warming was begun. The reflectivity increased very little up to 140°K, where a more rapid increase began; then at ~ 152°K the reflectivity started to decrease. At 153°K the sample temperature rose rapidly, an indication of crystallization, and the reflectivity showed a corresponding rapid but small increase. The reflectivity then continued to increase rapidly and reached a maximum at ~ 212°K. At this point the reflectivity was > 90 percent of that of a surface freshly coated with MgO smoke.

One may speculate that the decrease in reflectivity just before crystallization is due to the glass transition (*7*) where the amorphous solid becomes a viscous liquid. The further increase in reflectivity after crystallization is attributed to crystal growth from dimensions smaller than the wavelength of light to dimensions greater than the wavelength of light.

When the photomultiplier and its light pipe were moved to an angle of 45 deg from the surface to measure specular reflectivity, a thin layer of amorphous ice greatly reduced the light reflected from polished copper, an indication of a dull rather than a glassy surface for the amorphous ice.

Note added in proof: One of the referees of our revised manuscript called our attention to a technical comment by Seiber *et al.* (*8*) on the "Density of low temperature ice" with a reply by Delsemme and Wenger (*9*). Seiber *et al.* determined the density by measuring the thickness of the ice formed by the condensation of a known mass of water on a known surface area. The thickness was determined by interference techniques. They obtained a low value of 0.81 ± 0.02 g/cm^3 as compared with our value of 0.94 ± 0.02 g/cm^3. This difference is reasonable since their method gives the bulk density, an average that includes the density of pores and other voids.

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Chemically Induced Porphyria: Increased Microsomal Heme Turnover after Treatment with Allylisopropylacetamide

Abstract. *Excessive induction of δ-aminolevulinic acid synthetase in rats after treatment with porphyria-inducing chemicals, such as allylisopropylacetamide, is accompanied by a decrease in microsomal heme and cytochrome P450 concentrations. Measurement of the radioactive decay after labeling of the heme moiety of submicrosomal particles shows increased breakdown of heme in rats treated with allylisopropylacetamide. The effects of allylisopropylacetamide on heme synthesis and heme turnover may be interrelated.*

2-Allyl-2-isopropylacetamide (*1, 2*) and diethyl-1,4-dehydro-2,4,6-trimethylpyridine-3,5-dicarboxylate (DDC) and certain of its analogs (*1, 3*) produce a biochemical situation in rodents mimicking human hepatic porphyria—that is, the hepatic content and excretion of porphyrins and their precursors are increased. This abnormality in both human and chemically induced porphyria is an immediate consequence of the excessive de novo synthesis of hepatic δ-aminolevulinic acid synthetase (ALA-synthetase), the rate-controlling enzyme in porphyrin and heme biosynthesis (*1, 4*).

A larger number of chemicals, characterized by phenobarbital, induce more modest and circumscribed increases in ALA-synthetase in the same animals, seemingly providing heme for increased formation of microsomal cytochrome P450 (*5*). Because of the limited increase of ALA-synthetase, animals so treated do not exhibit the prolonged increases in urinary excretion of δ-aminolevulinic acid, porphobilinogen, and porphyrins which are characteristic of hepatic porphyria. The marked difference in the induction of ALA-synthetase between these drugs and porphyria-inducing chemicals like allylisopropylacetamide and DDC has remained unexplained.

The present studies are based on the following findings. (i) End product control of heme biosynthesis primarily by repression of ALA-synthetase constitutes an important regulatory mechanism in liver (*1, 5*). (ii) Administration of porphyria-inducing chemicals like allylisopropylacetamide and DDC decreases the concentration of heme and of cytochrome P450 in liver microsomes (*6*). We determined whether decreased synthesis or increased degradation is responsible for the decrease in cytochrome P450 and, further, whether this phenomenon is related to the induction of ALA-synthetase.

Male Sprague-Dawley rats of the Holtzman strain (180 to 220 g) were fasted for 24 hours before the beginning and throughout the course of the experiments. After the rats were decapitated, the livers were perfused with isotonic saline in situ; they were then excised, homogenized in 0.25*M* sucrose, and pooled. To assay ALA-synthetase activity in liver homogenates, the incorporation of [1,4-^{14}C]succinic acid (*7*) or [2,3-^{3}H]succinic acid (*8*) into δ-aminolevulinic acid (ALA) was determined and radioactive ALA was isolated by three consecutive chromatographic procedures (*8*). Cytochrome P450 was determined in liver microsomes by the method of Omura and Sato (*9*). δ[3,5-^{3}H]Aminolevulinic acid (588 mc/mmole, New England Nuclear) was injected intravenously in 0.9 percent NaCl in a dose of 10 μc per 100 g of body weight, and the animals were killed at various times after the injection. Microsomes were prepared by the method of Schneider (*10*). The final microsomal pellet was suspended in 0.1*M* KH_2PO_4–K_2HPO_4 (*p*H 7.0), containing 25 percent glycerol (by volume) and 2 m*M* ethylenediaminetetraacetate. The microsomes were then subjected to anaerobic digestion by treatment with subtilopeptidase (E.C. 3.4.4.16; Nagarse, Nagase

Company, Japan) as described by Nishibayashi *et al.* (*11*). Cytochrome b_5 is almost completely solubilized by this treatment and cytochrome P450 is quantitatively retained in the remaining submicrosomal particle without appreciable conversion to the modified P420 state (*11*). The labeled heme of this cytochrome P450 particle was isolated and recrystallized as described earlier (*5*). [^{3}H]Heme was then subjected to combustion (*12*), and the radioactivity was determined by liquid scintillation counting. Recrystallization of the heme did not reveal significant contamination by labeled nonheme compounds. Recoveries of heme as the pyridine hemochromogen (*9*) were 70 to 80 percent; the radioactivity of the heme was corrected for the losses during isolation. Protein concentrations were determined by the method of Lowry *et al.* (*13*).

The effects of a single intraperitoneal injection of phenobarbital or allylisopropylacetamide on concentrations of ALA-synthetase and microsomal cytochrome P450 are shown in Fig. 1. In contrast to phenobarbital, after injection of allylisopropylacetamide ALA-synthetase activity and cytochrome P450 concentration changed in a reciprocal fashion. The initial decrease in cytochrome P450 preceded a much greater and more prolonged increase in ALA-synthetase, with a slower initial rise.

Radioactive decay of the heme of submicrosomal particles after injection of δ[3,5-^{3}H]aminolevulinic acid was studied after fasting rats were treated for 5 days with allylisopropylacetamide; controls were treated with 0.9 percent NaCl (Fig. 2). The rate of decay was profoundly increased in rats treated with allylisopropylacetamide as compared to controls. In addition, the data indicate a biphasic disappearance of radioactivity, results that are similar to those of Levin and Kuntzman (*14*) who used a different methodology. In control rats the first phase with a half-life of 8 to 10 hours is followed by a slower phase with a half-life of 24 hours. Treatment with allylisopropylacetamide shortened the half-life of the first phase to 1 to 3 hours, producing a rapid initial decrease in radioactivity. Administration of a large dose of unlabeled ALA (3000 nmole/100 g) 3 hours after administration of radioactive acid had no effect on the isotope disappearance curve. Treatment of rats with phenobarbital (80 mg/kg per day, intraperitoneally, for 5 days) did not shorten the first phase of the decay curve.

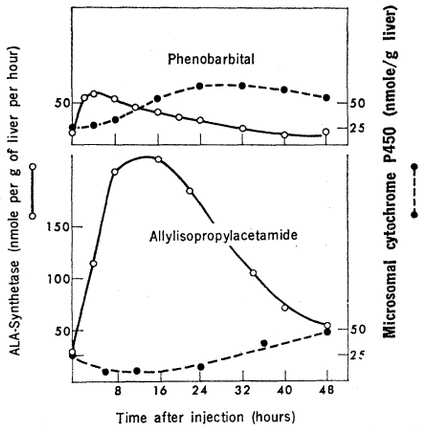

Fig. 1. Hepatic δ-aminolevulinic acid synthetase (ALA-synthetase) and microsomal cytochrome P450 after a single injection of phenobarbital or allylisopropylacetamide. Phenobarbital (80 mg/kg) and allylisopropylacetamide (300 mg/kg) were given intraperitoneally at zero time to fasted male rats. Groups of rats were killed at the times indicated, and enzyme activity was determined in homogenates and microsomes of pooled livers of four rats.

The decline in cytochrome P450 concentrations after administration of allylisopropylacetamide may be related to the rapid initial degradation of microsomal heme, as determined isotopically (Fig. 2). The increased fractional catabolic rate of the initial fast component in submicrosomal particles isolated from rats treated with allylisopropylacetamide does correspond, within the limitations of this study, with the fall in cytochrome P450 and total microsomal heme concentrations. Moreover, the radioactivity in "CO-binding particles" after the injection of labeled δ-aminolevulinic acid is associated with the CO-binding hemoprotein, as judged by column chromatography (*15*). More direct evidence for the association of cytochrome P450 with the rapidly turning over heme fraction must await more complete isolation techniques for this hemoprotein.

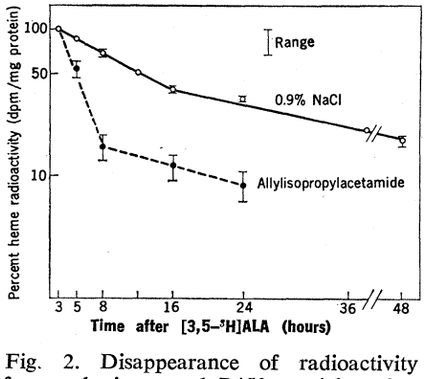

Fig. 2. Disappearance of radioactivity from submicrosomal P450 particles after injection of δ[3,5-^{3}H]aminolevulinic acid in control rats and rats treated with allylisopropylacetamide. Fasted rats were treated with allylisopropylacetamide (300 mg/kg per day) for 5 days; controls were treated with 0.9 percent NaCl. The P450 particles were prepared from pooled livers of four rats at various times after intravenous injection of δ[3,5-^{3}H]aminolevulinic acid (10 μc/100 g). Radioactivity was determined in isolated heme from P450 particles. For comparison, results are expressed as percent radioactivity (*dpm/mg protein*) of the initial value. Some determinations were made in duplicate pools for which mean value and range are given.

The mechanism whereby allylisopropylacetamide or its metabolites promote degradation of microsomal heme is unknown. Allylisopropylacetamide may affect the hemoprotein molecule per se or merely the heme moiety. An action on the heme-protein linkage could create labile pools of heme more susceptible to degradation. For example, heme is rapidly decomposed by lipid peroxides (*16*), which are present during reduced nicotinamide adenine dinucleotide phosphate–dependent cleavage of phospholipid-bound polyunsaturated fatty acids (*17*). Certain agents increase the susceptibility to lipid oxidation in microsomes with formation of peroxide intermediates (*18*).

In addition to these degradative effects on cytochrome P450, allylisopropylacetamide and DDC also diminish the concentrations of another hemoprotein, catalase, by apparently interfering with its synthesis (*19*). If a common mechanism is responsible for both effects, then the only explanation is that porphyria-inducing chemicals stimulate the breakdown of the precursor heme moiety (but not the holoprotein) of catalase, thus resulting in a block in synthesis, whereas at least one form of cytochrome P450-heme is subject to increased degradation even when combined with its apoprotein.

A possible relationship of the allylisopropylacetamide increased microsomal heme turnover and the excessive induction of ALA-synthetase is suggested. Reciprocal control mechanisms between ALA-synthetase and another hemoprotein, tryptophan oxygenase (E.C. 1.13.1.2), have been described (*20*). The rapid turnover of microsomal hemoprotein may divert heme from its repressor function in the regulation of ALA-synthetase. Such a direct causal relationship is uncertain at this time.

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Pulmonary Compliance: Alteration during Infection

Abstract. *Lungs excised from rats infected with* Mycoplasma pulmonis *are more difficult to inflate with air than those from uninfected animals; they show no significant differences from controls inflated with saline. The altered pulmonary function in lungs from infected rats is attributed to an increase in surface forces, implying disruption of the lung surfactant system.*

The cause of impaired respiration during pulmonary infection is not clear. Microbial infection may directly affect the lung tissues or indirectly affect function by altering the lung surfactant (*1*), a phospholipid that decreases the surface tension of the alveoli. Without surfactant, higher pressure would be required to inflate the lung, and lung stability would be impaired. Nothing is known regarding the status of surfactant in pneumonias of microbial origin.

We have used the rat as an animal model to show the effects of *Mycoplasma pulmonis* on pulmonary function. Respiratory function was assessed by the ability of freshly excised lungs to expand. Air and saline fillings were used to separate tissue effects from changes in surface tension.

Mycoplasma-free rats (*2*) were subdued by intramuscular injections of Innovar (*3*) prior to intranasal inoculation with 0.1 ml of a concentrated suspension of *M. pulmonis* (*4*). Control animals were similarly inoculated with growth medium (*5*). One to 5 days later, *M. pulmonis* was isolated from the throats of 90 percent of the animals inoculated with organisms. Throat cultures collected daily showed that, once animals became infected with *M. pulmonis*, they remained so until killed. Some infected animals exhibited either upper respiratory symptoms or middle ear involvement (manifested as labyrinthitis) or both. One rat had a sanguineous nasal exudate. However, most animals were asymptomatic until killed, and no animal died as a result of the infection.

Before they were killed with an overdose of sodium pentabarbital (*6*), infected and control animals were matched according to weight. The lungs were removed before cessation of heartbeat. A 5-mm section was sliced from the apical lobe, and the remaining portion was ligated. The section was minced and inoculated into mycoplasma culture media, cell cultures (*7*), and blood agar plates. Mycoplasmas were isolated from lung material as early as 8 days and recovered from one rat killed 39 days after inoculation. No mycoplasmas were isolated from lungs of control animals nor were bacteria or viruses recovered from the lungs of either the control or infected group.

Lung function was assessed by obtaining continuous pressure-volume (*P-V*) curves. By measuring the pressure needed to expand the freshly excised lungs with air, we calculated total compliance (C_L), the ratio of the change in volume to the change in pressure. The C_L consists of at least two components: (i) the contribution of tissue compliance (C_{tis}) due to elasticity and (ii) the surface compliance (C_{surf}) due to surface tension at the gas-liquid interface in the alveoli. To distinguish these factors, we first inflated and deflated the lungs with air to calculate C_L. An estimation of the tissue forces was determined from a *P-V* curve obtained with the use of saline instead of air, because the gas-liquid interface is presumably eliminated by filling with liquid. The net difference between C_L and C_{tis} is attributed to C_{surf} and was estimated by the relationship

$$\frac{1}{C_L} = \frac{1}{C_{tis}} + \frac{1}{C_{surf}}$$

The *P-V* technique was essentially as described by Beckman and Weiss (*8*). Lungs were attached to an infusion pump by a tracheal cannula (*9*) and inflated and deflated with air at a slow rate of 9.9 ml/min to a maximum pressure of 20 cm-H_2O. After the air cycles, lungs were degassed under vacuum, and the procedure was repeated with saline at 2.5 ml/min (*10*). Filling with saline proceeded to a volume at least equal to that achieved with air at 20 cm-H_2O. Volume and pressure changes were recorded automatically on an XY recorder, thus providing a continuous *P-V* curve.

In the comparison of compliance between infected and uninfected animals (Table 1), the criteria for infection were as follows: recovery of mycoplasmas from the lungs, or recovery from the throat along with serologic evidence of infection (*11*). Mycoplasmas were isolated from the throats of all 12 animals of the infected group. We needed to maintain lung integrity for *P-V* studies. Therefore, only a small segment (5 mm or less) from a single lobe was available for isolation of mycoplasmas from lungs. Even so, mycoplasmas were cultured from the lung biopsies from 6 of the 12 animals included in the infected group.

An analysis of the infected and comparable control group (Table 1) shows that infected animals tended to be smaller. Their lungs were heavier and contained less retained gas. However, none of these differences, including the higher lung weight/body weight ratio, were statistically significant ($P > .05$). Thus, while *M. pulmonis* may have slowed growth of the animals and caused slightly more pulmonary edema