

the acid secretory mechanisms are apparently operative in the late embryonic stages in man since premature babies secrete acid during the first 6 h of life<sup>25</sup>.

In conclusion, the occurrence of the histamine- and VIP-sensitive cyclic AMP systems in a human fetus at 15 weeks of age is temporally related to the respective appearance of parietal and mucoid cells in fetuses at 12 weeks of gestation<sup>1</sup>. Thus, these 2 cyclic AMP systems would seem to be appropriate tools for studying the early differentiation and the functional development of the gastric mucosa in man.

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## Evaporative resistance of pulmonary surfactant films<sup>1</sup>

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**Summary.** Films of surfactant from the lungs of rabbits, tortoises (*Testudo hermanni*) and frogs (*Rana pipiens*) offer resistance to the evaporation of water.

Pulmonary surfactant forms thin films at the air-fluid interfaces within the distal respiratory passages of vertebrate lungs and it is thought to be involved in the maintenance of a stable pulmonary architecture<sup>2-4</sup>. Surfactant films contribute to the bulk of the air-blood barrier and obviously offer some resistance to the diffusion of respiratory gases, water and anaesthetic agents. Direct measurement of the film resistance is rendered difficult by the inaccessibility of pulmonary alveoli. Consequently, as a first step towards quantifying this resistance, it was decided to determine what effect surfactant films have on the rate of evaporation from aqueous surfaces in vitro.

**Methods.** Surfactant was extracted from the lungs of 12 rabbits, 15 tortoises (*Testudo hermanni*) and 15 frogs (*Rana pipiens*) by saline lavage<sup>5</sup>. The lavage fluid obtained from each animal was cooled to 4°C and centrifuged at 800 × g for 10 min. The supernatant was then adjusted to a density of 1.10 with sodium chloride and recentrifuged at 100,000 × g for 1 h. The surfactant was recovered in visible bands at the top of the centrifuge tubes. The protein content of a sample of each extract was determined using the method of Lowry et al.<sup>6</sup>.

The evaporative resistance of surfactant films was measured using an evaporator of the type described by Archer and La Mer<sup>7</sup>. Essentially, the method determines the mass of water vapour absorbed by a desiccant (anhydrous lithium chloride) positioned just above an aqueous surface which bears a thin film. Buffered saline (0.145 M NaCl in 0.01 M Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.4) was used as the aqueous subphase and a measured quantity of surfactant extract was added to its surface using an Agla microsyringe. The quantity added was sufficient to form a complete

surface film; an interval of 10 min was allowed for spreading. A correction was made by a method previously described<sup>7</sup> to compensate for the mass of water vapour absorbed from the surrounding air.

**Results.** Interfacial films of rabbit surfactant offered significant resistance to evaporation (see table). At 37°C, for example, the presence of a surface film caused a 5% decrease in the overall mass of water lost by evaporation. Increasing the subphase temperature stepwise over the range 35–41°C resulted in a modest decrease in the value of the evaporative resistance. In contrast, the evaporative

Evaporative resistances of surfactant films at different temperatures

	Temperature (°C)	Evaporative resistance (sec cm <sup>-1</sup> × 10 <sup>2</sup> )*	Overall decrease in water loss due to presence of film (%)
Rabbit surfactant	25	87.9 ± 4.6	7.7
	35	42.1 ± 2.4	5.4
	37	37.2 ± 2.2	5.1
	39	13.9 ± 0.8	3.5
Tortoise surfactant	41	5.1 ± 0.5	1.4
	20	187.5 ± 9.3	16.3
	25	137.8 ± 8.2	12.0
	30	51.5 ± 3.6	8.4
Frog surfactant	15	276.5 ± 19.4	24.1
	20	188.3 ± 15.0	16.5
	25	144.3 ± 7.0	12.6

\* Mean ± SEM of measurements on 8 films. All films had a surface coverage of 8 cm<sup>2</sup>/μg surfactant protein.

resistance was independent of the surface coverage of the films within the range investigated ( $8\text{--}16\text{ cm}^2/\mu\text{g}$  surfactant protein).

Surfactants from tortoises and frogs also offer considerable resistance to evaporation. In each species, the evaporative resistance is temperature-dependent. For comparison, monomolecular films of palmitic acid (coverage  $24\text{ Å}^2/\text{mole}$ ), dipalmitoyl phosphatidyl choline (coverage  $50\text{ Å}^2/\text{mole}$ ) and ovalbumin (coverage  $40\text{ cm}^2/\mu\text{g}$ ) were also measured. Their evaporative resistances at  $25^\circ\text{C}$  were  $3.08 \pm 0.21\text{ sec cm}^{-1}$ ,  $0.97 \pm 0.05\text{ sec cm}^{-1}$  and  $1.36 \pm 0.11\text{ sec cm}^{-1}$ , respectively.

**Discussion.** The retardation of water evaporation by surfactant films is remarkable when one considers that the mean thickness of the films concerned has been estimated to be about  $50\text{ Å}$ . On the other hand, the classical studies of Rideal<sup>9</sup> demonstrated most convincingly that monomolecular layers of fatty acids can inhibit the evaporation of water. Furthermore, the present study has confirmed that monolayers of phospholipids and protein have evaporative resistance of the same order of magnitude as those of surfactant films.

The results of studies on the permeability of films of ox brain lecithin<sup>10</sup> and synthetic phospholipids<sup>11</sup> indicate that these materials behave as thin homogeneous layers in which water dissolves as discrete molecules and then moves across by simple diffusion; pores and fissures do not appear to make much contribution to film permeability. Films of

pulmonary surfactant are generally believed to consist of monomolecular layers of phospholipids (principally, dipalmitoyl phosphatidylcholine<sup>12,13</sup>). It seems likely therefore that the transfer of water molecules across surfactant films is due to a 'solubility-diffusion' mechanism.

The present data are only relevant to adsorbed films of pulmonary surfactant in vitro. However, it is tempting to speculate that surfactant may play some part in maintaining the moist nature of the respiratory epithelia in the lungs of amphibians and reptiles.

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## Autoradiographic analysis of alanine uptake by newborn pig intestine

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**Summary.** All villus enterocytes in the newborn pig intestine take up alanine by a process which is largely Na-dependent. Uptake in the adult rabbit intestine is confined to enterocytes near the tips of villi. Previous conclusions about how transport changes during development are reviewed on the basis of these findings.

There are now several reports in the literature showing that the ability of the small intestine to transport amino acids is maximal at or about the time of birth<sup>1-3</sup>. Postnatal development of transport function is considered, from these results, to be either negligible or nonexistent. To sustain this hypothesis it is necessary to prove that the functional properties of a single cell population are being studied throughout development and that the adult pattern of cell differentiation applies to the neonate.

The first assumption is questioned by the finding that enterocytes produced during late fetal and early postnatal life are structurally different from those found in the adult<sup>4-7</sup> and the subsequent observation that these differences can affect the way an intestine transports amino acids<sup>8,9</sup>. The assumption that normal cell differentiation takes place in the neonatal intestine remains untested. New techniques of autoradiography now allow one to identify the particular population of cells responsible for amino acid transport in an intestinal mucosa<sup>10</sup>. These techniques are used in the present work to check which cells transport alanine in the neonatal pig intestine.

Pieces of mid small intestine, taken from newborn and 1-day-old pigs, were used for autoradiographic measurement of alanine influx in an apparatus identical to that described previously for the measurements of isotope uptake using scintillation spectrometry<sup>11</sup>. Tritiated alanine

( $100\text{ }\mu\text{Ci/ml}$ ) was presented to the luminal side of the intestine at a concentration of  $1\text{ mM}$  for a period of 45 sec following a 10-min period of superfusion in Na-free medium. Glutaraldehyde fixation of the tissue and subsequent processing for autoradiography was as described previously<sup>10</sup>. Silver grains were enhanced physically by immersion in silver intensifier solution (IN-5, Kodak Ltd, London). Silver grain densities were then estimated on  $4\text{ }\mu\text{m}$  unstained sections using an automated microdensitometer (model M 85, Vickers Instruments Ltd, York). Scanning proceeded from the tip of each villus to a depth of  $150\text{ }\mu\text{m}$ . Experiments similar to those reported for newborn pig intestine were also carried out with adult rabbit ileum as control.

Alanine uptake in intestines taken from both newborn and 1-day-old pigs occurred along the whole length of the villus (fig. 1a and c respectively). This was in marked contrast to results found with the adult rabbit intestine where uptake was restricted to the tips of villi (fig. 1d). Some of the alanine transported into the mucosa of both newborn and 1-day-old pig intestines moved into the core of the villus during the 45-sec contact with isotope. Most of the alanine uptake by adult rabbit intestine remained within the mucosa. Both influx and efflux of alanine in newborn pig intestine was inhibited by the absence of Na (fig. 1b).

Quantitative comparisons between the amounts of alanine