Thorax

Editorial

Asthma: is there an airway receptor barrier?

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Clinically, asthma appears to be multifactorial as reflected by the many synonyms for the disease, but there is one feature common to all forms – namely, the hyperresponsiveness of airways to noxious stimuli.[1] If it were possible, one might consider introducing a physical barrier or "airway liner" (as distinct from the mucous layer), partially isolating bronchial air from the receptors which elicit these exclusion reflexes as a first line of defence. Maybe such a partial barrier is naturally present but is compromised in asthmatic subjects.

The possibility of a physical barrier was engendered some years ago upon discovery that normal tracheal or bronchial epithelium rinsed free of adhering mucus is not spontaneously wettable, but is quite hydrophobic.[2] This was surprising because, according to classical lipid bilayer theory,[3 4] the polar groups of the phospholipid molecules comprising the epithelial membrane are orientated outwards to form a hydrophilic surface. Even the intercalated protein is folded to confine any hydrophobic moieties to the central hydrophobic domain.[5] This raises the question of what agent could be present on the airway surface to render it so hydrophobic that a droplet of saline placed on the surface would "bead up" and display an appreciable contact angle.[2]

Surfactant

In an earlier editorial[6] it was pointed out how such hydrophilic to hydrophobic transitions can be effected by reversible binding (adsorption) of a surfactant to the surface, as widely exploited in the industrial use of surfactants where the adsorbed layer can impart many highly desirable properties. It was also pointed out how pulmonary surfactant – subsequently confirmed to be present in appreciable quantities in the upper airways[7] – possesses the same chemical groups conducive to strong adsorption, while the desirable properties include the ability of the adsorbed monolayer to act as a barrier – for example, as "corrosion inhibitors". This has led to the discovery of the very hydrophobic nature of the gastric mucosa[8] to which adsorbed surface active phospholipid (SAPL) offers a very effective barrier to hydrogen ions and other water soluble solutes,[9] as reviewed recently[10] with regard to the aetiology of peptic ulcer. It is interesting that patients with this disorder also display impaired pulmonary function for which surfactant deficiency has been offered as the common factor.[11]

Morphology

Apart from our own studies, pulmonary surfactant has been studied only with regard to its ability to reduce the surface tension of water, the conventional model[12 13] ignoring any potential function which cannot be related to the liquid-air interface. However, surfactant is highly osmiophilic[14] and is therefore easily recognised by electron microscopy. Thus it is highly pertinent that Weibel,[15] when summarising his many studies of the alveolar surface using conventional fixatives, describes the osmiophilic lining as "following the epithelial surface rather than the liquid-air interface", effectively confirming direct adsorption of surfactant to epithelium. A few years after our editorial[6] proposing direct binding of surfactant, Ueda and coworkers in Japan published a series of studies in which they produced electron micrographs of superb quality clearly demonstrating the surfactant layer immediately adjacent to the epithelial surface – not only in the alveolus[16] but also in the upper airways.[17] Moreover, this lining to airway epithelium appeared as oligolamellar layers almost identical to those demonstrated in the mucus-free oxyntic duct[18] where an SAPL barrier is believed to provide mucosal protection.[10]

The probable reason why earlier workers have not demonstrated an epithelial barrier by electron microscopy is their conventional choice of glutaraldehyde as fixative which is known to "destroy" hydrophobic surfaces.[19] Both Ueda *et al*[16] and ourselves[18] used tannic acid for fixing, as recommended for visualising adsorbed surfactant as lamellated SAPL.[14] Although tannic acid is capable of producing lamellated structures by phospholipid migration during fixation,[20] such artefactual layers would only be deposited onto a hydrophobic surface and especially one already hydrophobic by virtue of a naturally adsorbed monolayer of SAPL which, incidentally, is all that is needed to provide a barrier.[9 21] In any case, oligolamellar layers of SAPL have been confirmed on bronchial epithelium by epifluoresence microscopy using "hydrophobic probes" in the absence of tannic acid.[22]

Airway receptors

The same oligolamellar layers of SAPL described above have been demonstrated adjacent to the taste receptors of the tongue[23] leading to speculation[24] that the function of such a lining in the airways is to "mask" receptors. A partial physical barrier separating receptors on smooth muscle and neuroepithelial cells from bronchial air might moderate the bronchoconstrictive reflex to any noxious stimuli or other airborne "triggers".

In neurophysiology the concept of "unmasking"[25] or "uncovering"[26] of central receptors has come into vogue as a mechanism for explaining sensitisation of a particular reflex. Typically, only 1–5% of receptors are normally "unmasked" at any one time. The question of what is normally "masking" the remaining 95–99% is seldom addressed, but there is much indirect evidence[24] that this could be provided by adsorbed SAPL and could apply to peripheral receptors in the airways.

Source of surfactant

In the alveoli the source of surfactant is unequivocally lamellar bodies[27] produced in type II pneumocytes, so it is interesting to find such bodies in neuroepithelial cells in the upper airways.[28] Moreover, they have been observed on the epithelial surface[29] and in the Golgi complex associated with the cell's secretory mechanism.[28 29] As the only innervated cells directly exposed to any noxious agent in inspired air, they appear to be secreting SAPL to mask their own receptors, thus moderating the sensitivity of the constrictive reflex.

It is also interesting to find neuroepithelial cells encircled by overtowering Clara cells[28] because these have the capability to scavenge and recycle surfactant.[30] Moreover, the spatial distribution of cells observed under scanning electron microscopy[28] indicates that, upon bronchoconstriction, the Clara cells would normally "smother" neuroepithelial cells, excluding them from contact with bronchial air and any airborne stimuli, thus providing a mechanism for limiting constriction.

Chronic aspects

At the risk of being grossly oversimplistic, it could be said that the immensely complex immunology of this disease and the role of allergens is reflected in the underlying inflammation of the airways which is known[1] to persist at a reduced level in asthmatic subjects between attacks. One of the primary features of acute airway inflammation is exudation of fluid, often rich in plasma proteins.[31] At the alveolar level such proteins are regarded as inhibitory because they deplete the surface of surfactant.[32] As large water soluble molecules they can act as carriers for the small insoluble SAPL molecules by forming reversible phospholipoprotein bonds,[21] thus removing surfactant as occurs in ARDS.[33] This depletion would arise whether surfactant were located at an air-aqueous interface or adsorbed to epithelium as a barrier.

It is also easy to envisage how a non-specific blanket of SAPL, if directly adsorbed to airway receptors, might compromise their antigen recognition capabilities and their binding capability since SAPL is an excellent abhesive (release agent) when adsorbed.[21]

Anti-inflammatory agents

The highly effective role of steroids in treating and preventing asthma is generally attributed to their anti-inflammatory properties. Why, therefore, are non-steroidal anti-inflammatory drugs (NSAIDs) not only ineffective, but exacerbate the disease in 10% of asthmatics?[34] One explanation is that, just as NSAIDs are effective blood-borne "barrier breakers" in the stomach where the gastric mucosal barrier is claimed to be adsorbed SAPL,[8 10] so they could denude airway receptors of essentially the same barrier. Steroids, on the other hand, have been shown to promote the secretion of SAPL in the lung,[35] thus enhancing the receptor barrier by replenishing any deficiency in SAPL resulting from persistent inflammation or any other cause.

Diagnostic challenges

Diagnostic challenges in the form of histamine or methacholine can be interpreted on the basis of the surfactant model as simply testing the integrity of the SAPL barrier that separates the relevant receptors from bronchial air. However, methacholine and its isomer, acetylcholine, are particularly interesting in that their molecules possess the same terminal quaternary ammonium ion which, as a positively charged group, enables SAPL to bind to negatively charged epithelium.[21] Thus methacholine will compete with SAPL for adsorption sites on the receptors without providing the saturated fatty acid chains needed to complete the barrier – that is, disrupting the mosaic of adsorbed SAPL molecules as discussed in molecular detail elsewhere.[24] This is particularly pertinent because a methacholine challenge is capable of inducing acute asthma in non-asthmatic subjects, especially in children.[36]

Physical challenges

Just as methacholine can compete for adsorption sites on the barrier, so other adsorption sites could compete with the receptor barrier for a limited quantity of SAPL. Competing sites could be provided in the form of mineral surfaces which avidly adsorb surfactants,[21] especially α-quartz which can actually extract SAPL from solution in chloroform.[29] This could be a major factor in occupational asthma.

In fixing SAPL membranes (or barriers) for electron microscopy one must be particularly careful to avoid "osmotic shock"[37] – whether hypertonic or hypotonic – which can disrupt such structures including myelin which it resembles. Such disruption of the receptor barrier could explain bronchial hyperresponsiveness to non-isotonic aerosols.[38] Fluid shifting rapidly into the airways with sudden exercise could also "lift off" the barrier to expose more receptors to any "triggers" in bronchial air, thus adding another to the list of explanations for sensitisation of the airways with exercise.[39]

Any barrier deficiency of SAPL should be reflected in the adjacent mucous layer from which adsorption onto the epithelium must occur. Surfactant in physiological quantities has a major effect upon the rheology of mucus[40] with lecithin (a crude industrial form of SAPL) being much used in the food processing industry as a "viscosity modifier".[41] Thus SAPL deficiency could explain compaction of mucus and the viscid mucous plugs that are commonly found in the airways of asthmatic subjects.[42]

Mucus could have an important role in stabilising the hydrophobic layer of adsorbed SAPL by reducing its interfacial energy with bronchial fluid, just as gastric mucus would appear to assume a similar role adjacent to the hydrophobic mucosal barrier.[43]

Chemical agents

A potent "barrier breaker" in the stomach is bile which has a remarkable ability to remove SAPL barriers and the hydrophobicity imparted by them.[8] Hence it is interesting that gastro-oesophageal reflux has been claimed to potentiate asthma,[44] although this finding is controversial.

There could also be chemical attack of the barrier by enzymes such as those found in the faeces of the house dust mite,[45] although no report on the phospholipase content could be found.

Surfactant deficiency

Quantitative deficiencies in surfactant have been clearly demonstrated in the upper airways of asthmatic subjects.[46] While this is very important evidence for surfactant deficiency being viewed as a causative factor in asthma

based on the receptor barrier model, traditionalists reverse this line of reasoning to ask how asthma leads to surfactant deficiency.[46] This dichotomy of approach arises because, according to conventional dogma,[12 13] pulmonary surfactant acts only at liquid-air interfaces to reduce the surface tension (γ) and thus the pressure difference (ΔP) that causes air spaces to collapse or fluid to shift according to the Laplace equation ($\Delta P = 2\gamma/r$) where r is the radius of curvature. For airways as large as bronchioles, however, r is so large that ΔP is negligible whatever practical value one assumes for γ, and so the surface tension of any liquid lining is irrelevant.

Ameliorating the deficiency

If deficiency of surfactant causes airway hyperresponsiveness, protection should be afforded by administering exogenous surfactant or drugs to promote its secretion. Thus, it is particularly interesting that steroids promote surfactant secretion in the lung,[35] as witnessed clinically by their common application antepartum to reduce the incidence of respiratory distress syndrome at birth.[47] Although their role in asthma is traditionally attributed to their anti-inflammatory action, this explanation cannot be invoked for β_2 agonists which have been shown to have no effect on eosinophil, macrophage, lymphocyte, neutrophil, or mast cell counts.[48] However, they invoke the immediate and dramatic release of airway surfactant witnessed in many studies reviewed by Enhorning.[46] Moreover, unmasking might provide the answer to the "very puzzling question"[49] concerning what changes β adrenoreceptors in asthma.

Another agent which promotes surfactant secretion in the lung is ambroxol, which is marketed as a mucolytic agent on account of the ability of SAPL to modify the rheology of mucus as outlined above. Hence it could be pertinent that this drug has also been shown to offer protection against virus-induced airway hyperresponsiveness in recent animal trials.[50]

The prostaglandin PGE_2 has been shown to exert an inhibitory action on airway nerves in reducing the bronchoconstrictor response to an acetylcholine challenge.[36] This is interesting because PGE_2 is intimately involved in the control of surfactant synthesis and has been found to increase its concentration on the gastric mucosa and the hydrophobicity which it imparts as a barrier,[51] its anti-ulcer properties being well established.[52]

The provision of more surfactant to fortify the barrier should reduce the exudation of serous fluid eroding the barrier, just as it does in distal air spaces,[53] while the addition of exogenous SAPL to the dialysate has been shown to improve ultrafiltration of proteins during continuous ambulatory peritoneal dialysis.[54]

Dietary aspects

Much has been written on the dietary aspects and, more recently, the possible link between the switch from butter to margarine over the last decade – which has coincided with a tenfold increase in childhood asthma over this period[55] – as unsaturated phospholipids are less surface active.[21] It is interesting that the incidence of peptic ulcer and osteoarthritis[56] have followed the same trend because both are diseases in which SAPL could have major roles as either a barrier[10] or load-bearing joint lubricant.[57] The lower incidence of asthma claimed with higher fish oil consumption[58] is also interesting because these lipids tend to be more saturated.[59]

Exogenous surfactant

Direct administration of surfactant to the upper airways could be the modality of choice since it avoids the side effects of drugs, simply returning to the airways a substance that is normally present. Preliminary results from a pilot study in Japan[60] and a large clinical trial in Sydney[61] in which Exosurf is administered to asthmatic subjects have revealed appreciable amelioration of bronchoconstriction, assessed by lung function tests, and this success is continuing. Exosurf is a form of SAPL designed to spread rapidly over a liquid-air interface and has been successful in treating neonates with respiratory distress syndrome.[62] However, this may not be suitable for asthma if its real role is to mask receptors; a formulation designed for true adsorption would be preferable.

Conclusion

The concept of a partial barrier of surfactant separating bronchial air from the receptors capable of eliciting bronchoconstriction offers a novel physical model for asthma which complements the traditional emphasis on the immunological/allergenic aspects and the whole cell biology[1] of this disease.

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1 Holgate ST. Asthma: past, present and future. *Eur Respir J* 1993;**6**:1507–20.
2 Hills BA, Barrow RE. The contact angle induced by DPL at pulmonary epithelial surfaces. *Respir Physiol* 1979;**38**:173–83.
3 Gorter E, Grendel F. On bimolecular layers of lipoids on chromatocytes of blood. *J Exp Med* 1925;**41**:439–43.
4 Danielli JF, Davson H. A contribution to the theory of permeability of thin films. *J Cell Comp Physiol* 1935;**5**:495–508.
5 Tanford C. *The hydrophobic effect: formation of micelles and biological membranes*. 2nd ed. New York:Wiley, 1980:206.
6 Hills BA. What is the true role of surfactant in the lung? *Thorax* 1981;**36**:1–4.
7 Widdicombe JG. Role of surfactant in the upper respiratory tract. In: Ekelund L, Jonson B, Malm L, eds. *Surfactant and the respiratory tract*. Amsterdam:Elsevier, 1989:103–12.
8 Hills BA, Butler BD, Lichtenberger LM. Gastric mucosal barrier: the hydrophobic lining to the lumen of the stomach. *Am J Physiol:GI Liver Physiol* 1983 ;**6**:G561–8.
9 Hills BA, Kirwood CA. Gastric mucosal barrier: barrier to hydrogen ions imparted by gastric surfactant. *Gut* 1992;**33**:1039–41.
10 Hills BA. Gastric surfactant and the hydrophobic mucosal barrier. *Gut* 1996 (in press).
11 Kellow JE, Tao Z, Piper DW. Ventilatory function in chronic peptic ulcer: a study of ventilatory function in patients with gastric and duodenal ulcer. *Gastroenterology* 1986;**91**:590–5.
12 Clements JA. Surface phenomena in relation to pulmonary function. *Physiologist* 1962;**5**:11–28.
13 Hawgood S, Clements JA. Pulmonary surfactant and its proteins. *J Clin Invest* 1990;**86**:1–6.
14 Kalina M, Pease DC. The preservation of ultrastructure saturated in phosphatidyl cholines by tannic acid in model systems and type II pneumocytes. *J Cell Biol* 1977;**74**:726–41.
15 Weibel ER. Functional morphology of lung parenchyma. In Fishman AP, ed. *Handbook of Physiology Section 3*, Vol III. Washington:American Physiology Society, 1985:89–112.
16 Ueda S, Kawamura K, Ishii N, Matsumoto S, Hayashi K, Okayasu M, *et al.* Ultrastructural studies on surface lining layer of the lungs: Part IV. Resected human lungs. *J Jap Med Soc Biol Interface* 1995;**16**:34–60.
17 Ueda S, Ishii N, Matsumoto S, Hayashi K, Okayasu M. Ultrastructural studies on surface lining layer of the lungs (Part II). *J Jap Med Soc Biol* 1983;**14**:24–46.
18 Hills BA. A physical identity for the gastric mucosal barrier. *Med J Aust* 1990;**153**:76–81.
19 Untersee P, Gil J, Weibel ER. Visualization of extracellular lining layer of lung alveoli by freeze-etching. *Respir Physiol* 1971;**13**:171–85.
20 Schrijvers AHGJ, Frederik PM, Stuart MCA, Burger VV Th, Van Der Vusse GJ, Reneman RS. Formation of multilamellar vesicles by addition of tannic acid to phosphatidylcholine containing small unilamellar vesicles. *J Histochem Cytochem* 1989;**37**:1635–43.
21 Hills BA. *The biology of surfactant*. Cambridge:Cambridge University Press, 1988.
22 Ueda S, Kawamura K, Ishii N, Matsumoto S, Hayashi K, Okayasu M, *et al.* Ultrastructural studies on surface lining layer (SLL) of lungs: Part III. *J Jap Med Soc Biol* 1984;**15**:76–88.
23 Sbabarti A, Ceresi E, Accordini C. Surfactant-like material on the chemoreceptorial surface of the frog's taste organ: an ultrastructural and electron spectroscopic imaging study. *J Struct Biol* 1991;**107**:128–35.
24 Hills BA. A mechanism for masking receptors with particular application to asthma. *Med Hypoth* 1996;**46**:33–41.
25 Lynch G, Baudry M. The biochemistry of memory: a new and specific hypothesis. *Science* 1984;**224**:1057–63.
26 Nowak JZ, Sek B, Schorderet M. Dark-induced supersensitivity of dopamine D-1 and D-2 receptors in rat retina. *Neurol Report* 1991;**2**:429–32.
27 Stratton CJ. Morphology of surfactant producing cells and of the alveolar lining layer. In: Robertson B, Van Golde LMG, eds. *Pulmonary surfactant*. Amsterdam:Elsevier,1984:68–118.

28 Haller CJ. A scanning and transmission electron microscopic study of the development of the surface structures of neuroepithelial bodies in the mouse lung. *Micron* 1994;**25**:527–38.
29 Hills BA. Does adsorbed surfactant normally mask airway receptors? *Proc Ann Sci Mtg ANZ Thoracic Society* Hobart, 1995:47.
30 Geiger K, Gallagher ML, Hedley-White J. Cellular distribution and clearance of aerosolized dipalmitoyl lecithin. *J Appl Physiol* 1975;**39**:759–63.
31 Kay AB, Henson PM, Hunninghake GW, Irwin C, Lichtenstein LM, Nadel JA. Cellular mechanisms. In Holgate ST, ed. *The role of inflammatory processes in airway hyperresponsiveness*. Oxford:Blackwell, 1989:151–78.
32 Ikegami M, Jobe A, Jacobs H, Lam R. A protein from airways of premature lambs that inhibits surfactant function. *J Appl Physiol* 1984;**57**:1134–42.
33 Lachmann B. Intermediate and later changes in adult respiratory distress syndrome and their influence by surfactant replacement. In: Ekelund L, Johnson B. Malm L, eds. *Surfactant and the respiratory tract*. Amsterdam: Elsevier, 1989:177–83.
34 Samter M, Beers RF. Intolerance to aspirin. Clinical studies and consideration of its pathogenesis. *Ann Intern Med* 1986;**68**:975–83.
35 Torday JS, Smith BT, Giroud CJP. The rabbit fetal lung as a glucocorticoid target tissue. *Endocrinology* 1975;**96**:1462–7.
36 O'Bryne PM. Prostaglandins and thromboxane. In: Barnes PJ, Rodger IW, Thomson NC, eds. *Asthma*. London: Academic Press, 1992:225–33.
37 Banik NL, Davison AN. Enzyme activity and composition of myelin and subcellular fraction in the developing brain. *Biochem J* 1969;**115**:1051–62.
38 Smith CM, Anderson SD. Bronchial hyperresponsiveness as assessed by non-isotonic aerosols. *Progr Respir Res* 1990;**24**:105–16.
39 Makker HK, Holgate ST. Mechanisms of exercise-induced asthma. *Eur J Clin Invest* 1994;**24**:571–85.
40 Lopez-Vidriero M. Biochemical basis of physical properties of respiratory tract secretions. *Eur J Respir Dis* 1987;**71**(Suppl 153):130–5.
41 Schoen M. Confectionery and cacao products. In: Jacobs MD, ed. *Chemistry and technology of food and food products*. Vol III. New York:Interscience, 1951:2138–40.
42 Barnes PJ, Thomson NC. Other therapies used in asthma. In: Barnes PJ, Rodger IW, Thomson NC, eds. *Asthma*. London: Academic Press, 1992:659–66.
43 Hills BA. Gastric mucosal barrier: stabilization of hydrophobic lining to the stomach by mucus. *Am J Physiol* 1985;**244**:G561-342–9.
44 Ursechel HC, Paulson DL. Gastroesophageal reflux and hiatus hernia. *J Thor Cardiovasc Surg* 1967;**53**:21–8.
45 Woolcock AJ. Management of asthma in adults. In: Barnes PJ, Rodger IW, Thomson NC, eds. *Asthma*. London:Academic Press, 1992:679–99.
46 Enhorning G. Asthma, a condition of surfactant deficiency. In: Ekelund L, Jonson B, Malm L, eds. *Surfactant and the respiratory tract*. Amsterdam: Elsevier, 1989:273–81.
47 Liggins GD, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics* 1972;**50**:515–25.
48 Gardiner PV, Ward C, Booth H, Allison A, Walters EH. Effects of eight weeks of treatment with salmeterol on bronchoalveolar lavage inflammatory indices in asthmatics. *Am J Respir Crit Care Med* 1994:**150**:1106–11.
49 Szentivanyi A. The β adrenergic theory of the atopic abnormality in bronchial asthma. *J Allergy Clin Immunol* 1968;**42**:203–32.
50 So KL, Folkerts G, van der Linde H, Lachmann B, Nijkamp F. Ambroxol (Mucosolvan) prevents virus-induced airway hyperresponsiveness in guinea pigs. *Am J Respir Crit Care Med* 1995; **151**:A224.
51 Lichtenberger LM, Richards JE, Hills BA. Effects of prostaglandin PGE_2 on the surface hydrophobicity of aspirin-treated canine gastric mucosa. *Gastroenterology* 1985;**88**:308–14.
52 Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Gastric cytoprotective property of prostaglandins. *Gastroenterology* 1978;**72**:1121.
53 Robertson B. Surfactant substitution: effect of surfactant replacement. *Lung* 1980;**158**:57–68.
54 Krack G, Viglino G, Cavalli PL, Gandolfo C, Magliano G, Cantaluppi A, *et al.* Intraperitoneal administration of phosphatidylcholine improves ultrafiltration in continuous ambulatory peritoneal dialysis patients. *Peritoneal Dialysis Int* 1992;**12**:359–64.
55 Hodge L, Peat JK, Salome C. Increased consumption of polyunsaturated oils may be a cause of increased prevalence of childhood asthma. *Aust NZ J Med* 1994;**24**:727–8.
56 Australian Bureau of Statistics. *1989–90 National Health Survey – Musculoskeletal Conditions, Australia*. Catalogue No. 4374.0. Canberra: ABS,1991.
57 Hills BA. Synovial surfactant and the hydrophobic articular surface. *J Rheumatol* 1996 (in press) .
58 Hodge L, Salome CM, Peat JK, Haby MM. Oily fish consumption reduces the risk of childhood asthma. *Proc Ann Sci Mtg ANZ Thoracic Society* No.038, Hobart, 1995.
59 National Food Authority. *Nutritional values of Australian foods*. Canberra: Australian Government Public Service.
60 Kurashima K, Ogawa H, Ohka T. A pilot study of surfactant inhalation for the treatment of asthma attack. *Jap J Allergol* 1991;**40**:160–3.
61 Crawford ABH, Young IB. Effect of nebulised synthetic surfactant (Exosurf) on airway function and gas mixing in asthma. In: *Proc Ann Sci Mtg ANZ Thoracic Society*, Hobart, 1995.
62 Corbet A, Bucciavelli R, Goldman S, Mammel M, Wold D, Long W. Decreased mortality rate among small premature infants treated at birth with a single dose of synthetic surfactant: a multicenter controlled trial. *J Pediatr* 1991;**118**:277–84.