

extensive controlled study is needed, however, before this approach can be recommended for routine use in this common gastrointestinal manifestation of CF.

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#### REFERENCES

- Matseshe JW, Go VLW, DiMagno E. Meconium ileus equivalent complicating cystic fibrosis in post-neonatal children and young adults. *Gastroenterology* 1972; **72**: 732-36
- Park RW, Grand RJ. Gastrointestinal manifestation of cystic fibrosis: A review. *Gastroenterology* 1981; **81**: 1143-61
- Hanly JG, Fitzgerald MX. Meconium ileus equivalent in older patients with cystic fibrosis. *Br Med J* 1983; **286**: 1411-13.
- Rosentein BJ, Langbaum TS. Incidence of distal intestinal obstruction syndrome in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1983; **2**: 299-301.
- Lloyd-Still JD. Meconium. In: Textbook of cystic fibrosis. Boston. John Wright, 1983.
- Hodson ME, Mearns MB, Batten JC. Meconium ileus equivalent in adults with cystic fibrosis of the pancreas: A report of six cases. *Br Med J* 1976; **11**: 790-91
- Davis GR, Santa Ana CA, Morawski SG, Fordtran JS. Development of a lavage solution associated with minimal water and electrolyte absorption or secretion. *Gastroenterology* 1980; **78**: 991-95.
- Goldman J, Reichelderfer M. Evaluation of rapid colonoscopic preparation using a new gut lavage solution. *Gastrointest Endosc* 1981; **27**: 122-26.
- Thomas G, Brozinsky S, Isenberg JI. Patients acceptance and effectiveness of a balanced lavage solution (Golytely) versus the standard preparation for colonoscopy. *Gastroenterology* 1982; **82**: 435-37.
- Tolia V, Fleming S, Dubois RS. Use of Golytely in children and adolescents. *J Pediatr Gastroenterol Nutr* 1984; **3**: 468-70.
- Rhodes JB, Engstrom J, Stone KF. Metoclopramide reduces the distress associated with colon cleansing by an oral electrolyte overload. *Gastrointest Endosc* 1978; **24**: 162-63
- Zentler-Munro PL, Hodson ME, Batten JC. Meconium ileus equivalent in older patients with cystic fibrosis. *Br Med J* 1983; **287**: 501-02
- Wesley A, Forstner J, Qureshi R, Mantle M, Forstner G. Human intestinal mucin in cystic fibrosis. *Pediatr Res* 1983; **17**: 65-69.
- Morrissey SM, Tymvios MC. Acid mucin in human intestinal goblet cells. *J Pathol* 1978; **126**: 197-208.

## OXIDANT ACTIVITY IN EXPIRED BREATH OF PATIENTS WITH ADULT RESPIRATORY DISTRESS SYNDROME

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**Summary** Hydrogen peroxide levels were measured in the breath condensate of 43 patients receiving mechanical ventilation. In 16 patients the mean breath condensate peroxide level was  $1.68 \pm 0.35 \mu\text{mol/l}$  on the day they met diagnostic criteria for adult respiratory distress syndrome (ARDS). The peak breath condensate peroxide level in the 27 patients in whom ARDS did not develop was significantly lower ( $0.34 \pm 0.08 \mu\text{mol/l}$ ). Plasma lysozyme, a measure of in-vivo neutrophil turnover, was significantly higher in ARDS than in non-ARDS patients ( $9.2 \pm 2.2 \text{ U/ml}$  v  $3.4 \pm 1.1 \text{ U/ml}$ ). These findings support the hypothesis that neutrophil activation and oxidant production are involved in the pathogenesis of ARDS.

### Introduction

THE mechanism of lung injury in adult respiratory distress syndrome (ARDS) is not yet certain. Data from animal models<sup>1-7</sup> and indirect evidence from studies in human beings<sup>8-12</sup> have suggested that toxic oxygen metabolites

produced by stimulated neutrophils are a possible agent of the alveolar injury. Cochrane et al<sup>10</sup> demonstrated increased oxidant activity in the lung in ARDS; a protein present in bronchoalveolar lavage fluid,  $\alpha_1$ -proteinase inhibitor, was oxidatively inactivated.

Hydrogen peroxide, a volatile oxygen metabolite produced by stimulated neutrophils, can enter the gas phase at physiological temperatures.<sup>13</sup> Williams and colleagues have shown spontaneous chemiluminescence in human breath which correlated with hydrogen peroxide content.<sup>13,14</sup> On the basis of the theory that toxic oxygen metabolites are involved in the damaging process of acute lung injury, we postulated that volatile oxygen metabolites would be present in increased amounts in the expired breath of patients with ARDS. To test the hypothesis, we measured the hydrogen peroxide concentration of breath condensates from patients receiving mechanical ventilation for various indications including ARDS. Other studies were carried out to correlate breath peroxide levels with measurements of neutrophil activity.

### Patients and Methods

#### Patients

We studied 43 patients requiring mechanical ventilation for various reasons. During study periods, all patients receiving mechanical ventilation in the medical and surgical intensive-care units at University Hospital were studied daily. The study was approved by the Human Subject Review Committee of the University of Michigan. Patients who met all our criteria (modified from those of Fowler et al<sup>15</sup>) were considered to have ARDS: acute respiratory failure requiring mechanical ventilation; diffuse, bilateral alveolar infiltrates on chest X-ray; static lung compliance less than 50 ml/cm water; arterial to alveolar  $\text{PO}_2$  ratio  $\leq 0.2$ ; absence of left ventricular failure (pulmonary artery wedge pressure  $\leq 16 \text{ mm Hg}$ ).

#### Methods

**Breath collection.**—Breath condensate was obtained by passing expired gas through 90 cm 'Tygon' tubing (internal diameter 15 mm, Norton Co) submerged in an ice-water bath. The tubing was connected to the expiratory limb of the ventilator tubing distal to the exhalation valve. Liquid that had previously pooled within the ventilator circuit was prevented from entering the condenser tubing. Expired gas was collected until a condensate of approximately 1 ml had formed; this was always accomplished within 5 min. The condensate was then immediately transferred to a polystyrene tube and placed on ice.

**Hydrogen peroxide** in breath condensate was assayed by the scopoletin/horseradish peroxidase method.<sup>16</sup> The person doing the assays was unaware of the clinical status of the patient. Hydrogen peroxide causes a fall in scopoletin fluorescence by oxidising it in the presence of horseradish peroxidase. The hydrogen peroxide in breath condensate was quantified by comparing the fall in scopoletin fluorescence with the falls caused by standard amounts of reagent hydrogen peroxide. The specificity of the assay was confirmed by pretreatment of the sample with catalase (1000 U/ml) which caused a loss of all measured peroxide-like activity. For the assay, 500  $\mu\text{l}$  breath condensate was added within 1 h of collection to 600  $\mu\text{l}$  5 mmol/l scopoletin containing 144 U horseradish peroxidase in phosphate-buffered saline (pH 7.4) and frozen at  $-20^\circ\text{C}$ . Samples processed in this way were stable for at least a month.

**Neutrophil activity.**—At the time of breath condensate collection, a sample of peripheral venous blood was taken and anticoagulated with edetic acid. White-blood-cell counts were made with a Coulter counter. Plasma was separated and frozen at  $-70^\circ\text{C}$  within 1 h of collection. Plasma lysozyme activity, an indicator of in-vivo neutrophil turnover,<sup>17</sup> was measured.<sup>18</sup>

**Statistical analysis.**—Group data are expressed as mean  $\pm$  SEM. Data from ARDS and non-ARDS groups were compared by the

Mann-Whitney U test.<sup>19</sup> Paired data were evaluated by the paired Student's *t* test. The presence of linear correlation between two sets of measurements was determined by one-way analysis of variance. Probability values less than 0.05 were considered significant.

### Results

We studied 16 patients with ARDS and 27 without (see table); 7 patients had two or more diagnoses. The ARDS patient listed under "Other" had been successfully resuscitated from cardiopulmonary arrest. 1 ARDS patient had no other associated diagnosis. Diagnoses of non-ARDS patients designated "Other" included postoperative respiratory insufficiency (2), amiodarone poisoning (1), disseminated carcinomatosis (1), resuscitation from cardiopulmonary arrest (1), and pancreatitis (1). Patients were studied for a mean of 3 days (range 1–10 days).

The hydrogen peroxide content of breath condensate from patients with ARDS was higher than that of the control group (fig 1). The mean ( $\pm$ SEM) level in the 16 ARDS patients on

ASSOCIATED DIAGNOSES OF PATIENTS

	ARDS (n=16)	Non-ARDS (n=27)
Sepsis*	6	4
Shock†	4	1
Aspiration pneumonia‡	2	5
Other pneumonia§	3	3
COPD with acute infection	..	4
Neurological dysfunction	..	7
Hypertransfusion¶	3	..
Lung contusion	1	2
Long-bone fracture	1	..
Other	1	6

\*Fever with bacterial growth in blood culture.

†Systemic systolic blood pressure <90 mm Hg.

‡Pneumonia after witnessed aspiration or suction of gastric contents from trachea.

§Fever, new localised infiltrates, pathogen isolated from sputum.

¶Twelve or more units of blood given in 12 h.

COPD=chronic obstructive pulmonary disease.

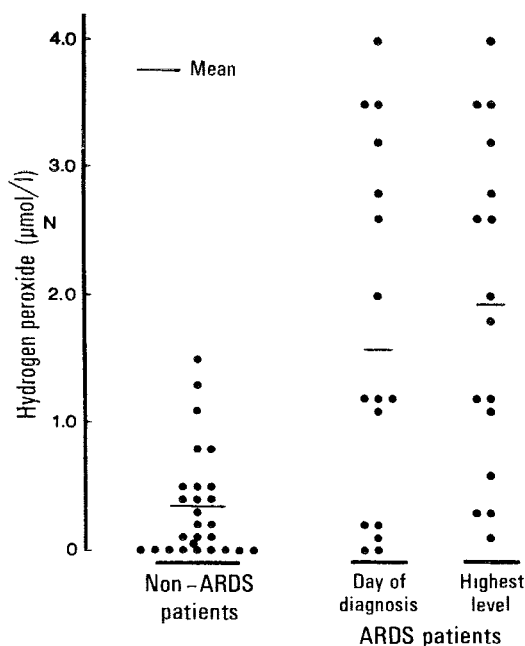


Fig 1—Hydrogen peroxide concentration in breath condensate.

Levels for ARDS patients shown on the day diagnostic criteria for ARDS were met and peak concentration measured during the study period; levels for non-ARDS are highest measured.

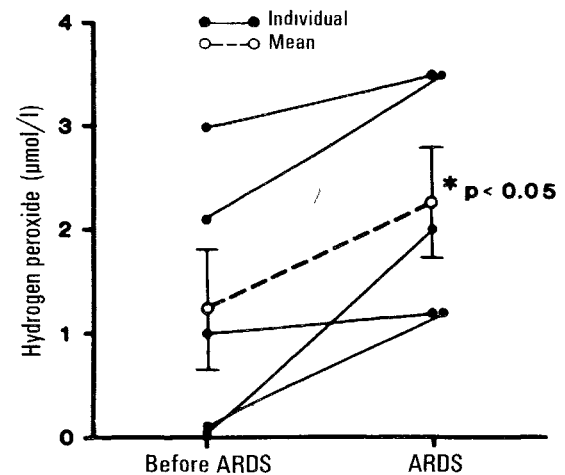


Fig 2—Evolution of breath condensate hydrogen peroxide concentration during development of ARDS in 5 patients.

the day they first met ARDS criteria was  $1.68 \pm 0.35 \mu\text{mol/l}$ . In 5 of the ARDS patients the hydrogen peroxide levels rose further during the course of their illness. The mean peak level for the ARDS group was  $1.93 \pm 0.32 \mu\text{mol/l}$ . Both initial and peak peroxide levels were significantly higher ( $p < 0.005$ ) than the peak levels of the non-ARDS group ( $0.34 \pm 0.08 \mu\text{mol/l}$ ).

11 of the 16 ARDS patients met the diagnostic criteria on day 1 of the study, and 5 on subsequent study days. 24–48 h before these 5 patients met ARDS criteria, their mean breath condensate peroxide concentration was  $1.24 \pm 0.58 \mu\text{mol/l}$ . It increased significantly ( $p < 0.05$ ) to  $2.28 \pm 0.52 \mu\text{mol/l}$  on the day that ARDS criteria were satisfied (fig 2). Subsequent breath peroxide measurements were available for 7 ARDS patients who, on the day of diagnosis, had levels more than one standard deviation above the mean non-ARDS level. 1 day after meeting ARDS criteria 5 of them still had raised levels. 5 ARDS patients survived and breath hydrogen peroxide was repeatedly measured until extubation. In all 5 the level fell as they improved clinically. The peak breath concentration of these patients was  $2.04 \pm 0.60 \mu\text{mol/l}$  and it decreased significantly ( $p < 0.05$ ) to  $0.64 \pm 0.29 \mu\text{mol/l}$  within 24 h of extubation.

Six patients with ARDS and 7 without had received glucocorticoids (>60 mg methylprednisolone equivalent per day). There was no significant difference in hydrogen peroxide levels between those who did and those who did not receive glucocorticoids.

Williams and colleagues<sup>13</sup> showed that breath chemiluminescence increased when the subject inhaled 100% oxygen. To see whether the difference in expired hydrogen peroxide between the groups was due to the higher fraction of inspired oxygen ( $\text{FiO}_2$ ) requirements of the ARDS patients, we compared these measurements for the two groups (fig 3). No correlation was found by least squares linear regression between breath condensate hydrogen peroxide and  $\text{FiO}_2$  in either ARDS ( $r = 0.05$ ) or non-ARDS patients ( $r = 0.02$ ).

Plasma lysozyme was measured in 13 ARDS and 21 non-ARDS patients. The ARDS group had a mean level of  $9.2 \pm 2.2 \text{ U/ml}$  on the day they met ARDS criteria, which was significantly higher ( $p < 0.02$ ) than the value of  $3.4 \pm 1.1 \text{ U/ml}$  measured in the non-ARDS patients on the day of highest breath condensate hydrogen peroxide. Plasma lysozyme level correlated with breath condensate peroxide in

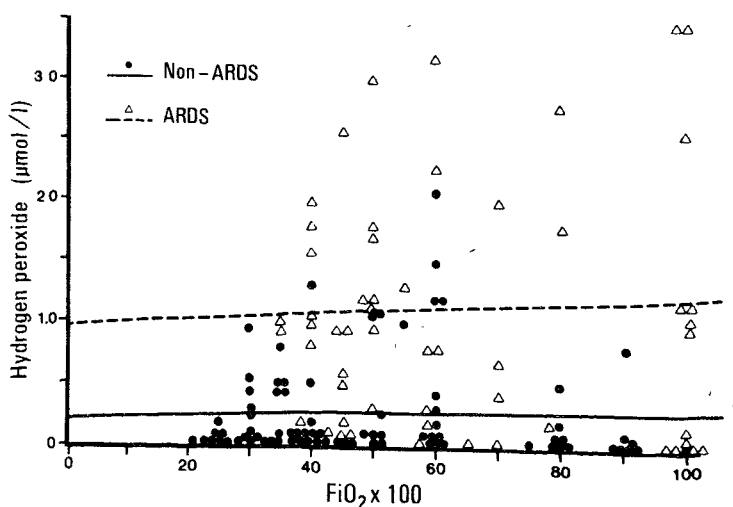


Fig 3—Breath condensate hydrogen peroxide concentration and oxygen content of inspired gas.

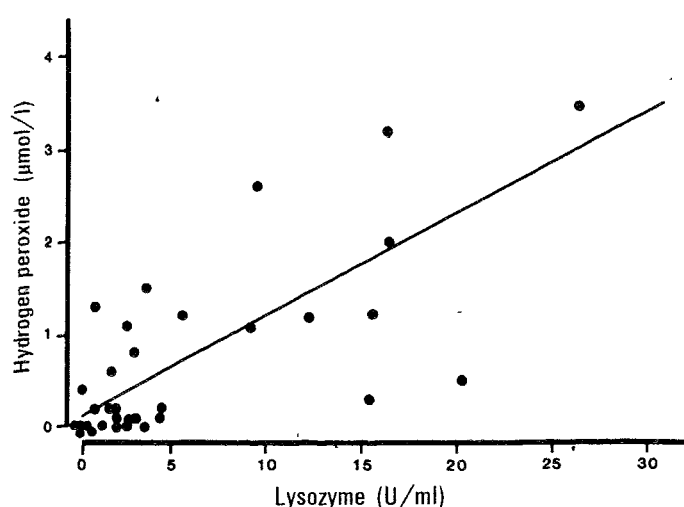


Fig 4—Breath condensate hydrogen peroxide and plasma lysozyme.

both groups ( $r=0.71$ ,  $p<0.001$ ; fig 4). The highest breath condensate peroxide measured during daily sampling occurred within 24 h of the highest plasma lysozyme in 10 of 13 ARDS and in 17 of 21 non-ARDS patients. Serial measurements of plasma lysozyme were available in 4 patients who did not meet ARDS criteria on day 1 of study but subsequently did so. Their mean level rose significantly from  $7.0 \pm 2.4$  U/ml 24–48 h before criteria for ARDS were satisfied to  $17.1 \pm 3.1$  U/ml on the day diagnostic criteria were met ( $p<0.05$ ).

On the day patients met ARDS criteria the mean white-blood-cell count was  $20.4 \pm 2.0 \times 10^9/l$ , significantly ( $p<0.01$ ) greater than the value in non-ARDS patients on the day of peak breath condensate peroxide ( $12.7 \pm 1.3 \times 10^9/l$ ). There was no significant correlation ( $r=0.10$ ) between white-blood-cell count and plasma lysozyme, so the differences in plasma lysozyme between the groups were not due to the difference in white-blood-cell counts.

### Discussion

Animal studies have suggested that toxic oxygen metabolites might be an important damaging agent in some types of acute lung injury.<sup>1-7</sup> Many of these studies have implicated activated neutrophils as the main instigator of tissue injury, possibly by their production of oxygen metabolites. Substances which inactivate or inhibit the

formation of toxic oxygen species have reduced the lung injury in some of these models. The presence of oxygen metabolites in one rabbit model of lung injury was suggested by the demonstration that endogenous lung catalase was inactivated if the animals were pretreated with aminotriazole.<sup>7</sup> This aminotriazole-dependent inactivation of catalase is known to be dependent on the presence of hydrogen peroxide. The pathogenesis of human ARDS is thought to involve processes similar to those demonstrated in animal models.  $\alpha_1$ -proteinase inhibitor in bronchoalveolar lavage fluid of patients with ARDS is oxidatively inactivated,<sup>10</sup> which suggests that ARDS is associated with the generation of oxidants within the lung.

Patients in the non-ARDS group with pneumonia had low breath peroxide concentrations despite the probable accumulation of activated neutrophils in their lungs. The reason for high peroxide levels in ARDS but low levels in these patients is unknown, but it may be due to differences in the pattern of ventilation to diseased areas, in the local activity of oxygen metabolite scavengers, or in the intensity or duration of neutrophil peroxide production.

Unlike Williams et al<sup>13</sup> we found no relation between inspired oxygen concentration and breath peroxide level, but their subjects were not intratracheally intubated. Certain bacteria commonly present in the mouth produce greater amounts of peroxide when exposed to increased oxygen tension.<sup>20</sup> In addition, the enzyme lactoperoxidase is present in saliva<sup>21</sup> and may cause some of the chemiluminescence detected by Williams et al. Chemiluminescence, which is produced by a variety of complex reactions, may depend on several factors in addition to peroxide concentration. Because we studied only intubated patients, we eliminated the contribution of oral flora to peroxide in expired breath.

Because stimulated neutrophils release lysozyme, plasma lysozyme concentration has been used as an indicator of in-vivo neutrophil turnover.<sup>17</sup> High concentrations of neutrophil granule enzymes have been found in the bronchoalveolar lavage fluid of patients with ARDS.<sup>8,9</sup> We found significantly higher plasma lysozyme levels in ARDS than in non-ARDS patients, which were not due merely to the higher white-blood-cell counts in ARDS patients. The lysozyme levels in our ARDS patients were temporally and quantitatively correlated with breath peroxide. Although lysozyme can come from many sources, including macrophages, we speculate that the high peroxide and lysozyme levels are due to neutrophil activation in ARDS.

Serial breath condensate peroxide measurements appeared to fluctuate in accordance with the clinical status of the ARDS patients. We believe these findings support the theory that activated neutrophils producing toxic oxygen metabolites are involved in the pathogenesis of ARDS. Furthermore, we speculate that measurement of breath condensate peroxide could potentially serve as a means to detect oxidant-mediated lung injury in critically ill patients.

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## LONG-TERM SKIN ALLOGRAFT SURVIVAL AFTER SHORT-TERM CYCLOSPORIN TREATMENT IN A PATIENT WITH MASSIVE BURNS

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**Summary** In a child with extensive burns, cyclosporin was given to extend the survival of cadaveric skin allografts obtained from numerous unmatched donors. No evidence of graft rejection was seen, either during treatment or in the 2 years after cyclosporin was withdrawn.

### Introduction

WHEN used as a wound dressing in cases of extensive burn injury, skin allografts prolong survival by reducing fluid and protein losses and providing a temporary barrier to microbes.<sup>1</sup> Subsequently, they must be replaced by autografts, and in cases of massive thermal injury the patient's remaining skin may be insufficient. Autologous cultured epithelium may prove useful in these circumstances,<sup>2</sup> but this takes three or four weeks to prepare in quantity.<sup>3</sup> Might immunosuppression prolong the life of the allograft? Burke et

al<sup>4,5</sup> had some success with azathioprine and later antithymocyte globulin, but this approach was abandoned: the resultant neutropenia and immunodeficiency necessitated treatment in a complex protective environment. We hypothesised that cyclosporin might prove a less hazardous means of immunosuppression, since its principal action is on thymus-dependent antigens<sup>6,7</sup> and the responses to several bacterial products are T-cell independent.<sup>8</sup> Renal transplant patients receiving cyclosporin are less prone to infection than those receiving conventional immunosuppression.<sup>9</sup> After establishing that cyclosporin extends the survival of skin allografts in rats after thermal injury and early excision,<sup>10</sup> we tried this agent in a child whose extensive burns required cover with skin allografts from several unmatched donors.

### Case Report

An 11-year-old boy sustained 85% body surface burns and inhalation injury. Primary excision of both legs to the fascial level was performed 72 h post-burn. Frozen banked-skin allografts were meshed at 1.5 to 1 and applied over widely meshed autografts. No attempt was made to match cadaver donor grafts with the recipient. The regional organ procurement agency was able to supply information on HLA type for three of the allograft donors:

	Blood group	Rh	HLA A	B	C	DR
Donor 1	B	-	2,31	7		2,7
Donor 2	O	+	2	49,35		1,5
Donor 3	A	+	3,28	35	2,4	2,3

The recipient was A rhesus positive; A 2, 1; B 39, 60; Cw 3. DR typing was not done.

Cyclosporin was started one day before initial primary excision and was given orally when possible, 8mg/kg per day. When intravenous administration was needed, the dose was 2.6 mg/kg per day. He also received nafcillin and gentamicin. Additionally, eight days post-burn both upper extremities were excised and allografted; and eighteen days post-burn the back was debrided and allografted with fresh skin from a kidney transplant donor. At one month, autografts were placed on both upper extremities and the buttocks were allografted. Further upper-extremity autografts were placed at two months post-burn. Cyclosporin was stopped 120 days after the original injury. During the course of treatment, the child had the equivalent of about three total body blood transfusions.

### Results

Fig 1, a and b, shows the lower limbs at three months and six months. Because the allografts came from people of various races, they give a patchwork appearance (the amount of pigment in histological sections of the dark areas was consistent with black skin). In the 2 years since cyclosporin treatment was stopped, no signs of skin graft rejection have been observed (fig 2); and at no time have the allografts shown classic histopathological evidence of rejection.

Monitoring of cyclosporin levels revealed fluctuations, independent of dose changes, of the kind reported by others. As judged from serum creatinine and bilirubin, the drug did not seriously diminish kidney or liver function. Positive blood cultures were obtained at 10 days (*Pseudomonas aeruginosa*), 40 days (*Escherichia coli*), and 60 days (*Klebsiella pneumoniae* and enterococcus), and at these times there seemed to be a depression of white cell count followed by an appropriate increase. IgG concentrations averaged 499±92 mg/dl, slightly below the normal range in our laboratory; IgM (71±15 mg/dl) and IgA (174 mg/dl, day 35 only) were within normal limits. A mumps skin test, performed four months after the end of cyclosporin therapy, was positive.

### S R BALDWIN AND OTHERS: REFERENCES

- Craddock PR, Fehr J, Brigham KL, Kronenberg RS, Jacob HS. Complement and leukocytes mediate pulmonary dysfunction in hemodialysis. *N Engl J Med* 1977; **296**: 769-74.
- Brigham KL, Bowers RE, Haynes J. Increased sheep lung vascular permeability caused by *Escherichia coli* endotoxin. *Circ Res* 1979; **45**: 292-97.
- Hosea S, Brown E, Hammer C, Frank M. Role of complement activation in a model of adult respiratory distress syndrome. *J Clin Invest* 1980; **66**: 375-82.
- Till GO, Johnson KJ, Kunkel R, Ward PA. Intravascular activation of complement and acute lung injury dependency on neutrophils and toxic oxygen metabolites. *J Clin Invest* 1981; **69**: 1126-35.
- Johnson KJ, Ward PA. Role of oxygen metabolites in immune complex injury of lung. *J Immunol* 1981; **126**: 2365-69.
- Till GO, Beauchamp C, Menapace D, et al. Oxygen radical dependent lung damage following thermal injury of rat skin. *J Trauma* 1983; **23**: 269-76.
- Schraufstatter IU, Revak SD, Cochrane CG. Proteases and oxidants in experimental pulmonary inflammatory injury. *J Clin Invest* 1984; **73**: 1175-84.
- Lee CT, Fein AM, Lippman M, Holtzman H, Kimbel P, Weinbaum G. Elastolytic activity in pulmonary lavage fluid from patients with adult respiratory-distress syndrome. *N Engl J Med* 1981; **304**: 192-96.
- McGuire WW, Spragg RG, Cohen AB, Cochrane CG. Studies on the pathogenesis of the adult respiratory distress syndrome. *J Clin Invest* 1982; **69**: 543-53.
- Cochrane CG, Spragg R, Revak SD. Pathogenesis of the adult respiratory distress syndrome: evidence of oxidant activity in bronchoalveolar lavage fluid. *J Clin Invest* 1983; **71**: 754-61.
- Zimmerman GA, Renzetti AD, Hill HR. Functional and metabolic activity of granulocytes from patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1983; **127**: 290-300.
- Thommasen HV, Boyko WJ, Russell JA, Hogg JC. Transient leucopenia associated with adult respiratory distress syndrome. *Lancet* 1984; **i**: 809-12.
- Williams MD, Leigh JS Jr, Chance B. Hydrogen peroxide in human breath and its probable role in spontaneous breath luminescence. *Ann NY Acad Sci* 1982; **386**: 478-83.
- Williams MD, Chance B. Spontaneous chemiluminescence of human breath. *J Biol Chem* 1983; **258**: 3628-31.
- Fowler AA, Hamman RF, Good JT, et al. Adult respiratory distress syndrome: risk with common predispositions. *Ann Intern Med* 1983; **98**: 593-97.
- Root RK, Metcalf J, Oshino N, Chance B. H<sub>2</sub>O<sub>2</sub> release from human granulocytes during phagocytosis: I. Documentation, quantitation, and some regulating factors. *J Clin Invest* 1975; **55**: 945-55.
- Hansen NE. The relationship between the turnover rate of neutrophilic granulocytes and plasma lysozyme levels. *Br J Haematol* 1973; **25**: 771-82.
- Worthington Enzyme Manual. Worthington Biochemical Corp: Freehold NJ, 1972: 100-01.
- Zar JH. Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall, 1974.
- Thomas EL, Pera KA. Oxygen metabolism of *Streptococcus mutans*: uptake of oxygen and release of superoxide and hydrogen peroxide. *J Bacteriol* 1983; **154**: 1236-44.
- Hamon CB, Klebanoff SJ. A peroxidase-mediated *Streptococcus mutans* dependent antimicrobial system in saliva. *J Exp Med* 1973; **137**: 438-50.