchiectatic changes was Pa > Hi > St at all time points. No changes were found for controls. Mean (SD) data are shown in the table. Persistence of these viable bacteria within the bronchial tree irreversibly damages bronchial wall components to a relative severity as is observed clinically in human Bx.

Exp'tal group	*% Bronchial wall muscle*	*% Bronchial wall vessel*	*% Vessel wall muscle*	*% Bronchial wall collagen*	*% Elastin degradation*
Pa	2·56 (0·6)	24·1 (7·2)	7·3 (0·5)	51·5 (4·5)	10·2 (1·0)
Hi	8·2 (3·1)	14·6 (6·3)	7·1 (2·2)	45·7 (3·8)	9·3 (2·0)
St	20·2 (5·5)	13·8 (2·4)	5·3 (1·1)	41·3 (4·4)	14·7 (1·1)
N	30·6 (6·0)	5·8 (2·0)	2·2 (0·7)	29·1 (4·9)	23·2 (2·8)

Pa *v* N $p < 0·0001$ at all sites.

Effects of subminimal inhibitory concentration (MIC) of antibiotics on the morphology of non-typable *Haemophilus influenzae* (NTHi)

A RUTMAN, KWT TSANG, A PALMER, D ROBERTS, G TILLOTSON, PJ COLE, R WILSON *Host Defence Unit, Royal Brompton National Heart and Lung Institute, and Royal Postgraduate Medical School, London* The effects of subMIC antibiotics on NTHi morphology have not been investigated. We have studied the effects of 0·25 MIC of amoxycillin (AMO), loracarbef (LC) and ciprofloxacin (CIP) on SH9, a clinical isolate of NTHi. Bacteria were obtained by centrifugation of the minimal essential medium (with or without 0·25 MIC of antibiotics) that immersed adenoid organ cultures whose cut surfaces were embedded in agar. After 24 h incubation at 37°C bacterial viable counts rose from 8×10^5 to $4(2) \times 10^7$ colony forming units/ml. The pellets were smeared onto sterile glass coverslips and were critically point dried for scanning electron microscopy (SEM). At SEM (10 000 ×), the first 100 non-dividing bacteria seen at random were electronically captured and assessed for the following parameters: perimeter, major elliptical axis (EA) and minor EA, and elongation

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factor (mean maximum intercept/mean perpendicular intercept i.e. the more elongated the shape the greater the factor). We conclude that subMIC of antibiotics alter the morphology of NTHi which may influence bacterial adherence to the mucosal surface.

Bacterial morphology	*Control (no antibiotic)*	*0·25 MIC AMO*	*0·25 MIC LC*	*0·25 MIC CIP*
Perimeter (μm)	3·1 (0·7)	3·3 (1·4)	3·4 (1·9)	3·5 (1·3)*
Major EA (μm)	1·4 (0·4)	1·5 (0·7)	1·6 (0·9)	1·6 (0·6)*
Minor EA (μm)	0·4 (0·1)	0·4 (0·1)	0·4 (0·2)	0·4 (0·1)
Elongation factor	6·4 (17·8)	3·4 (5·3)	3·1 (4·7)	2·9 (0·9)

Data = mean (SD) of 100 bacteria. *$p < 0.01$ *v* control (Friedman's test).

How virulent strains of *Moraxella catarrhalis* evade serum killing

S MURPHY, T SCOTT, CT KEANE, JB WALSH AND D COAKLEY *Mercer's Institute for Research on Ageing; Department of Clinical Microbiology, St James's Hospital; and Department of Biological Sciences, Institute of Technology, Dublin, Ireland* Strains of *Moraxella catarrhalis* (*M catarrhalis*) associated with clinical infection are typically resistant to the complement mediated bactericidal effect of normal human serum. Asymptomatic colonising strains of the organism, however, are usually sensitive to serum killing. The aim was to study the mechanism by which virulent strains of *M catarrhalis* evade the bactericidal effect of serum. This was done by studying phagocytosis of serum sensitive and serum resistant strains in the presence of normal serum. Phagocytosis requires pre-opsonisation. A component of the complement cascade (C_{3b}) is the main opsonin of serum. A microscopical assay was used to quantify ingestion of bacteria by neutrophils. Results are expressed as the percentage of neutrophils ingesting bacteria. 12 strains of *M catarrhalis* were studied: six serum sensitive strains and six serum resistant strains. There was no significant difference in the degree to which serum sensitive and serum resistant strains were phagocytosed by neutrophils in the presence of normal serum (34 + 13% *v* 37 + 14% $p > 0.05$). These results show that both serum sensitive and resistant strains of *M catarrhalis* are opsonised and phagocytosed to an equal extent. Evasion of complement-mediated bactericidal killing must then occur beyond the C_{3b} stage in the complement cascade. Evasion may involve blockage of insertion of the membrane attack complex due to surface virulence factors.

T cell receptor Vβ gene usage is not restricted in tuberculous pleural effusions

TK LIM, NK CHIN, BW LEE, CC SEAH, FL SIN *Departments of Medicine and Pediatrics, National University and Alexander Hospitals, Singapore* T cell receptors (TCR) are major antigen recognising sites on T cells. We have shown, in tuberculous pleural effusions, that there is compartmentalisation of helper, cytotoxic/natural killer and memory T cells in the pleural fluid (*Am Rev Respir Dis* 1992;**145**:A474). However, we found no preferential use of the γδ TCR over the αβ TCR by pleural T cells (*Chest* 1993;**103**:198S). Restriction of Vβ family gene usage by T cells has been reported in sarcoidosis, some autoimmune diseases, and toxic shock syndromes. This study examines the repertoire of the TCR Vβ (variable segment) using mAbs to detect gene products from 6 Vβ families (Vβ 5·1, 5·2, 5·3, 6, 8, and 12) in T cells from pleural fluid (PF) and peripheral blood (PB) of 13 patients with pleural tuberculosis. The Vβ gene usage in PF for the group [expressed as mean (SD)% CD4+ T cells] was Vβ 5·1 = 4·5 (2·9)%, 5·2 = 0·6 (0·7)%, 5·3 = 2·2 (1·2)%, 6 = 3·1(1·8)%, 8 = 3·5 (1·7)%, 12 = 3·5 (3·3)%. In the PB Vβ 5·1 = 2·9 (1·3)%, 5·2 = 1 (1)%, 5·3 = 1·04 (0·8)%, 6 = 1·6 (1·3)%, 8 = 1·9 (0·6)%, 12 = 2·08 (2)%. For individual patients, the Vβ expression did not exceed 2 SD from the overall mean for the whole group in either PB or PF. We conclude that Vβ gene use by T cells was not restricted in tuberculous pleural effusions.

Cellular tropism of HIV-1 strains isolated simultaneously from peripheral blood leucocytes and bronchoalveolar lavage cells of AIDS patients

JOHN R CLARKE, ADELINE N NUKUNA, JOHN D WILLIAMSON, DAVID M MITCHELL *Department of Medical Microbiology and Department of Respiratory Medicine, St Mary's Hospital, Paddington, London* Primary isolation of HIV-1 from matched PBL and BAL samples from 10 AIDS patients with respiratory symptoms was attempted by cocultivation of 2×10^6 patient PBL or BAL cells with an equal number of indicator cells. The target cells used in this study were cord blood leucocytes (CBL), the lymphoblastoid cell lines CEM, H9, and MOLT-4, and the monoblastoid cell lines HL-60, THP-1, and U937. We successfully isolated HIV-1 from nine out of 10 PBL and seven out of 10 BAL samples. Matched PBL and BAL virus isolates were obtained from six patients in this study. In all instances the cellular tropism of the HIV-1 strains obtained from BAL differed from the matched PBL strain. Six out of nine (66%) HIV-1 strains obtained from PBL were pantropic and grew in most or all cell lines. Only two out of seven (29%) BAL viruses could be considered pantropic and those viruses were isolated from individuals with repeated respiratory episodes. Interestingly, three out of seven (43%) BAL isolates grew only in lymphoblastoid cell lines, whereas one out of seven (14%) replicated only in monoblastoid cell lines. Only the two pantropic BAL viruses were successfully recovered from CBL whereas seven out of nine (78%) PBL viruses grew in CBL. This study strongly shows that HIV-1 strains from the lung represent a phenotypically distinct sub-population of virus from AIDS patients.

HIV-1 proviral DNA copy number in peripheral blood leucocytes and bronchoalveolar lavage cells of AIDS patients

JOHN R CLARKE, JOHN D WILLIAMSON, DAVID M MITCHELL *Department of Medical Microbiology and Department of Respiratory Medicine, St Mary's Hospital, London* In this study we have determined the quantity of HIV-1 proviral DNA by PCR in cells obtained from the lung by bronchoalveolar lavage (BAL). This has been compared with the proviral DNA in peripheral blood leucocytes (PBL) obtained simultaneously from the same patients. The mean HIV DNA copy number per 10^6 cells was 391 for PBL with a range of 1 to 9000 and 2971 for BAL cells with a range of < 1 to 70 000. The quantity of HIV DNA detected in BAL cells was higher than that detected in the corresponding PBL samples in 44 out of 78 (56%) patients whereas more HIV DNA was detected in the PBL compared with BAL cells in 14 out of 78 (18%) patients. In this study in both BAL and PBL higher levels of HIV DNA were detected in the adherent (monocyte/macrophage) enriched cell populations compared with other non-adherent cells (leucocytes). Patients known to be receiving azidothymidine treatment had a lower mean HIV DNA load in all cell fractions compared with those patients on no antiretroviral therapy. A relation between HIV DNA copy number and ability to recover infectious HIV progeny in vitro by cocultivation with cord blood leucocytes was found for both PBL and BAL cells.

Absorption, accumulation, and metabolism of [^{14}C]-pentamidine in the isolated perfused rabbit lung

A BELLO, A HUTT, C MARRIOTT, V FACHINI, M JOHNSON *Department of Pharmacy, King's College, London; Rhone Poulenc Rorer UK, Dagenham, Essex* Pentamidine isethionate, used for the treatment of infections of *Pneumocystis carinii*, is often administered by inhalation. However, little is known concerning the absorpton, accumulation, or metabolism of the drug when given by this route. In an attempt to address these issues we have examined the fate of [^{14}C]-pentamidine using an isolated perfused rabbit lung (IPL) model. [^{14}C]-Pentamidine isethionate (1·0 mg, 14·4 μCi) was given as an aqueous solution via the trachea using a single pass rabbit IPL model (perfusate flow rate 10 ml/min for 90 minutes). The perfusate was analysed for total radioactivity and the concentration of pentamidine determined by high performance liquid chromatography (HPLC). The maximal perfusate drug concentration (C_{max}) and time to C_{max}, (T_{max}) were 1·1 μg/ml (n = 3) and 9·33 minutes respectively. The area under the perfusate concentration time curve (AUC) was 33·04 μg/ml/min with 47.4% of the radioactivity being eliminated in the perfusate over 90 minutes. [^{14}C]-pentamidine isethionate (0·25 mg, 3·6 μCi) was also given as a bolus to a recirculating IPL model (60 ml of perfusate, flow rate 10 ml/min). The drug perfusate concentration declined in a biexponential manner with 27·8% (n = 4) of recovered radioactivity being taken up in the tissue over 90 minutes. HPLC and radiochemical analysis of lung samples from two of the three single pass experiments revealed a radioactive peak which did not correspond to the parent compound. Only [^{14}C]-pentamidine was detected in the lungs from the recirculating IPL experiments and perfusate samples from both the single pass and recirculating experiments. These data suggest that the biotransformation of pentamidine only occurs when the compound is presented to the lumenal surface of the lung.

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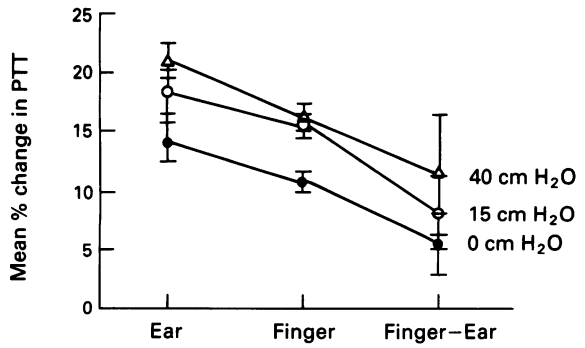
Surfactant protein D interacts with *Pneumocystis carinii*

DM O'RIORDAN, J STANDING, E CROUCH, AH LIMPER *Thoracic Diseases Research Unit, Mayo Clinic, Rochester, Minnesota, USA* Surfactant protein D (SP-D) is a recently described surfactant component, which possesses a calcium (Ca^{2+}) dependent lectin binding domain common to the C-type lectins; surfactant protein A, mannose binding protein and conglutinin, each of which has a role in host defence. *Pneumocystis carinii* (PC) is a carbohydrate rich organism possessing several surface glycoproteins the most abundant of which is gp 120. We hypothesised that SP-D might interact with PC through binding of the lectin moiety to PC surface glycoproteins. We, therefore, investigated binding of SP-D to PC component proteins. SP-D was purified and radiolabelled with $Na^{125}I$. PC were obtained from the lungs of steroid-treated rats. Solubilised PC proteins were resolved by SDS-PAGE, transferred to nitrocellulose membranes, incubated with ^{125}I-SP-D, and binding of ^{125}I-SP-D to PC proteins was assessed. SP-D demonstrated consistent binding to a 120 kDa molecule in the presence of Ca^{2+} (2 mM). Incubation either in the presence of 100 mM glucose or in the absence of Ca^{2+} (20 mM EDTA) totally inhibited SP-D binding. Purified gp 120 bound ^{125}I-SP-D in an identical manner to that for whole organism extracts. We further questioned whether increased amounts of SP-D were present in the lung during PC pneumonia. Immunohistochemical staining revealed substantial amounts of SP-D around clusters of intra-alveolar organisms in the lungs of rats with PC pneumonia. Increased amounts of SP-D were consistently demonstrated in BAL fluid from PC infected animals compared with uninfected controls. We conclude that SP-D production is enhanced in the lung during PC pneumonia and that SP-D interacts with gp 120 present on the surface of the organism. SP-D may represent an additional host defence during PC pneumonia.

Mechanism of pulse transit time lengthening during obstructed inspiration

DJ PITSON, S KNIJN, M VAN HERWAARDEN, JR STRADLING *Osler Chest Unit, Churchill Hospital, Oxford* Pulse transit time (PTT) (time of propagation of pulse wave from EGG R wave to detection at the finger) is inversely proportional to blood pressure (BP). The size of the rises in PTT with inspiration correlate well with the degree of inspiratory effort and this relation is as good as that between inspiratory effort and arterial BP (Stradling *et al. ERS* 1993). This study explores the mechanism of PTT lengthening during inspiration to explain its sensitivity to changes in inspiratory effort. PTT measured from the ECG R wave has two components: the cardiac pre-ejection systolic period (PEP) and actual pulse wave transit time from aortic valve opening (APTT). Two simultaneous PTT measurements were made in six subjects breathing through a mask with three inspiratory loads (0,15, and 40 cm H_2O). One measured PTT to the ear (mainly PEP), the other measured PTT to the finger (PEP+APTT). The mean inspiratory PTT rise and the % change from preinspiratory PTT was calculated for ear and finger and for their difference (mainly APTT). The graph shows the changes in PTT at the two sites and their difference for each of the inspiratory loads. The mean % change in PTT during an obstructed inspiration was always greatest at the ear suggesting that increases in PEP (left ventricular ejection time) are the greatest contributor to the lengthening in measured PTT at the finger. Hence measuring PTT from the ECG R wave (which includes PEP) proves to be a sensitive method of identifying inspiratory effort.



Mechanical determinants of apnoea duration in obstructive sleep apnoea (OSA)

K REES, DPS SPENCE, PMA CALVERLEY *Aintree Chest Centre, Liverpool* The anatomical factors that predispose patients to obstructive sleep apnoea are well recognised but there is less information about why apnoeas end. Studies in small numbers of patients suggest that respiratory muscles may achieve fatiguing levels of effort at apnoea termination but this has been disputed. The consistency of the inspiratory response to the apnoea has not been examined and differences in the timing and duration of inspiratory effort during apnoeas might explain differences in apnoea duration. We have studied 15 patients with OSA of variable severity (mean age 47·2 (2·8), mean BMI 32·5 (2·2), mean AHI (38·6)) in an invasive one night polysomnographic study. In addition to the normal variables we recorded PPL from an osophageal balloon. Fifteen apnoeic events were analysed for each patient from stable stage 2 sleep. Timing was established from the PPL trace and total inspiratory effort from the product of PPL and Ti (PTi). Mean apnoea duration varied from 13·8 to 32·1 seconds with four to seven efforts. Inspiratory time increased significantly throughout the apnoeas (first, 1·76, mid, 1·95, last, 2·05 efforts (s)) while Ttot shortened from mid apnoea onwards (first, 4·13, mid, 4·15, last, 3·55 efforts (s)). These changes were consistent between patients. PTi increased from 42·2 (6·6) to 85·2 (11·9) during the apnoea but the PTi at apnoea termination was very variable. Final PTi was related to apnoea duration ($r = 0.75$, $p < 0.01$) and the change in PTi during the apnoea was related to total AHI ($r = 0.56$, $p < 0.01$). However, apnoea duration was unrelated to AHI ($r = 0.13$). Thus the respiratory timing response to apnoea is consistent and shortening of Ttot precedes arousal. Overall load compensatory mechanisms appear intact and respiratory effort is appropriate to the length of the apnoea. These data suggest that factors other than the intensity of inspiratory muscle contraction determine apnoea duration in OSA.

Effect of age and sex on upper airway calibre

SE MARTIN, R MATHUR, NJ DOUGLAS *Rayne Laboratory, University of Edinburth; City Hospital, Greenbank Drive, Edinburgh* Eight five per cent of patients with the sleep apnoea/hypopnoea syndrome (SAHS) are men and most are middle aged. Patients with SAHS have smaller upper airway areas (Brown *et al. Am Rev Respir Dis* 1987;**136**:628) than normal subjects. We used acoustic reflection to measure upper airway calibre in a pool of normal subjects, aged 18–75, recruited from the hospital workforce, to look at the effect of age and sex on upper airway calibre. Subjects breathed normally through a mouthpiece connected to an acoustic reflection system (Marshall *et al. Physiol Meas* 1993;**14**:157), both sitting upright and supine while 10 measurements of upper airway calibre were made. A plot of airway area against distance was reconstructed and an average measurement of airway area at the oropharyngeal junction (OPJ) was obtained. Thirty one men (median age 31, range 16 to 70), and 30 women (median age 37, range 22 to 74) with BMI < 26 were studied. Men had significantly larger OPJ areas (1·8 cm^2 (SE 0·1)) than women (1·6 (0·1); $p = 0.05$) when sitting but on lying down average airway areas were similar (1·3 (0·1); $p = 0.4$). Men showed a larger percentage change in airway area on lying down (25%, 13%; $p < 0.02$) than women. Age did not correlate with airway area in either sex or when both groups were combined (r values < 0.25, $p > 0.1$). BMI was not significantly different between sexes (23·0) (SD 1·9) male, 22·4 (2·4) female; $p = 0.2$). Men had a larger neck circumference (37·0 cm (SD 1·7) male, 32·0 (1·4) female, $p < 0.0001$) which correlated significantly with BMI ($r = 0.58$, $p < 0.01$ male, $r = 0.6$, $p < 0.001$ female). Men had significantly higher neck circumferences in relation to BMI than women. This mass loading may explain the greater upper airway narrowing seen in the men on lying down and may contribute to the increased prevalence of SAHS in men.

Clinical usefulness of the Epworth sleepiness scale (ESS) in suspected sleep apnoea

AH KENDRICK, N WILTSHIRE, JR CATTERALL *Sleep Unit and Respiratory Department, Bristol Royal Infirmary, Bristol* The ESS (John. *Sleep* 1991;**14**:540–5) is a self administered questionnaire giving a subjective assessment of daytime sleepiness. The total range is 0 to 10, with a maximum of 24. We have assessed the usefulness of the questionnaire. 180 patients were referred for assessment. All had pulse oximetry. 108/180 were assessed with thoraco-abdominal movement and nasal/oral airflow. The desaturation frequency (DF) was calculated as the number of desaturations (>4%) per hour and the apnoea/hypopnoea index (AH) as the number of events per hour with a desaturation of >4%. Patients were divided into four subgroups – (1) AH or DF < 5; (2) AH or DF 5–15; (3) AH or DF 15–30; (4) AH or DF > 30. In 19 patients, ESS was obtained before and after establishment on nasal CPAP. The results are given as mean (SD) or mean (range). For the group, a weak correlation of ESS to DF ($r = 0.3$, $p < 0.001$) but not to AH was obtained. Analysis of variance

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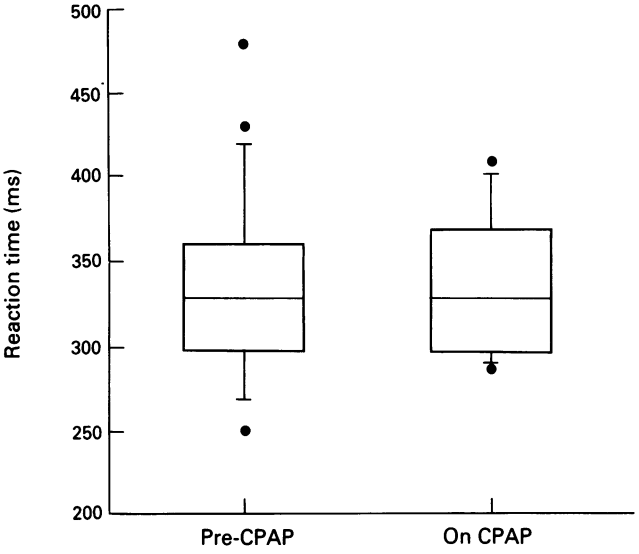
Group	n	Age (y)	M/F	ESS
1	90	47·0 (11·5)	73/17	10·1 (0 to 24)
2	46	50·5 (10·6)	40/6	9·6 (2 to 19)
3	21	50·7 (10·5)	21/0	10·6 (0 to 18)
4	23	52·2 (10·8)	22/1	14·5 (8 to 22)

showed a significant difference in ESS between the subgroups ($p < 0.01$). 40/90 patients in group 1, 18/46 in group 2, 11/21 in group 3, and 20/23 in group 4 had an ESS > 10. In the patients on CPAP, ESS fell significantly ($p < 0.001$) from 17·4 (3·6) to 5·8 (4·4). We conclude that (1) a significant number of patients with a DF or AH of < 30 have pronounced increases in subjective daytime sleepiness and (2) the effectiveness of CPAP treatment on daytime sleepiness can be assessed.

Daytime vigilance in patients with obstructive sleep apnoea before and after CPAP treatment

JM PARTLETT, DJ PITSON, RJO DAVIES, JR STRADLING *Osler Chest Unit, Churchill Hospital, Oxford* The multiple unprepared reaction time (MUPRT) test is an objective measure of impaired vigilance in patients with obstructive sleep apnoea (OSA) manifesting daytime sleepiness. When patients are successfully treated with continuous positive airway pressure (CPAP), subjective sleepiness improves. This study assesses whether the MUPRT test improves in parallel with symptoms. Vigilance was tested in a group of patients (n = 18) with sleep study confirmed OSA using the MUPRT test. The same patients were retested after being successfully treated with nasal CPAP (mean compliance 4·35 hours/night). The Box and Whisker plot (fig) shows the median reaction times and distribution, both



before and after treatment with nasal CPAP during the second half of the MUPRT test. The Wilcoxon test for paired sample data suggests that there is no significant difference between the two groups ($p = 0.8$). All patients had reported a subjective improvement in daytime sleepiness. However, objective testing with the MUPRT test showed no significant improvement in vigilance. We conclude that the MUPRT test is not a useful index of symptom relief and thus cognitive function. It is therefore not a useful objective measure of the effectiveness of nasal CPAP treatment.

Effects of CPAP on daytime function in the sleep apnoea/hypopnoea syndrome: a placebo controlled study

HM ENGLEMAN, SE MARTIN, IJ DEARY, NJ DOUGLAS *Respiratory Medicine Unit, City Hospital, Greenbank Drive, Edinburgh* CPAP may improve mood state (Derderian *et al. Chest* 1988;**94**:1023–7) and daytime sleepiness in the sleep apnoea/hypopnoea syndrome (SAHS) (Lamphere *et al. Chest* 1989;**96**:1364–7), but objective evidence from controlled trials of enhanced cognitive performance after CPAP is lacking. We therefore performed a randomised, placebo-controlled crossover study of daytime function in 32 consecutive SAHS patients (mean 36 apnoeas ± hypopnoeas/hour slept, range 7-129) comparing four weeks with CPAP with four weeks with a placebo tablet which the patients were told might improve upper airway muscle function. Before treatment each patient had a CPAP titration study and a psychometric familiarisation session to reduce learning effects. On the last day of each treatment patients attended for a full day of assessments comprising a multiple sleep latency test to quantify daytime sleepiness and a comprehensive psychometric battery evaluating psychological wellbeing, mood, symptoms, and cognitive performance. Mean CPAP compliance was 3·5 (SE 0·4) hours/night, but even at this relatively low level of CPAP use, two-way repeated measures ANOVA found improved subjective symptoms ($p < 0.001$), psychological wellbeing ($p < 0.05$), energy levels ($p = 0.001$), and objective daytime sleepiness ($p < 0.05$) on CPAP compared with placebo. Cognitive performance was significantly better on CPAP than on placebo, with improved scores on tests of vigilance (steerclear, $p < 0.01$), mental flexibility (trailmaking B, $p < 0.02$), attention (digit symbol substitution, $p < 0.05$), and in estimated intellectual deficit (IQ decrement, $p < 0.05$). This study provides objective evidence of improvement in symptoms, psychological distress, energetic arousal, and cognitive deficit after the commencement of CPAP therapy in SAHS.

Prediction of nasal continuous positive applied pressure (CPAP) in the treatment of obstructive sleep apnoea

AH KENDRICK, N WILTSHIRE, JR CATTERALL *Sleep Unit and Respiratory Department, Bristol Royal Infirmary, Bristol* Nasal CPAP is a highly effective treatment for obstructive sleep apnoea (OSA). To reduce both snoring and apnoeas, it is important to provide the correct pressure for each patient. The purpose of this study was to derive a simple equation to predict this pressure. 31 patients (27 male; age 38–71; body mass index (BMI) 21·3–54·3 kg/m^2) with a clinical history of OSA had polysomnography, excluding EEG, which confirmed the presence of apnoeas. The number of desaturations of > 4% (DS_4) ranged from 17 to 879 (mean 226) per night, with the lowest saturation (LS) ranging from 30% to 88% (mean 67%). On three subsequent nights, CPAP was applied in increasing levels and the effects assessed with pulse oximetry. On the third night, the final pressure produced a DS_4 ranging from 0 to 64 (mean 11). Stepwise regression analysis was applied to the final pressure (cm H_2O) *v* initial DS_4, LS, age, height, mass, and BMI to obtain the prediction equation:

$$\text{Pressure} = 4{\cdot}44 + 0{\cdot}0075DS_4 + 0{\cdot}084\text{BMI};\ \text{SD} = 0{\cdot}95;\ r = 0{\cdot}91$$

This equation has been applied to five male patients (age 40–58; BMI 27·6–46·2 kg/m^2) who are now established on CPAP therapy. The predicted pressure reduced the DS_4 from a mean of 241 (range 88–542) to a mean of 9·6 (range 1–25). We conclude that this simple equation provides a useful guide to the pressure most likely to achieve the reduction in apnoeas required for the successful establishment of patients on nasal CPAP.

Comparison of nine nasal CPAP systems

N WILTSHIRE, AH KENDRICK, JR CATTERALL *Sleep Unit and Respiratory Department, Bristol Royal Infirmary, Bristol* We have compared nine nasal CPAP systems available in the United Kingdom. The systems (origin) were (1) Taema CP90 (France), (2) Healthdyne Tranquility$^+$ (USA), (3) Devilbiss Revitalizer (USA), (4) Remstar Choice (USA), (5) Remstar LS (USA), (6) Puritan Bennett C318 (USA), (7) Thomas (UK), (8) Si-Plan CPG.011 (UK) and (9) Sullivan APD2 (Australia). We (a) assessed, at a CPAP pressure of 10 cm H_2O, sound level (SL_{10}; dB), pressure drop (cm H_2O) along a 547 cm tube (PD_{10}) and flow rate (FL_{10}; l/min); (b) measured pressure range (cm H_2O) and mass (kg); (c) estimated electric consumption (EC; kW/h) for one year at 8 h/night. Cost (£) is for a complete system. All complied with a current European safety standard, had a

System	SL_{10}	PD_{10}	FL_{10}	Range	EC	Mass	Cost (£)
1	43	0·3	122	4·0–18·1	204	3·5	795
2	45	0·4	170	3·0–17·6	302	3·8	671
3	52	0·1	163	3·9–24·9	277	4·2	495
4	47	0·2	163	2·5–25·1	365	2·8	800
5	52	0·2	160	2·3–22·7	365	2·7	730
6	51	0·4	140	2·7–24·5	321	3·2	651
7	44	0·1	153	1·4–25·0	146	6·2	650
8	47	0·2	213	3·8–16·7	146	8·2	740
9	49	0·1	138	4·1–21·2	204	2·2	616

110/240 volt switch and, except system 3, a DC adaptor. An hour meter was not available on systems 2, 3, and 5 and ramping was unavailable on systems 1 and 7. Warranty was one year except system

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3 (three years) and 9 (two years). We conclude that (a) differences occur in cost, sound level, electric consumption, flow rate and mass, and (b) increasing the tube length from blower to mask has little effect on the set pressure.

Natural history of snoring/sleep disordered breathing and behaviour problems in young children

NJ ALI, D PITSON, JR STRADLING *Osler Chest Unit, Churchill Hospital, Oxford* There are no prospective studies of the natural history of snoring and associated behaviour problems in children, so 2·5 years after our original survey of 4–5 year olds (*Arch Dis Child* 1993;**68**:60–6) we asked the same parents to complete another questionnaire with the aims of collecting information on the natural history of snoring and testing the robustness of the associations between snoring and behaviour problems we found and reported on then. Of the original 789 children in the 1989/90 study we received responses from 507 (64%). These 507 were a representative sample of the 789 in terms of sex ratio and responses to the original survey. The overall prevalence of habitual snoring (most nights) at 11·2% in 1992 was the same as in 1989 for these children, but more than half of the 1992 habitual snorers had developed it since the first survey (it had resolved in about half of the original group). Trend analysis (χ^2) showed that the increasing prevalences of sleepiness, hyperactivity, and restless sleep across the snoring categories were significant (see table, all $p < 0·002$), but not so for abdominal pain (a question to look for spurious associations). As in our 1989 survey we found that children who snore *most nights* (compared with those who *never* snore) had an increased risk of daytime sleepiness (relative risk (RR) = 6·3, 95% CI 2·6–15), hyperactivity (RR = 2·7, 95% CI 1·6–4·6), and restless sleep (RR = 2·3, 95% CI 1·7–3·3), but not of having frequent attacks of abdominal pain (RR = 1·3, 95% CI 0·9–1·9).

	Snoring category (%)			
	Never (n = 129)	*Rarely (n = 166)*	*Sometimes (n = 140)*	*Most nights (n = 55)*
Sleepy in day	5	10	10	29
Hyperactive	15	12	12	40
Restless sleep	27	31	48	65
Abdominal pain	33	44	44	46

Sleep disordered breathing, behaviour, and psychological functioning in children: effects of adenotonsillectomy

NJ ALI, D PITSON, JR STRADLING *Osler Chest Unit, Churchill Hospital, Oxford* We have previously reported a strong association between snoring/sleep disordered breathing (SDB) and behaviour problems in a community study of children (*Arch Dis Child* 1993;**68**:360–6). Here we present the results of a prospective controlled study of the effects on behaviour and psychological functioning of treating SDB by adenotonsillectomy (AD). Three groups of children were studied; (1) SDB group, n = 12, mean age 7·6, (2) snorer group (snorers without obvious SDB) n = 11 mean age 7·8 and (3) control group, n = 10, mean age 7·5. The children were studied before and 4–6 months after surgery (AD for groups 1 and 2, another surgical procedure for 6/10 of group 3). After AD the SDB group showed significant improvements in all three subscales of the Parent Conners behaviour scale, whereas the snorer group improved on the hyperactivity subscale only. There were no significant changes in the control scores (see table, median scores are shown. *p = 0·04, **p = 0·003). Attention measured objectively by the continuous performance test also improved in SDB and snorers (p = 0·02 and p = 0·006 respectively). The improvements seen suggest that even mild SDB has an impact on children's behaviour. The snorers occupy an intermediate position between the SDB and controls suggesting a dose/response relation between sleep disruption and behaviour/attention problems.

		Aggression	*Inattention*	*Hyperactivity*
SDB	preop	0·85	1·0	0·91
	postop	0·67*	0·67**	0·58**
Snorer	preop	0·56	0·66	0·83
	postop	0·56	0·50	0·67*
Control	preop	0·53	0·53	0·58
	postop	0·61	0·78	0·66

Health status measured by SF-36 in recipients of domiciliary nasal intermittent positive pressure ventilation (NIPPV)

N POTTER, MW ELLIOTT, AK SIMONDS *Royal Brompton National Heart and Lung Hospital, London* Five year actuarial survival in 176 Royal Brompton Hospital patients receiving domiciliary NIPPV is: 85% in idiopathic scoliosis, 92% previous TB, 100% previous poliomyelitis, 70% neuromuscular disease, and 43% COPD. Health related quality of life is a necessary adjunct to these data and has been examined with the SF-36 measure of health status in 116 patients (mean age 54·2 years) receiving domiciliary NIPPV for six months to seven years. Patient categories were (*a*) early onset scoliosis n = 35, (*b*) previous poliomyelitis n = 21, (*c*) previous TB n = 16, (*d*) neuromuscular disorders (excluding polio) n = 14, (*e*) COPD n = 12, (*f*) miscellaneous n = 7. The SF-36 examines eight aspects of health (physical function, role limitation due to physical and mental problems, social function, mental health, energy and vitality, pain, and health perception). The questionnaire was administered by post with a follow up telephone call. Responses were anonymous and coded by diagnostic category. Response rate was 90·5% (105/116). Mean (SE) results for the whole group were: physical function 30·4 (2·58)%, role limitation: physical 46·6 (4·0)%, social function 65·0 (2·9)%, mental health 72·6 (1·9)%, energy and vitality 47·8 (2·0)%, pain 72·9 (2·7)%, health perception 47·2 (2·3)%. These results are comparable with SF-36 data from patients with serious medical conditions (diabetes, hypertension, recent MI, cardiac failure) (McHorney *et al. Med Care* 1993:**31**;247–63). Physical function was significantly higher in groups (*a*), (*b*), and (*c*) compared with group (*e*). Patients with COPD and neuromuscular disease had a similar level of physical function, but role limitation was greater in the COPD group (18·7 *v* 65·0% p = 0·006). This finding is likely to reflect the younger age of their handicap and higher mental health score (p = 0·048). Overall, although health status is reduced, quality of life seems comparable with ambulant patients with other chronic disorders.

Randomised controlled study of nasal pressure support ventilation compared with oxygen therapy in stable hypercapnic chronic obstructive pulmonary disease (COPD): preliminary results

DJ MEECHAM JONES, EA PAUL, JA WEDZICHA *Department of Thoracic Medicine, The London Chest Hospital, London* Long term oxygen therapy (LTOT) has been shown to improve survival in patients with chronic respiratory failure due to COPD. Nasal positive pressure ventilation has been proposed as a treatment for these patients; however the comparison of nasal ventilation with established LTOT has not been previously determined. We report a study of the effect of the addition of nasal pressure support ventilation (NPSV) to domiciliary oxygen therapy compared to that of oxygen alone in a group of patients with stable hypercapnic respiratory failure due to COPD. 18 patients with hypercapnic COPD were studied (15 M, 3 F, median age 69, range 43–74 years). All were established on long term domiciliary oxygen therapy (median duration 20, range 3–70 months) and in a stable clinical state prior to entry, mean (SD) Pao_2 5·99 (0·70) kPa, $Paco_2$ 7·36 (0·38) kPa, FEV_1 0·81 (0·30) l, FVC 2·12 (0·60) l. Following a four week run in period each patient underwent two consecutive three month periods of (*a*) oxygen therapy alone and (*b*) oxygen therapy plus NPSV in randomised order. NPSV was delivered with a BIPAP ventilator. Lung function, arterial blood gases, and exercise tolerance were assessed during run in and at the end of each study period. 14 of the 18 patients completed the study, with only one patient withdrawn due to inability to tolerate the equipment. There were significant improvements in blood gases at three months following the addition of NPSV: mean (SD) Pao_2 rose from 5·90 (0·89) kPa (O_2 alone), to 6·69 (0·93) kPa (O_2 + NPSV), mean change (95% CI) + 0·79 kPa (+ 0·39 to + 1·20). Mean (SD) $Paco_2$ fell from 7·60 (0·72) kPa (O_2 alone), to 7·03 (0·60) kPa (O_2 + NPSV), mean change − 0·57 kPa (− 0·84 to − 0·29). There was no significant change in exercise tolerance in the two treatment periods: median (range) six-minute walking distance O_2 alone 235 (80 − 440) m, O_2 + NPSV 240 (100–450) m (p = 0·44). In this group of patients with advanced but clinically stable hypercapnic respiratory failure due to COPD, the addition of NPSV to domiciliary oxygen therapy produced significant improvements in resting daytime blood gases at three months. This mode of ventilation proved easy to establish and was well tolerated. Nasal positive pressure ventilation may be a useful addition to LTOT in the long term management of this group of patients. [This work is supported by The British Lung Foundation.]

Non-invasive assisted ventilation in the management of respiratory failure in acute severe asthma

VHF MAK, C HARVEY, DF TREACHER *Department of Thoracic Medicine and Intensive Therapy Unit, St Thomas' Hospital, London* The presence of hypercapnia in a patient with acute severe asthma demands urgent action, and may require intubation and mechanical

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ventilation. There are, unfortunately, significant risks associated with mechanical ventilation in asthmatic patients. The use of non-invasive assisted ventilation (NIAV), and in particular nasal intermittent positive pressure ventilation (NIPPV), in the management of acute hypercapnic respiratory failure in patients with chronic respiratory disease is becoming common. However, the use of NIAV in the management of hypercapnia in acute severe asthma has not been reported. We describe our experience of NIAV in three patients (two female) admitted with acute asthma and hypercapnia. All three (aged 25–29) had a short history of worsening asthma unresponsive to repeated doses of inhaled bronchodilators. On admission they were distressed, able to speak only a few words, their peak flows were unrecordable, respiratory rate over 40 breaths/min, hypercapnic relative to their degree of respiratory distress (P_{CO_2} 5·73–8·53 kPa), and mildly acidotic (pH 7·24–7·29). They were treated initially with high dose nebulised bronchodilators, intravenous corticosteroids, and aminophylline but did not respond. They were therefore transferred to intensive care where they were considered to need urgent ventilatory assistance. Rather than formal ventilaton, they were commenced on intermittent positive pressure ventilation using a NIPPY ventilation and full face mask (Thomas Respiratory Systems), with inspiratory pressure support of 17–20 cm H_2O, and supplemental oxygen. They continued regular nebulisers and aminophylline infusions. All three responded rapidly, returning to relative normocapnia (P_{CO_2} < 4·5 kPa) within four hours of initiation of NIAV and continued to require NIAV for 8–24 hours. They were well enough to be discharged from the ITU after 12–36 hours. Where facilities for NIAV exist, their initial use in the management of hypercapnia in severe acute asthma may abort the need for formal ventilation. Obviously, these treatments need to be carried out in a controlled environment where intubation and ventilation can be carried out rapidly if NIAV fails.

Changes in functional residual capacity (FRC) and pattern of resting breathing with the "Hayek oscillator"

E JACKSON, R LANE, A LAVERTY, G SLADE, J RAE, Z HAYEK *Institute of Child Health, 30 Guilford St, London* The "Hayek Oscillator" is a cuirass ventilator, capable of providing high frequency ventilation against a background of continuous negative extrathoracic pressure (CNEP) allowing control of FRC. The cuirass consists of a rigid, moulded plastic shell that covers the chest and upper abdomen, and is secured to either a soft (SB) or hard (HB) back plate. The aims of the present study were: (1) to assess the effect of wearing the cuirass on FRC and the pattern of resting breathing; (2) to measure the effect on FRC of applying CNEP. We studied 16 healthy, adult volunteers (age 22–58 years, 10 female). Subjects were studied with no cuirass, cuirass alone, and cuirass maintained at −6 cm H_2O (CNEP), for each of the SB and HB cuirasses. FRC (helium dilution), and respiratory pattern (uncalibrated respiratory inductance plethysmography) were measured for each experimental condition. The mean (range) changes in FRC with cuirass alone were −0·011 (−0·31 to +0·19) for SB cuirass and −0·071 (−0·28 to +0·20) for HB cuirass. With CNEP, the changes in FRC relative to baseline were +0·151 (−0·10 to +0·42) and +0·111 (−0·13 to +0·62) for SB and HB respectively. There were no consistent changes in the patterns of resting breathing between conditions. The group mean changes in FRC with the cuirass alone were not statistically significant. However, 3/16 subjects showed a reduction in their FRC of greater than 10%. With CNEP, the increase in group mean FRC over baseline was statistically significant with both SB and HB cuirasses ($p < 0·05$). We conclude that the cuirass in itself may result in a pronounced fall in FRC in some subjects. However, the oscillator allows FRC to be modified by application of CNEP.

In vivo and in vitro electrophysiological characteristics of the airways of the insertional mutant mouse model of cystic fibrosis (CF)

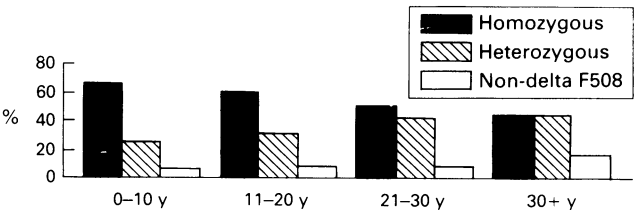
SN SMITH, DM STEEL, JR DORIN, DJ PORTEOUS, DM GEDDES, EWFW ALTON *Ion Transport Laboratory, National Heart and Lung Institute, London; MRC Human Genetics Unit, Edinburgh* We have previously described the preliminary electrophysiological characteristics of the CF mouse generated by exon 10 targeted insertional mutagenesis (*cf/cf*) (*Nature* 1992;**359**:211). We have now studied the detailed in vivo and in vitro bioelectric properties of the nasal and lower airway epithelium of this model to assess how closely it reproduces the relevant characteristics found in patients with CF. The in vivo nasal PD of *cf/cf* mice was significantly greater than in wild type animals, as was the response to amiloride (100 μM). Thus as in humans sodium absorption is likely to be increased in the nasal epithelium of the mutant animals. Furthermore, as in CF patients cAMP mediated chloride secretion was reduced, but calcium related responses were preserved. As previously reported, this pattern of responses also characterised the trachea and bronchi, with the exception of the response to amiloride, which was significantly reduced in the *cf cf* animals. This pattern of tracheal responses has also been reported in the Cambridge replacement CF mouse model (*Nature Genetics*; **4**:35). The virtual absence of submucosal glands in the murine trachea, and the lack of an increase in sodium absorption suggest that any pulmonary pathology developing in these animals relates to reduced surface epithelial cAMP dependent chloride secretion.

Assessment of chloride secretion across human nasal epithelium using ^{125}I efflux

DM STEEL, SN SMITH, N CAPLEN, DM GEDDES, EWFW ALTON *Ion Transport Laboratory, National Heart and Lung Institute; Department of Biochemistry, St Mary's Hospital Medical School, London* Cystic fibrosis (CF) is characterised by reduced cAMP mediated chloride secretion across epithelial cells. Poor supplies of both CF and non-CF human lower airways necessitates the use of alternative tissues for airway ion transport studies. Human nasal epithelial cells provide an easily accessible source, which can provide multiple samples of cells that may have been treated in vivo with pharmacological or gene transfer agents. ^{125}I efflux is well recognised to reflect chloride movement across epithelial cells. Therefore, this study aimed to optimise the conditions for the use of ^{125}I efflux across human airway cells obtained from nasal brushings. Human nasal cells were obtained by brushing the floor of the nasal cavity about 1–3 cm distal to the anterior tip of the inferior turbinate. Cells were plated on to matrigel and studied using standard ^{125}I efflux techniques. When cells were seeded on to 24 well (22 mm diameter) plates no response to stimulation with either agents elevating intracellular cAMP or calcium was seen. However, when cell density was increased by seeding on to 96 well (0·9 mm diameter) plates, ^{125}I efflux in response to both types of stimulation was seen. In paired studies the mean (SE) optimum response occurred on day 2 after seeding (cAMP: 1·4 (0·8) k^{-1}, Ca^{2+}: 1·0 (0·2) k^{-1}, n = 4) in comparison with day 1 (cAMP: 0·3 (0·1) k^{-1}, Ca^{2+}: 0·3 (0·1) k^{-1}, n = 4), day 4 (cAMP: 0·5 (0·4) k^{-1}, Ca^{2+}: 0·7 (0·4) k^{-1}, n = 4) or day 7 (cAMP: 0·5 (0·2) k^{-1}, Ca^{2+}: 0·3 (0·3) k^{-1}). We conclude that nasal brushings provide a convenient source of airway epithelial cells for studies of chloride secretion relevant to the assessment of new treatments for CF.

Prevalence of the delta F508 mutation in cystic fibrosis in the West Midlands

PJ RYAN, N HARRISON, D SPENCER, M GILES, P FARNDON, P WELLER, DE STABLEFORTH *Birmingham Children's and Birmingham Heartlands Hospital; West Midlands Regional Clinical Genetics Service* The delta F508 gene is the most common mutation causing cystic fibrosis. In the United Kingdom the delta F508 mutation accounts for 76% of all cystic fibrosis alleles (Super M. *J R Soc Med* 1992;**85** (suppl 19): 6–8). As the delta F508 homozygote is associated with a poorer prognosis one would expect that it would be under-represented in the older age groups. Of 461 cystic fibrosis patients currently attending the regional paediatric and adult cystic fibrosis centres 413 have been examined by mutational analysis. The frequency of delta F508 homozygotes in the paediatric clinic was 64% (n = 185) compared with the adult clinic (55%, n = 68). The data are analysed in 10 year age bands and show a decreasing proportion of delta F508 homozygotes in the older age groups and an increasing proportion of compound heterozygotes and less common mutations. This is due to two main reasons: earlier specialist referral and earlier death reflecting the increased severity of the delta F508 genotype. There was no significant difference between the frequency of male and female delta F508 homozygotes.



Serum IgG response to an 80 kDa outer membrane protein of *Pseudomonas cepacia* in patients with cystic fibrosis

DE LACY, AW SMITH, PA LAMBERT, DE STABLEFORTH, EG SMITH, PH WELLER, MRW BROWN *Birmingham Children's Hospital; Department*

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of Pharmaceutical Sciences, Aston University; Birmingham Heartlands Hospital, Birmingham There is growing evidence that transmission of *P cepacia* (Pc) can occur by patient to patient contact (Govan *et al. Lancet* 1993;**342**:15–9), raising issues of patient segregation in cystic fibrosis clinics. Early diagnosis of Pc is therefore important if segregation is to be effective. Nelson *et al* (*J Med Microbiol* 1993;**39**:39–47) have recently reported raised IgG titres to a Pc-specific lipopolysaccharide core determinant in colonised patients. Independently, we have developed an ELISA based serodiagnostic test using an 80 kDa outer membrane protein (OMP). This protein was recovered by electroelution from OMPs separated by SDS-PAGE, coated onto ELISA plates which were then reacted with patient sera, protein A-peroxidase, and chromogenic substrate. The endpoint was taken as the optical density (OD) obtained with a 1:200 dilution of serum. Serum from 14/16 CF patients colonised with Pc showed high OD values (mean 1·37, range 1·02–1·72), 2/16 gave intermediate values (0·62 and 0·77), whereas 43/43 non-colonised patients showed a lower OD (mean 0·56, range 0·23–0·92). Our results suggest that by using an endpoint value of OD 1·0 we can distinguish between Pc-colonised and non-colonised patients.

Plasma immunoreactive interleukin 8 (IL-8) in cystic fibrosis

SC BELL, L DAVIES, JS ELBORN, DJ SHALE *Section of Respiratory Medicine, University of Wales College of Medicine, Llandough Hospital, Cardiff* Chronic *Pseudomonas aeruginosa* infection occurs in most adults with cystic fibrosis (CF) and causes an injurious inflammatory response which is mainly neutrophil mediated. Cytokines – for example, TNFα, IL-1β, IL-6, and IL-8 – are implicated in injury. IL-8 may be particularly important as it is a major neutrophil chemotactic and activation factor, which causes release of elastase and myeloperidoxase and stimulates superoxide production. We investigated the pattern of circulating immunoreactive IL-8 in 10 clinically stable CF adults; 10 patients receiving antibiotics for a respiratory exacerbation (days 1 and 15); and in 10 age and sex matched controls. IL-8 was measured by ELISA (Medgenix, Belgium) and was detected in the plasma of 8/10 clinically stable patients, 10/10 patients in pulmonary exacerbation, and in none of the controls. IL-8 levels were greater in those patients in exacerbation compared with the stable group ($p<0·05$) and there was a trend to reduced IL-8 levels after a course of antibiotics (table). Pooled IL-8 data for patients with CF were negatively correlated with FEV_1 ($r=0·67$, $p<0·001$) and positively with the peripheral neutrophil count ($r=0·58$, $p=0·008$).

	Mean (range) IL-8 (pg/ml)
Healthy subjects	<0·7
CF-clinically stable	1·46 (<0·7–2·99)
CF (exacerbation-day 1)	3·21 (0·9–5·53)
CF (exacerbation-day 15)	2·56 (1·1-6·6)

IL-8 is detectable in the plasma of patients with CF. Levels are correlated with peripheral blood neutrophil count, and are greater in those with more severe lung disease.

Sensitisation to *Aspergillus fumigatus* in adult patients with cystic fibrosis

M HAYES, CP MAGUIRE, JP HAYES, J MASTERSON, MX FITZGERALD *Departments of Respiratory Medicine and Diagnostic Imaging, St Vincent's Hospital/University College Dublin, Dublin 4, Ireland* Subclinical allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis may be associated with pulmonary inflammation. We assessed the prevalence of sensitisation to *A fumigatus* (*Af*) in relation to clinical status in 73 patients attending our outpatients over a three month period. Patients completed an interview directed questionnaire and underwent clinical assessment. There were 38 (51%) males and 35 (49%) females (age 20·9 (0·58) years, mean (SE)). There were 28 (38%) patients with atopy, and 32 (43·2%) with a positive skin prick test to *Af*. Asthma (defined as a positive response to two of three questions: wheeze or shortness of breath on climbing stairs, early in the morning or on waking at night) was found in 24 (32%). Transient infiltrates were present in 30 (44%) patients, proximal bronchiectasis in 28 (41%), and an aspergilloma was found in one patient. There were nine (13·2%) patients with a combination of asthma, positive skin test to *Af* and transient infiltrates. Of these only six had specific IgG antibodies to *Af*. Five of these six patients were taking oral steroids and two had raised eosinophil counts. Patients with a positive skin prick test to *Af* had significantly lower %pred FEV_1 (53·8 (4·6) compared with 66·5 (3·7); $p<0·05$) and Swachmann score (71·1 (2·7) compared with 79·5 (2·0), $p<0·05$)) but did not differ in age, sex, age of diagnosis, or number of years with pseudomonas. There was a significant relation between a positive skin test to *Af* and atopy but not asthma. Sensitisation to *Af* may be associated with more severe disease in patients with cystic fibrosis.

Early focal *x* ray film changes and later morbidity and mortality in cystic fibrosis

ML KIRBY, S MUKHOPADYAY, A DUNCAN, F CARSWELL *University of Bristol and Bristol Royal Hospital for Sick Children, St Michael's Hill, Bristol* The variable rate of progression of respiratory disease in cystic fibrosis (CF) is not predicted by genotype, and reliable prognostic markers would be useful in individual management. Focal chest *x* ray (CXR) film changes were studied retrospectively to assess their value as markers. Forty eight children born between 1965 and 1979, with confirmed CF and complete records available, were identified from the Bristol Children's Hospital cystic fibrosis clinic. Of these, 18 were excluded from analysis because of late diagnosis or management elsewhere, leaving 30 patients for study. Nineteen of these had lobar or segmental focal opacification on CXR before six years of age. Routine spirometry and radiological scores at 7 and 13 years were compared, and survival curves constructed, for groups with and without early changes. The groups were comparable with regard to presentation, age at diagnosis, year of birth, genotype, and routine respiratory management. There were more girls among those with early CXR changes ($p<0·05$). The peak expiratory flow (PEF) at 7 years was significantly lower in those with early focal changes ($p<0·05$); the mean values for all expiratory data (FEV_1, FVC, and PEF) were lower and median CXR scores higher (suggesting more severe disease) at 7 and 13 years in those with previous early changes, although not significantly. There were seven deaths before the age of 13, all in the group with early CXR changes ($p<0·01$). These results suggest that focal CSR changes before 6 years may indicate a worse prognosis in children with CF, and imply a need for more aggressive treatment in this group. [MLK and SM were funded by the Cystic Fibrosis Trust, UK.]

Fibrosing alveolitis (FA) and desquamative interstitial pneumonitis (DIP)

N SHARIEF, OF CRAWFORD, R DINWIDDIE *Respiratory Unit, Hospital for Sick Children, Great Ormond Street, London* The experience and evaluation of treatment strategies in FA/DIP over the last 16 years by a review of all cases referred to a tertiary referral centre is reported. There were 25 cases including 16 boys and nine girls. Mean age of onset was 2·25 years (range 7 days to 11·6 years). In each case the diagnosis was confirmed by open lung biopsy at a mean age of 3·29 years (range 7 weeks to 15·1 years). Presenting features were tachypnoea (19), cyanosis (15), cough (12), exertional dyspnoea (7), recurrent chest infections with or without wheeze (9), and clubbing (8). Four patients recovered without anti-inflammatory medication. The others received specific treatment. Eleven were given prednisolone only; six improved, there was no improvement in two, and three died despite treatment. Five patients received chloroquine only and four responded. Five patients received both prednisolone and chloroquine. One patient died despite treatment with both drugs and there was a variable response in the remaining four. Of the 10 patients who were given chloroquine six (60%) showed a good response. A younger presentation was associated with a worse prognosis but there was no relation between chest radiology at presentation and outcome. Those with mild histological changes all survived but when there was severe desquamation or fibrosis on the biopsy, there was no relation between severity and outcome. There was a family history in four cases (16%). FA/DIP probably represents a disease spectrum of multiple aetiology with a variable prognosis and response to treatment.

Predicting the severity of airways disease from arterial alveolar oxygen difference in children with bronchopulmonary dysplasia

RW ILES, AT EDMUNDS *The Respiratory Laboratory, The Royal Hospital For Sick Children Edinburgh* Partial forced expiratory flow volume curves (PFEFVC) were taken in infants with bronchopulmonary dysplasia (BPD) less than 5 months of age (age range 63–120 days). Measurement of the arterial oxygen tension while breathing 50% oxygen in a steady state headbox was taken within two hours of

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the PFEFVC. Measurements were taken on or around the child's expected date of delivery (EDD) (n = 21) and between three and four months later (n = 15). All infants had been born prematurely (23–30 weeks' gestation). All infants had been ventilated, were oxygen dependent at 28 days of age, and had chest x ray film findings compatible with BPD. Calculations of the maximum flow at functional residual capacity (VmaxFRC), and arterial–alveolar oxygen difference in 50% oxygen were made for each patient. Regression analysis was performed using arterial–alveolar oxygen difference to model an equation to predict the Vmax FRC. Comparison showed no significant correlation at term. Significant correlation was found at three months with values of $p < 0.05$ and $r = 0.56$. The findings at term may reflect the difficulty in obtaining accurate PFEFVC in small babies with severe airways disease. Alveolar–arterial difference may represent a simple way of assessing the severity of lung disease in infants with BPD.

Oxygen therapy and maturational differences in infants with bronchiolitis

DC ANDREWS, S LAWREY, A THOMSON, P JOHNSON *Departments of Obstetrics and Pediatrics, John Radcliffe Hospital, Oxford* Fifteen infants (3 weeks to 8 months old) treated for bronchiolitis without IPPV, were monitored while in head box oxygen (total of 840 hours) Either (A) Sao_2, pulse rate, breathing and activity (pad sensor), and microphone; or (B) A plus ECG heart rate, chest, and abdominal excursion, were recorded to a computer aided display and analysis system (CARDAS). Eight were term and seven were expreterm infants. Sleep disruption was pronouncd. Term infants had tachypnoea, range in quiet sleep (QS): 49–71 (normal infants: 38-25 between 1 and 6 months); obstruction (usually partial) with increased thoracoabdominal phase angle (TAPA; normals QS: 11 (1); term bronchiolitis: 40 (13); active sleep (AS): 130 (19)). Arousals periodically relieved progressive hypoxaemia/hypoventilation (up to 50/hour during AS). Infants born preterm also hypoventilated with progressive hypoxia but with much less obstruction (TAPA less). All had periodic breathing (PB) usually terminating the hypoventilation, and increasing the Sao_2. The PB cycle length was 12–14 s with < 10 s apnoeas (similar to healthy term infants). Two infants had periods of long central or mixed apnoea (some > 30 s). In these the fall in Sao_2 from a "flat" 100% (hyperoxia) and bradycardia were delayed suggesting that hyperoxia was promoting the length of apnoea, before arousal and/or onset of breathing occurred. (1) The responses of expreterm infants are consistent with inhibitory mechanisms prevailing over excitatory ones from obstruction or airway irritation as they had minimal chest wall distortion (less effort) and more apnoea, apparently promoted by hyperoxia in some of them. (2) Clinical staff were unaware of these events, in particular the occurrence of apnoea in expreterm infants, and were misled by intermittent Sao_2 measurements, not recognising hyperoxia or true from false hypoxaemia. High dependency care was probably unnecessarily prolonged by relying on intermittent pulse oximetry.

Tidal breathing parameters and specific airway conductance in early life

CAROL DEZATEUX, I DUNDAS, E JACKSON, ME FLETCHER, J STOCKS *Unit of Epidemiology and Biostatistics and Portex Unit of Anaesthesia, Institute of Child Health, London* There has been increasing interest in tidal breathing parameters in early life. However, their relation to more established measures of airway function at this stage remains unclear. This study compares plethysmographic measurements of specific airway conductance (sGaw) and the ratio of time to reach peak tidal expiratory flow to total expiratory time ($t_{PTEF}:t_E$) in 108 infants (1–18 months of age), of whom 45 had been diagnosed with recurrent wheezing, bronchiolitis, or asthma by a physician (group A), and 63 of whom were healthy infants recruited from the community (group B). Similar measurements were made in 27 infants eight months after hospital admission for acute bronchiolitis (group C), and a community group of healthy age and sex matched controls (group D). Results (mean (SD)) are summarised in the table. $t_{PTEF}:t_E$ was significantly lower in recurrently wheezy compared to healthy infants, and in infants with prior bronchiolitis when compared with controls. sGaw and $t_{PTEF}:t_E$ were significantly correlated in group A and group B ($A:F_{1,62} = 5.28$, $p < 0.05$; $B:F_{1,43} = 9.77$, $p < 0.01$). These data confirm the association between low values of $t_{PTEF}:t_E$ and diminished airway function that has been reported previously.

Group	n	$t_{PTEF}:t_E$	sGaw ($s^{-1}.kPa^{-1}$)	p ($t_{PTEF}:t_E$)
A Healthy infants	63	0·31 (0·07)	2·30 (0·87)	< 0·05
B Wheezy infants	45	0·27 (0·08)	1·39 (0·65)	
C Postbronchiolitic	27	0·24 (0·06)	2·02 (0·82)	< 0·05)
D Controls	27	0·28 (0·06)	1·93 (0·69)	

Evidence for changing risk factors for chronic obstructive lung disease during the last 150 years

GB MARKS, PGJ BURNEY *Department of Public Health Medicine, UMDS, London* To investigate the influence of smoking and other factors on time trends in mortality due to chronic obstructive lung disease (COLD), we analysed the historical mortality dataset for England and Wales for the period 1901 to 1991. Deaths in 15 to 74 year olds attributed to all forms of COLD except asthma were analysed. Data were adjusted to correct for important changes in the method of coding that were introduced in 1921 and 1940. Analysis of age, sex, and year of death specific mortality rates by iteratively reweighted least squares regression revealed significant ($p < 0.0001$) independent effects of age, period of death, and cohort of birth. The death rate in 55 to 74 year old males declined from 9·1/1000 in 1901 to 1·4/1000 in 1991. The decline was greater if analysed by year of birth: 13·1/1000 for subjects born near 1841 decreasing to 0·7/1000 for subjects born near 1921. Among 55 to 74 year old men there was an increase in the mortality rate among those born between 1871 and 1911 and among females in this age group the downward trend ends with those born in 1881. These interruptions to the downward trend are consistent with the effect of cigarette smoking which peaked in the early 1950s among males and continues to rise among females. The overall downward trend, however, implies that other important risk factors for COLD operated before and during the epidemic of smoking. Potential risk factors for which time trends are consistent include prenatal nutrition and occupational dust exposures (males). In conclusion, analysis of the time trend for COLD mortality during the 20th century shows that smoking is the latest in a changing sequence of risk factors for this disease. [Data for this analysis were provided by OPCS.]

Relation of serum IgE, eosinophils, smoking, and bronchial responsiveness to FEV_1/FVC in a middle aged and elderly population

DS RENWICK, MJ CONNOLLY *University Department of Geriatrics, Barnes Hospital, Manchester* There is evidence of a role of serum immunoglobulin E (IgE) in pathogenesis of chronic airway obstruction but debate on its import in the aged (*Am Rev Respir Dis* 1992;**146**:402; *Chest* 1986;**90**:416). We examined baseline FEV_1/FVC, IgE, serum eosinophils (EO), and bronchial response (BR) to methacholine (log slope FEV_1 dose response; *Am Rev Respir Dis* 1992;**146**:592) in 196 (83 male) subjects aged 45–86 (mean 63) years in an epidemiological survey. IgE was higher in smokers (geometric mean (GM) = 17·3 IU/ml; 95% CI = 9–33 IU/ml) than ex/non-smokers (GM = 6·9 IU/ml; 95% CI = 4–11 IU/ml: $p = 0.02$). Log IgE correlated with pack-years (PY) smoked ($r = 0.22$, $p = 0.002$). There was no relation between EO and smoking. Multiple regression showed an independent negative relation between FEV_1/FVC and PY ($p < 0.001$), BR ($p < 0.001$), and EO ($p = 0.05$), with a negative trend for age ($p = 0.1$) but not log IgE ($p = 0.64$), ($r = 0.42$). Conversely log IgE was independently positively related to BR ($p = 0.04$), as were PY ($p < 0.001$) and EO ($p = 0.07$). Baseline FEV_1 was an independent positive predictor of BR ($p < 0.001$) ($r = 0.61$). Despite lack of an independent relation between IgE and airway function, analysis of variance revealed a synergistic effect ($p = 0.04$) of current smoking and IgE (categorised: 0–10, 10·1–20 and > 20 IU/ml) on FEV_1/FVC. There was no synergism between smoking status and EO. Immunological factors may affect lung function in mid–late life. IgE effects may be by intrinsic mechanisms increasing BR and/or potentiation of pathways allowing smoking related airflow limitation.

Differential cell counts are more reproducible than total cell counts in induced sputum from patients with chronic obstructive pulmonary disease

VM KEATINGS, PJ BARNES *Department of Thoracic Medicine, National Heart and Lung Institute, London* Thirteen patients with chronic obstructive pulmonary disease (COPD) (nine men and four women,

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mean age 72·2 years, mean FEV_1 47·5% predicted), underwent sputum induction on two occasions, 14 days apart. Sputum samples were homogenised with dithiothreitol to facilitate total cell counting. Slide preparations were stained with May Grunwald Giemsa for differential cell counts which were carried out in a blind fashion. Mean (SD) total cell count was 3·7 (2·5) $\times 10^6$ per ml of sputum. Mean (SD) differential counts were as follows: macrophages 29·2 (11·5)%, neutrophils 57·3 (18·5)%, eosinophils 3·2 (5·6)%, lymphocytes 0·4 (0·9)%, epithelial cells 10 (11·7)%. Total cell counts are similar to those previously found in asthma and normal samples (*Am Rev Respir Dis* 1993;**147**:1126–31), but with a much higher percentage of neutrophils. Within patient total cell counts varied widely over the two week period (mean variability 60%) but differential cell counts remained relatively constant (mean variability 16%). It is likely that, although every effort is made to minimise it, varying degrees of salivary contamination of sputum are responsible for the variations in total cell numbers.

Effect of correction of hypoxia on proteinuria in chronic obstructive lung disease (COLD)

BG MARSHALL, JS MILLEDGE, M LANDON *Northwick Park Hospital and MRC Clinical Research Centre, Watford Road, Harrow* Our previous studies have demonstrated an association between chronic obstructive lung disease and proteinuria. Changes in renal function have been reported in patients with COLD, and histological changes at the level of the glomerular apparatus described. Proteinuria seen in patients with the obstructive sleep apnoea syndrome corrects with improvement of oxygenation; and in healthy male mountaineers at altitude, a correlation has been reported between quantity of proteinuria and both the degree of hypoxia experienced, and the height attained, which reverses with correction of hypoxia. We have explored the hypothesis that the proteinuria in COLD can be reversed by oxygen therapy for three hours. A heterogenous group of outpatients with COLD, four with and three without clinical and ECG evidence of cor pulmonale (all free from oedema at the time of the study) were selected for investigation, all of whom exhibited a level of proteinuria > 15 mg/mmol creatinine in our previous study. No patient had a history of hypertension, diabetes mellitus or renal disease. A three hour urine specimen was collected from the patient, in the semirecumbent position and breathing air, and was repeated on a subsequent day with the patient breathing oxygen at a concentration sufficient to increase oxygen saturation to a level >95% throughout the study period. The total protein level was measured on each specimen and compared on and off oxygen therapy. Pulmonary function, arterial blood gases before and at the end of oxygen therapy, serum and urinary creatinine, urinary sodium concentration, and urinary γ-glutamyltransferase concentration were measured. Arterial oxygen saturation was monitored throughout oxygen administration, via pulse oximetry. Proteinuria was not reduced in any patient by three hours of supplementary oxygen. This suggests that the underlying

Protein excretion (mg) in three hours (n=7)

	Age (y)	*FEV_1 (litres)*	*Air Pao_2 (kPa)*	*Oxy Pao_2 (kPa)*	*Air $Paco_2$ (kPa)*	*Oxy $Paco_2$ (kPa)*	*Air Prot (mg)*	*Oxy Prot (mg)*
Mean	74·6	0·73	7·67	14·1	5·57	6·4	25·59	21·34
SD	5·3	0·3	1·36	3·31	1·24	2·30	26·7	24·9
Range	69–83	0·4–1·4	6–9·9	9·8–18·8	4·0–7·3	4·2–10·5	5·3–85·1	4·0–76·4
Median	73	0·7	7·7	13·6	5·3	5·6	18·2	14·5

renal defect in patients with COLD may be more than just a functional, reversible increase in glomerular permeability, reflecting tissue hypoxia; more likely it is related to irreversible morphological changes in the glomerular apparatus, or to a mechanism unrelated to the underlying hypoxic state. Further studies are needed, with more prolonged exposure to oxygen, to establish the exact pathophysiological mechanisms involved, and indeed whether the renal leak is modifiable at all in COLD patients.

Validation of the breathing problems questionnaire by physiological measures

J BOTT, ME HYLAND, S SINGH, CAP KENYON *The London Chest Hospital, London; University of Plymouth, Devon; Glenfield Hospital, Leicester* The breathing problems questionnaire (BPQ) is a 33 item self report scale that measures quality of life in patients with chronic obstructive pulmonary disease (COPD). Principal components analysis has revealed a two factor structure, one factor of functional status and a second factor of COPD on affective states. This study explores the correlations between factor scores and objective measures of morbidity. Forty stable outpatients with COPD were studied: mean (SD) FEV_1 1·1 (0·41) l, FVC 2·5 (0·65) l, and PEF 214 (74) l; all performed a shuttle walking test (SWT), mean 321 m (SD 205), and completed a BPQ. Pulse rate (PR) and blood pressure (BP) were measured before and after the SWT. Using Pearson product moments, the functional status factor of the BPQ correlated with the shuttle walking test ($r=0·58$, $p<0·001$), log change in PR ($r=0·68$, $p<0·001$) and log change in systolic BP ($r=0·55$, $p<0·001$), but was uncorrelated with percentage predicted measures of FEV_1, FVC, or PEF. The emotional reaction factor of the BPQ failed to correlate with any physiological or exercise variables. The shuttle walking test failed to correlate with any of the percentage predicted measures of respiratory function or change in systolic blood pressure, but did correlate with log change in pulse rate ($r=0·62$, $p<0·001$). The results confirm the separation of the functional status and emotional reaction factors of the BPQ and validate the functional status factor against measures associated with exercise tolerance, but not respiratory function, in this group of patients.

Quality of life (QoL) in chronic airflow limitation (CAL)

JC WATERHOUSE, R HARPER, F MARSH, NMB JONES, J BRAZIER, P HOWARD *Department of Medicine and Medical Care Research Unit, University of Sheffield, Royal Hallamshire Hospital, Sheffield* Over a four month period adults attending a chest clinic were invited to participate in a study of quality of life (QoL). They were aged 35 and over, and according to their medical records had FEV < 70% FVC, a diagnosis of irreversible or poorly reversible airways obstruction, and no mention of carcinoma. 160 patients accepted and 13 refused. We measured spirometry, pulse oximetry, six minute walking test with oximetry, and assessed QoL with two disease specific measures (St George's Hospital respiratory questionnaire, and McMaster's chronic respiratory questionnaire) and two general health measures (SF-36 and Euroqol). One hundred and fifty four patients completed the SF-36, 140 St George's, 143 Euroqol, and 70 McMaster's. Of particular interest are the results with the SF-36 (a self completion measure comprising 36 items) as it is increasingly being used in clinical trials and health services research. In our study we were able to compare patients' scores with those of an age and sex matched sample from the general population in Sheffield. Those with CAL had substantially worse scores than the general population in seven of the eight dimensions of health measured by the SF-36 – for example, mean scores of 30·3 compared with 72·5 for physical function; 46·0 and 83·0 for social function respectively. Surprisingly, for mental health, the difference was less than might be expected for a chronically disabled group (mean scores of 65·1 and 71·2 respectively). In CAL females scored significantly lower than males in four of the eight dimensions (social function, vitality, pain and general health perception) ($p<0·05$, 2 sample t test). Patients' scores on the Euroqol were worse than for the general population. Scores on the SF-36 showed good agreement with comparable dimensions in the disease specific questionnaires – for example, "energy" in SF-36 and McMasters. 93 people were Fletcher grade 4 or more (breathless when walking on the flat at their own pace). Eleven were on long term oxygen therapy by concentrator (13–18 hours) and nine others used oxygen on an occasional basis (one cylinder lasting on average seven days). In spite of their degree of disability, these patients received little support from health and social service workers – only six had home helps, and only three were visited regularly by a community or district nurse.

Inhaled steroids in obstructive lung disease in Europe: the ISOLDE trial; protocol and progress

P SHERWOOD BURGE AND PETER CALVERLEY FOR THE ISOLDE STUDY GROUP *Birmingham Heartlands Hospital, Birmingham* FEV_1 declines with age, and the rate of decline is faster in patients with COAD. The ISOLDE trial compares the effect of inhaled fluticasone propionate (500 μg) twice daily with placebo. The primary endpoint is annual decline in post bronchodilator FEV_1. The study emphasises quality control of spirometry and quality of life assessment. 960 patients, aged 40–75, will be studied for three years. Entry criteria include smokers and ex-smokers with a post bronchodilator FEV_1 <70% predicted and >0·791 with FEV_1/FVC <60%. Asthmatics are excluded on clinical diagnosis or ΔFEV_1 >10% following 400 μg salbutamol (ΔFEV_1/predicted FEV_1). Following an eight week run in without any form of steroid treatment all patients receive a two week course of prednisolone (0·6 mg/kg/day) before randomisation to fluti-

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casone or placebo. All patients receive salbutamol and ipratropium bromide as required rather than regularly. So far 327 patients have been recruited. 165 patients have reached randomisation and 76 have been withdrawn before randomisation, mainly due to exacerbations of COAD. The mean FEV_1 on entry is 1·1 litres and FVC 2·46 litres. The average age is 65 (range 41–75) years. The ISOLDE patient group have more severe disease, are older, and are receiving a higher effective dose of inhaled steroid than other ongoing or contemplated studies in COAD.

Comparison of prednisolone and mianserin on ventilatory exercise and psychometric variables in COPD

A GROVE, BJ LIPWORTH, CG INGRAM, RA CLARK, DP DHILLON *Department of Respiratory Medicine and Clinical Pharmacology, Ninewells and King's Cross Hospital, Dundee* There is controversy as to whether effects on mood play a part in mediating the response to prednisolone in COPD. Following an initial two week placebo run in, 12 patients with COPD: age 62·5 (1·5) years, FEV_1 29 (3·2)% of predicted, <15% reversibility, were given 3 wk of either prednisolone (40 mg/day) or mianserin (60–90 mg/day) separated by a three week placebo washout. Measurements were made at the end of each period. FVC was increased ($p<0·05$) by prednisolone (mean and 95% CI): 2·42 (2·28–2·55) l compared with placebo: 2·17 (2·03–2·30) l or mianserin: 2·19 (2·06–2·32) l. There were no significant differences between treatments for FEV_1, lung volumes, 6 MW or Borg scale. Exercise testing showed that prednisolone produced a greater HR_{max} than placebo or mianserin ($p<0·05$), and produced a greater VE_{max} than placebo ($p<0·05$). Vo_{2max} was greater ($p<0·05$) with prednisolone: 0·89 (0·81–0·94) l/min compared with mianserin: 0·75 (0·68–0·82) l/min. Sao_2 pre and post-exercise did not differ between treatments. Psychological testing showed that prednisolone produced a lower anxiety score than placebo ($p<0·05$), whereas there was no difference between treatments in scores for daily activities or social adjustment. Thus prednisolone, unlike mianserin, produced significant effects on ventilatory and exercise parameters and also reduced anxiety levels, but had no impact on walking distance or daily activities. This suggests that the effects of prednisolone in COPD are unlikely to be due to changes in mood, and that mianserin has no therapeutic role.

North East Thames oxygen register: a pilot for a regional register

AA OKUBADEJO, G BRAID, EA PAUL, JA WEDZICHA *Department of Thoracic Medicine, London Chest Hospital, London* A register of patients using long term oxygen therapy (LTOT) at home could monitor assessment, prescription, and follow up; however, there is no oxygen register available in the United Kingdom. A pilot study for such a register was undertaken in the North East Thames region. All new oxygen concentrator installations were reported to us weekly for four months by the contractors, a form was then sent to the prescribing doctor, and the information returned entered into a register database. Patients were visited at home after six weeks by a respiratory nurse and pulse oximetry performed with the patient breathing air and oxygen (O_2), the results being sent to the patients' general practitioner and hospital doctor. There were 130 patients (73M:57F) entered into the register, median age 69 (range 20–98); forms were returned for 110 (85%) patients. A diagnosis was received for 100 patients (77%), in 67 the diagnosis was COPD, the prescription was initiated by a physician in 84 cases, only 41 had been assessed on two separate occasions as is recommended. The mean (SD) Pao_2 was 7·2 (1·94) kPa, mean (SD) $Paco_2$ 6·6 (1·5) kPa, and mean (SD) FEV_1 0·81 (0·44) l. After six weeks, 27 (20%) patients had died, 32 patients could not be contacted despite three attempts, Sao_2 was measured at home in 71 patients, 7 (10%) had Sao_2 <92% breathing O_2 and required a higher O_2 flow rate. This study has shown that with adequate funding a regional register of patients using LTOT could be set up. Audit of the use of domiciliary LTOT and of adherence to guidelines would be simplified, and communication between general practitioner and hospital improved. Although there was some difficulty in contacting patients at six weeks, the follow up visit would enable patients with undercorrected Sao_2 to be identified and would provide much needed advice and support for these patients.

Ambulatory oxygen therapy in kyphoscoliosis

DJ MEECHAM JONES, JH BELL, JA WEDZICHA *Department of Thoracic Medicine, The London Chest Hospital, London* Patients with COPD may benefit from ambulatory oxygen, with improvement in exercise capacity, the changes being related to the fall in Sao_2 (Lock *et al. Respir Med* 1991;85:407–12). However, the effect of exercise on Sao_2 in patients with kyphoscoliosis and of correction with ambulatory oxygen have not been previously reported. We have investigated the effects of ambulatory oxygen in 10 patients (5M, 5F; median age 59 (range 43–78) years) with stable kyphoscoliosis (mean (SD) Pao_2 air 8·76 (1·23) kPa, $Paco_2$ 6·49 (0·82), FEV_1 0·93 (0·19) l, FVC 1·58 (0·72) l). Six patients were established on domiciliary NIPPV. Six minute walking tests were performed (a) on air (baseline), (b) with a cylinder of air at 2 l/min and (c) with oxygen at 2 l/min in random order, the patient being blinded as to the cylinder content. Oximetry was performed during the walks and visual analogue scores (VAS) for breathlessness completed. There was no significant difference in the decrease in Sao_2, distance walked, or VAS scores between the baseline and the air cylinder walks. However, there was a significant fall in median Sao_2 on the baseline walk on air; from 94% (86–96%) at rest to 81·5% (59–91%), median (range) change 12·5% (3–27%) $p=0·0002$. With ambulatory oxygen there was a significant improvement ($p=0·016$) in the Sao_2 on exercise compared with baseline, median (range) fall in Sao_2 4·5% (1–16%), $p=0·004$. There were also significant improvements ($p=0·03$) in VAS scores for breathlessness between the oxygen and air cylinder walks. Median recovery time improved from 95 (30–210) s with air to 25 (0–110) s with oxygen ($p=0·002$). However, there was no significant difference in the median walking distance between the air (350 (265–430) m) or oxygen walks (375 (290–430) m; $p=0·84$) and no relation with the changes in Sao_2. Patients with kyphoscoliosis showed a fall in Sao_2 on exercise, as occurs in patients with COPD. The exercise desaturation, breathlessness, and recovery times were significantly improved with ambulatory oxygen at 2 l/min, although walking distance was unaffected. Patients with kyphoscoliosis should have oximetry performed on exercise and assessment for suitability for ambulatory oxygen therapy. [This work is supported by The British Lung Foundation.]

Respiratory symptoms and functional status of 20–45 year olds attending general practice

M MORRIS, R GALLACHER, RA CLARK *Department of Respiratory Diseases, Univerity of Dundee, King's Cross Hospital, Dundee* Patients with COAD often present with significant symptoms and functional abnormalities in their late 40s. Little is known of the early clinical development of COAD which, if defined, might allow prophylactic intervention before clinically significant functional damage has occurred. Under their new contracts general practitioners are charged with regular health checks on all their patients. If a simple screening programme for early COAD can be devised then it could be used in the routine assessment of younger adults. The results of a pilot study in 20–45 year olds in a rural Tayside practice (970 patients in this age range) are reported. 102 case notes stratified by age and sex were randomly selected and reviewed – eight cases were exempt by way of having pre-existing respiratory diseases – 70 of the remaining 94 (24 males and 46 females) attended for assessment consisting of completing the St Georges respiratory questionnaire modified to exclude significant URT disease, ventilatory function testing (Compact Vitalograph and PEF) and completing a two week diary card. There were 27 smokers, 30 non-smokers, and 13 ex-smokers. 44% reported significant non-URT respiratory symptoms (a score of greater than 2·0 on the St Georges Scale) and 40% had at least one index of respiratory function less than 80% predicted. About 50% of both smokers and non-smokers had either symptomatic or functional abnormalities or both. There was no difference between the sexes, nor was there a clear relation suggesting increasing abnormality with age. Fourteen (20%) had a body mass index of greater than 30. These results will form the basis for a more extensive study with long term follow up.

A system to study factors influencing the destructive potential of normal human neutrophils

A BARTON, C BUNCE, P HARRISON, C LLEWELLYN-JONES, RA GRANT, G BROWN, RA STOCKLEY *Department of Immunology, University of Birmingham and the General Hospital, Birmingham* There is substantial evidence to support the role of neutrophils in the pathogenesis of destructive lung disease. The potential for tissue destruction is largely determined during the 10–14 days required for differentiation and maturation in the bone marrow before release into the circulation. We have developed a system to study normal human neutrophil differentiation in vitro. Primitive human myeloid blast cells were isolated from 18–22 week fetal liver derived mononuclear prepara-

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tions by negative selection and counter flow cell elutriation. The resultant cells (74% blasts) were seeded at 2×10^5/well (n = 5) and cultured for five days in 10% fetal calf serum with IL-3 (100 μ/ml). At this point the IL-3 was reduced to 10 μ/ml and G-CSF was added (30 ng/ml) and culture continued until day 14. Over this period the cell number increased from 4·33 (0·98) $\times 10^5$ cells/well to 5·49 (0·74) $\times 10^6$. By the sixth day the cells were still predominantly blasts (73·1(3·0)% of total cells) and 13·7(2·1)% were promyelocytes. Myelocytes (2·0(0·5)%) appeared on day 6 rising to 9·4(1·2)% by day 14. Metamyelocytes were rarely seen until day 8 when they accounted for 4·8(0·7)% of the cells rising to 18·6(2·0)% by day 14. More mature cells (segmental and band neutrophils) increased to 51·1(2·8)% by day 14. This change was associated with an increase in the proportion of cells becoming phagocytic (46·4%) and staining for neutrophil elastase (74·3%). In addition the cells developed the ability to degranulate and digest fibronectin (0·66 (0·16) μg/3×10^5 cells) and respond to stimulation with 10^{-6} molar F-met-leu-phe (1·12 (0·2) μg). This was associated with expression of F-met-leu-phe receptors (162·8 (41·3) $\times 10^3$ receptors/cell). This system will enable us to study factors which influence the destructive potential of neutrophils and its modulation.

Oesophagogastrectomy (OG) and whole blood rheology: leucocyte priming in vivo?

C SELBY, E DROST, E CAMERON, W MACNEE *Units of Respiratory Medicine and Thoracic Surgery, City Hospital, Edinburgh* Neutrophil (PMN and other leucocyte) sequestration in the pulmonary microcirculation is partially determined by their deformability measured in vitro as filterability: the pressures generated when 10^5 PMN/ml pass at constant flow through a microscope membrane (Selby *et al. JAP* 1991). In a model of acute lung injury PMNs harvested in vitro from venous blood 24 h after OG are consistently less deformable than before (Selby *et al. ARRD* 1992). To reduce potential pertubations of cell harvesting, whole blood rheology was now measured which reflects the deformability of the more rigid though less numerous leucocytes. Peripheral arterial, pulmonary efferent, blood was obtained via an indwelling cannula from six patients (3M; median age 60) undergoing OG, diluted with buffer to a leucocyte concentration of $1{\cdot}5 \times 10^5$/ml whereupon deformability was measured within 15 minutes of acquisition. Due to their pronounced preponderance after OG, PMN deformability was the major determinant of whole blood leucocyte rheology. Plateau filtration pressures after surgery were lower compared with before OG (median pre 2·1 (range 1·4–5·7) cm H_2O, 62–89% PMN; post 1·4 (0·7–2·1) cm H_2O, 87–96% PMN; $p < 0{\cdot}05$), not compatible with activation of circulating leucocytes. Neither were leucocytes more adhesive nor expressed increased surface CD18 antigen. Diluted whole blood from patients post OG stimulated in vitro by 10^{-7}M fMLP resulted in greater increases of peak over basal pressures compared with blood obtained before OG (pre OG 6·0 × (3·5–6·0 ×); 24 hours post-OG 12 × (8–20 ×) $p < 0{\cdot}05$). This response to fMLP was present 0·48 hours after OG and is compatible with leucocyte priming in vivo. Primed leucocytes have the potential for greatly enhanced pulmonary sequestration and are potentially injurious to capillary-alveolar integrity. [Supported by the British Lung Foundation.]

F-met-leu-phe receptors are increased in neutrophils from subjects with emphysema who continue to smoke

RA STOCKLEY, RA GRANT, CG LLEWELLYN-JONES, SL HILL, D BURNETT *The General Hospital, Steelhouse Lane, Birmingham* It is generally believed that destruction of lung elastin is central to the pathogenesis of emphysema. As neutrophils can destroy lung elastin even in the presence of inhibitors such as α_1 antitrypsin the number and activity of neutrophils recruited to the lung is likely to determine the development and severity of disease. We have shown that neutrophils from patients with emphysema show increased chemotactic response to formyl peptides (*Lancet* 1987;ii:1043–6) indicating a potential clue to susceptibility. However, progression of emphysema is largely dependent on continued smoking. Because formyl peptide receptor numbers relate to chemotactic response we have studied the number of neutrophils from age matched healthy non-smokers (n = 7) and smokers (n = 4) and compared the results with current smokers (n = 67) and ex-smokers (n = 7) with emphysema. The receptor numbers in the healthy non-smokers (median = 222; range 114-885 $\times 10^3$/cell) were similar (NS) to the healthy smokers (median = 332; range 243–411). On the other hand the receptor numbers for the current smokers with emphysema (median = 463); range 362–1080) were significantly higher than for the age matched smokers ($p < 0{\cdot}001$) and non-smokers ($p < 0{\cdot}001$). On the other hand receptor numbers for ex-smokers with emphysema (median = 343; range 207-789) were similar (NS) to healthy smokers and non-smokers. These results show the first clear evidence of a difference between smokers with and without emphysema which is not present in ex-smokers with emphysema. The data provide a major clue to the pathogenesis of emphysema in patients without α_1 antitrypsin deficiency.

Effect of mediators from normal and fibrotic lung fibroblasts on neutrophil activation

A SOUTHEY, CM O'CONNOR, MX FITZGERALD *Department of Medicine, University College Dublin and St Vincent's Hospital, Dublin* Continued tissue damage by oxidants and degradative enzymes released by neutrophils is thought to contribute to fibrosis in lung disease. Recent in vitro studies suggest that cytokines released by fibroblasts may contribute to the accumulation of neutrophils at inflammatory sites. It is also suggested that selection of a subset of phenotypically distinct fibroblasts may be important in the fibrotic process. The objectives of this study were to determine (1) if fibroblast-derived cytokines enhance neutrophil activation and (2) if mediators released by lung fibroblasts from normal and fibrotic sources differed in their effects on neutrophils. Conditioned media (FCM) from two normal and two fibrotic human lung fibroblast cell lines were examined for their effects on neutrophil survival, superoxide production, and degranulation of both primary and secondary granules (by measurement of lactoferrin and elastase release). Results indicated that FCM from both normal and fibrotic lung fibroblasts enhanced neutrophil survival to 40% after 48 hours compared with 13% in control medium ($p < 0{\cdot}02$). No increase in superoxide, lactoferrin, or elastase release was observed after exposure of neutrophils to FCM from normal or fibrotic cell lines. While confirming that mediators released by fibroblasts enhance neutrophil survival, these results indicate that, under the same conditions, neutrophil activation is not increased. No difference was observed between the effects of FCM from normal or fibrotic lung fibroblast lines on neutrophil function. Thus in situ stimulation of fibroblasts, rather than selection of phenotypic subtypes, may be of significance in the fibroblast–neutrophil interaction observed in lung fibrosis. [This work was supported by The Health Research Board of Ireland.]

Neutrophil adhesion and migration in a child with leucocyte adhesion deficiency

CM MORELAND, CG LLEWELLYN-JONES, PJ DARBYSHIRE, RA STOCKLEY *Lung Immunobiochemical Research Group, The General Hospital, and The Children's Hospital, Birmingham* Leucocyte adhesion deficiency (LAD) is associated with recurrent life threatening respiratory infections. Patients have severely reduced expression of the leucocyte integrin molecules LFA-1, MAC-1, and p150,95 (CD 11a/CD 18, CD 11b/CD 18, CD 11c/CD 18) that mediate cell-cell interactions important in inflammatory reactions. We report investigations of neutrophils from a 3 month old child with LAD. Flow cytometry indicated a phenotype of severe CD 11a, CD 11b, CD 11c, and CD 18 deficiency. Adherence to unstimulated human umbilical vein endothelial cells (HUVECs) was found (on three occasions) to be severely reduced (5·9%, SD (0·6%). Adherence to LPS-stimulated HUVECs (four hour preincubation with 1 μg/ml LPS, 37°C) was increased for the CD 18-deficient neutrophils (10·7% (1·3%)) but still reduced compared with normal neutrophil adherence to LPS-stimulated HUVECs (27·2% (0·1)%). Transendothelial migration of CD 18-deficient neutrophils through unstimulated HUVECs was severely reduced (6·1% (1·8%), n = 3) compared with normal random neutrophil migration (15·4% (7·2%), n = 5). Minimal transendothelial migration was also seen in response to the chemoattractant FMLP (10^{-8}M) placed beneath the endothelial cell layer (3·63% (1·6%), n = 3) compared to normal neutrophils (28·0% (2·6%), n = 4)). By contrast, the CD 18-deficient neutrophils did undergo migration in response to both IL-8 (5×10^{-9}M) (23·7% (1·8%), n = 3) and pooled sputum from bronchiectasis patients (26·3% (11·3%), n = 3) although this was still less than the response of normal cells (36·5% (11·3%) and 53·2% (9·8%) respectively). Antibody to CD 18 reduced normal neutrophil migration to IL-8 and sputum (14·2% (5%) and 25·3% (2·4%), respectively) but had no effect on the LAD neutrophil response (17% (4·2%) and 25·4% (8·3%) respectively). These results confirm the role of CD 18-dependent transendothelial migration for normal neutrophils, but show that LAD neutrophils are able to migrate through endothelium in response to IL-8 by a CD 18-independent mechanism.

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Neutrophil elastase and cathepsin G mRNA and protein in Chediak-Higashi bone marrow

D BURNETT, CJ WARD, J CROCKER, RA STOCKLEY *Lung Immunobiochemical Research Laboratory, The General Hospital and Department of Histopathology, Heartlands Hospital, Birmingham* Patients with Chediak-Higashi disease (CHD) have recurrent infections, including those of the respiratory tract. The blood neutrophils (PMNs) from patients with CHD are deficient in elastase (NE) and cathepsin G (CG) but do contain other azurophil granule proteins (for example, defensins and myeloperoxidase). We wished to determine whether transcription and synthesis of NE and CG are normal in CHD. Blood PMNs were isolated from three patients with CHD and bone marrow was collected from one. Immunohistochemistry was used to localise CG and NE in marrow cells and cell lysates were assayed for NE and CG by ELISA and by Western immunoblotting. The mRNA for NE, CG and β-actin in bone marrow was detected by Northern blotting. Blood PMNs from the patients with CHD were devoid of CG and NE but the enzymes were present in bone marrow cells, although immunohistochemistry showed they were not within cytoplasmic granules. The enzyme content of NE and CG in CHD bone marrow, compared with normal marrow, was 24% and 16%, respectively. Quantitative scintillation scanning of Northern blots showed that NE and CG mRNA (corrected for β-actin mRNA) were expressed equally to normal marrow. The results suggest that transcription of NE and CG mRNA in promyelocytes of CHD is normal, but that the protein products are deficient in these cells and absent in mature PMN. This suggests that the translated proteins are not packaged into azurophil granules but are degraded or secreted.

Effects of indomethacin on peripheral neutrophil function and lung secretions in bronchiectasis

CG LLEWELLYN-JONES, SL HILL, RA STOCKLEY *The General Hospital, Steelhouse Lane, Birmingham* Bronchiectasis is often associated with the production of purulent sputum as a result of neutrophil (PMN) influx, while neutrophil elastase (NE) is known to cause many of the pathological features of the disease. Therefore, agents which inhibit PMN migration and degranulation may have a beneficial effect on disease progression. We have studied the effects of the non-steroidal anti-inflammatory agent indomethacin on PMN chemotaxis, degradation of fibronectin (FN), and superoxide anion generation in vitro, as well as secretion elastase, myeloperoxidase levels, and chemotactic activity. Nine patients with clinically stable bronchiectasis were treated with indomethacin at 25 mg three times daily for a total of four weeks, followed by a six week washout period. All nine patients remained stable during the study period. PMN chemotaxis fell from a mean (SE) of 19·86 (1·35) cells/field at day 0, to 9·74 (1·15) after 14 days ($p < 0.001$), and to 8·46 (0·68) after 28 days ($p < 0.001$) returning to 19·16 (1·67) after the washout period. There was a reduction in FN degrading by both resting PMN and PMN stimulated with 1 mM F-met-leu-phe; with resting cells from a mean value of 1·90 (0·19) mg at day 0 to 1·70 (0·10) mg at day 14 ($P < 0.01$) and 0·87 (0·08) mg at day 28 ($p < 0.01$) returning to 2·87 (0·21) mg after the washout period; and for stimulated cells from a mean of 3·17 (0·35) mg at day 0, to 2·15 (0·13) mg at day 14 ($p < 0.01$), and 1·48 (0·05) mg at day 28 ($p < 0.01$), returning to 2·87 (0·21) mg after washout. However, the treatment had no effect on in vitro PMN superoxide anion generation by both resting and stimulated PMN. During this four week treatment there was no significant change in lung secretions although there was a small reduction in sputum sol phase chemotactic activity from a mean value of 18·0 (1·46) cells/field at day 0, to 16·9 (1·65) at day 14 and 16·2 (1·27) at day 28 returning to 19·9 (1·82) after the washout period. These data suggest that non-steroidal anti-inflammatory agents have a major effect on peripheral neutrophil function but do not alter the lung inflammation over a four week period.

Antitrypsin S_{iiyama} forms spontaneous intermolecular loop-sheet polymers like those of the Z variant

DA LOMAS, J FINCH, K SEYAMA, T NUKIWA, RW CARRELL *University of Cambridge Department of Haematology, MRC Centre, Cambridge; LMB, MRC Centre, Cambridge; Department of Respiratory Medicine, Juntendo University, Japan* Three mutations of α_1-antitrypsin are associated with the formation of hepatic inclusions: Z (^{342} Glu- > Lys), S_{iiyama} (^{53}Ser- > Phe) and M_{malton} (^{52}Phe deleted). We have previously shown that Z antitrypsin accumulates in the liver of homozygotes by loop-sheet polymerisation in which the reactive centre loop of one molecule is inserted into the A sheet of a second. These polymers form spontaneously on incubating isolated plasma Z, but not M, antitrypsin at 37°C and have structural identity to antitrypsin isolated from hepatocytes of a Z homozygote (*Nature* 1992;357,605). To show that loop-sheet polymerisation is a more general mechanism, antitrypsin was isolated from the plasma of a 43 year old male with mild emphysema who was a homozygote for the S_{iiyama} mutation. The mutation was confirmed by DNA sequencing and isoelectric focusing analysis. Ammonium sulphate fractionation and two chromatographic steps yielded 1·5 mg of antitrypsin S_{iiyama} from 150 ml plasma. The material was 30% pure as it eluted with other proteins at a much higher NaCl concentration than either M or Z antitrypsin. Antitrypsin S_{iiyama} was inactive as an inhibitor of chymotrypsin and non-denaturing PAGE and Western blot analysis showed that more than 95% of the material was present as high molecular mass polymers. These polymers failed to form complexes with, or be cleaved by, chymotrypsin indicating that the reactive centre loop is inaccessible to proteolytic cleavage. Electron microscopy and immunogold labelling showed antitrypsin S_{iiyama} to be formed of filaments of 10–15 molecules, identical in appearance to polymers of Z antitrypsin formed in vitro. These results support the proposal that loop-sheet polymerisation is the basic defect responsible for the plasma deficiency associated with the accumulation of antitrypsin in the liver.

Possible role of interleukin 10 (IL-10) in regulating the functional capacity of alveolar macrophages (AMs)

MCI LIPMAN, P DEBENHAM, S SREENAN, A VAUGHAN, MA JOHNSON, LW POULTER *The Royal Free Hospital and School of Medicine, London* We have previously shown that AMs represent a functionally heterogenous cell population. These may be differentiated phenotypically using monoclonal antibodies (Mo Abs) RFD1 and RFD7. We have also demonstrated that the proportion of AMs with "suppressor" phenotype may alter during disease. The present study was designed to investigate whether IL-10 (reported to down regulate T cell function) might be responsible for increasing the suppressor activity of AMs. Bronchoalveolar lavage (BAL) and peripheral blood monocytes (PBMs) were obtained from normal volunteers. Cytospins were made at time = 0 and after 24 hours' culture with or without IL-10. Expression of RFD1+ (antigen presenting), RFD7+ (phagocytic) and RFD1+RFD7+ (suppressor) cells were as shown in the table (denoted as ratios D1:D7:D1D7): Further studies

		PBMs		*BAL*
t=0 h		59:24:17		55:20:25
t=24 h	+IL-10	36:56:8	+IL-10	41:29:30
	−IL-10	40:45:5	−IL-10	53:28:19

using allogeneic mixed lymphocyte reactions (MLRs) revealed that increased proportions of D1+ cells enhanced T cell stimulation; whereas increased D1+D7+ cells suppressed T cell reactivity. The addition of Mo Abs RFD1 or RFD7 to the MLRs confirmed this finding. It is concluded that the changing expression of antigens seen by Mo Abs RFD1 and RFD7 influences AM function. This expression may be modulated by IL-10.

Priming effects of hypoxia on function of rat alveolar macrophages

AP GREENING, M GORDON, MH BAIN, F BÖLLERT, MB TUOHY *Rayne Laboratory, Respiratory Medicine Unit, University of Edinburgh, Edinburgh* We have previously shown that human and rat alveolar macrophages (AMs) can be primed by hypoxia for an enhanced release of hydrogen peroxide (H_2O_2) when stimulated by phorbol ester. We have explored whether hypoxia primes for enhanced H_2O_2 release using a more physiological trigger and whether cytokine release may also be affected, using rat AMs. AMs were obtained by tracheobronchial lavage of female germ-free Wistar rats (ex-breeders) and enriched by plastic adherence for one hour. They were cultured in gassed sandwich boxes to provide ambient O_2 of 21% or 2·5%, the difference being N_2. The AMs were cultured for four hours and eight hours (half of the eight hour cultures were stimulated by 1000 ng LPS for the second four hours), the culture supernatants stored at −80°C until assay, the AMs washed and their release of H_2O_2, stimulated by fMLP (10^{-8}M to 10^{-6}M) and opsonised zymosan (0·40 and 0·75 μg/ml) determined by the phenol red spectrophotometric assay. Supernatants of the cultured AMs (pre-stimulus) were assayed for TNF α (in-house ELISA). AM cultures for eight hours at 2·5% O_2 showed enhanced release of H_2O_2 (nmol/10^6 AMs/hour) compared with AMs

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cultured at 21% O_2 when stimulated with 10^{-6}M fMLP (n=7; mean (SE) 2·11 (0·58) *v* 1·42 (0·48); p=0·02) or opsonised zymosan, 0·75 µg/ml (n=5; 2·69 (0·63) *v* 1·67 (0·50); p=0·05). AMs cultured for four hours or eight hours at 2·5% O_2 showed an enhanced spontaneous and LPS-stimulated release of TNFα (pg/10^6 AMs) compared with AMs cultured at 21% O_2 (mean (SE), n=7)4 hours spontaneous, 1203 (476) *v* 889 (331), p<0·02; eight hours spontaneous, 1400 (529) *v* 818 (309), p<0·02; eight hours LPS 1000 ng, 1559 (589) *v* 965 (364), p<0·05. In conclusion, hypoxia can prime AMs for an enhanced release of reactive oxygen species in response to physiological stimuli, and for an enhanced release of tumour necrosis factor.

Monocyte interleukin-8 responses to lipopolysaccharide stimulation and dexamethasone inhibition: a single cell analysis

P PANTELIDIS, AM SOUTHCOTT, RM DU BOIS *Royal Brompton Hospital, London* Interleukin-8 (IL-8), an 8·4 kDa chemokine, is a potent chemoattractant and activator of neutrophils. Modulation of neutrophil traffic to the lung in chronic inflammation could limit lung injury. Corticosteroids (S) are first line treatment for chronic lung disease. The aims of this study were (1) to determine if IL-8 secretion by monocytes (PBM) is reduced by S; (2) to examine whether the effect on PBM cells was selective using the reverse haemolytic plaque assay (RHPA) to identify IL-8 secretion by single cells. Monolayers of peripheral blood mononuclear cells (PBM) and protein G-coupled sheep red blood cells (sRBC) were cultured for 12 hours in the presence of polyclonal anti IL-8 antibody + varying concentrations of lipopolysaccharide (LPS) (10 ng–10 µg/ml) and dexamethasone (DXM) (10^{-10}–10^{-6}M). IL-8 secretion by individual PBM was quantified after complement lysis of the IL-8/anti IL-8 complexes bound to sRBC. IL-8 was spontaneously secreted by 30% of the PBM in a dose dependent manner increasing to a maximum of 60% with the addition of ⩾1 µg/ml LPS. The average plaque size of the plaque forming cells was also increased from 1064 (114) µm² to 1915 (53) µm². At DXM concentrations ⩾10^{-8}M the per cent of plaque forming cells decreased to 6% in unstimulated cultures and 8% in LPS stimulated cultures with a threefold decrease in the average plaque area. We conclude (1) IL-8 is spontaneously produced by subsets of monocytes; (2) LPS increases both the amount of IL-8 secreted per monocyte and the number of monocytes that secrete IL-8; (3) DXM down regulates the number of IL-8 secreting cells and IL-8 secretion per cell.

Transcriptional regulation of IL-5 gene expression in human T cells

DJ COUSINS, DZ STAYNOV, TH LEE *Department of Allergy and Allied Respiratory Disorders, UMDS, Guys Hospital, London* The cytokines IL-3, IL-5, and GM-CSF can all cause eosinophil differentiation; however, IL-5 is the only specific eosinophil differentiating factor. Because it is not expressed by bone marrow stromal cells it is not involved in constitutive but in inducible hematopoesis. Whereas the function of IL-5 is extensively investigated the available data for the regulation of its expression are very limited. The IL-5 gene is on chromosome 5q31 in close proximity with IL-3, GM-CSF, M-CSF, and IL-9, which suggests that there may be a common mechanism of their regulation. Using DNase I footprinting and band retardation assays we have studied the transcription factors involved in the activation of IL-5 gene expression. We have found several *cis* acting elements in the promoter of the IL-5 gene: (1) A 15 nucleotide sequence ATTATTCATTTCCTC. It is fully conserved between mouse and human IL-5 and differs by only one nucleotide (underlined T) from GM-CSF. Although this sequence is not found in other genes it binds transcription factors found in many different cells. (2) AAGATTCTTC, which is present with low homology in the promoters of many lymphokine genes was also found to be occupied by a DNA-binding protein(s). (3) GAGGAA, which binds transcription factors of the ets family, different to the ets-1/ets-2 factors found in activated T cells. This sequence is not present in the GM-CSF promoter.

Effect of intravenous salbutamol on the activity of the renin–angiotensin system in asthma

EA MILLAR, RM ANGUS, NC THOMSON *Department of Respiratory Medicine, Western Infirmary, Glasgow* We have reported that the renin–angiotensin system is activated in acute severe asthma, and also by nebulised β_2 agonist therapy. We have now studied the effect of intravenous salbutamol on the activity of the renin-angiotensin system in eight mild asthmatic patients (mean (SD) age 35·4 (9·1) years, FEV_1 82 (12·8)%). After a 20 minute rest, baseline blood samples were withdrawn for estimation of plasma renin and angiotensin II (AII). Patients then received either salbutamol (5, 10, and 20 µg/min) in increasing increments at 30 minute intervals, or placebo (0·9% saline). The drugs were given intravenously according to a randomised double blind crossover study design. Plasma renin and AII were measured at the end of each infusion period, and 30 minutes after completion of the final infusion. Patients remained supine for the duration of the study. There was no significant difference between baseline values of renin and AII on either study day (mean (SE) plasma renin of 21·7 (4·5) and 19·0 (6·3) µU/ml and AII of 6·7 (2·3) and 7·5 (2·3) pg/ml before salbutamol and placebo respectively). After the 10 and 20 µg/min infusion periods and 30 minutes after completion of the final infusion, plasma renin levels were significantly greater than placebo (p<0·05) (mean (SE) absolute change in renin of 6·0 (3·5), 5·1 (4·1), and 3·7 (4·1) µU/ml respectively after salbutamol and −0·43 (3·1), −2·7 (2·7) and −1·7 (1·4) µU/ml after placebo). AII levels were significantly greater than placebo (p<0·05) 30 minutes after completion of the final infusion (mean (SE) absolute change in AII of 5·1 (4·3) pg/ml and −0·4 (0·69) pg/ml respectively). We conclude that there is only minor activation of the renin–angiotensin system by intravenous salbutamol, by contrast to the more pronounced activation we previously observed following a single nebulised dose of salbutamol. [EM supported by the National Asthma Campaign.]

Effect of intravenous angiotensin II on airway function in asthma

EA MILLAR, RM ANGUS, JJ MORTON, JMC CONNELL, NC THOMSON *Department of Respiratory Medicine, Western Infirmary, Glasgow* In a previous study we demonstrated activation of the renin–angiotensin system in acute severe asthma. Although recent in vitro work has shown that angiotensin II (AII) can potentiate vagal mediated bronchoconstriction of rabbit airway smooth muscle (Yamawaki *et al. Am Rev Respir Dis* 1991;**143**:A343), the effect of AII on bronchomotor tone in vivo in humans is unknown. In a randomised double blind study we have examined the effect of AII (administered as an intravenous infusion) on lung function in eight mildly asthmatic patients. On one study day angiotensin II (prepared in 0·9% saline) 2, 4, and 8 ng/kg/min was infused in increasing increments every 30 minutes. On the other day placebo (0·9% saline) was administered. FEV_1 (the best of three readings) was measured every 30 minutes by dry wedge spirometer and blood pressure recorded every 15 minutes. Blood samples were taken at baseline and thereafter at 30 minute intervals for estimation of plasma AII. At 0, 30, 60, and 90 minutes mean (SD) plasma AII levels were 12·4 (5·3), 25·1 (11·3), 55·9 (28·8), and 121·3(53·3) pg/ml respectively after infusion of AII (2,4 and 8 ng/kg/min) and 12·8 (7·9), 12·2 (8·1), 11·1 (6·9) and 11·5 (6·3) pg/ml after placebo. There was no significant difference between baseline FEV_1 on either study day (mean (SE) baseline values of FEV_1 were 2·55 (0·64) and 2·63 (0·34) litres prior to infusion of AII and placebo respectively). However, a significant bronchoconstrictor response to AII was observed at 60 and 90 minutes, with a mean (SE) fall in FEV_1 from baseline of 0·18 (0·03) and 0·34 (0·13) litres or 7·8 (2·02) and 12·4 (3·3)% respectively, compared with 0·02 (0·09) and 0·01 (0·09) litres or 0·3 (1·3) and 0·6 (1·2)% after placebo. In addition five out of eight patients reported cough and chest tightness during the high dose infusion of AII. It is of interest that the range of plasma levels of AII achieved during the AII infusion are comparable with those observed in acute asthma suggesting that this hormone may contribute to bronchoconstriction in acute severe asthma. [EM supported by the National Asthma Campaign.]

Effect of the oral neutral endopeptidase inhibitor candoxatril on bronchomotor tone and histamine reactivity in asthma

RM ANGUS, MJA MCCALLUM, NC THOMSON *Department of Respiratory Medicine, Western Infirmary, Glasgow* Neutral endopeptidase (NEP) is found in many tissues in humans including the lung. Metabolism by NEP is one of the main mechanisms for the clearance of atrial natriuretic peptide (ANP), a hormone that causes bronchodilation and reduces non-specific bronchial reactivity in man. Candoxatril (Pfizer, UK), an oral NEP inhibitor, has been shown to raise circulating ANP levels; such a drug might therefore allow the beneficial effects of ANP on the airway to be exploited. We have

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sought to determine if the administration of candoxatril will alter bronchomotor tone (FEV_1) and histamine reactivity. Ten male asthmatic patients with stable asthma were enrolled (mean (SD) age 31·6 (10) years, FEV_1 92·1(10·7)% predicted) in a randomised double blind placebo controlled study. On each study day, after baseline spirometry, patients received 200 mg of candoxatril or placebo. Spirometry was repeated at half hourly intervals. After two hours an histamine inhalation test was performed. There was no significant difference in the FEV_1 values at baseline or at two hours after dosing between active and placebo study days with mean (SE) FEV_1 (l) at baseline and two hours of 3·71 (0·29) and 3·85 (0·29) on the placebo day and 3·89 (0·27) and 4·05 (0·82) on the active day. The geometric mean (range) PC_{20} on the placebo day and active day did not differ significantly at 0·93 (0·13–32) mg/ml and 1·17 (0·25–25·8) mg/ml respectively. ANP levels rose significantly on the active day from a mean (SE) baseline value of 12·1 (2·1) to 29·1 (4·8) pg/ml at two hours ($p < 0·005$), but did not change on the placebo day (baseline 11·54 (2), two hour level 10·8 (1·9) pg/ml). Plasma drug levels were maximally increased between two and three hours (mean (SE) plasma candoxatril at one hour 161 (53), two hours 846 (136), three hours 896 (68), four hours 626 (60) ng/ml). No side effects were observed or noted by the patients. We conclude that the acute administration of the oral NEP inhibitor candoxatril does not alter bronchomotor tone or bronchial reactivity to histamine in stable asthmatic patients. [RMA supported by the British Lung Foundation.]

Inhibition of neutral endopeptidase enhances the effects of inhaled atrial natriuretic peptide

RM ANGUS, EA MILLAR, GC CHALMERS, NC THOMSON *Department of Respiratory Medicine, Western Infirmary, Glasgow* The hormone atrial natriuretic peptide (ANP) causes bronchodilation and partially protects against direct and indirect bronchial challenges. ANP is thought to be inactivated by two routes: degradation by the enzyme neutral endopeptidase-24·11 (NEP) and binding to a non-guanylyl cyclase clearance receptor. We have found in studies on human airway that phosphoramidon, the actions of which include inhibition of NEP, enhances the in vitro protectant effect of ANP on methacholine induced contraction. We therefore hypothesised that NEP may be inhibiting the in vivo effects of inhaled ANP. To test this we examined the effects of inhaled thiorphan, an NEP inhibitor, on the protective effect of ANP against histamine induced bronchoconstriction. In a randomised, double blind, placebo controlled, crossover study 10 mild asthmatic patients were studied (six female), mean (SD) age 31 (8·4) years and FEV_1 3·42 (0·71) l, 85 (30)% predicted. Patients were asked to withhold β_2 agonists for eight hours and antihistamines for 48 hours before each visit while inhaled corticosteroids were continued unaltered. On study visits they received either thiorphan 1 mg (in 2 ml) or placebo (saline) followed by ANP (1 mg) or placebo (saline). Spirometry was measured after each inhalation after which they received a dose of histamine calculated to cause a 30% fall in their FEV_1 at a prestudy visit. Spirometry was then repeated at 0·5, 1·5, 3, 5, 10, 15, 20, 25, and 30 minutes. Pretreatment with ANP alone caused a significant reduction ($p < 0·05$) in the mean (SE) maximum % fall in FEV_1 after histamine (15·9 (2·9)) compared with the fall after placebo (24 (2·9)) or thiorphan alone (24 (4)). Prior inhalation of thiorphan greatly enhanced the ANP effect: mean (SE) maximum % fall (5·1 (2·3), $p < 0·01$). Our results confirm that airway NEP is important in modulating the effect of inhaled ANP. [RMA supported by the British Lung Foundation.]

Magnitude and duration of the inhibitory effects of SK&F 106203 on LTD_4 induced bronchoconstriction in healthy volunteers

RD MURDOCH, J UPWARD, C COMPTON, K FITZPATRICK, A NICHOLS *Clinical Pharmacology Unit, SmithKline Beecham Pharmaceuticals, Harlow* The aim was to assess the potency and duration of a novel leukotriene antagonist, SK&F 106203, on leukotriene D_4 induced bronchoconstriction. A previous study had determined the reproducibility and safety of repeated leukotriene challenge in healthy volunteers over a period of 12 hours (Compton *et al. Thorax* 1993;**48**:457). The study was conducted as a randomised, single blind, parallel group design with active and placebo groups. Subjects were randomised within each of six groups (six subjects per group). Each subject received, on a single occasion, an oral dose of SK&F 106203 (50, 100, 200, 300, 400 mg) or placebo. A cumulative LTD_4 ($2 \times 10^{-7} - 2 \times 10^{-4}$M) bronchial provocation test, preceded by a saline challenge, conducted according to the tidal breathing method of Cockcroft was performed 4, 8, and 12 hours after SK&F 106203 or placebo. The primary end point was a 40% fall in $PEFR_{70}$ (PC_{40}). A plasma pharmacokinetic profile was obtained from each subject. The study was approved by an independent ethics committee. Data in the table represent the parametric analysis of PC_{40} for $PEFR_{70}$ for each challenge time by dose showing the ratio relative to placebo. The

Dose time	*Placebo*	*50 mg*	*100 mg*	*200 mg*	*300 mg*	*400 mg*
+4 h	1·0	1·65	3·90	5·63	4·84	2·34
+8 h	1·0	2·91	5·73	7·20	5·99	2·13
+12 h	1·0	2·67	7·93	2·08	3·27	0·99

increases in plasma concentrations of SK&F 106203 were linear with dose but there was no clear relation between plasma concentration and the attenuation of LTD_4 induced bronchoconstriction, seen maximally with the 200 mg dose. The compound was generally well tolerated; the most commonly reported symptoms were those expected in association with LTD_4 challenge. Thus SK&F 106203 demonstrated attenuation of LTD_4 induced bronchoconstriction. The maximum observed effect was eight hours after dosing: there was no clear relation to plasma concentration or to dose in this study.

Equipotency of budesonide and fluticasone propionate in the vasoconstriction assay

N ANDERSSON, S KLINT, G RANDWALL, J-E WIRÉN *Clinical Research and Development, Astra Draco AB, Lund, Sweden* The vasoconstriction assay (McKenzie and Stoughton *Arch Dermatol* 1962;**86**:608–10; Place *Arch Dermatol* 1970;**101**:531–7) is an accepted method for assessing the potency of topical corticosteroids. In vasoconstriction assays, budesonide has been shown to be at least twice as potent as beclomethasone dipropionate (BDP) (Johansson *et al. Eur J Clin Pharmacol* 1982;**22**:523–9). Fluticasone propionate is a recently registered corticosteroid intended for the treatment of rhinitis and asthma. It has been tested previously in a vasoconstriction assay (Phillips *Respir Med* 1990;**84**:suppl A:19–23) and was also shown to be twice as potent as BDP. The present study was conducted to compare directly the topical potency of fluticasone and budesonide. Nineteen subjects (age 19–49, nine men) entered and completed the study, which was of a placebo controlled, double blind design. The two corticosteroids were dissolved in 95% ethanol and tested at five different dilutions. Ten µl of each substance and concentration (including placebo) were applied, in a randomised order, to two sites within an outlined area of the skin of one forearm. The test areas were occluded for 18 hours. After removal of the occlusion, the blanching intensity was assessed at 1, 2, 3, 6, 12, and 24 hours. The blanching was graded visually using a scale of 0–3 (in steps of 0·5) and AUC_{1-24} (area under the curve, 1–24 hours) was determined. In the relative potency calculations, assuming parallel regression lines, budesonide was assigned the value of 1 and fluticasone propionate had a relative potency of 0·98 (95% CI 0·63–1·53). Thus in the present vasoconstriction assay, budesonide and fluticasone were found to be equipotent.

Soluble ICAM-1 (sICAM-1) release is preferentially higher in the asthmatic lung than peripheral blood, but is not altered by allergen challenge

M SARNO, C PRIOR, S ROTONDETTO, J ALLEN, G OTT, A PEZZA, MONICA SPITERI *Lung Cell Biology Group, School of Postgraduate Medicine, Keele University, Staffordshire, UK; Department of Medicine, University Hospital, Innsbruck, Austria* Cell surface adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) could have an important role in allergen-induced airway inflammatory reactions in asthma (Wegner C *et al. Science* 1990;**247**:456–9). Inflammatory mediators can modulate this cellular expression of ICAM-1. We have demonstrated that the spontaneous release of IL-1, IL-6, TNF-α, and GMCSF by asthmatic lavage cells (BAC) is increased on exposure to relevant allergen. In this study we tested the effect of in vitro relevant allergen stimulation on sICAM-1 production by BAC and peripheral blood mononuclear cells (PBMC) obtained from 10 atopic asthmatic patients and eight atopic rhinitic patients. Measurements of sICAM-1 (ELISA, Bender MedSystems) were performed on supernatants from 24-hour serum-free cultures of unstimulated and allergen-stimulated unfractionated BAC and PBMC (1×10^6 cells/well). Asthmatic BAC released significantly more sICAM-1 (1·71 (0·38) ng/ml) than autologous PBMC (0·47 (0·19) ng/ml) ($p < 0·005$). Furthermore, spontaneous production of sICAM-1 by asthmatic BAC was higher than rhinitic BAC (0·65 ng/ml) ($p = 0·01$). No difference in release of sICAM-1 was shown between BAC and PBMC in the

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rhinitic subjects. No significant increase in sICAM-1 was detected in either asthmatic/rhinitic BAC or PBMC after relevant allergen. Our data suggest that although there is a preferential increase in sICAM-1 in the asthmatic lung, its production is unaltered on exposure to relevant allergen despite the enhanced release of specific cytokines.

Use of inulin to compare volumes of lung fluid sampled by bronchoalveolar lavage (BAL) in atopic asthmatic patients (AA) and normal subjects (N)

LJ RESTRICK, AP SAMPSON, PJ PIPER, JF COSTELLO *Department of Thoracic Medicine, King's College School of Medicine and Dentistry and Department of Pharmacology, Royal College of Surgeons, London* BAL fluid (BALF) is a variable mixture of instilled fluid and lung fluid (epithelial lining fluid and fluid which enters the lung during BAL). This makes the interpretation of solute concentrations in BALF difficult, particularly if the contribution of lung fluid is altered as a consequence of increased epithelial permeability, as occurs in inflammation – for example, asthma. We describe a new method for measuring the contribution of lung fluid to BALF in AA and N using inulin added to the instilled lavage fluid as an external marker of dilution. BAL (3×60 ml isotonic saline with 0·5 mg/ml inulin at 37°C) was performed in 13 mild AA using β_2-agonists only (mean (SD) methacholine PC_{20} 2·19 (2·29) mg/ml; median (range)% predicted FEV_1 100 (90–112)) and in 11 non-smoker N (% predicted FEV_1 102 (85–145)). Inulin concentration ([inulin]) was assayed in BALF by its conversion into fructose, reaction with resorcinol and spectrophotometric assay of the product at 413 nm. The inulin dilution factor (DF; BALF [inulin]/instilled fluid [inulin] ratio), and the percentage and volume of lung fluid were calculated:

$$\text{Lung fluid volume (ml)} = (1 - \text{DF}) \times \text{BALF volume (ml)}$$

There was no significant difference between the inulin DF of 0·931 (0·825–0·952) for AA and of 0·907 (0·768–0·985) for N ($p = 0·77$). The coefficient of repeatibility for the DF was 0·076. Lung fluid accounted for 7·0 (4·8–17·5)% of BALF from AA and 9·4 (1·6–23·2)% from N. There was no significant difference between the volume of lung fluid sampled of 8·1 (5·4–22·2) ml in AA and 12·3 (1·9–30·6) ml in N ($p = 0·56$), validating comparison of solute concentrations per ml of BALF between these AA and N, and also enabling solute concentrations to be expressed and compared per ml of lung fluid. The use of inulin in BAL demonstrates the large variation in the contribution of lung fluid to BALF between subjects and has potential for the standardisation and validation of solute concentrations in BALF.

Receptor subtypes mediating contraction to endothelin-1 in bovine bronchi

JE NALLY, R MCCALL, LC YOUNG, MJO WAKELAM, JC MCGRATH, NC THOMSON *Institute of Physiology and Biochemistry Department, University of Glasgow; Department of Respiratory Medicine, Western Infirmary, Glasgow* Endothelin-1 (Et-1) is the most potent bronchoconstrictor yet isolated. Levels of Et-1 are raised in patients with acute severe asthma, suggesting a role for this peptide in airway disease. It is thought to act on at least two subtypes of receptor, Et_A and Et_B, and these have been demonstrated in a number of tissues. The receptor type through which Et-1 acts in bovine bronchi is, however, not known. The present study sought to examine this question. Contractions of rings of bronchi in response to Et-1 were measured isometrically. Cumulative concentration–response curves (CRCs) were constructed to Et-1 and the -log of the concentration evoking a 200 mg contraction ($-\log EC_{200\,mg}$ (SE)) calculated). Et-1 ($10^{-10} - 3 \times 10^{-7}$M) evoked concentration dependent contractions. Preincubation of tissues with the Et_A antagonist FR139317 ($10^{-7} - 10^{-6}$M) evoked a significant ($p < 0·001$) rightward shift of the CRC ($-\log EC_{200\,mg}$, 7·63 (0·4) compared with control $-\log EC_{200\,mg}$ (9·1 (0·3), $n = 7$). In the absence of a suitable antagonist of ET_B receptors, desensitisation of these receptors using prolonged exposure to the slowly dissociating Et_B agonist, sarafotoxin (10^{-6}M) was undertaken. In the presence of such desensitisation, the CRC evoked by Et-1 was significantly ($p < 0·05$) shifted leftward at all except the highest concentration ($-\log EC_{200\,mg}$ (8·8 (0·3) compared with control ($-\log EC_{200\,mg}$, 7·9 (0·3), $n = 7$). In bovine bronchial smooth muscle, two endothelin receptor subtypes exist. Et-1 evokes contraction via an Et_A receptor type. This contraction is offset by parallel activation of an inhibitory Et_B-type receptor, the inhibition of which substantially enhances the contraction. [This work was supported by the NAC.]

Transfection of cultured human airway smooth muscle cells with a β-galactosidase reporter construct

IP HALL, MI KOTLIKOFF *Department of Therapeutics, University Hospital of Nottingham, Nottingham; University of Pennsylvania, Philadelphia, PA, USA* Cultured human airway smooth muscle (HASM) cells provide a useful model system for the study of airway responses. One potential way of modulating responses is by transfection with foreign genes. In this study a range of transfection methods were assessed in cultured HASM cells. HASM cells from passage 4–12 were grown as previously described and seeded into 100 mm^3 petri dishes. COS cells were used as a control. The eukaryotic expression vector pCMVβGAL and an appropriate control vector were purified by CsCl density gradient centrifugation. At 50% confluence cells were transfected by one of three methods: (1) calcium phosphate precipitation, (2) calcium phosphate plus glycerol shock or (3) lipofection. A range of concentrations of vector/carrier DNA were used between 1 µg and 10 µg. All experiments were performed in duplicate. Cells were harvested after 48 hours and β-galactosidase (β-gal) activity measured in the cell extracts. COS cells transfected with pCMVβGAL expressed high levels of β-gal activity regardless of the method used for transfection. HASM and COS cells transfected with the control plasmid expressed no detectable β-gal activity. HASM cells transfected with pCMVβGAL showed uniformly low, but detectable β-gal activity by the calcium phosphate precipitation method. Transfection efficiency appeared to be independent of DNA concentration and was not markedly enhanced by the use of either of the other two methods of transfection. This study shows that while HASM cells can be transfected with a construct containing the gene for β-gal driven by a CMV promoter, the levels of expression achieved are much lower than those achieved in an immortalised cell line. [Both vectors were kindly provided by Professor N Avadhani. The financial assistance of the MRC and the NAC is gratefully acknowledged.]

Pulmonary vascular impedance and resistance in hyperoxia and in hypoxia: dogs *v* pigs

P WAUTHY, D DE CANNIERE, C STEFANIDIS, M DELCROIX, S BRIMIOULLE, R NAEIJE *Laboratory of Cardiovascular and Respiratory Physiology, Erasme Campus, Free University of Brussels, Belgium* Higher pulmonary artery pressures (Ppa) and more vigorous hypoxic pulmonary vasoconstriction (HPV) have been reported in pigs compared with other species such as dogs or humans. We investigated mean Ppa *v* pulmonary blood flow (Q) relations and pulmonary vascular impedance (PVZ) spectra in six minipigs (mean weight 28 kg) and in six weight-matched dogs. The animals were anaesthetised with morphine and α-chloralose, and ventilated in hyperoxia (fraction of inspired O_2, FIO_2, 0·4), or in hypoxia (FIO_2 0·12). Instantaneous Ppa and Q were measured in the main pulmonary artery using an ultrasonic flow probe and a manometer tipped catheter. Plots of mean Ppa *v* Q were generated by a manipulation of venous return. PVZ was computed from the complete Fourier series expressions for Ppa and for Q. The pigs in hyperoxia had an arterial Po_2 (Pao_2) of 16·0 (1·9) kPa (mean (SE), a Ppa of 28 (2) mm Hg, a Q of 3·3 (0·3) l/min, a 0 Hz impedance (Z_0) of 686 (86) dyne s cm^{-5}, and a characteristic impedance (Zc) of 161 (24) dyne s cm^{-5}. Hypoxia in the pigs decreased Pao_2 to 5·2 (0·4) kPa, increased Ppa by an average of 11 mm Hg over the entire range of Q studied, from 3·5 to 1·5 l/min, and increased Z_0 and Zc to 987 (188) and 189 (26) dyne s cm^{-5} respectively. The dogs in hyperoxia had a Pao_2 of 16·8 (2·0) kPa, a Ppa of 20 (2) mm Hg, a Q of 4·9 (0·5) l/min, a 0 Hz impedance of 440 (44) dyne s cm^{-5}, and a Zc of 113 (10) dyne s cm^{-5} Hypoxia in the dogs decreased Pao_2 to 5·7 (0·4) kPa, increased Ppa by an average of 2 mm Hg over the range of Q studied from 4·5 to 1·5 l/min, and did not significantly affect Z_0 nor Zc. All pulmonary haemodynamic determinations were significantly different ($p < 0·001$) in pigs compared with dogs. We conclude that the pig is a natural intact animal model of raised pulmonary arterial resistance and elastance in hyperoxia and in hypoxia.

Effects of chronic aorta-pulmonary shunting on pulmonary haemodynamics in growing pigs

D DE CANNIERE, C STEFANIDIS, S BRIMIOULLE, R NAEIJE *Laboratory of Cardiovascular and Respiratory Physiology, Erasme Campus, Free University of Brussels, Belgium* Systemic to pulmonary shunting in growing pigs is a recognised experimental model of high flow

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pulmonary hypertension associated with congenital heart defects. We investigated multipoint mean pulmonary artery pressure (Ppa) *v* cardiac output (Q) plots and pulmonary vascular impedance (PVZ) spectra in 13 piglets aged around 4 months and ventilated alternatively in hyperoxia (fraction of inspired O_2, F_{IO_2} 0·4) and in hypoxia (F_{IO_2} 0·12). These measurements were done eight weeks after either an anastomosis between the thoracic aorta and the pulmonary trunk (n = 7 piglets) or a sham operation (n = 6). Instantaneous Ppa and Q were measured in the main pulmonary artery with an ultrasonic flow probe and a manometer tipped catheter. Plots of mean Ppa versus Q were generated by a manipulation of venous return. PVZ was computed from the complete Fourier series expressions for Ppa and for Q. In the sham operated piglets, in hyperoxia, Ppa was 20 (1) mm Hg (mean (SE), Q 114 (7) ml/kg, 0 Hz impedance (Z_0) 535 (56) dyne s cm^{-5}, and characteristic impedance (Zc) 124 (14) dyne s cm^{-5}. Hypoxia in the sham operated piglets increased Ppa by an average of 12 mm Hg over the entire range of Q studied, from 60 to 120 ml/kg and increased both Z_0 and Zc, to 870 (80) and 204 (60) dyne s cm^{-5} respectively. In the shunted piglets, in hyperoxia, Ppa was 26 (2) mm Hg, Q 120 (10) ml/kg, Z_0 456 (54) dyne s cm^{-5}, and Zc 180 (10) dyne s cm^{-5}. Hypoxia in the shunted piglets increased Ppa by an average of 3 mm Hg over the entire range of Q studied, from 60 to 120 ml/kg, and had no effect on Z_0 or on Zc. We conclude that an aorta-pulmonary shunting of two-month duration in growing pigs induces a moderate increase in both pulmonary vascular resistance and elastance, and is associated with a blunting of pulmonary vascular reactivity to hypoxia.

Effect of superoxide dismutase on hypoxic pulmonary vasoconstriction (HPV) after ischaemia-reperfusion (I-R) in the isolated blood perfused rat lung

M MESSENT, MJD GRIFFITHS, TW EVANS *Department of Anaesthesia and Intensive Care, Royal Brompton National Heart and Lung Hospital, London* We previously demonstrated that I-R in the isolated, blood perfused rat lung results in augmented HPV. We hypothesised that augmentation was due to scavenging of endothelially derived relaxant factor (NO) by superoxide following I-R. To test this the isolated, blood perfused rat lung was used as described (*Clin Sci* 1993;**85**:71–5). The preparation was stabilised for 0·5 hours, twice exposed to hypoxia (F_{IO_2} 0·3) and the rise in pulmonary artery pressure (Ppa) recorded. After ischaemia for 0·5 hours, two groups of rats (n = 5) were reperfused for 0·5 or one hour. Prior to reperfusion 100 IU of superoxide dismutase (SOD) was added to the perfusate to scavenge superoxide. After reperfusion of either 0·5 or one hour the lungs were twice exposed to hypoxia and the pressor response measured. The control groups (n = 5) used the same protocols substituting saline 0·2 ml for SOD. A paired Student's *t* test was used to compare Ppa rise before and after I-R. Results are mean (SE) mm Hg. The control

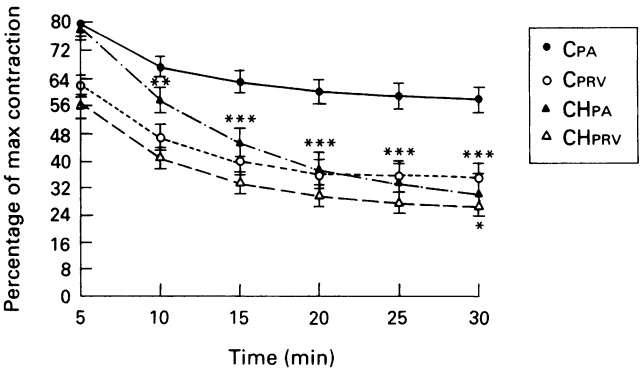
	0·5 h I-R + SOD	*1 h I-R + SOD*	*0·5 h I-R (control)*	*1 h I-R (control)*
HPV before I-R	13 (3·03)	5·2 (0·97)	5·8 (1·2)	6·75 (0·9)
HPV after I-R	12·8 (3·2)	7·0 (1·14)	8·8 (1·71)*	14·8 (3)*

* $p < 0.05$.

group demonstrates that HPV is increased after I-R. SOD prevented this increase of HPV due to I-R indicating that modulating NO release during HPV reacts with and is scavenged by superoxide produced during I-R. [M Messent is supported by the BUPA medical trust.]

Effects of the Ca^{2+} antagonist nifedipine on isolated pulmonary arteries from normoxic and chronically hypoxic rats

F ZHANG, AH MORICE *Departments of Medicine and Pharmacology, Royal Hallamshire Hospital, University of Sheffield, Sheffield* Contraction of vascular smooth muscle depends, at least in part, on calcium influx through transmembrane voltage-sensitive channels. Nifedipine, a potent slow channel Ca^{2+} antagonist, dilates isolated pulmonary arteries. In this study, isolated large and small rat pulmonary arteries were maximally precontracted with 100 mM KCl and vasorelaxation by nifedipine was compared between large pulmonary arteries (PA, diameter > 800 μm) and pulmonary resistance vessels (PRV, diameter < 300 μm) from both normoxic (C) animals and animals exposed to chronic hypoxia (CH). Vessels were dissected and mounted on an automated myograph, and maintained in oxygenated physiological saline solution (5 ml) at 37°C. Resting tensions were equivalent to intramural pressures of 17·5 or 35 mm Hg. Nifedipine caused a dose dependent vasorelaxation which was greater in CPRV than CPA. In CHPA, nifedipine was more potent and vasorelaxation was similar in CHPA, CHPRV and CPRV (fig). These results show that



*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with controls.

nifedipine is a potent vasodilator of isolated pulmonary vessels, with chronic hypoxia leading to an increase in potency in PA.

Neutral endopeptidase inhibition in rats with hypoxic pulmonary hypertension

JS THOMPSON, AH MORICE *Department of Medicine, University of Sheffield, Sheffield* Atrial natriuretic peptide (ANP) relaxes pulmonary vessels. ANP levels are raised in conditions characterised by pulmonary hypertension and it has been hypothesised that ANP may be autoregulatory in the pulmonary circulation. ANP is metabolised by neutral endopeptidase (NEP). We have studied the effects of the NEP inhibitor, SCH 42495, in rats with established hypoxic pulmonary hypertension. Rats (n = 32) were divided into four groups: chronic hypoxic controls in hypoxia for 10 days (CHC10), normoxic controls in air for 10 days (NC10), chronic hypoxic SCH 42495-treated rats in hypoxia for 20 days (CHT20), and chronic hypoxic vehicle treated rats in hypoxia for 20 days (CHV20). After 10 days hypoxia, oral treatment with drug (30 mg/kg, twice daily) or methyl cellulose vehicle (0·4%, twice daily) was started. Evidence of right ventricular hypertrophy (RVH) and pulmonary vascular remodelling (PVR) was determined, and plasma ANP and haematocrit (Hct) measured. CHC10 rats had significant ($p < 0.05$) RVH (0·53 (0·08)) and PVR (29·0 (0·01)% and had gained significantly less body weight (33·2 (5·5)g) than NC10 rats (0·31 (0·04), 10·9(0·01)%, and 59·2 (11·9)g). CHC10 rats had raised plasma ANP (58·4 (9·9) pM) and Hct (42 (4·0)%) compared with NC10 rats (23·9 (3·2) pM and 26(1·5)%). Treatment with SCH 42495 caused a significant reduction in PVR (25·0 (0·01)%) and RVH (0·52 (0·09)) in CHT20 rats with established pulmonary hypertension compared with CHV20 controls (33·0 (0·02)% and 0·61 (0·09). CHT20 rats also had significantly lower PVR than CHC10 rats. Thus treatment caused a partial reversal of PVR after 10 days. Short term inhibition of NEP causes regression of established PVR.

Brain natriuretic peptide (BNP) is as potent a vasodilator as atrial natriuretic peptide (ANP) in isolated rat pulmonary resistance vessels (PRVs)

W SHEEDY, AH MORICE *Department of Medicine, University of Sheffield, Sheffield* Atrial and brain natriuretic peptides have been shown to possess many similarities in their structure and functions. We have previously shown that ANP is a vasodilator of prostaglandin $F_{2\alpha}$-contracted pulmonary resistance vessels (PRVs) and that this effect is increased on adaptation to hypoxia. In this experiment we have studied the effects of BNP on similar vessels also contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). PRVs from control (C) (n = 4) and chronic hypoxic (CH) (n = 8) animals were dissected and mounted as ring preparations between the jaws of an automated myograph. Vessels were then precontracted with 100 μM $PGF_{2\alpha}$; 30 minutes later when the contraction had plateaued either BNP or ANP was added to

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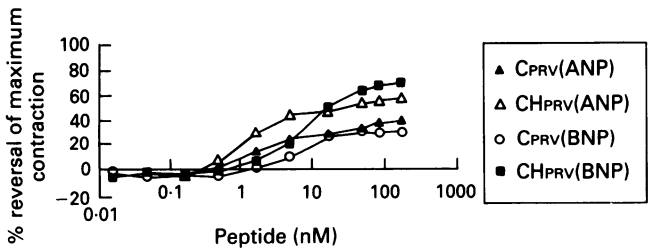

produce cumulative dose–response curves. Both ANP and BNP produced dose dependent relaxation in C and CH vessels. Maximum relaxation using either ANP or BNP was significantly greater in CH vessels than in C vessels. ANP had a significantly lower EC_{50} in CH vessels when compared with BNP; (2·2 (0·9) *v* 8·0 (1·6) nM respectively), $p < 0.05$, suggesting ANP is about four times more potent in CH vessels.

Contractile responses of sumatriptan and 5-HT in human pulmonary arteries

AGB TEMPLETON, RA CLAYTON, MR MACLEAN *Institute of Physiology, University of Glasgow, Glasgow* Sumatriptan is an agonist showing a degree of selectivity for 5-HT1D receptors mediating smooth muscle contraction, which is typically four to 10-fold less potent than 5-HT as a vasoconstrictor (Humphrey *et al. Br J Pharmacol* 1988;**94**:1123–32; Parsons and Whalley. *Eur J Pharmacol* 1989;**174**:189–96; Templeton *et al. Br J Pharmacol* 1993;**108**:249P). A 5-HT-like receptor mediated response equipotent to that of 5-HT has not yet been reported in a human artery. We describe here such a receptor mediating contraction of human pulmonary artery. Human lung tissue was obtained from patients undergoing surgery for bronchial carcinoma. Arteries used were normally between 3–5 mm internal diameter and cut into rings 2–3 mm in length. Rings were set up under 1·5 g tension, maintained at 37°C, bubbled with 16% O_2; 5% CO_2 balance N_2. Tissues were exposed to 50 mM KCl, after which cumulative concentration response curves to the agonists were constructed either in the absence or presence of antagonist. Sumatriptan and 5-HT were equipotent at inducing contractile responses in the human pulmonary artery with -log EC_{50} values of 6·59 (0·1) and 6·56 (0·14). The maximum response achieved to sumatriptan was some 30–40% less than that to 5-HT. Ketanserin (10 nM) antagonised the responses to both agonists with -log EC_{50} for 5-HT of 5·81 (0·23) and sumatriptan of 6·27 (0·17). Methiothepin reduced the maximum response to 5-HT but had no significant effect on potency. However, it did reduce the sensitivity to sumatriptan with -log EC_{50} of 6·08 (0·15). These results suggest that the human pulmonary artery has a functional population of 5-HT1D-like receptors which may play an important part in the contractile response to 5-HT.

Serotonin and pulmonary artery vasoconstrictor responses in humans

AY BUTT, M TAKAO, Y KATAYAMA, J PEPKE-ZABA, G CREMONA, TW HIGENBOTTAM *Respiratory Physiology Department, Papworth Hospital, Papworth Everard, Cambridge* Serotonin (5-HT) may have an important role in pathogenesis of pulmonary hypertension but little is known of the mechanism. To study the phenomenon we have used 31 rings of human pulmonary arteries from five patients; one patient with cystic fibrosis and one with Eisenmenger's syndrome received heart/lung transplantation, two patients with emphysema received single lung transplantation and one patient with bronchiectasis underwent lobectomy. Segmental and subsegmental arteries were dissected and rings (2–5 mm in length and 2–4 mm in diameter) were mounted in 20 ml organ baths filled with Krebs-Ringers solution at 37°C and continuously bubbled with 95% O_2 and 5% CO_2. Rings were studied in pairs with (E_+) and without (E_-) endothelium and their isometric tension was recorded. After obtaining a stable basal tone rings were treated with 5-HT (10^{-8} to 10^{-4}M) followed by washouts and were allowed to relax. Then an optimum precontraction was achieved with prostaglandin $F_{2\alpha}$ (10^{-8} to 10^{5}M) and at plateau of contraction rings were again treated with 5-HT (10^{-8} to 10^{-4}M). Some of the rings (n = 4) were presented with ketanserin (10^{-4}M) before contraction with $PGF_{2\alpha}$. There was a marked vasoconstrictor response to 5-HT from basal tone (rise in tension: 142·5 (32·6)% in E_+ rings and 65·8 (14·4)% in E_- rings; mean (SE); $p < 0.05$). From precontracted state there was a further rise in tension with 5-HT (48·9 (23·6)% in E_+ and 48·2 (16·5)% in E_- rings). In rings pretreated with ketanserin further rise in tension was smaller with 5-HT (15·5 (2·1)% in E_+ and 17·8 (4·5)% in E_- rings). We conclude that 5-HT is a potent vasoconstrictor of diseased human pulmonary arteries from patients with chronic lung disease. Such vasoconstriction may be partly due to the direct stimulation of 5-HT_2 receptors and partly due to the release of a vasoconstrictor substance from endothelium.

Use of a chemiluminescent method to study the chemical reaction of nitric oxide (NO) with biologically important molecules under physiological conditions

CDR BORLAND, EAG DEMONCHEAUX, EC CONSTABLE, MB DAVIES, TW HIGENBOTTAM *Department of Respiratory Physiology, Papworth Hospital, Cambridge; University Chemical Laboratory, University of Cambridge; Applied Sciences, Anglia Polytechnic University, Cambridge* The study of the reactions of nitric oxide (NO) in aqueous solutions under physiological conditions is difficult. We have devised a method for directly studying the reactions of NO (1 nM) with a range of biologically important molecules continuously circulated in Krebs buffer equilibrated with 95% O_2/5% CO_2. 100 ppm NO in 95% O_2/5% CO_2 was passed through a flask containing Krebs buffer at 37°C connected to a second flask through which nitrogen was passed under reduced pressure to a chemiluminescent analyser. Solutions of interest in increasing concentrations (n = 15) are injected into the first flask. The most dilute solutions to react with NO and estimated concentrations were: haemoglobin $10^{-6}(9 \times 10^{-9}$M), human red cells $10^{-5}(9 \times 10^{-8}$M), human plasma $> 10^{-2}$ (−), $FeSO_4$ 10^{-9}M, amino acid solution $10^{-6}(5 \times 10^{-9})$, cysteine 10^{-8}M. The second order rate constants for these reactions should be inversely proportional to minimum molar concentrations. The reaction of cysteine with NO was inhibited by human plasma (n = 3). The half lives of NO in Krebs buffer and distilled water with nitrogen and CO_2/N_2 (n = 5) were measured by connecting a third flask in series with headspace excluded. Measured $t^{\frac{1}{2}}$NO/H_2O N_2 = 6·51 (0·23) min, NO/H_2O CO_2 O_2 = 6·89 (0·3) min, NO/Krebs N_2 = 1·9 (0·72) min, NO/Krebs CO_2 O_2 = 1·74 (0·35) min. (1) NO reactions under physiological conditions can readily be studied by this method. (2) The reactions of NO with haemoglobin and cysteine are likely to be of great physiological importance. (3) The reaction of NO with oxygenated Krebs solution seems to be with species other than oxygen.

Comparison of the rate of reaction of nitric oxide (NO) with human whole blood and plasma: gaseous NO reacts more rapidly with red cells than plasma

CDR BORLAND, EAG DEMONCHEAUX, EC CONSTABLE, MB DAVIES, TW HIGENBOTTAM *Department of Respiratory Physiology, Papworth Hospital, Cambridge; University Chemical Laboratory, University of Cambridge; Applied Sciences, Anglia Polytechnic University, Cambridge* NO is known to react rapidly with red cells (Carlsen, Comroe. *J Gen Physiol* 1959;**42**:83–107). Endothelium-derived relaxing factor (EFRF) is inhibited by constituents of plasma (Edwards *et al. Cardiovasc Res* 1986;**20**:549–56). We have compared the rate of reaction of NO with human whole blood and plasma by measuring the rate of disappearance of NO gas (10 ppm) in 95% oxygen and 5% CO_2 from the headspace of a 500 ml flask containing 10 ml of freshly drawn human whole blood or plasma at 37°C. NO reacted with whole blood (half life = 9·0; 95% CI: 6·6-14·3 min; n = 3) whereas the rate of disappearance with plasma was much slower (half life = 40·5; 95% CI: 28·6–70 min; n = 3). In fact the reaction with plasma was no faster than for NO in contact with distilled water. It is concluded that the main fate of gaseous NO entering the blood (for example, when inhaled) is reaction with red cells. The long half life of NO in a gas mixture above all blood will reflect not only the reaction of NO with the red cells but also the transfer kinetics from gas to liquid.

Difference between basal release of nitric oxide (NO) and response to acetylcholine in isolated lungs from patients with chronic obstructive lung disease (COLD)

G CREMONA, EA BOWER, T HIGENBOTTAM *Papworth Hospital; Physiological Laboratory, Cambridge* We have previously demonstrated impaired endothelium-dependent relaxation in isolated conduit pulmonary arteries from patients with COLD (Dinh-Xuan *et al. N Engl J Med* 1991;**324**:1539–47). To investigate the effects of endothelium-

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dependent relaxation of resistance vessels, we have studied the response to acetylcholine (ACh; n=4) and to N^G-nitro-L-arginine methyl ester (L-NAME; n=4) in isolated lungs from COLD patients undergoing heart/lung transplantation. As a control group, lungs from heart donors not used for transplantation were studied. The lungs were obtained immediately after excision, ventilated with air and 5% CO_2, and perfused at constant flow with buffered Krebs-Henseleit solution plus dextran (3·5%) and albumin (1%) at 37°C. Indomethacin (10^{-5}M) was added to block the cyclo-oxygenase pathway. The change in perfusion pressure was measured after addition of either L-NAME (10^{-5}M) or to ACh (10^{-8} to 10^{-4}M), the last after precontraction with UA46619. L-NAME increased mean (SD) perfusion pressure by 13·5 (4·8) mm Hg in COLD compared with 9·6 (3·4) mm Hg in the control lungs ($P=0.09$). After precontraction by UA46619 (mean increase 14·2 mm Hg), ACh reduced perfusion pressure by 3·2 (2·6) mm Hg in COLD and by 10 (3·6) mm Hg in control lungs (p=0·01). We conclude that impairment of pulmonary endothelium-dependent relaxation in COLD is a general phenomenon affecting both conduit and resistance vessels. Basal release of NO, however, seems to be conserved in COLD lungs.

Effect of nitric oxide synthase inhibition on contractile responses to sumatriptan in bovine pulmonary arterioles

KM MCCULLOCH, AGB TEMPLETON, MR MACLEAN *Institute of Physiology, University of Glasgow, Glasgow* It has previously been shown that the selective 5HT1D agonist sumatriptan contracts bovine pulmonary arteries but not mesenteric arteries (Templeton *et al. Br J Pharmacol* 1993;**108**:249P). In this series of experiments we studied responses to sumatriptan in isolated bovine pulmonary arterioles and the effect of nitric oxide synthase inhibition on these responses. Pulmonary arterioles (internal diameter 206 (25) μm, n=10) were mounted on a Mulvany wire myograph in Krebs saline at 37°C and bubbled with 16% O_2 5% CO_2 balance N_2. After one hour equilibration, cumulative concentration–response curves (CCRC) to sumatriptan were conducted, and subsequent to this a second CCRC to sumatriptan was carried out in the presence or absence of N-nitro-L-arginine methylester (L-NAME nitric oxide synthase inhibitor). Only 70% of vessels contracted to sumatriptan, (max contraction = 203 (65) mg, $-\log EC_{50} = 5{\cdot}6$ (0·2), n=8). In these vessels the presence of 10^{-4} M L-NAME markedly potentiated responses to sumatriptan without altering tissue sensitivity (max contraction 1378 (427) mg, $-\log EC_{50} = 5{\cdot}5$ (0·2), n=8). Inclusion of 2×10^{-4} M L-arginine prevented this effect of L-NAME. In addition we have recently shown that raising vascular tone has a similar effect on these vessels increasing the maximum contraction (from 128 (19) mg to 733 (133) mg) with no change in tissue sensitivity ($-\log EC_{50}$ 6·0 (0·3) with no tone, and 6·1 (0·1) with tone present, n=4). These results have potential implications in pulmonary hypertension where vessels are subjected to an increase in vascular tone with a decrease in endogenous nitric oxide production.

Effect of chronic inhibition of NO synthase on pulmonary and renal resistance vessels in rats

GQ TENG *Department of Medicine, Royal Hallamshire Hospital, Sheffield* NO is continuously released in the systemic circulation and attenuates pressure. However, in the normal, normoxic blood-perfused rat pulmonary circulation, there is little or no release of NO except during vasoconstriction (Barer *et al. J Physiol* 1993;**463**:1–16). The effect of chronic inhibition of NO synthase by the L-arginine analogue L-NAME on pulmonary and renal resistance vessels was studied in 6 LN rats receiving 300 μg/ml in drinking water compared with 6 W rats receiving plain water. Vessels 250–380 μm diameter were mounted in a Cambustion myograph in physiological saline in the isometric mode and gassed with O_2 or N_2, both +5% CO_2. Transmural pressure was 20 mm Hg in pulmonary, 100 mm Hg in renal vessels and lung vessels were given a control resting tension 100% of the internal pressure, renal vessels 90%. Tests were made during preconstriction with $PGF_{2\alpha}$ ($10^{-5}-10^{-6}$M in different tests). L-NAME treatment was effective, as the dilator effect of ACh was significantly reduced: for lung vessels the reduction of $PGF_{2\alpha}$ tension was, for Ach (10^{-5}M), 56·7 (9·3)% in W and 33·1 (7·3)% in LN rats, p<0·02 and for renal vessels (ACh 10^{-6}) 69·0 (3·2)% compared with 42·2 (6·3)%, p<0·003. Also, acute L-NAME caused a lesser rise in tension in LN than W rats. For lung vessels the tension rise in mN was 0·5 (0·2) *v* 1·5 (0·5), p<0·03 and for renal vessels 0·8 (0·2) *v* 2·7 (0·2), p<0·001. Lung and renal vessels contract and relax respectively in hypoxia; contraction is biphasic in lung vessels and in renal vessels there is a brief early contraction before dilatation. In LN rats both contraction phases were reduced in lung vessels: phase one, 1·2 (0·5) *v* 5·5 (1·8) mN, p<0·03, phase two, 0·8 (0·2) *v* 1·8 (0·4), p<0·01. In LN rats the contraction phase was abolished in renal vessels. There is no obvious explanation for the reduced contraction phases in LN rats. Perhaps chronic loss of NO may alter a balance between vasoconstrictor and dilator mechanisms.

Aminoguanidine selectivity inhibits inducible nitric oxide synthase

MJD GRIFFITHS, M MESSENT, TW EVANS *National Heart and Lung Institute, London* Aminoguanidine (AG) incorporates the guanidino group of L-arginine linked to hydrazine. Studies with non-vascular tissue have suggested that AG is a more potent inhibitor of inducible compared with constitutive nitric oxide synthase (NOS) (*Diabetes* 1993;**42**:221–32). We have shown that NOS is induced in rat pulmonary artery after pre-treatment with endotoxin (LPS) (*Thorax* 1993;**48**:467) but the effects of AG on vascular NOS in this model are unknown. Male Wistar rats were treated with LPS (20 mg/kg, intraperitoneally), or vehicle (1 ml NaCl, intraperitoneally) four hours before killing. Pulmonary artery rings were dissected and suspended in organ baths containing oxygenated Krebs solution. The endothelium was removed by abrasion, confirmed by failure to relax to acetylcholine. Concentration–response curves to phenylephrine (PE) were constructed and the EC_{50} for PE calculated. After contraction with the ring's EC_{50} of PE, AG concentration–response curves were constructed in vessels from sham and LPS-treated rats. Separate experiments were performed in the presence of L and D-arginine (2 mM). AG caused dose-dependent contraction in vessels from LPS-treated rats (EC_{50} $1{\cdot}51\ (0{\cdot}17)\times10^{-4}$ M), that was abolished by pre-incubation with L but not D-arginine (EC_{50} $7{\cdot}41\ (0{\cdot}05)\times10^{-5}$ M, p>0·05). Results shown are mean (SE) of six observations. AG had no significant effect on vessels from sham treated rats. Relaxation to acetylcholine after PE induced contraction was preserved in endothelium intact AG treated rings (results not shown). These findings are consistent with the hypothesis that AG selectively inhibits inducible NOS, but further work is required to exclude other actions of this compound. [MG is a Wellcome Clinical Research Fellow.]

Effects of chronic oral treatment with a nitric oxide blocker, L-NAME, on hypoxic pulmonary vasoconstriction in the rat

CELIA EMERY, GUO QUI TENG *Department of Medicine, University of Sheffield, Sheffield* Whether nitric oxide (NO) is continuously released to maintain low pulmonary vascular resistance or is only released in response to a rise in pressure, as during hypoxia, is debatable. Acute doses of the NO synthase inhibitor, L-NAME, enhanced the rise in pulmonary artery pressure (Ppa) (hypoxic pulmonary vasoconstriction; HPV) in isolated perfused lungs (IPL) of both normal rats (C) and chronically hypoxic rats (CH; 10%O_2, three weeks) (Barer *et al. J Physiol* 1993;**463**:1–16). Here we report the effect of chronic oral treatment with L-NAME (LN; 45 mg/kg/day for three weeks) on HPV in anaesthetised rats, with Ppa measured by cardiac catheter (3CLN and 8CHLN), and in isolated rat lungs, perfused with blood at constant flow (9CLN and 10CHLN); controls drank water (W). In both CLN and CHLN systemic blood pressure was raised (BPsys range 140–180 mm Hg *v* 100–140 mm Hg in W controls). However, there was no effect on normoxic Ppa in either anaesthetised rats or in IPLs, CH rats developed pulmonary hypertension. In anaesthetised rats, ventilation with 10–12%O_2 caused a rise in Ppa which was significantly greater (p<0·05) in 8CHLN than 7CHW (mean (SD) 7·3 (4·2) *v* 3·4 (2·2) mm Hg) and was also increased in 3CLN *v* 3CW. The fall in BPsys during hypoxia was not altered in LN treated rats. The range of arterial oxygen values was similar in all groups. In IPLs the rise in Ppa with 10%O_2 was significantly greater (p<0·01) in 9CLN than in 10CW (18·5 (7·5) *v* 8·8 (4·2) mm Hg) but not significantly different in 10CHLN *v* 9CHW (22·4 (12·8) *v* 19·0 (15·6) mm Hg). Thus in rats treated chronically with a blocker of NO synthesis, sufficient to raise BPsys, HPV was enhanced but normoxic Ppa was not altered, suggesting that NO may be released in response to hypoxia but not continuously released under normoxic conditions.

Amitriptyline induced lung injury in rats

X LIU, GR BARER *Department of Medicine, Royal Hallamshire Hospital, Sheffield* Acute respiratory distress syndrome (ARDS) has

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been recorded after amitriptyline (AMI) overdose. We have previously shown that acute doses of AMI caused large pulmonary pressor effects and oedema in cats in vivo and in isolated blood perfused rat lungs; electron microscopy in rats showed lesions in alveolar and capillary membranes. We have studied the nature of the endothelial damage caused in 19 blood-perfused rat lungs (constant flow), ventilated with air (normoxia) or 2% O_2 (hypoxia) both with 5% CO_2. AMI caused dose dependent increases in pulmonary artery pressure (Ppa). For cumulative doses of 0·6, 1·0, 1·5, and 2·5 mg AMI, the cumulative increases in Ppa in seven rats were 1·1 (0·4), 4 (1·4), 5·3 (2·0), and 17·4 (3·3) mm Hg (mean (SE)), terminating in oedema. Often, when Ppa was substantially raised, the next dose caused profound dilatation. The following causes of constriction/dilatation were excluded; release of histamine, 5-hydroxytryptamine (5-HT), noradrenaline (NA) or cyclo-oxygenase products because the effects remained after appropriate doses of chlorpheniramine, methysergide, phentolamine, meclofenamate, or cimetidine. Some endothelial functions were retained after AMI, others impaired. NO synthase and ACE were unimpaired since, after Ppa was substantially raised by AMI, the L-arginine analogue and NO synthase inhibitor L-NAME caused a rise in Ppa and angiotensin 1 caused a pressor effect. Amine uptake was, however, impaired; pressor effects of 5-HT and noradrenaline were almost abolished (5HT +8·9 (2·3) mm Hg before, +1·5 (0·3) mm Hg after, $p=0·02$; NA +5·0(1·0) mm Hg before, +0·3(0·2) mm Hg after, $p=0·005$). Hypoxic vasoconstriction was reduced but not abolished by AMI. Thus AMI provides a model of lung injury but the precipating factors have not yet been found.

Carcinoma of the bronchus in Yorkshire 1976 to 1990: trends since 1984

CK CONNOLLY, SM CRAWFORD, PL RIDER, ADM SMITH, MF MUERS *Friarage Hospital, Northallerton; Yorkshire Cancer Registry, Cookridge Hospital, Leeds* Changes in epidemiology and management of carcinoma of the bronchus, as recorded by the Yorkshire Cancer Registry between 1976 and 1983 have been reported previously (Connolly CK *et al, Br J Cancer* 1990;**61**:579–83). This report extends the study to 1990. 18 212 (2580 per year) cases were reported during life between 1984 and 1990, 20 155 (2519) between 1976 and 1983. Average age at presentation increased from 66·1 (1976) to 68·3 (1983) and 69·2 (1990). The male/female ratio fell from 3·61:1 (1976) to 2·28:1 (1983) and 2·1:1 (1990). The histological confirmation rate and mean age of confirmed cases also increased (45·4%, 60·8 years, (1976); 58·4%, 63·5 years, (1983); 61·7%, 64·5 years, (1990)). The ratio of small to squamous cell carcinoma was unaltered at 3:7. Known cases of adenocarcinoma increased at an accelerating rate (108 in 1976 to 230 in 1990). The surgical rate did not alter overall but increased from 5% to 8% in the 70–74 age group. Prognosis after radical surgery improved over the age of 70, so that two year survival is now independent of age over 60. There was no further improvement in the outcome of small cell carcinoma after 1983. Overall two year survival improved significantly ($p<0·04$) from 6·2% (1976) to 9·4% (1987). Improvement was greater in patients with better prognosis. 75% survival increased from about 22 to 24 days (annual percentage trend 0·7%, $p<0·02$); median survival from 79 to 91 days (1·1%, $p<0·01$), and 25% survival from 224 to 266 days (1·3%, $p<0·001$). Major contributions to this improvement were the introduction of chemotherapy for small cell carcinoma in the first five years, and a more aggressive approach to surgery in the elderly throughout the period. These results emphasise the need for proper evaluation of all patients with carcinoma of the bronchus.

Increased prevalence of adenocarcinoma of the bronchus in Yorkshire

SM CRAWFORD, CK CONNOLLY, PL RIDER, ADM SMITH, MF MUERS *Cancer Medical Research Unit, University of Bradford, Bradford* All cases with carcinoma of the bronchus diagnosed in the Yorkshire Region of the National Health Service are reported to the Regional Cancer Registry. 38 275 subjects were notified between 1976 and 1990. The histological confirmation rate rose from 45·4% to 61·7%. The male/female ratio decreased from 3·6 to 1 to 2·1 to 1 during the period. Absolute numbers of patients known to have adenocarcinoma increased progressively from 108 in 1976, by 43% to 153 in 1983, and by a further 57% to 230 in 1990. The proportion of those that were female rose progressively from 32·2% in 1976–8 to 38·1% in 1987–90. Detailed analysis by sex and age group did not suggest that the increased proportion of females was responsible for the increase in numbers. The observed total of adenocarcinoma was 579 in the five-year period 1976–80. It would have been expected to increase to 775 in 1986–90, allowing for the increased histological confirmation rate and the changes in the age/sex distribution of the population. The observed number was 1061, an excess of 286 (37%). Analysis by district confirmed that it was unlikely that the changes were entirely due to differing alterations in diagnostic practice, although the districts with the highest proportion of adenocarcinomas, 17% and 21%, respectively, accounted for 87 of the excess cases. The proportion of adenocarcinoma in the remaining districts was 9–16% (median 11%). The findings suggest a real increase in the incidence of adenocarcinoma of the bronchus in Yorkshire in the decade to 1988.

Atypical alveolar hyperplasia and adenocarcinoma of the lung

KM KERR, FA CAREY, D LAMB *Department of Pathology, Aberdeen University Medical School, Foresterhill, Aberdeen; Department of Pathology, Edinburgh University Medical School, Teviot Place, Edinburgh* Foci of atypical bronchioloalveolar cells have been found in cancer bearing lungs raising the possibility of an adenoma-carcinoma sequence in the lung (*Cancer* 1988;**61**:1009–14). We have identified 19 cases of atypical alveolar hyperplasia (AAH) which conform to the lesions described in the literature. All patients except one had resection for peripheral adenocarcinoma, all were smokers, and 14/19 (74%) were female. Between 1986–1990, 175 resections performed in Edinburgh for adenocarcinoma yielded 10 cases (5·7%) while 90 resections from Aberdeen provided nine cases (10%). Foci of AAH and the associated tumours were examined for expression of proliferating cell nuclear antigen (PCNA) and products of the p53 and c-erbB-2 genes using immunohistochemistry and paraffin embedded material. All the tumours and AAH foci showed increased expression of PCNA. Nine cases had material stained for p53 and c-erbB-2. In three cases both tumour and some AAH foci showed stainable p53 protein, two showed positivity in tumour or AAH foci but not both. Four cases showed no staining for p53. C-erbB-2 was detected in four tumours but in only one of these was the AAH also positive. A further case showed c-erbB-2 in the AAH only. Positivity for p53 and c-erbB-2 related poorly. These results confirm the proliferative nature of AAH while the presence of stainable p53 and c-erbB-2 proteins shows that AAH can exhibit genetic changes linked with malignant transformation.

Gastrin in small cell lung cancer: cloning of the receptor, Ca^{2+} mobilisation and growth

TJ SETHI *Respiratory Medicine Unit, Department of Medicine (RIE), Edinburgh* Compelling evidence that gastrin acts as a cellular growth factor or as an autocrine factor in tumours has been difficult to document using clonal cell populations, due to the lack of a convenient model system. This study demonstrates that addition of gastrin at nanomolar concentrations to the SCLC line H510 causes a rapid mobilisation of Ca^{2+} from intracellular stores. This SCLC cell line provides a model system in which to study gastrin. Gastrin is shown to act as a direct growth factor acting through CCK_B/gastrin receptors. To extend these findings, the expression of the CCK_B/gastrin receptor was isolated, cloned, and sequenced. Surprisingly, Ala/Thr-Ala/Gly-Pro-Gly-Pro (residues 272–276) of the canine/rat gastrin receptor corresponding to the third cytosolic loop, a region thought to play a critical part in signal transduction, was absent in the amplified human sequence. A full length cDNA clone was isolated and divergencies of the human brain CCK_B/gastrin receptor to the canine/rat receptors were confirmed. The complete coding region predicts a protein of 447 amino acid residues including seven putative transmembrane spanning regions. No differences were noted between the human brain and SCLC cell CCK_B/gastrin receptor sequences. Northern blot analyses revealed a single transcript of 2·4 kb in SCLC. The levels of RNA expression match directly with the ability of SCLC cells to respond to gastrin. This is the first time that gastrin/CCK_B receptors are shown to stimulate growth outside the gastrointestinal system. Interestingly, SCLC cells have been shown to express gastrin and CCK peptides, further extending the hypothesis that SCLC growth is regulated by multiple autocrine and paracrine loops involving neuropeptides.

Increased responsiveness to neuropeptide agonists and antagonists of small cell lung cancer cells that progressed from sensitive to resistant to chemotherapeutic drugs

TJ SETHI *Respiratory Medicine Unit, Department of Medicine (RIE), Edinburgh* SCLC (small cell lung cancer) is characterised by the

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presence of intracytoplasmic neurosecretory granules and by its ability to secrete many peptides. The Ca^{2+}-mobilising neuropeptides bradykinin, cholecystokinin (CCK), gastrin-releasing peptide (GRP), neurotensin, and vasopressin have been shown to be growth factors in SCLC cell lines, suggesting that these neuropeptides stimulate SCLC growth by an extensive network of autocrine and paracrine interactions. The SCLC cell lines GLC-14, GLC-16, and GLC-19 were established from one patient during longitudinal follow up when the tumour changed from sensitive to completely resistant to chemotherapy. The responsiveness of these SCLC cells to neuropeptide agonists and antagonists were investigated. The results show that the SCLC cell lines GLC-14, GLC-16, and GLC-19 exhibit a progressive increase in responsiveness to neuropeptides both in terms of their ability to mobilise Ca^{2+} and to stimulate clonal growth. The broad-spectrum neuropeptide antagonists (D-Arg[1],D-Phe[5],D-Trp[7,9],Leu[11]) substance P and (Arg[6],D-Trp[7,9],MePhe[8]) substance P (6–11) block bradykinin, CCK, galanin, gastrin, GRP, neurotensin, and vasopressin mediated signals and growth in SCLC cell lines, and inhibit SCLC growth in vitro and in vivo. It is also shown that the growth of all three GLC SCLC cell lines is inhibited by broad spectrum neuropeptide antagonists. Strikingly, the GLC-19 cell line which is derived from a tumour completely resistant to chemotherapy displays increased sensitivity to broad spectrum antagonists. These findings support the hypothesis that the clinically aggressive drug-resistant SCLC cells that emerge after chemotherapy might have a more extensive network of neuropeptide regulation. Thus broad spectrum neuropeptide antagonists are a logical adjunct to conventional chemotherapy.

Imaging of small cell lung cancer with a radiolabelled somatostatin analogue

KJ O'BYRNE, JT ENNIS, PJ FREYNE, LJ CLANCY, JS PRICHARD, DN CARNEY *Mater Misericordiae Hospital and St James' Hospital, Dublin* Recent work suggests that between 50% and 75% of small cell lung cancer (SCLC) tumours have specific high affinity binding sites for somatostatin. This study evaluated the potential role of the radiolabelled somatostatin analogue, ^{111}In-pentetreotide, in the detection and staging of SCLC in patients before and after chemotherapy with scintigraphic imaging techniques. Thirteen patients were studied before chemotherapy. After standard staging six patients had limited stage disease (LD) and seven extensive disease (ED). ^{111}In-pentetreotide imaging led to the detection of all primary sites of disease including a primary site of disease not detectable with chest x ray film or CT of the thorax. All sites of metastatic disease detected by standard staging were imaged in three of seven patients. Furthermore a cerebellar metastasis was detected in a patient thought to have disease confined to the right hemithorax. This was subsequently confirmed with a CT brain scan. Following chemotherapy three patients with LD and two with ED prior to treatment were evaluated. Standard staging suggested complete remissions (CR) in the LD patients. ^{111}In-pentetreotide imaging detected residual intrathoracic disease in two CR patients and in the patients with ED both of whom had had a partial response to chemotherapy. These results demonstrate that ^{111}In-pentetreotide imaging has a role in the clinical evaluation of patients with SCLC. This technique may be of particular value in detecting residual intrathoracic disease in patients thought to have CR by conventional staging methods.

Feasibility of using glycosylated recombinant human granulocyte colony stimulating factor (G-CSF) to increase the dose intensity of doxorubicin, cyclophosphamide and etoposide (ACE) in small cell lung cancer (SCLC)

N THATCHER, NM BLEEHEN, DJ GIRLING, G LALLEMAND, D MACHIN, RJ STEPHENS ON BEHALF OF THE MEDICAL RESEARCH COUNCIL LUNG CANCER WORKING PARTY *MRC Cancer Trials Office, 5 Shaftesbury Road, Cambridge* The purpose of this phase II study was to test the feasibility of shortening the interval between cycles of ACE from the usual three weeks to two weeks in the treatment of SCLC, by adding G-CSF to reduce the duration of neutropenia following a cycle. 20 patients with SCLC and good performance status (WHO grade 0–2) were prescribed six cycles of two-weekly ACE (doxorubicin (50 mg/m^2), cyclophosphamide (1 g/m^2), and etoposide (120 mg/m^2) intravenously on day 1, and etoposide (240 mg/m^2) orally on days 2 and 3), plus G-CSF (5 μg/kg/day) subcutaneously on days 4 to 14 of each cycle. The G-CSF (Ienograstim) was provided free by Chugai-Rhône-Poulenc. Three patients died during the treatment period and a further five had ACE terminated before the 6th cycle, three for toxicity and two for progressive disease. Of 71 intervals between cycles, 42(59%) were of the prescribed 14 days, nine (13%) of 15 to 20 days, 15 (21%) of 21 days, and five (7%) longer; but during the first four cycles, 36 (77%) of 47 intervals were of 14 days and a further three (6%) of up to 17 days. The reason for delay was thrombocytopenia in 20(69%) of the 29 instances. 18 patients experienced WHO grade 3 or 4 neutropenia at some time, but at two weeks after a cycle only one had grade 3 and none grade 4. These levels of neutropenia are similar to those reported with standard three-weekly ACE. 17 patients required blood transfusion and 12 platelet transfusion. The only serious adverse reaction to G-CSF was an episode of transient rash with facial and periorbital oedema. In conclusion, the addition of G-CSF made it feasible to give ACE at two-week intervals. The MRC Lung Cancer Working Party is now comparing this policy v the standard regimen of ACE at three-week intervals in a randomised trial (protocol LU19), to establish whether increasing the dose intensity in this way improves survival.

Confirmatory activity of gemcitabine in non-small cell lung cancer (NSCLC)

B COTTIER, T LE CHEVALIER, M GOTTFRIED, U GATZEMEIER, FA SHEPHERD, P WEYNANTS, HJM GROEN, R ROSSO, K MATTSON, H CORTES-FUNES, M TONATO, M VOI *Clatterbridge Centre for Oncology, UK; Institute Gustave Roussy, France; Krankenhause Grosshandsdorf, Germany; Toronto General Hospital, Canada; Clinique Universitaires, UCL de MontGodinne, Belgium; Academisch Ziekenhuis Groningen, Netherlands; Instituto Natioale per la Ricerca sul Cancro, Italy; Helsinki University Central Hospital, Finland; Hospital 12 de Octubre, Spain; Ospedale Regionale, Italy; Lilly Research Centre, Windlesham, UK* Gemcitabine is a novel pyrimidine analogue with activity against various solid tumours including NSCLC with reported response rates of >20%. Between January and December 1992, 161 chemonaive patients with inoperable NSCLC were recruited in nine countries to confirm the results obtained in earlier phase II trials. The dose of gemcitabine was 1250 mg/m^2 over 30 minutes weekly × 3 with one week rest. All patients are evaluable for toxicity and 153 who completed one cycle of therapy and had tumour assessment are evaluable for tumour response: 124 male and 37 female, median age 59 years (range 35–75). Performance status was 0 in 11%, 1 in 84%, and 2 in 5%. Histology was squamous in 44%, adenocarcinoma in 52%, and mixed adenosquamous in 4%. Five per cent of the patients were stage IIIa, 31% IIIb and 64% were IV. Doses were reduced in 4% of injections and omitted in another 6% due to toxicity but escalated doses were given in 26% of injections (≤20% increase at each course) with a maximum dose given so far of 2592 mg/m^2. Three (2%) complete responses and 28 partial (18·3%) have been independently validated by an external review board, giving an overall response rate of 20·3% (95% CI: 10–36%). Overall the drug was well tolerated with WHO grade III and IV neutropenia seen in 5% and 1% of injections respectively, and grade III/IV transaminase elevation occurred in 2%. WHO grade III/IV non-haematological toxicity with an incidence >1% was nausea and vomiting observed in 4% injections. The results of this study confirm the efficacy and mild toxicity of gemcitabine in the treatment of NSCLC, which together with original mode of action make it an exciting agent for use in combination therapy.

Preoperative staging of patients with non-small cell lung cancer

RFH TAYLOR PD WILKINSON, RF MILLER, RM RUDD *The London Chest Hospital, Bonner Road, London* Clinicians vary in their approach to the preoperative staging of patients with non-small cell lung cancer. We surveyed the differing practices and related them to the frequency of non-curative surgery and long term survival. Patients were traced through the National Health Service Register in March 1992 to determine their survival. Staging tests and results of surgery were recorded in 281 consecutive patients who had an operation for non-small cell lung cancer between January 1984 and August 1988. Full staging was carried out on 94 (33%) of the 281 patients; chest evaluation (CT chest and/or mediastinal exploration) with incomplete imaging assessment of extra-thoracic disease on 74 (26%); and evaluation of metastatic disease alone on 36 (13%). No staging procedure beyond clinical evaluation, chest radiography, and bronchoscopy was performed on 77 (27%) patients. Twelve (9%) of 137 patients referred to clinicians who routinely used full staging had non-curative operations; contrasting with 26 (18%) of 144 referred to clinicians who rarely used full staging (χ^2 5·19, p=0·02). Patients in the last group had correspondingly more advanced disease (21% with postoperative pathological stages IIIB-IV against 15% in patients

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routinely staged). The overall survival of patients partially staged was only marginally poorer than those fully evaluated (40·3% at three years against 47·0%; logrank χ^2 0·93, p = 0·34). Preoperative staging practices varied widely within this unit, and incomplete staging was associated with a higher frequency of non-curative surgery. Expert clinical judgement alone may not reliably exclude patients unsuitable for surgery.

Investigation of pleural effusion: the new Olympus LTF semi-flexible fibreoptic thoracoscope in comparison with Abram's needle biopsy

SR BICKNELL, LG MCALPINE, AJ PEACOCK *Department of Respiratory Medicine, Western Infirmary, Glasgow* Recently physicians have performed thoracoscopy under local anaesthetic using rigid thoracoscopes or flexible bronchoscopes. The second instrument allows a greater range of access within the pleural cavity but is more difficult to manipulate. The Olympus LTF semiflexible fibreoptic thoracoscope combines features of both instruments having a solid body and a flexible terminal section. In the first study with this instrument we have compared the diagnostic yield with closed needle biopsy. We investigated 20 patients aged 45–85 with pleural effusion. Patients were admitted to hospital and thoracoscopy was performed in a ward side room after premedication with atropine and papaveretum. After infiltration with local anesthetic a standard Abram's biopsy was performed. An Olympus flexible trocar was then introduced by blunt dissection and the LTF thoracoscope was inserted through the flexible cannula. The final diagnosis was malignant pleural effusion in 13/20 patients. Nine of 13 were detected by Abram's biopsy giving a sensitivity of 69%. Eleven of 13 were detected by fibreoptic thoracoscopy giving an improved sensitivity of 84%. In the two patients with malignant effusions not confirmed by thoracoscopic biopsy the appearances were recorded as clearly malignant but biopsies taken contained only reactive mesothelial tissue. The procedure was well tolerated by all patients and no complications were encountered. The LTF fibreoptic thoracoscope allows excellent visualisation and access to all pleural areas but we think that provision of a larger biopsy channel (currently 2 mm) would increase the accuracy of diagnosis.

Fluid volume in pleural aspiration cytology: how much is enough?

G CHRISTIE, ME MCKEAN, C DUNCAN, JG DOUGLAS, DJ GODDEN *Departments of Thoracic Medicine and Pathology, Aberdeen Royal Hospitals NHS Trust* Pleural aspiration cytology is of value in the investigation of pleural effusion. The optimum volume of aspirate required for diagnosis is unknown, and, in practice, small volumes of aspirate are often submitted (Walshe *et al. Thorax* 1992;**47**:734–7). The objective of this study was to investigate the diagnostic accuracy of cytology for malignant disease using varying volumes of aspirate. We studied 50 procedures in 43 patients (mean age 70·5 years). 100 ml of aspirate was separated into 10 ml, 30 ml, and 50 ml aliquots for conventional centrifugation. Cytocentrifugation was also performed on the 30 ml aliquot. Results for each aliquot were compared with the final clinical diagnosis, established at subsequent review of case records. 25 patients (27 procedures) had a final diagnosis of malignancy (14 extrathoracic orgin, eight bronchial carcinoma, and three mesothelioma). The 10 ml samples yielded 16 true positive, 22 true negative, eight false negative and four suspicious results. Sensitivity was 67% and specificity was 100%. In no case where the 10 ml sample was negative were either the 30 or 50 ml samples positive. Analysis of the larger fluid volumes in the four cases with suspicious 10 ml samples yielded two further true positives, one true negative, and one false negative. Cytocentrifugation did not improve sensitivity or specificity. We conclude that 10 ml is a sufficient volume for initial screening of pleural aspirate for malignancy. If the appearances are suspicious, examination of a larger volume may be helpful. Alternative routine use of cytocentrifugation, which requires increased preparation time, provides no additional diagnostic benefit. [Supported by Grampian Health Board Area Medical Audit Committee.]

Comparison of the effects of salmeterol and salbutamol on the endurance exercise capacity of men with mild asthma

W ROBERTSON, J SIMKINS, SP O'HICKEY, S FREEMAN, RM CAYTON *Department of Respiratory Medicine, Birmingham Heartlands Hospital, Birmingham* Although the effects of salmeterol on exercise induced asthma have been well described (Anderson, *Chest* 1991;**100**:1254) there are no reports of its effect on exercise performance. The pre-exercise administration of 50 µg salmeterol, 200 µg salbutamol, or placebo on the endurance cycle capacity at a work load of 70% Vo_2 max were compared in a double blind, randomised cross over design trial of eight asthmatic men, aged 33 (11) years, FEV_1 81 (31)% pred (mean (SD)). After a familiarisation test, subjects performed the three tests at weekly intervals. β agonists were withheld for six hours, and the treatments were given 30 minutes before exercise. The results

Variable	*Salmeterol*	*Salbutamol*	*Placebo*
FEV_1 (1) at rest	3·23 (1·19)	3·26 (1·12)	3·15 (1·11)
Post treatment	3·60 (1·19)*	3·59 (1·15)*	3·19 (1·11)
Post exercise	3·49 (1·21)*	3·48 (1·21)*	2·97 (1·14)
Endurance time (min)	48·1 (23·2)	53·6 (19·8)	48·8 (25·7)
Pulse at rest (bpm)	70 (9)	78 (12)	72 (12)
at 15 min exercise	130(20)*	136 (25)	136 (22)
at end of exercise	144 (29)*	153 (27)	151 (26)
Lactate at rest (mM)	0·67 (0·38)	0·98 (0·37)*	0·66 (0·38)
at 15 min exercise	3·03 (1·64)	3·64 (1·84)*	2·95 (1·69)
at end of exercise	2·46 (1·83)	2·54 (2·32)	2·32(1·52)

All results given as mean (SD); * $p < 0·05$.

show that salmeterol, despite increasing airway calibre, does not significantly affect endurance capacity. Salbutamol but not salmeterol increased blood lactate during exercise. No adverse significant cardiorespiratory or metabolic effects during exercise were seen with salmeterol.

Within subject reproducibility of the bronchoconstrictive response to exercise when heat loss is kept constant

BS SHACKELL, EA COCKER, CY MAN, GP VENTRESCA, SA OSBORNE, MR CROSS *Phase 1 (Clinical Trials Unit), Fort Bovisand, Plymouth; Clinical Pharmacology Division, Glaxo Group Research Ltd, Greenford* Exercise-induced asthma (EIA) occurs in 70% to 80% of asthmatic patients. Bronchoconstriction reaches a peak in the first five to 10 minutes after exercise (Freedman. *Clin Sci* 1992;**83**:383). The major stimulus for EIA may be heat loss from the respiratory mucosa, resulting from the hyperpnoea of exercise (Chandler *et al. J Appl Physiol* 1977;**46**:476). The coefficient of variation (CV) for EIA is 20 to 60% for tests repeated at one week and one month respectively (Strauss *et al. J Clin Invest* 1978;**61**:433). The present study was designed to assess the within subject reproducibility of the bronchoconstrictive response to exercise when heat loss is kept constant. Eleven male asthmatic patients (mean age 22, range 18–28) with a pre-exercise $FEV_1 \geqslant 75\%$ predicted and a fall in $FEV_1 \geqslant 14\%$ after exercise, took part in the study. Three exercise tests were performed, mostly one week apart. In exercise testing, treadmill running at a 10° slope was used while breathing air at 2 to 8°C. The sequence was: (a) two minutes to raise heart rate (HR) by 30 bpm; (b) two minutes with HR at 130–160 bpm; (c) about six minutes at 85% of predicted maximum HR (defined as 210–0·65 (age). FEV_1, FEF_{25}, FEF_{75}, and PEF were measured pre-exercise and at 2, 4, 6, 8, 10, 12, 14, and 16 minutes post-exercise. Minute volume, expired air humidity, and inspired and expired air temperatures were measured every 15 seconds. Accumulated heat loss was kept constant in visits 2 and 3 compared with visit 1 by adjusting the duration of the third part of the exercise test. Heat loss in visit 1 ranged from 4565 cal to 8523 cal with a CV adjusted for visits 1, 2, and 3 of 3·8%. The mean (SD) maximum post-exercise falls in FEV_1 for visits, 1, 2, and 3 were 27·8% (13·2), 32·7% (12·2) and 34·0% (14·0) respectively, with an adjusted CV of 17·9%. For PEF, the mean maximum falls in visits 1, 2, and 3 were 36·5% (19·1), 47·1% (16·0) and 44·7% (19·8) respectively, with an adjusted CV of 24·2%. By keeping heat loss constant during exercise, the CV of EIA as expressed by the maximum fall in FEV_1 and PEF were at the lower end of published values. This method of inducing bronchoconstriction provides a reproducible tool for assessing the efficacy of asthma therapy.

Slowing of sniff oesophageal maximal relaxation rate (MRR) can be detected non-invasively

D KYROUSSIS, GH MILLS, C HAMNEGARD, S WRAGG, J ROAD, M GREEN, J MOXHAM *Royal Brompton Hospital, London* We have previously shown that sniff oesophageal maximal relaxation rate slows after maximal isocapnic ventilation (MIV) (*J Appl Physiol* 1991;**70**:2173). Inspiratory pressure in the nares (Pnasal) during a sniff through the contralateral nostril provides a non-invasive estimation of sniff oesophageal pressure (Poes) both in normals (Heretier *et al. Eur Respir J*

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1991;4:392S) and patients. We have developed a nasal cast to minimise pressure loss in the instrumented nostril and thus enhance the agreement of sniff Pnasal with sniff Poes. The aim of this study was to investigate whether the slowing of sniff Poes MRR after a two minute MIV could be detected from sniff Pnasal. We studied three well trained normal subjects. Each subject performed 10 sniffs before a two minute MIV and again at intervals to 10 minutes post MIV. Poes and Pnasal were recorded during sniffs and the MRR for each sniff was determined both for the Poes and the Pnasal. Before MIV the mean sniff Poes MRR was 9·3% per 10 ms and for Pnasal MRR was 9·9% per 10 ms. The subjects showed a mean decrease in sniff Poes MRR of 32% (range 28–36%) immediately after MIV, which returned to control values within five minutes ($p < 0.02$). A similar reduction in sniff nasal MRR was observed with a mean fall of 33% (range 29–39%) ($p < 0.03$). We conclude that the slowing of sniff Poes MRR following MIV is reflected in sniff Pnasal MRR in normal subjects. Pnasal MRR may be a useful method for non-invasive measurement of inspiratory muscle relaxation rate.

High resolution computed tomography (HRCT) scanning to evaluate structural changes in lungs of patients with severe chronic asthma

DH CARR, S HIBON, KF CHUNG *Departments of Radiology and Thoracic Medicine, Royal Brompton Hospital, London* Longstanding asthma with chronic symptoms may be associated with structural remodelling of the airways through repair and fibrosis. To investigate the degree of these potential changes, we performed HRCT with an Imatron Ultrafast CT scanner using 3 mm thick sections at 10 mm scan intervals in 12 asthmatic patients (10 females; mean age 46; range 15–66 years; never smokers). All scans were performed on full inspiration and eight of the patients also had scans in expiration. The mean duration of asthma was 30 (range 2–58 years) with FEV_1 (mean 46% (range 26–74%) of predicted). According to the score of Aas, all patients had severe to very severe asthma. All patients were on high dose inhaled corticosteroid therapy with 11 on frequent doses or chronic prednisolone therapy. None of the patients had a history of chronic purulent sputum production. Bronchial wall thickening and dilatation was noted in segmental and subsegmental bronchi on HRCT in eight of 12 patients with mucus plugging in two. Emphysema was noted in two patients. Of the eight expiratory scans, five showed evidence of patchy airway trapping. HRCT is a sensitive method for detecting bronchial wall thickening and dilatation in patients with chronic severe asthma, confirming a previous report (*Am Rev Respir Dis* 1992;**146**:1084–7). The mechanism of bronchial wall thickening and dilatation in asthmatic patients may be related to the repair process of chronic airway inflammation in asthma. HRCT may be used to follow up overall structural changes in the lungs of patients with chronic asthma.

Effect of lung volume on the transdiaphragmatic twitch pressure

CH HAMNEGARD, S WRAGG, D KYROUSSIS, GH MILLS, J MOXHAM, M GREEN *Royal Brompton Hospital, London* We have previously shown that a single inspiratory manoeuvre potentiates the diaphragm twitch (*Am Rev Respir Dis* 1993;**147**:A699). Smith and Bellemare (*J Appl Physiol* 1987;**62**:1893) showed that the transdiaphragmatic twitch pressure varied with lung volume but they did not control for twitch potentiation. We evaluated the relation between the unpotentiated $P_{di}tw$ and lung volume in four male volunteers. Twitch stimulation of the phrenic nerves was applied to the relaxed seated subjects by cervical magnetic stimulation. Static elastic properties of the lungs and chest wall between TLC and RV were determined from oesophageal and mouth pressure. To avoid potentiation of the twitch by preceding muscle activity, the subjects breathed quietly, inspiration was slow, and airway pressure was applied to inflate the lungs to TLC. Five measurements were made at each lung volume. At FRC mean $P_{di}tw$ was 31·8 for the group (range, 26·5 to 38·1) cm H_2O; at 15% (mean 0·9 l) above FRC the $P_{di}tw$ decreased by a mean of 6·3 (range, 3·7 to 5·9) cm H_2O and at TLC by 21·2 (range, 17·9 to 29·9) cm H_2O. This was mainly due to a decrease in oesophageal pressure. At 15% below FRC (mean 0·9 l) the $P_{di}tw$ increased by a mean of 4.1 (range, 8·6 to 2·4) cm H_2O; at a volume close to RV by 9·2 (range, 5·5 to 8·7) cm H_2O. The values for $P_{di}tw$ were less than previous reports as the diaphragm was not potentiated but nevertheless there was a close linear relation between the diaphragm contractility as judged by $P_{di}tw$ and lung volume. As the diaphragm shortens with increasing lung volumes it generates less pressure for a given neural output.

Diaphragmatic excursion correlated with transdiaphragmatic pressure during slow inspirations and sniffs

E COHEN, A MIER, K MURPHY, A GUZ *Department of Medicine, Charing Cross and Westminster Medical School, London* Diaphragmatic function is generally assessed by measuring transdiaphragmatic pressure (Pdi). More recently, using ultrasound, we have shown a linear relation between diaphragmatic excursion (DE) and different inspired volumes (V_T) (BTS summer meeting 1993). This study was performed to assess the relation between DE and Pdi in four normal subjects (three males), during slow inspirations and sniffs of increasing size. DE was recorded by M-mode ultrasonography of the right posterior hemidiaphragm in the supine posture. The transducer was firmly fixed by a specially built frame connected to the bed. Pdi was recorded with balloon catheters. Subjects were instructed to (a) take slow inspirations of increasing size between FRC and TLC, and then (b) to perform sniffs of small, medium and maximal intensity. Results [mean (SD)] are summarised in the table. We conclude that (1) a linear relation exists between DE and Pdi during the manoeuvres described. (2) Pdi during a sniff is high whereas the DE is small. A radiologist using the sniff test to screen diaphragmatic movement may come to erroneous conclusions about diaphragmatic function.

	DE v Pdi				
Manoeuvre	*Slope (cm/cm H_2O)*	*y intercept (cm)*	*r (range)*	*Max DE (cm)*	*Max Pdi (cm H_2O)*
Graded V_T	0·146 (0·024)	0·48 (1·38)	0·931–0·990	6·4 (0·8)	40 (5)
Graded sniffs	0·013 (0·009)	0·85 (0·36)	0·861–0·942	2·1 (0·5)	84 (11)

Ventilatory chemosensitivity in patients with intrapulmonary shunts

BL BAKER, JMB HUGHES, S PURI, JGF CLELAND *Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London* Do patients with severe arterial hypoxaemia from an early age have a blunted ventilatory response to hypoxia? We studied the ventilatory response to isocapnic hypoxaemia and iso-oxic hypercapnia using an open circuit technique in nine patients with HHT and pulmonary arteriovenous malformations (PAVMs) and in 10 age matched controls. Pre-test arterial oxygen saturation (Sao_2) on pulse oximetry in the PAVM group (erect posture) was 78·1 (SE 7)%. For the hypoxic ventilatory response (HVR) subjects breathed 50% O_2 (with 0·2 l/min CO_2 added) with a mouth piece and nose clip in the semirecumbent position for five minutes before switching to 10·5% O_2, additional CO_2 being added to maintain a constant end-tidal P_{CO_2}. For the hypercapnic response (HCVR) subjects breathed air for three minutes before addition of CO_2 in two minute steps at rates of 0·2, 0·4, and 0·6 l/min. Ventilation, P_{ETCO_2} and Sao_2 were measured continuously. All PAVM patients had HVR and HCVR measured twice and the results were averaged. The slope of VE against Sao_2% or P_{ETCO_2} was calculated by linear regression from breath by breath data. Mean regression values (r) for patients with PAVM were 0·55 (0·05) for HVR and 0·66 (0·16) for HCVR. The mean HVR for PAVMs was −0·83 (0·19) l/min/Sao_2% at a mean P_{ETCO_2} of 28·9 (1·2) mm Hg, not significantly different from controls (−0·76 (0·10) at P_{ETCO_2} 41·7 (0·89)). HCVR at Sao_2 of 91·6% (1·1) for PAVMs was 2·2 (0·6) l/min/mm Hg, which was significantly ($p > 0.0001$) higher than HCVR for controls (1·3 (0·1) at Sao_2 98·4% (0·5)). Patients with PAVM have a normal HVR despite a lower P_{CO_2} but a higher HCVR due to the accompanying hypoxaemia.

D(V − aP_{CO_2}) reflects both peripheral metabolism and changes in pulmonary ventilation in the pig hind limb under cardiopulmonary bypass

LCM PALLARÉS, N MOAT, P MACNAUGHTON, M MESSENT, J SHEPHARD, TW EVANS *Department of Anaesthesia and Intensive Care, National Heart and Lung Institute, London* The difference between venous and arterial P_{CO_2}, D(V − aP_{CO_2}), has been used in cardiorespiratory failure

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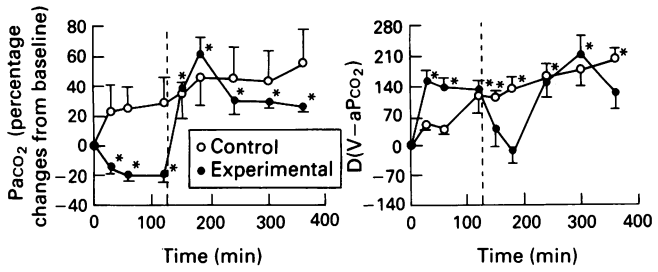


as an index of the adequacy of peripheral oxygenation. However, it has been shown that in addition to tissue oxygen availability, the $D(V-aPco_2)$ may also be influenced by lung perfusion and ventilation. To evaluate the effects produced in the $D(V-aPco_2)$ by changes in $Paco_2$ induced by different ventilatory patterns during 120 minutes of cardiopulmonary bypass (CPB), we studied the hind limb of six experimental and three control pigs. The $D(V-aPco_2)$ was assessed at two timepoints before (baseline), during (30, 60, and 120 minutes) and after (150, 180, 240, 300, and 360 minutes) CPB. Control pigs were studied at the same timepoints but received sham operations without CPB. Changes in $Paco_2$ induced by CPB and mechanical ventilation were recorded during the experiment. Control pigs had their ventilation kept stable so that all changes observed could be attributable to peripheral metabolic changes induced by anaesthesia. The swings in the $D(V-aPco_2)$ observed in the experimental group mirrored changes in $Paco_2$. In the control group a stable increase in the difference was observed, while no significant changes were observed for $Paco_2$. This may reflect an influence of anaesthesia on peripheral oxygen utilisation. We conclude that the $D(V-aPco_2)$ reflects changes in ventilation as well as peripheral CO_2 production. [LCMP is funded by the Brazilian Research Council (CNPq) and The Ministry of Health.]

Measurement of right to left shunt in patients with the hepatopulmonary syndrome

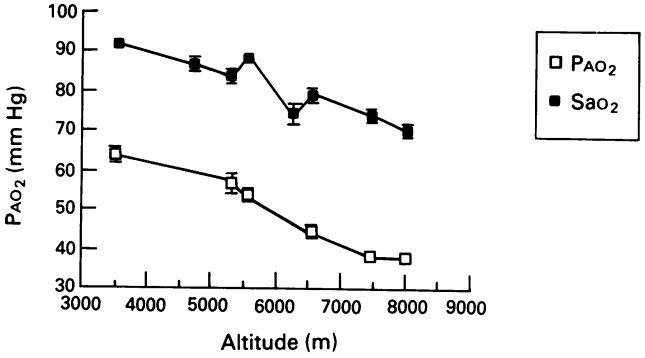
MKB WHYTE, JMB HUGHES, GJ BELLINGAN, AM PETERS, J UEKI, W USSOV *Departments of Medicine (Respiratory Division) and Diagnostic Radiology, RPMS, Hammersmith Hospital, London* The hepatopulmonary syndrome (profound arterial hypoxaemia in patients with liver cirrhosis) has been ascribed to pulmonary microvascular dilatation, resulting in an effective right to left shunt. The physiological right to left shunt has been measured by 100% O_2 breathing but a comparison with a true anatomic shunt using radiolabelled MAA has only been attempted in a single patient (Genovesi *et al. Am Rev Respir Dis* 114:59). We have studied five patients age 35–54 (mean 43) years, four with cirrhosis and one with postsinusoidal portal hypertension. None had evidence of macroscopical arteriovenous malformations on pulmonary angiography or CT scan. Diffusing capacity, arterial oxygen saturation, and arterial blood gases were obtained. Right to left shunt was measured simultaneously by two methods: injection of ^{99m}Tc-MAA (particle size 10–55 μm) and the 100% oxygen breathing technique. All patients had impaired diffusing capacity (Kco (mean (SE) 56 (4)% predicted, Tlco 47 (5)%). Oxygen saturation fell significantly in the erect posture, from 92 (2)% supine to 82 (4)% when erect ($p<0.02$, $n=4$). Mean Pao_2 was 7·2 (0·5) kPa. Right to left shunt measured by the MAA technique was 34 (5)% of cardiac output. Shunt measured by 100% oxygen was smaller in all cases, mean 17 (3)%. The mean difference was 16·6 (4·8)% ($p<0.03$, $n=4$). These results confirm that significant anatomic right to left shunting occurs in patients with the hepatopulmonary syndrome. The smaller physiological shunt suggests that abnormal vascular channels are small enough for oxygen diffusion to occur at high Pao_2.

Correlation of echocardiography and right heart catheter in systemic sclerosis

GD PHILLIPS, JB CAILES, AU WELLS, CM BLACK, TW EVANS, RM DU BOIS *Royal Brompton National Heart and Lung Hospital and Department of Rheumatology Royal Free Hospital, London* Pulmonary hypertension (PHT), either alone or in association with pulmonary fibrosis, is the most common cause of death in systemic sclerosis (SSc). Treatment of PHT is often unsuccessful. Early diagnosis offers hope of implementing treatment when the disease may be reversed. The gold standard for diagnosis of PHT is the right heart catheter (RHC); however, this is an expensive and invasive procedure. This study aimed to compare the diagnosis and quantification of PHT by echocardiography with RHC in patients with SSc in whom either clinical assessment, echocardiogram, or other non-invasive tests (ECG, chest *x* ray, and gas transfer) suggested PHT. Twenty four patients were enrolled between 1987 and 1993. All received an echocardiogram and RHC within a six month period of each other (mean 1·2 months). The echocardiogram assessed cardiac anatomy, and where possible (16 of 24 patients) Doppler estimates of the pressure gradient between right atrium and ventricle, allowing calculation of pulmonary artery (PA) systolic pressure. In addition, to include patients without tricuspid regurgitation (Doppler estimation not possible) all patients had PHT graded as either absent, mild, moderate, or severe on the basis of either Doppler measurements or right ventricular characteristics. On RHC pulmonary hypertension was defined as PA systolic pressure greater than 30 mm Hg. Grading of PHT on echocardiogram correlated highly with RHC, $r=0.84$ (Spearman's rho), $p<0.001$. Doppler estimates of PA systolic pressures were also highly correlated with the values obtained at RHC, $r=0.90$ (Pearson's correlation coefficient). The positive predictive value of the echocardiogram for the diagnosis of PHT in this setting was 0·88, rising to 0·92 where a Doppler estimate of PA pressure was available. We conclude that echocardiography correlates highly with RHC in SSc. We suggest that typical echocardiographic findings may reduce the need for RHC in many patients.

Respiratory function at extreme altitude: results from the British 40th anniversary Everest expedition

ANDREW PEACOCK, PETER JONES *Department of Respiratory Medicine, Western Infirmary, Glasgow; Department of Anaesthetics, University Hospital of Wales* The success of the 1953 Everest expedition was due in large part to the physiological studies of Griffith Pugh who calculated the likely alveolar Po_2 that would be encountered by the climbers and therefore the appropriate levels of oxygen that should be used. Since then there have been many physiological studies but none in which there has been a comparison of alveolar Po_2 and arterial oxygen saturation at altitudes as high as 8000 m. As a consequence, climbers on Everest tend to follow Pugh's guidelines for oxygen usage. As part of the studies performed on the British 40th Everest expedition we wished to determine spirometry and the relation between Pao_2 and Sao_2 in nine elite climbers (aged 32–53; eight F, one M) attempting the summit. We measured Sao_2 using a portable pulse oximeter (pneuPAC Ltd Luton, UK), Pao_2 using an oxygen fuel cell (Teledyne Keighley, UK) attached to a digital voltmeter and FEV_1/FVC using a portable spirometer (Escort, Vitalograph, UK). Spirometry was performed at altitudes up to Camp 2 at 6550 m. Measurements of Sao_2 and Pao_2 were made at rest breathing air up to and including the South Col (8000 m). None of the climbers developed high altitude pulmonary oedema and none demonstrated any change in FEV_1 or FVC. The changes in Pao_2 and Sao_2 with altitude are shown (figure). The graph shows a parallel decline in Pao_2 and Sao_2



down to mean levels of 38 (SD 1·3) mm Hg and 70 (2·5)% respectively. It is astonishing that people are able to climb carrying heavy packs at these levels of oxygenation and even more astonishing that a very few (including one of our climbers) can climb a further 848 m to the summit without oxygen. We hope that these results will help future expeditions to plan their oxygen requirements.

Analysis of deaths due to pneumonia in Scottish subjects aged 15–64 years

JH WINTER, MR KERR, D HENDERSON, S KENDRICK, J CLARK *King's Cross Hospital, Dundeee: Information and Statistics Division, Common Ser-*

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vices Agency, Edinburgh We identified deaths from pneumonia in the Scottish population during 1991 from death certification records. The Scottish record linkage system then enabled us to identify all recorded hospital admissions for these patients during the period 1981–91. The information was entered onto a database and analysed. 244 deaths were recorded as being due to pneumonia in the age group 15–64 years in 1991. Most deaths occurred in the older age groups but 10·7% occurred at less than 40 years and 24% at less than 50 years. The number of hospital admissions recorded in the previous 10 years varied from none to 58. Only 11% had no record of previous hospital admission. 50% of subjects had between one and six admissions to hospital over the previous 10 years. The major discharge diagnoses of previous hospital admission were cardiovascular, respiratory and neurological diseases, trauma, and alcohol related disorders. These data indicate that most young adults recorded as dying from pneumonia have had previous hospital admissions and significant underlying disease. As most deaths from pneumonia are due to pneumococcal disease the administration of pneumococcal vaccine to patients who are admitted to hospital may prevent some deaths from pneumonia in young people.

Lung function in bronchiectasis: the influence of chronic colonisation with *Pseudomonas aeruginosa*

S EVANS, S TURNER, B BOSCH, C HARDY, M WOODHEAD *Manchester Royal Infirmary, Oxford Road, Manchester* Sputum isolation of *Pseudomonas aeruginosa* (PA) in bronchiectasis is associated with extensive disease (*Am Rev Respir Dis* 1993;**147**:A645). Colonisation with PA may be the result or the cause of severe disease. We have compared spirometry before and after PA colonisation with that of patients colonised by other organisms. All patients (n = 10) with chronic PA colonisation, defined as >3 isolations of PA over >3 months, were studied (PA group). All were women, mean age 60 (range 52–82) years. These were compared with all other female patients with bronchiectasis aged >50, with no isolations of PA (n = 20, mean age 61 (51–79), (non-PA group)). Follow up for the PA group was 10·2 (SD 5·5) years, including 4·9 (4·4) years since PA colonisation, and 8·1 (5·6) years for the non-PA group. Two of the non-PA and none of the PA group were smokers during the follow up period. The PA group FEV_1 (% predicted) and FVC (% predicted) were lower than the non-PA group (FEV_1, 33 (15) *v* 50 (13), p = 0·005 and 50 (14) *v* 74 (19), p = 0·001). The PA group also had lower values at the time of initial colonisation with PA than the present values for the non-PA group (FEV_1, 37 (13) *v* 50 (13), p = 0·028 and FVC 52 (17) *v* 74 (19), p = 0·004). Change of FEV_1 and FVC was faster in the PA group (−3·0 (4·7)%/y *v* −0·1 (1·6)%/y, p = 0·002 and −4·0 (7·4)%/y *v* +0·13 (2·04)%/y respectively). Reduction of FEV_1 and FVC in the PA group before PA colonisation was intermediate, not being statistically different from either value above (FEV_1, −0·8 (2·7)%/y and FVC, −1·94 (4·0)%/y). We conclude that patients with bronchiectasis who become colonised by PA have poorer lung function at time of colonisation than those colonised by other organisms. Decline in lung function is faster in those colonised by PA than those colonised by other organisms. It is unclear whether PA colonisation causes an accelerated decline in lung function or if it is only a marker of those whose lung function is already declining rapidly.

Survival in HIV positive intravenous drug users after a first episode of *Pneumocystis carinii* pneumonia

GE PACKE, CL LEEN, RP BRETTLE *Regional Infectious Diseases Unit, City Hospital, Edinburgh* There is a lack of United Kingdom data on survival after an episode of *Pneumocystis carinii* pneumonia (PCP) in intravenous drug users (IVDUs) who are seropositive for human immunodeficiency virus (HIV). The case notes of those HIV positive IVDUs who developed PCP during the period 1989–92 were examined. All were treated in the infection unit at the City Hospital, Edinburgh. Data were collected on 34 patients in whom the development of PCP defined the onset of acquired immune deficiency syndrome (AIDS). Survival distributions were estimated by the Kaplan-Meier method. The equality of survival curves was assessed by calculating the Mantel-Cox statistic. The median (range) time patients were known to be infected with HIV before the episode of PCP was 5·33 (0 to 9·25) years. The median age at the time of developing PCP was 31·25 (24–40) years. One patient died acutely of respiratory failure; the other 33 patients recovered from the first episode of PCP. Twenty seven patients were started on PCP prophylaxis. Zidovudine was commenced in 13 patients, and in six it was continued. Two patients were given dideoxyinosine. Over the period of observation 23/24 patients died. Median survival time was 22 months (95% CI 12·5 to 30 months). Median survival time in the female patients (30 months) was longer than that seen in the male patients (22 months), but not significantly so (p = 0·85). These results indicate better survival in IVDUs with AIDS in the United Kingdom compared with similar patients in North America (*N Engl J Med* 1991;**324**:1412–6). This may be due to differing demographic factors or different models of care for HIV positive patients.

Open lung biopsy in HIV positive patients with acute respiratory episodes and negative bronchoscopic investigations

RF MILLER, WB PUGSLEY, MH GRIFFITHS *UCLMS, Middlesex Hospital, London* Open lung biopsy (OLB) in HIV positive patients with acute respiratory episodes is rarely necessary because of the high yield from fibreoptic bronchoscopy (FOB) and bronchoalveolar lavage (BAL). In this study we report our experience of OLB in 19 HIV positive patients (all had ⩾1 negative FOB and BAL; four also had negative transbronchial biopsy). Indications for OLB were, group A, 14 patients thought clinically to have pneumocystis pneumonia (PCP) but not responding to treatment; group B, four patients with focal chest *x* ray film changes; group C, one patient thought to have pulmonary Kaposi's sarcoma (PKS). No patient was mechanically ventilated (MV); Pao_2 (on air) ranged from 4·4 to 13·9 (mean = 9·7) kPa. OLB was performed via a lateral thoracotomy and tissue was stained histochemically and cultured for bacteria, mycobacteria, and fungi. In group A five patients had non-specific interstitial pneumonitis (NIP) (one also had PKS), three had PCP, two had PKS, one had bronchiolitis obliterans organising pneumonia (BOOP) and emphysema, one had BOOP and PCP, one had widespread necrosis and no infection, and one had normal lung tissue; in group B one had B cell lymphoma, one occult alveolar haemorrhage, one had NIP, and one had *Pseudomonas aeruginosa* and BOOP; in group C the patient had NIP. All patients survived OLB; none required MV. Two patients had postoperative bacterial chest infection (one also had persistent pneumothorax). OLB results significantly affected management; in group A inappropriate treatment was discontinued in 10 patients, all four in group B began specific therapy, and the patient in group C avoided chemotherapy. OLB in HIV positive patients has a high diagnostic yield and low morbidity; it should be considered in those with negative bronchoscopic results.

Mycobacterial infection in Newcastle, 1990–1993: an analysis from the Newcastle TB register

M MOHAN, J BERKLEY, P TATUM, K FISHWICK, A CANT, SJ BOURKE, DJ HENDRICK *Chest Unit and Regional Unit for Occupational Lung Disease, Newcastle General Hospital; University of Newcastle upon Tyne* The TB register was set up in 1989 to record from 1 January 1990 all new cases of mycobacterial infection and to coordinate testing of close contacts. A computer program allows an updated analysis of the cumulative database at any time. Up to 31 August 1993 the total of cases reported was 317 and there were 1282 close contacts. The reported cases were classified as shown in the table. Annual statistics

	Culture +ve MTB (92)	*Culture +ve atypical* (43)	*Smear +ve* (17)	*Histology +ve* (12)	*Clinical only* (30)	*Skin +ve* (123)	*Total* (317)
0–5 years	2	4	3	0	3	23	35
6–15 years	0	4	0	1	2	89	96
16+ years	88	31	14	11	23	8	175
Age unknown	2	4	0	0	2	3	11
Female	32	13	10	6	13	58	132
Male	60	30	7	6	17	65	185
Caucasian	69	41	15	6	12	61	204
Non-Caucasian	21	2	2	6	16	62	109
Pulmonary	58	12	10	2	22	0	104
Non-pulmonary	34	31	7	10	8	123	213
Close contacts	1	0	0	0	5	38	44

from 1990 to 1992 show a rise of 53% (77% to 118%) in the total number of cases. However, if atypical organisms or "tuberculin skin reactivity only" are excluded the annual incidence of clinically active disease actually fell by 36% from 58% to 37%. This suggests that the increase in incidence of tuberculosis reported in a number of Western cities over recent years has not yet affected Newcastle. The contact tracing procedure identified 44 new "cases"; of these only one was culture positive, the others having only clinical evidence or tuberculin skin reactivity. However, as many as 43·6% defaulted.

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Increase in tuberculosis notifications: younger women show the greatest increase

M DOHERTY, DPS SPENCE, PDO DAVIES *Tuberculosis Research Centre and Aintree Chest Centre, Liverpool* Notifications of tuberculosis have been increasing in England and Wales since 1987. Earlier data presented to this society (*Thorax* 1991;**46**:778P) suggested that this increase was mainly due to an increase among elderly patients. Notifications of tuberculosis have continued to rise (total cases: 1987, 5085; 1988, 5161; 1989, 5432; 1990, 5204; 1991, 5436; 1992, 5861; 1993, 6166 (estimate from first six months)) but little is known of the demographic distribution of the continued increase. We have examined the notifications of tuberculosis by age and sex between 1987 and 1991 (the last year in which an age breakdown is available). Summary data are presented in the table. These data show that the

	Age					
	0–14	*15–24*	*25–44*	*45–64*	*65–74*	*75+*
Females:						
1987	222	331	670	487	226	206
1991	227	331	822	554	264	193
% change	+4·6	0	+22·7	+13·7	+16·8	−6·3
Males:						
1987	217	279	822	797	416	326
1991	227	291	919	722	402	307
% change	+4·6	+4·3	+11·8	−9·4	−3·3	−5·8

increase in notifications in tuberculosis has a widespread age distribution. The early increase in disease in the elderly has not been maintained. Reasons for the striking increase in younger women are unclear but HIV is not implicated. Immigration and worsening social circumstances may be contributory (*Thorax* 1992;**47**:849)

Tuberculosis in refugee populations in East London

NAUREEN BHATTI, MALCOLM LAW, JOHN MOORE-GILLON *Directorate of Public Health, East London and City Health Authority; Department of Respiratory Medicine, St Bartholomew's Hospital, London* Hackney is a socioeconomically deprived inner London borough (population 200 000). From 1984 to 1988 TB notifications averaged 40/year. They have since steadily risen, reaching 100 in 1992. Over the same time, some 23 700 refugees (principally Kurds 14 000, Somalis and Ethiopians 6500) have arrived in the borough and now constitute 12·5% of the population. A review of all 232 TB notifications 1990–1992 was performed with the help of local health advocates, who identified individuals with Kurdish, African, Indian sub-continent, and SE Asian names. Before 1988 there were few refugees in Hackney. TB in refugees is thus likely to account for over half the increase in notifications between 1984–88 and 1990–92. Refugees are contributing significantly to increasing TB in our area, and current healthcare provision is not meeting the needs of this group.

Ethnic group	*% of borough population*	*No (%) of notifications*	*Rate relative to England and Wales (95% CI)*
White	60	59 (25)	1·5 (1·1–1·9)
Black Caribbean	14	20 (8)	2·6 (1·4–3·7)
Indian	6	71 (31)	25 (19–31)
Black African	6	29 (13)	8·7 (5·5–12)
Turkish kurds	6	28 (12)	6·1 (3·9–8·4)
Ethiopian/Somalian	3	10 (4)	4·9 (1·9–7·9)
Vietnamese	1	11 (5)	18 (7·5–29)
Other	4	4 (2)	2·0 (0·0–4·1)
Total		232 (100)	3·7 (3·2–4·2)

Increase of tuberculosis in overseas students of higher education in Edinburgh, Scotland in 1991: a taste of things to come?

J FACCENDA, AG LEITCH *Respiratory Medicine Unit, Department of Medicine (RIE), University of Edinburgh; Royal Victoria Chest Clinic, Chalmers Hospital, Edinburgh* The incidence of tuberculosis (TB) in the 15–40 year age group in Edinburgh has been monitored closely since the relatively high prevalence of HIV infection (predominantly in intravenous drug abusers) in the population has been appreciated. In 1991 a relative excess of cases of TB in this age group was notified which was entirely accounted for by disease in overseas students of higher education. Five of 17 notifications in the 15–40 year age group were of overseas students: three aged 21–29 years from central Africa, one 27 year old Asian, and one 26 year old western European. One of the African students was also seropositive for HIV infection. Disease in the five students presented clinically on average 26 (range 10 to 48) months after entry to the United Kingdom. Three of the five students had smear positive disease and were highly infectious. Two patients had had normal chest radiographs one and two years previously on entry to the United Kingdom. Three patients had not been screened for TB at any time since entering the country. The British Thoracic Society has described the deficiencies of the present system for radiologically screening overseas immigrants for the presence of tuberculous disease on entry to the United Kingdom (*BMJ* 1990;**300**:995–8). The increasing prevalence of HIV infection in countries with high annual TB infection rates has led to an increase in risk of reactivation of tuberculous disease in their populations. Universities and other institutions of higher learning should devise and implement a programme of screening for tuberculous disease in students from these countries. A low threshold of suspicion of tuberculous disease in such students should be maintained even for those who are negative on initial screening.

Evaluation of TB screening for overseas students

S SEBUGWAWO, MEM KIRKUP, AVJ BUTLER, GH STEWART, JR CATTERALL *Control of Infection Department, Bristol and District Health Authority; Student Health Service, Bristol University; Respiratory Department, Bristol Royal Infirmary, Bristol* The aims of this study were (1) to evaluate Bristol University's policy of screening overseas students for tuberculosis (TB); (2) to determine how many overseas students had either been screened before leaving their countries of origin or been examined by United Kingdom immigration port medical inspectors before entry; (3) to investigate current policies and procedures employed by other district health authorities and universities to screen newly arrived overseas students. 842 new overseas students were admitted for the 1992/93 academic year. 383 (46%) came from countries for which the university policy required TB screening. Only 170 (44%) of the 383 eligible students complied with the requirement to be screened. Six (4%) were offered chemoprophylaxis, one (1%) was offered BCG vaccination, and 11 needed follow up examinations. 273 (71%) of the students responded to a questionnaire regarding screening at the port of entry and in their country of origin. 226 (83%) had entered the United Kingdom via Heathrow Airport. 23 (8%) had had a medical interview/examination at a United Kingdom port of entry but only 10 (4%) had had chest radiography (CXR). Three port medical forms had not been received by the consultant in communicable disease control. 23 major universities and their district health authorities were surveyed. Of the 20 districts that replied, 14 had a policy of screening overseas students that relied on the immigration port health notification system. Two had a separate policy, four had no policy. In the 16 districts with a policy three used CXR and a tuberculin skin test for screening. Four screened by CXR. Nine used CXR if indicated by the results of the skin test. The screening of overseas students for TB is variable and incomplete. Consideration should be given to the development of national guidelines, particularly as the number of overseas students is increasing.

Analysis of the frequency of drug reactions to antituberculosis drugs

LP ORMEROD *Chest Clinic, Blackburn Royal Infirmary, Blackburn, Lancashire* The records of 1277 patients treated for all forms of tuberculosis in Blackburn between 1978–92, which included drug regimens and durations, were analysed. 69 drug reactions occurred in 67 patients (5·2%) which required a change of treatment. The total duration of treatment in months was known for each of the main antituberculosis drugs, and the rates of reaction were compared. There were 35 reactions to pyrazinamide (rash 26: hepatic nine) in 1448 patient-months (m) (1/41·4 m), six to streptomycin (all vertigo) in 353 months (1/58 m), 21 to rifampicin (rash three: hepatic 17: thrombocytopenia one) in 104·28 months (1/496 m), six to isoniazid (four hepatic: two neuropathy) in 12 128 months (1/202 m) and two to ethambutol (rash one; ?ocular one). The ocular reaction was not confirmed on full ophthalmological assessment giving one reaction in 5009 patient-months. Reactions occurred significantly more in females (χ^2 7·23; $0{\cdot}01 > p > 0{\cdot}001$), this being due to higher reaction rates in white (χ^2 5·89; $0{\cdot}05 > p > 0{\cdot}01$) and Pakistani ($\chi^2$ 6·20; $0{\cdot}05 < p > 0{\cdot}01$), but not Indian ethnic patients. The reaction rate increased with age from 1·7% (6/259) at age 0–19, to 4·6% (26/569) for age 20–39, 7·7% (20/260) at age 40–59, and 8·8% (17/193) for age 60+. This trend was particularly pronounced in females; white

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ethnic patients had a significantly greater reaction rate than Pakistani patients (χ^2 7·11; 0·01 > p > 0·001), but not Indian patients (χ^2 3·39; p > 0·05). There were no differences between Indian and Pakistani patients overall but Pakistani males had lower rates than females (χ^2 6·20; 0·05 > p > 0·01). The differences between white and Asian patients can be largely explained by the differences in the age and sex structures of the two populations.

Mycobacterial drug resistance and the use of combined preparation chemotherapy in North America and the United Kingdom

DPS SPENCE, PDO DAVIES *Aintree Chest Centre and Tuberculosis Research Unit, Liverpool* The incidence of drug resistant tuberculosis is higher in North America (United States 8·8%, Canada 6·3%) than in the United Kingdom (4·1%). Of most concern is the increasing number of cases of multidrug resistant tuberculosis reported from the United States and New York in particular. Multidrug resistant disease is extremely uncommon in the United Kingdom, most cases probably acquiring infection with resistant organisms while abroad. An important cause of drug resistant organisms may be single agent antituberculosis chemotherapy which may be self administered by patients prescribed single rather than combined preparations such as Rifinah, Rimactazid, or Rifater. Evidence from pharmaceutical companies supplying the United States shows that between 10% and 20% of rifampicin is given to patients as a combined preparation [(Rifamate (isoniazid and rifampicin)]. Rifater is not currently available in the United States. Combined preparations are not currently available in Canada. In the United Kingdom data collected by each FHSA and detailed in PACT analyses for the year 1992 showed that 60% of rifampacin and 25% of pyrazinamide were prescribed as a combined preparation. Data for hospital prescribing are not available nationally. The two pharmaceutical companies distributing antituberculous chemotherapy were asked for details of combined product sales in the United Kingdom and reported that 73% and 79% of rifampacin was prescribed as a combined product. The apparent disparity between PACT data and pharmaceutical company data may be explained by the fact that hospital prescribing of rifampicin is almost 100% as a combined product. The use of combined preparation therapy for tuberculosis seems to be associated with a lower incidence of mycobacterial drug resistance when the United Kingdom and North America are compared. Combination chemotherapy may have an important role in preventing the emergence of drug resistant tuberculosis.

Drug resistant tuberculosis in south east England, 1981–92, in HIV and non-HIV populations

D BUCHANAN, JM GRANGE, MD YATES *Department of Medicine, King's College Hospital, London; PHLS Regional Tuberculosis Centre, Dulwich Hospital* Morbidity rates from tuberculosis worldwide are increasing. The number of bacteriologically proved cases confirmed by the PHLS Regional Tuberculosis Centre at Dulwich decreased from 1477 in 1981 to 1028 in 1988 but has steadily increased to 1245 in 1992. This compares with similar trends in other areas. The percentage of cases resistant to one or more antituberculosis drugs has remained constant, but the incidence is greater in immigrants from the Indian sub-continent (ISC) and Africa (between 7·6 and 11·5%) than in Europeans (between 1·8 and 5·2%). In both groups the main drug resistance was to isoniazid (H) alone, streptomycin (S) alone, and then to H + S. Rifampicin (R) resistance was only encountered in five European patients (0·07%) and eight ISC/African patients (0·15%). Resistance to H + R occurred in only four European patients (0·07%) and 15 ISC/African patients (0·28%). In south east England the first known case of tuberculosis in an HIV positive patient occurred in 1984. The total number of cases had increased to 167 cases by 1992. Nine (5%) of the 167 isolates of *M tuberculosis* were resistant to one or more of the tested drugs. Of the 38 African patients, six (16%) showed drug resistance (three H, one S, two H + S); of the 124 European patients, three (2%) showed drug resistance (two H, one R). Although the incidence of drug resistance does not seem to be increasing at the moment, continued careful surveillance must be maintained.

Characteristics of patients with drug resistant and drug sensitive tuberculosis in east London

N AL JARAD, S PARASTATIDES, CD SHELDON, H GAYA, RM RUDD, DW EMPEY *East London Tuberculosis Centre, The London Chest Hospital, Bonner Road, London* The aim of the study is to investigate the characteristics of patients with drug resistant tuberculosis diagnosed at the London Chest Hospital between 1984 and 1992 and to compare them with a control group of patients with drug sensitive tuberculosis diagnosed within the same period. We reviewed the microbiology results between 1984–92. 292 patients had culture positive pulmonary tuberculosis. Drug resistant strains were isolated from 20 patients (6·8%). We reviewed the case notes of all patients with drug resistant tuberculosis and of 101 with drug sensitive pulmonary tuberculosis as a control group. Ten patients out of 292 (3·4%) had strains resistant to a single drug and nine (3·1%) had resistance to more than one first line drug. One patient had strains resistant to isoniazid plus capreomycin. Strains resistant to more than one drug were all resistant to isoniazid and rifampicin. In five patients these were also resistant to pyrazinamide and in two they were resistant to streptomycin. Single drug resistant strains were resistant to isoniazid (eight patients), streptomycin (one patient). Previous treatment for tuberculosis was the most significant association with drug resistant tuberculosis (7/9; 78%) for patients with resistance to more than one drug; 5/10 (50%) for single drug resistance compared with six patients (6%) in the drug sensitive group, p < 0·0001. Extensive disease affecting both lungs at presentation was found more commonly in patients with drug resistant tuberculosis, 16/20 (80%) compared with 36/101 (36%) patients from the drug sensitive group (p = 0·031). Homelessness was not reported in any patient in either group, but living in hostels or care homes was reported in 6/20 (30%) in the drug resistant group compared with 11/101 (11%) patients in the drug sensitive group (p = 0·025). Fourteen patients (70%) with drug resistant tuberculosis were foreign born compared with 46 patients (46%) with drug sensitive tuberculosis (p = 0·047). We conclude that in east London the main factors associated with the emergence of drug resistant tuberculosis are previous treatment for tuberculosis, extensive disease at presentation, disease at a young age, foreign birth, and living in hostels or care homes.

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