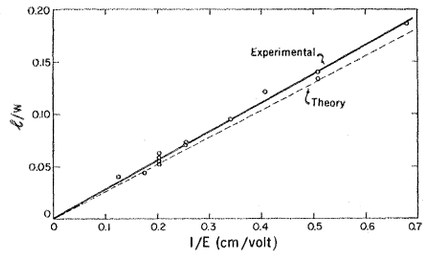

Fig. 3. Linear plot showing the effect of the field strength E on the thickness l of the solute layer.

sec for D (*5*) and -6.5×10^{-5} cm²/sec volt for μ (*6*).

The agreement between theory and experiment in Fig. 3 is exceptionally good. Some results for different systems show a comparable agreement with theory, whereas others are in accord only with the trend of the theoretical curve, and show departure in some important detail. Hemoglobin at *p*H 8.0, for instance, produced a line intercepting the positive ordinate rather than the origin. All proteins at *p*H 4.5 generated linear plots of approximately correct slope but with negative intercepts.

The reasonable agreement between theory and experiment makes it possible to use theory as a tool in tailoring experimental conditions for optimal EFFF separations.

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Induction of Pulmonary Edema and Emphysema in Cattle and Goats with 3-Methylindole

Abstract. *Microorganisms from rumen converted* L-*tryptophan and indoleacetic acid to 3-methylindole in vitro. Oral doses of 3-methylindole caused interstitial pulmonary edema and emphysema in cattle and goats. Intravenous infusion of this metabolite also induced pulmonary disease in cattle. These results demonstrate than an end product of ruminal fermentation of tryptophan can induce acute pulmonary disease in cattle and goats.*

Acute bovine pulmonary emphysema (ABPE) has been recognized as a naturally occurring disease of cattle for over 100 years (*1*). Despite substantial research, the etiology of the disease remains unknown. Our investigations have shown that oral doses of DL-tryptophan (0.57 to 0.70 g per kilogram of body weight) cause interstitial pulmonary edema and emphysema in cattle (*2, 3*). The onset, clinical signs, and pulmonary lesions of the tryptophan-induced disease are similar to those seen in naturally occurring ABPE. Cattle affected by the naturally occurring or experimentally induced disease have labored, irregular breathing with an increased respiration rate; they often die from interstitial pulmonary edema and emphysema within 1 to 7 days after exposure to inducing agents. Necropsy reveals over-inflated lungs, dark red in color and firmer than normal; alveoli are edematous and ruptured, and there is proliferation of alveolar epithelial cells with variable infiltration of neutrophils or eosinophils (*4, 5*).

Only oral doses of DL-tryptophan are effective in causing pulmonary disease even though comparable increases in tryptophan concentrations in plasma are obtained after intraperitoneal injection or intravenous infusion of tryptophan (*3*). These results suggest that a product of tryptophan fermentation in the rumen may be a causative factor in the development of tryptophan-induced pulmonary disease in cattle. Oral doses of tryptophan do not cause pulmonary disease in sheep (*3, 5*) or in goats (*5*). The objective of our experiments was to identify a product of ruminal tryptophan fermentation that is capable of causing pulmonary edema and emphysema in cattle. Identification of this metabolite may be significant in the search for causative agents in pulmonary disease of other species.

We tested the effect of D- and L-tryptophan on the development of pulmonary edema and emphysema in cattle. Three steers given an oral dose of D-tryptophan (0.4 g/kg) and one steer given 0.35 g/kg orally did not develop clinical signs of pulmonary disease. An oral dose of L-tryptophan (0.35 g/kg) caused pulmonary disease in two steers. The results indicate that L-tryptophan is the effective isomer, and we have subsequently used L-tryptophan (0.35 g/kg) for experimental induction of pulmonary disease in cattle. Oral doses of indoleacetic acid also cause pulmonary disease in cattle (*5*). Three of six cows developed clinical signs of pulmonary disease, and two died after being given an oral dose of indoleacetic acid (0.6 g/kg).

Our experiments were designed to identify the products of ruminal fermentation of L-tryptophan, D-tryptophan, and indoleacetic acid. We incubated these compounds (10 mg) in vitro for 8 hours under anaerobic conditions with strained rumen fluid (25 ml) obtained from a fistulated steer fed only hay. The D and L isomers of [^{14}C]tryptophan were isolated from DL-[^{14}C]-tryptophan (uniformly labeled in the benzene ring) by paper chromatography according to the method of Loh and Berg (*6*) except that *N*-butanol was substituted for 2-pentanol in the solvent system. The compounds L-[^{14}C]-tryptophan (0.55 μc), D-[^{14}C]tryptophan (0.35 μc), and [2-^{14}C]indoleacetic acid (1.0 μc) were added to the appropriate flasks of rumen fluid. Fermentation metabolites were separated by ion-exchange chromatography (*7*) and were identified by thin layer chromatography on silica gel plates with three solvent systems: (i) isopropanol, ammonia, water (20 : 1 : 2), (ii) butanol, acetic acid, water (12 : 3 : 5), and (iii) 6 percent benzene/chloroform. Ehrlich reagent (*8*) was used to locate the tryptophan metabolites.

The chief product of L-tryptophan fermentation was 3-methylindole (3MI); indole and indoleacetic acid were also detected as end products. D-Tryptophan was not converted to any of these metabolites under the experimental conditions. Indoleacetic acid was also converted to 3MI by the microorganisms of the rumen. Indole was present in control rumen fluid incubations without added substrate. An Ehrlich-positive metabolite, tentatively identi-

fied as 5-hydroxy-3-methylindole, was produced when 5-hydroxyindoleacetic acid was used as the substrate. The results indicate that L-tryptophan and indoleacetic acid are converted in vitro to 3MI by microorganisms in the rumen.

We tested the ability of 3MI to induce pulmonary edema and emphysema in cattle and goats. Oral doses of 3MI were given as a water slurry with a stomach tube and hand pump. Necropsies were conducted on all animals. Two goats given an oral dose of 3MI (0.3 g/kg) in an early experiment died as a result of diffuse pulmonary edema at 36 and 54 hours after being given the dose. The morning feed was withheld before giving the same oral dose to four additional goats. These goats then developed clinical signs of pulmonary disease within 4 hours, and they died from severe pulmonary edema between 5 and 11 hours after administration of 3MI. No significant pulmonary lesions were present in two control goats given an equal volume of water. The earliest clinical signs of pulmonary disease appeared between 6 and 12 hours after giving three cows an oral dose of 3MI (0.2 g/kg). These cows died from pulmonary edema and emphysema at 33, 69, and 73 hours after administration of 3MI. Two cows given 3MI orally (0.1 g/kg) developed clinical signs of pulmonary disease within 12 to 24 hours; pulmonary lesions were present at necropsy 4 days after administration of the dose. No significant pulmonary lesions were present in one control cow.

We gave 3MI (0.06 g/kg) to three cows as 12-hour intravenous infusions. The infusion solution consisted of 0.11 g of 3MI per milliliter of propylene glycol. Clinical signs of pulmonary disease appeared between 6 and 12 hours after beginning the infusion in all cows, and one cow died from pulmonary edema and emphysema at 56 hours. Pulmonary edema and emphysema were present in the remaining two cows at necropsy 3 days after the beginning of infusion. Propylene glycol infusion caused no significant pulmonary lesions in two control cows. Some hemolysis and hematuria was noted in cows infused with 3MI, but not in cows given oral doses of 3MI. The results indicate that 3MI can cause pulmonary edema and emphysema in cattle and goats when given either as a single oral dose or as an intravenous infusion.

Oral doses of 3MI are more effective in inducing pulmonary edema and emphysema in cattle than are oral tryptophan doses. Clinical signs of pulmonary disease and death occur earlier with 3MI, and with lower molar equivalent doses, than with tryptophan. Also, a lower intravenous dose of 3MI induced more severe pulmonary disease than did the lowest oral dose. These results suggest that 3MI is the ruminal metabolite of tryptophan responsible for the development of pulmonary emphysema and edema in cattle. Since 3MI can cause severe pulmonary edema and emphysema when given intravenously, it is probable that it, or a metabolite, acts directly on the lung to cause pulmonary disease. The ability of 3MI and other indoles to disrupt biological membranes is related to the lipophilic properties of these compounds (*9*). Aryl molecules, including 3MI and related indoles, hemolyze erythrocytes and cause disintegration of certain protozoa in the rumen (*10*). When injected into rabbit knee joints, 3MI and related compounds such as indole, 5-methylindole, 5-cyanoindole, 5-nitroindole, 5-bromoindole, 1,2-dimethylindole, and others can also cause acute arthritis (*9*). Of these, 3MI and 5-bromoindole cause the most severe lesions. The lesions include hypertrophy and proliferation of synovial lining cells along with congestion and edema of the synovial membrane. These lesions resemble those induced by intraarticular injections of streptolysin S which ruptures lysosomes (*11*).

Aerosols of homogenized leukocytes and papain have been used to experimentally induce emphysema in dogs and other animals; the lesions resemble those in human pulmonary emphysema (*12*). A theory that pulmonary emphysema results from the release of proteolytic enzymes in the lung has been proposed (*12, 13*) as follows: (i) the deficiency of α_1-globulin with trypsin-inhibiting properties in serum of individuals susceptible to emphysema might allow proteolytic destruction of pulmonary cell membranes; (ii) adverse effects of noxious substances may cause immobilization of leukocytes and degradation of cellular membranes of leukocytes, alveolar macrophages, or other cells.

High doses of 3MI cause acute pulmonary disease in cattle and goats; since 3MI is capable of disrupting cellular membranes, it is possible that the presence of indole derivatives may be involved in the etiology of pulmonary emphysema in animals. The relationship, if any, between experimentally induced pulmonary edema and emphysema in cattle and naturally occurring pulmonary disease in humans is not known, but substantial quantities of 3MI, indole, and other neutral indole derivatives have been identified in cigarette smoke (*14*). Cigarette smoke residue is also toxic to ciliated protozoa and results in degradation of internal mitochondrial membranes, loss of ciliary activity, and cell death (*15*). These findings, along with our experimental results, raise the question of whether cronic forms of human pulmonary emphysema could result from long-term exposure to the neutral indole derivatives present in cigarette smoke.

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