resistance was independent of the surface coverage of the films within the range investigated (8–16 $cm^2/\mu g$ 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 $A^2$/mole), dipalmitoyl phosphatidyl choline (coverage 50 $A^2$/mole) and ovalbumin (coverage 40 $cm^2/\mu g$) were also measured. Their evaporative resistances at 25 °C were $3.08 \pm 0.21$ sec $cm^{-1}$, $0.97 \pm 0.05$ sec $cm^{-1}$ and $1.36 \pm 0.11$ 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 Å[8]. On the other hand, the classical studies of Rideal[9] demonstrated most convincingly that monomolecular layers of fatty acids can inhibit the evaporation of water. Furhtermore, 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[10] and synthetic phospholipids[11] 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[12,13]). 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 cinfined 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[1-3]. 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[4-7] and the subsequent observation that these differences can affect the way an intestine transports amino acids[8,9]. 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[10]. 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[11]. Tritiated alanine (100 μCi/ml) was presented to the luminal side of the intestine at a concentration of 1 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[10]. Silver grains were enhanced physically by immersion in silver intensifier solution (IN-5, Kodak Ltd, London). Silver grain densities were then estimated on 4 μm 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 μm. 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

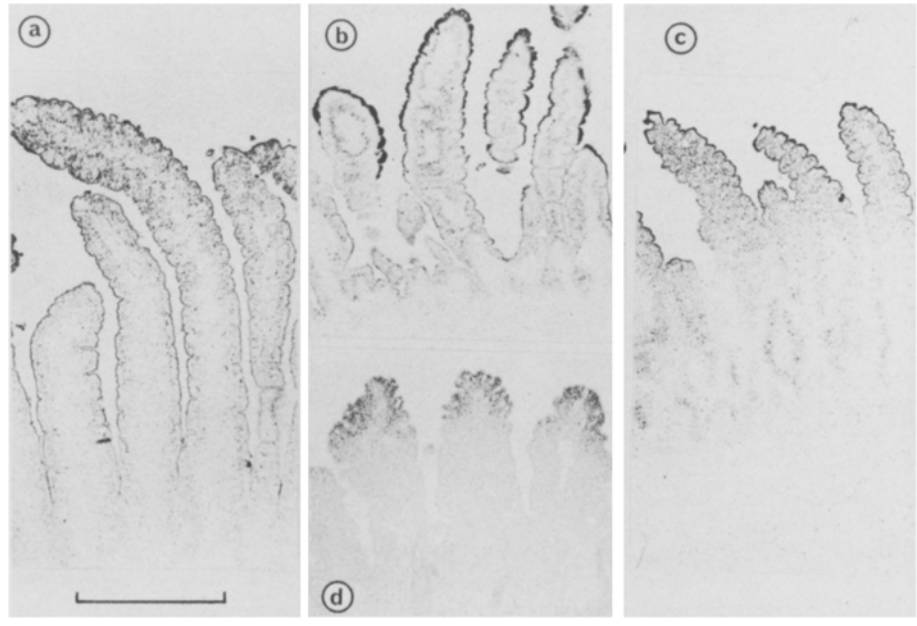


Fig. 1. Autoradiographic localization of tritiated alanine in intestinal villi. Pieces of small intestine taken from newborn pigs (a, b), a 1-day-old pig (c) and an adult rabbit (d) were incubated with tritiated alanine for 45 sec as described in the text. Newborn pig intestine was also incubated in the presence (a) and absence (b) of Na. The black outline around the villi, particularly noticeable in figure 1 (b) is an artefact produced during physical development of Ag grains. Scale bar: 250 μm.

entering the neonatal pig intestine under these various conditions were carried out by summing individual densities recorded along equal lengths of villus mucosa. Removing Na reduced the total grain density by 66% (values of 234 and 79 for Na-containing and Na-free media respectively). Alanine uptake by the 1-day-old pig intestine was slightly less than that recorded for the newborn pig (234 for the newborn compared with 177 for the 1-day-old animal). Previous experiments using rabbit intestine show a close correspondence between the uptakes of different amino acids measured by autoradiography and scintillation spectrometry[11]. Distribution of alanine uptake along the villus of the newborn pig intestine is compared quantitatively with that for adult rabbit intestine in figure 2. Uptake of alanine by the newborn pig intestine falls slightly on moving down the villus (average fall in density reading 0.13 $\mu m^{-1}$). This was negligible compared with that seen in the rabbit (average fall in density reading 1.26 $\mu m^{-1}$ recorded over the villus 25–75 μm from the tip). The ability of the newborn intestine to transport alanine appears to be expressed generally throughout the whole population of enterocytes. The results for adult rabbit intestine confirm those reported previously[11].

Many of the absorptive functions of enterocytes taken from adult intestines only appear during the later stages of cell migration. Present results with the rabbit, which agree with those published previously for the hamster[13], substantiate this finding. Much less is known about the migration and maturation of enterocytes in the neonatal animal. Present results suggest that these processes are very different from those seen in the adult. Negligible cell migration in the neonatal intestine may be associated with slow maturation of transport processes in all villus enterocytes or normal cell migration might occur coupled with early expression of amino acid absorptive capacity. In either case the final structure of enterocytes produced in the neonate is significantly different from that found in the adult. The sparseness of the terminal web and the presence of a special system of tubules in the apical cytoplasm allows these cells to take up ingested proteins. It could also make them freely permeable to amino acids, though the Na-dependency of uptake and the large net absorption of amino acids measured in vitro[14], both suggest that a large part of transport takes place through an energy driven process.

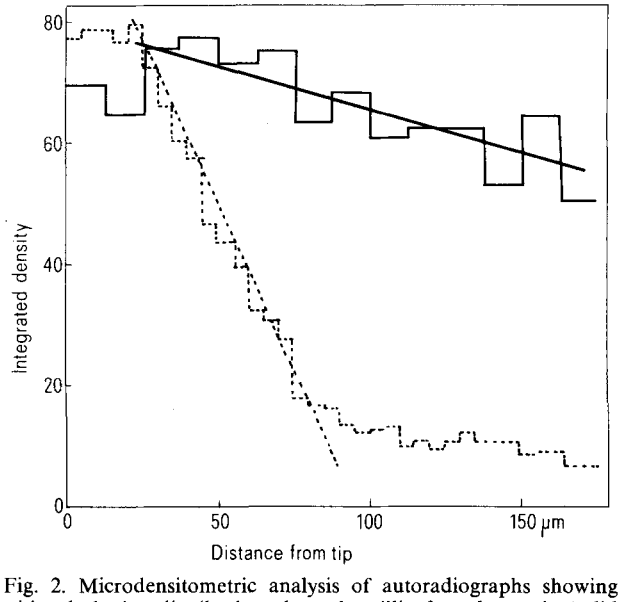


Fig. 2. Microdensitometric analysis of autoradiographs showing tritiated alanine distribution along the villi of newborn pig (solid lines) and adult rabbit (broken lines) intestines. Values for newborn pig and adult rabbits give means of estimates carried out on 5 and 25 villi respectively.

The high rate of amino acid transport seen previously across neonatal intestines probably arises from the large number of cells involved in absorption rather than from any specific maturation of a single transport process. Postnatal inhibition of transport probably results from the physiological state of the tissue at this stage in development, endocytosed protein having caused cellular distortion and general inhibition of Na transport[15]. Further delayed changes in amino acid transport are more likely the result of changes initiated in the postnatal pattern of cell replacement and differentiation.

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## Early ultrastructural changes in the cerebral cortex of albino rats subjected to 3-aminopyridine seizures

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*Summary.* The ultrastructural effects of the convulsant 3-aminopyridine were studied. It was found that astrocytic processes suffered swelling, while pyramidal cells underwent shrinkage and condensation in the 2nd and 3rd layers of rat cerebral cortex.

Recent electrophysiological investigations demonstrated that 3-aminopyridine (3-AP) applied locally on the cerebral cortex of adult cats caused convulsive phenomena within 5 min[2]. The present study concerning the ultrastructure of the 3-AP seizure focus, has been initiated because clear-cut morphological changes indicating facilitated transmitter release were found at spinal cord synapses after i.v. 4-aminopyridine administration[3,4]. To our knowledge, no electron microscopic studies have analyzed so far the morphological changes in neocortical aminopyridine seizures.

*Materials and methods.* Experiments were carried out on CFY strain male and female albino rats weighing 300-450 g. The fronto-parietal border region of the neocortex[5] was exposed on 1 side under chloralose-urethane anesthesia. We placed Gelaspon (VEB Jenapharm) soaked in 37 °C 0.9% NaCl on the meningeal surface and put a 2 mg 3-AP crystal (Koch-Light Labs Ltd) on the wet Gelaspon. Spontaneous jerks of the head and neck musculature occurred 2-4 min after the 3-AP application. Control animals were operated in the same manner except for the application of 3-AP. 3-AP treated animals were perfused 10 min after the beginning of the manifest muscle jerks, control animals 10 min after the Gelaspon application, both with a formaldehyde-glutaraldehyde fixative solution[6] for 20 min. Animals were decapitated after perfusion and their heads immersed in the same fixative overnight. After fixation small pieces were excised from the cerebral cortex, osmicated and embedded in araldite (Fluka). Ultrathin sections stained with lead citrate[6] were examined with a Tesla BS 500 electron microscope. Ultrastructural changes found in the cortical tissue treated with 3-AP (referred to as the primary focus) were compared to the control operated side.

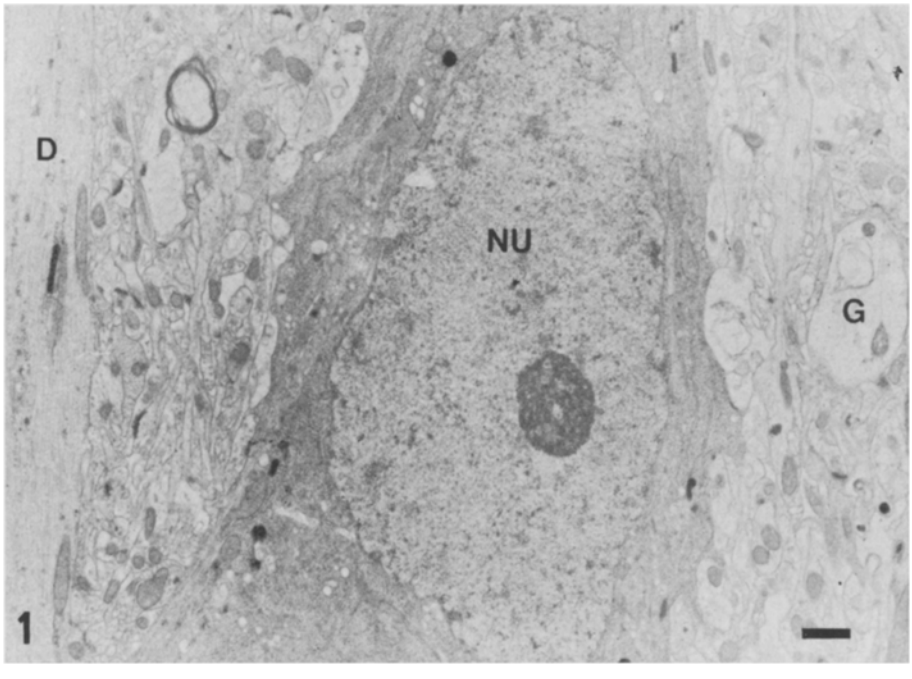


Fig. 1. Shrunken pyramidal cell in the 3rd layer of the cerebral cortex in the primary focus. Note the increased electron density of the neuronal cytoplasm and the swollen glial process at G. D, dendrite; NU, pyramidal cell nucleus. Bar represents 1.0 µm.