development (*11*); it is higher at 11 days in HAS duodenal phosphatase than in LAS (Table 1).

This reduction in duodenal phosphatase activity in HAS mice indicates that some strain-specific difference distinguishes the milks of the two strains. Since changing litter size (and milk availability) does not alter phosphatase activity within either strain, it appears that the milk of one strain contains a constituent that is absent from (or present in ineffective concentration in) the milk of the other. Attempts to identify the factor by injecting likely substances (serine phosphate, glycerophosphate, glucose, hypertonic saline) into the stomachs of young mice have so far proved unsuccessful.

In normally developing young mice, the relatively low-ratio, low-activity phosphatase present at 11 days is replaced in the third week by two isozymes of higher ratio and activity (*14*). The replacement may be elicited precociously by actinomycin D, puromycin, cycloheximide, and ethionine, and thus appears to depend on conversion of low- to high-ratio forms rather than to *de novo* synthesis of the latter (*15*). It has been proposed that the inhibitors of protein synthesis act by removing a regulatory protein that restrains the conversion reaction, perhaps by limiting the rate at which the phosphatase-containing microvillar surface is allowed to mature (*16*). Since the normal tendency in development is toward higher ratio and higher activity (*11*), it seems reasonable to assume that the postulated restraining mechanism is weaker during the first 11 days in HAS duodenum than in LAS; thus the alkaline phosphatase activity is permitted to rise to higher levels in HAS infants than in LAS. If this is so, the role of the milk factor may be to oppose the action of the regulatory protein in the HAS, which strain loses activity when the young are taken off the milk of their natural mothers. Only the HAS is susceptible to the influence of the milk factor; since both strains are inbred, this distinction seems to be basically genotypic.

Another significant observation is that the reduced enzyme activity in HAS mice nursed by LAS mothers remains almost double the activity in the LAS. The obvious inference is that the difference between the two strains reflects at least two regulatory factors: in part the higher level of activity in the HAS must result from inherent genetic differences with respect to the determination of alkaline phophatase; but in part it reflects the action of a strain-specific milk factor that functions as an environmental determinant of variation.

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Metabolism of Alveolar Cells: Histochemical Evidence and Relation to Pulmonary Surfactant

Abstract. *Large alveolar cells of normal dog lung are rich in enzymes concerned with oxidative and synthetic pathways. In three experimental situations where ability of the lung to produce surfactant was impaired, the enzyme-rich cells were lacking or absent. Findings support the concept that these cells are sites of active metabolism, possibly including production of surfactant.*

There is evidence that the lung is not a passive organ merely for exchanging oxygen and carbon dioxide, but that it also performs active metabolic functions (*1*). One function that has received special attention is the synthesis of fatty acids and phospholipids that are important ingredients of the surface-active lining, essential to alveolar stability (*2*).

To learn more about metabolic pathways in lung tissue and to identify the cellular elements most active biochemically, we examined fresh, quick-frozen lung sections for oxidative enzyme activity. To test the hypothesis that alveolar cells may have a special role in the production of pulmonary surfactant, we compared these enzyme reactions in lungs known to synthesize phospholipids and to contain surfactant (normal dog) with those in lungs whose ability to lower surface tension is either questionable (chicken) or lacking (frog, and in dogs after pulmonary artery ligation). Alveolar cells in lungs of normal dogs showed evidence of considerable metabolic activity, consistent with a biosynthetic function. The large enzyme-rich cells were lacking or absent in the other lungs, which suggests a correlation with capacity of the lung to form pulmonary surfactant.

Under anesthesia and through an open chest incision, a segment of lung was infiltrated with liquid gelatin introduced into the airway. Gelatin was allowed to solidify by pouring cold saline on the surface of the lung (*3*). A sample of lung was then removed; it was immediately quenched in isopentane cooled to about −150°C by

Table 1. Oxidative enzymes in alveolar cells of normal dog. Abbreviations: DH, dehydrogenase; NAD, nicotinamide-adenine dinucleotide; and NADP, nicotinamide-adenine dinucleotide phosphate.

Enzyme *	Coenzyme	Intensity of reaction †
NAD and NADP diaphorases		+ + + +
Isocitrate DH	NAD	+ + +
Succinate DH		+
Malate DH	NAD	+ + + +
α-Glycero-phosphate DH	NAD	+ +
Lactate DH	NAD	+ + +
Glucose-6-phosphate DH	NADP	+ +
6-Phospho-gluconate DH	NADP	+ +
Cytochrome oxidase		+ +

* Technique for diaphorase reactions was that used by Novikoff *et al.* (*4*); for dehydrogenases, as described in Pearse (*5*); and for cytochrome oxidase, according to Burstone (*6*). † Graded without count of granules.

liquid nitrogen and sectioned in a cryostat (Harris International) maintained at −15°C. All sections were 6 μm thick and were incubated for 60 minutes in the appropriate medium (Table 1) with nitro-blue tetrazolium. Usefulness of tetrazolium salts is based on their reduction by accepting electrons from substrate through dehydrogenase and its coenzyme. The reduced forms (formazans), colored and insoluble, serve as markers for the presence of enzyme reactions and for the cellular site. Controls were done without substrate, without coenzyme, and after acetone extraction to remove formazan deposits on lipid (*4*).

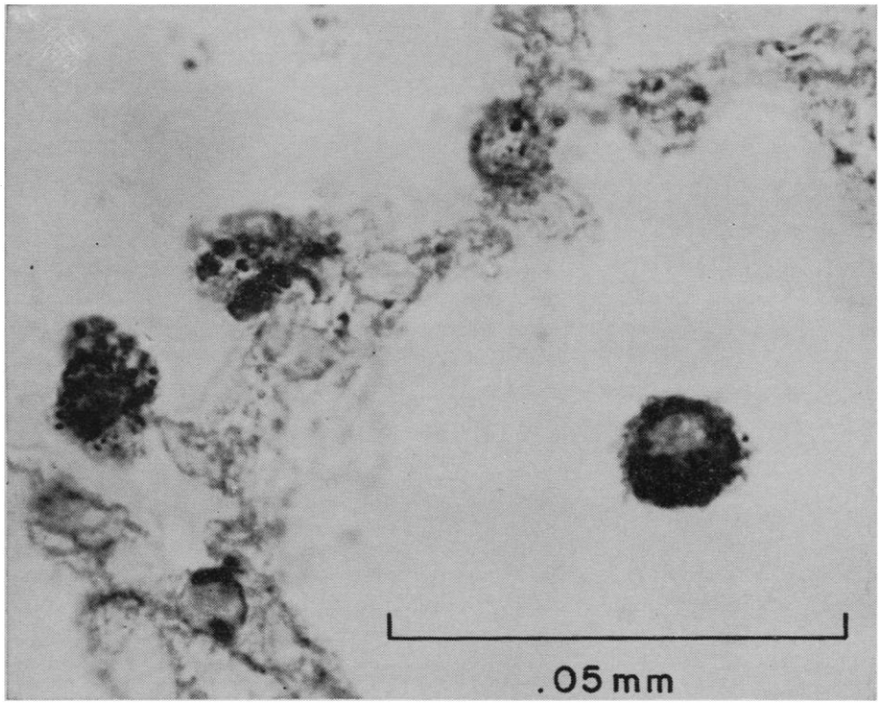

Fig. 1. Nicotinamide-adenine dinucleotide phosphate diaphorase reaction in lung of normal dog (× 1000). Large alveolar cell and free alveolar macrophage show strong reaction; remainder of alveolar wall shows little reaction.

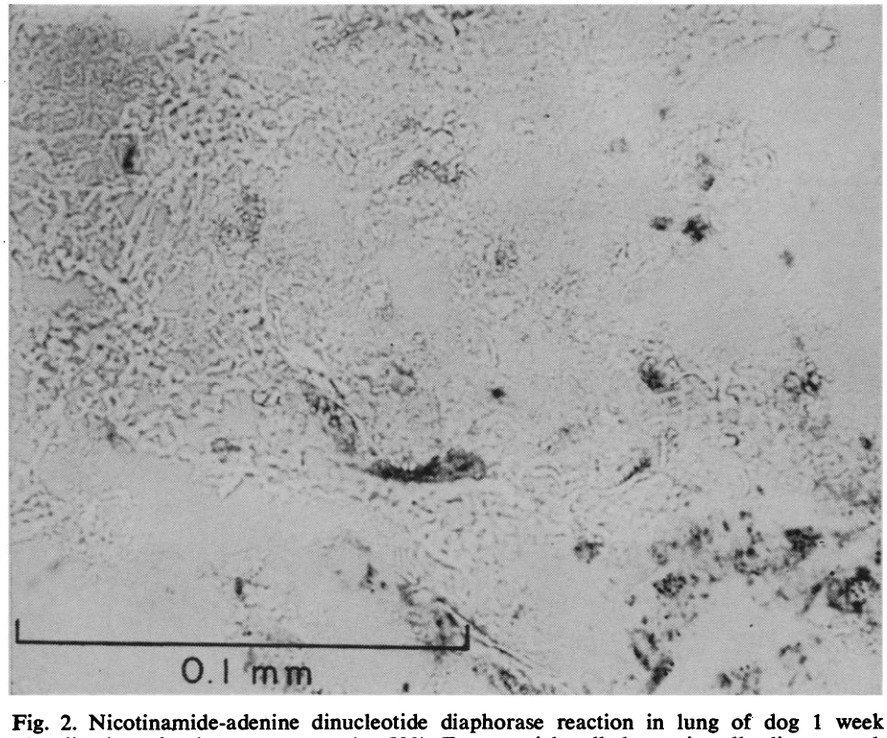

Fig. 2. Nicotinamide-adenine dinucleotide diaphorase reaction in lung of dog 1 week after ligation of pulmonary artery (× 500). Enzyme-rich cells have virtually disappeared.

Alveolar cells (*7*) (great alveolar cells, septal cells, corner cells, granular pneumonocytes, or type II cells) showed strong or moderately strong reactions to almost all enzymes that were tested (Table 1). "Free" alveolar cells (macrophages) gave similar reactions, but other cellular elements in the alveolar wall, that is, squamous epithelial (type I) cells and endothelial cells, reacted weakly or not at all (Fig. 1).

The relative abundance of enzymes of the Krebs cycle and respiratory chain (isocitrate and malate dehydrogenases, and cytochrome oxidase) probably reflects a high level of oxidative metabolic activity (*8*). The presence of these enzymes also correlates with in vitro studies of the alveolar macrophage which reveal that this cell depends to a considerable extent on oxidative phosphorylation and cytochrome-linked respiration to provide energy for phagocytosis (*9*) and that it has a particularly high rate of oxygen consumption (*9*, *10*). The glycolytic pathway, evidenced by lactate and α-glycerophosphate dehydrogenase reactions, is also an important source of energy for phagocytosis (*9*). These similarities between alveolar cell and alveolar macrophage support the notion that they are variants of the same cell (*11*).

A biosynthetic role for the alveolar cell is suggested by the strong reactions for nicotinamide-adenine dinucleotide phosphate (NADP) diaphorase and for dehydrogenases (glucose-6-phosphate and 6-phosphogluconate) of the hexose monophosphate shunt (*8*). This pathway, already demonstrated biochemically in rabbit lung slices (*12*), is an important generator of reduced NADP necessary for the synthesis of fatty acids, and is a source of ribose, required for the synthesis of lecithin (*13*).

Tyler and Pearse (*3*) have recently reported the presence of a large number of oxidative enzymes in the alveolar cells of rat lung. Our results on normal dog lung are in general agreement with theirs, except that they obtained no reaction for succinate dehydrogenase. This relative weakness or absence of succinate dehydrogenase reaction in lung sections is difficult to explain. Sorokin (*14*) found that, as the mammalian lung matures, alveolar

cells contain increasing amounts of glucose-6-phosphate and succinate dehydrogenases. Dannenberg *et al.*, using histochemical methods in vitro, showed the latter enzyme to be active in the alveolar macrophage (*10*).

In preparations of lungs from three animals associated with deficient production of pulmonary surfactant (chickens, frogs, and dogs 1 week after pulmonary artery ligation) the large enzyme-rich alveolar cells were lacking or absent (Fig. 2). Compared to lungs from dogs, lungs from chicken or frogs have lower surface activity as determined on the surface balance (*15*), although bubbles obtained from chicken lungs appear to have normal stability (*16*). Morphologically, the osmiophilic lamellar bodies of mammalian alveolar cells are absent in amphibian lung (*17*) and are present only in restricted areas of avian lung, but not in the air-capillary epithelium (*18*).

That the large alveolar cells are rich in enzymes of oxidative and synthetic metabolism, and that their absence correlates with diminished pulmonary surface activity lend further support to the concept that they are sites of elaboration of alveolar surfactant. Since these enzymes are located in the mitochondria, the findings also correlate with evidence that the synthesis of fattty acids by the lung is most active in the mitochondrial fraction (*19*).

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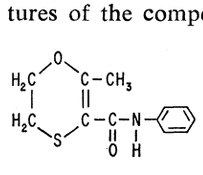
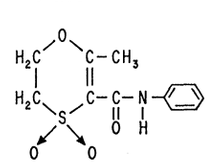
Systemic Fungicidal Activity of 1,4-Oxathiin Derivatives

Abstract. *Treatment of pinto bean and barley seed with 1,4-oxathiin derivatives gave disease control by systemic fungicidal action of such pathogenic fungi as* Uromyces phaseoli *and* Ustilago nuda. *The two chemicals, D735 and F461, were highly specific and selective against the pathogens without injury of the hosts.*

Two compounds, 2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin (D735) and its sulfone analog (F461), were tested for fungicidal activity and found to act systemically against several fungus species when used as foliar sprays or for treating soil or seed. The structures of the compounds are



D735



F461

These chemicals (*1*) appear to be particularly effective in controlling plant pathogenic fungi such as wheat leaf rust, *Puccinia rubigo-vera tritici* (Eriks) Carleton; bean rust, *Uromyces phaseoli typica* Arth.; loose smut of barley, *Ustilago nuda* (Jens.) Rostr. and *Rhizoctonia solani* Kühn. In general, various analogs showed no, or considerably less, fungitoxicity; that is, the free carboxylic acid and its esters and *N*-alkylamides were quite ineffective.

Table 1. Loose smut control of barley caused by *Ustilago nuda* in field experiments with seed treatments of 1,4-oxathiin derivatives at three dosages.

Treatment	Chemical (% by wt)	Seed heads (total No.)	Seeds infected	
			No.	Percentage
D735	0.125	745	14	1.9
D735	.25	723	5	0.7
D735	.5	701	0	.0
F461	.125	736	107	14.5
F461	.25	718	90	12.5
F461	.5	693	62	8.9
None		656	114	17.5

Compared with protectants relatively little progress has been made in the development of systemic fungicides (*2*), and there are numerous reviews on this subject (*3*). We now describe the systemic fungicidal activity of compounds D735 and F461 against bean rust and loose smut.

Seeds of pinto beans, *Phaseolus vulgaris* (l.), were treated with D735 and F461 by tumbling the finely ground chemical with the seed in a glass jar, 0.25 percent of chemical by weight of seed being used. The seeds were planted in 4-inch pots in the greenhouse, and then the separate sets of plants were inoculated, at 1- and 2-week intervals, with uredospores of bean rust, *Uromyces phaseoli.* Bean plants grown from untreated seeds were included in the test. Both chemicals effectively controlled the development of rust symptoms on the primary bean leaves when inoculated 7 days after planting. At this interval D735 gave 99 percent and F461 100 percent disease control. In the 2-week interval between planting and inoculation, F461 gave 99 percent control on the primary leaves and 96 percent on the trifoliolates, whereas D735 failed to control rust on any of the leaves. In these tests the untreated plants had an average of 12 pustules per square centimeter. One can speculate from these results that the sulfone is the more stable form in the plant.

Foliar disease control was obtained by seed treatment without causing injury to the plants. Both materials are fairly water soluble (D735 approximately 170 parts per million and F461 approximately 1000 parts per million) and appear to be readily translocated in the transpiration stream (xylem) to the site of the pathogen. Similar disease control was obtained by foliar