nerve supply led to Wedensky's inhibition of transmission of the nerve impulse at the motor end plates;[5] that lactic acid caused Zenker's waxy degeneration of muscle;[6] that the time of onset of rigor was influenced by the intact peripheral nerves;[7] that occlusion of circulation and muscle activity produced pain and "explosive" changes in the electric potential of the hyperexcitable sensory spindles;[8] that increased hydrogen ion concentration in muscle resulted in the discharge of augmented amounts of acetylcholine at the motor end plates;[9] and that nerve cell proteolysis was produced by lactic acid.[10]

The observations in this paper suggest that the abnormal accumulation of acid metabolites of muscle activity first increase the permeability of the end plates to the gold staining axonic substance, and that with increasing hydrogen ion concentration the end plates undergo complete liquefaction and act like chemical safety fuses.

Summary. Lactic acid in concentrations of 0.05 to 0.3% injected into the exposed zone of innervation of the sternomastoid muscle in the white rat produced sudden destruction of most of the motor end plates within 30 seconds to 25 minutes dependent upon the concentration of the acid. This liquefaction of the hypolemmal axons of the end plates was preceded by a first phase of retraction and a second phase of expansion and fragmentation. Lactic acid caused a progressive depletion, in both the hypolemmal and the epilemmal axons, of auriphilous substances. The blood vessels were enlarged, hyperemic, and contained clumps of agglutinated red blood cells in the rigorous muscle paralyzed by lactic acid. It is suggested that the acid metabolites which accumulate during rigor mortis of muscle are responsible for the destruction of the end plates and that during life certain processes that result in the intramuscular accumulation of acid, such as vascular congestion and anoxia, likewise may result in the destruction of the motor end plates.

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Excretion of Metabolic Products of Sulfapyridine in the Dog.

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It was suggested that, following the oral administration of sulfapyridine in man, water-soluble detoxication products of the drug appeared in the urine.[1] Later it was reported that administration of the drug caused a marked increase in the urinary glucuronides, and the glucuronic acid output paralleled the sulfapyridine levels.[2] The isolation of a hydroxysulfapyridine and a water-soluble hydroxysulfapyridine glucuronide was announced.[3] Details of this work are presented here.

Experimental. Daily doses of 0.5 g per kg body weight of sulfapyridine were fed to dogs and urine samples, collected from time to time, were worked up as follows:

Isolation of the hydroxysulfapyridine: 2400 cc of urine containing 18.0 g of sulfa-

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3 Scudi, J. V., *Science*, 1940, **91**, 486.

pyridine as measured by the method of Bratton and Marshall[4] was acidified with acetic acid and cleared with lead acetate. The filtrate was neutralized with dilute ammonia and treated with a saturated solution of basic lead acetate to maximum precipitation. The filtrate, containing 4.5 g of sulfapyridine, was discarded. The basic lead was removed as the sulfide. The filtrate was saturated again with hydrogen sulfide to complete the removal of lead, and the filtrate (pH 5.0-5.5) was concentrated *in vacuo* at 30° to 35° to a thick syrup. Extraction with chloroform gave 125 mg of crystals. Recrystallized several times from water and dilute alcohol, the hydroxysulfapyridine appeared as pale yellow needles and melted at 180-181° corr. It depressed the melting point of sulfapyridine; gave a positive diazo, ferric chloride, and indophenol reaction.[5] The ultra-violet absorption data obtained with aqueous solutions of the hydroxysulfapyridine have been published.[2]

Calculated for $C_{11}H_{11}O_3N_3S$:
C = 49.81; H = 4.15; N = 15.84
Found:
C = 50.33; H = 4.26; N = 15.96

Isolation of the Silver Salt of the Hydroxysulfapyridine Glucuronide: 250 cc of urine containing 5.0 g of sulfapyridine as measured by the Marshall method[4] was treated with powdered lead acetate to maximum precipitation and the precipitate was discarded. The filtrate, neutralized with dilute ammonia, was treated with saturated basic lead acetate to maximum precipitation. The precipitate was slurried in water and the lead was removed as the sulfide. The filtrate was freed of excess hydrogen sulfide by aeration and the product was precipitated with an excess of mercuric acetate. The mercury precipitate was mixed to a paste with small volumes of water and the mercury was removed with hydrogen sulfide. After removing excess hydrogen sulfide, the product was precipitated by the addition of excess 10% silver nitrate. The silver salt of the hydroxysulfapyridine glucuronide was immediately removed by centrifugation and was thoroughly washed with water, alcohol and ether. Dried at 100° it weighed 154 mg. The product, a pale yellow powder, gave a diazo[4] and a naphthoresorcinol value[6] corresponding to approximately one molecule of glucuronic acid per molecule of sulfapyridine. The indophenol reaction was negative but became positive after acid hydrolysis.

Calculated for
$C_{11}H_9O_2N_3S\ Ag \cdot O \cdot C_6H_8O_6Ag \cdot 3H_2O$:*
C = 28.77; H = 3.24; N = 5.92;
Ag = 30.46; H_2O = 7.61
Found:
C = 28.98; H = 3.38; N = 5.73;
Ag = 30.16; H_2O = 6.85

Isolation of the Brucine Salt of the Hydroxysulfapyridine Glucuronide: On several occasions it was not possible to isolate analytically pure samples of the silver salt. On one occasion the above procedure gave a silver salt containing 32% silver (1.75 g of the silver salt were obtained from 1350 cc of urine containing 13 g of arylamine). This was powdered and suspended in 10 cc of water and the silver was removed as the sulfide. The filtrate was concentrated to a syrup *in vacuo*, and the syrup was taken up in 95% alcohol. Addition of an excess of brucine in alcohol yielded the brucine salt of the hydroxysulfapyridine glucuronide. The product, pale yellow needles, melted with decomposition at 215°

Calculated for
$C_{17}H_{18}O_9N_3S \cdot C_{23}H_{26}N_2O_4 \cdot 4H_2O$:
C = 52.98; H = 5.74; N = 7.72
Found:
C = 53.03; H = 6.03; N = 7.84

The position of the hydroxyl group in the hydroxysulfapyridine has not been completely established. The indophenol reaction[5] is given only by those phenols possessing a free para

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* The sulfonamide hydrogen forms a silver salt. Adding one molecule of silver nitrate to a hot ammoniacal solution of sulfapyridine gives excellent yields of the white crystalline silver salt. Calculated for $C_{11}H_{10}O_2N_3S$ Ag: Ag = 30.33. Found: Ag = 30.07.

position, and it is not given by pyridones. Hence the hydroxyl group can only be in the 3-position of the pyridine ring or in the benzene ring ortho or meta to the primary amine. Through the courtesy of Dr. A. R. Day, who prepared the ortho hydroxy derivative of sulfapyridine, we have been able to rule out this possibility. The meta hydroxy derivative has not been ruled out, but we have been inclined to favor the 3-position in the pyridine ring as being the more plausible. This postulation is in agreement with the recent work of Weber *et al.*[7] which definitely places the hydroxyl group in the pyridine ring.

Discussion. These results indicate that the urinary elimination of sulfapyridine is more complex than hitherto recognized. Since the hydroxysulfapyridine and the water-soluble glucuronide possess free arylamino groups they are measured by diazotization procedures along with the "free" sulfapyridine. Their therapeutic efficiency may not be equivalent to that of the unchanged drug. Further, the excretion of a part of the drug in a highly soluble form is important in the etiology of sulfapyridine urolithiasis.[8]

Summary. Following oral administration of sulfapyridine, a hydroxysulfapyridine and a water-soluble hydroxysulfapyridine glucuronide have been isolated from dog urine.

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Microbiological Differentiation of Pyridoxine and Pseudopyridoxine.*

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Snell, Guirard, and Williams[1] obtained abnormally high pyridoxine values when urine was assayed with *Streptococcus lactis R* against a pyridoxine standard. These workers demonstrated that the effect was due to the presence in urine of a metabolite of pyridoxine which they called pseudopyridoxine. It was found that pseudopyridoxine could be formed by the interaction of pyridoxine and amino acids during autoclaving.[2]

Using yeasts and molds for pyridoxine assay, other workers (Robbins and Ma,[3] Carpenter *et al.*,[4] Atkin *et al.*,[5] and Stokes *et al.*,[6]) did not obtain abnormally high values on materials known to contain pseudopyridoxine. It has therefore been postulated that these organisms do not respond to pseudopyridoxine.

While Snell *et al.*[2] obtained evidence that *S. lactis* utilizes only pseudopyridoxine and not pyridoxine, no conclusive evidence has been obtained that yeasts do not utilize both pyridoxine and pseudopyridoxine. The high assay values obtained with *S. lactis* merely reflect the small conversion of pyridoxine to pseudopyridoxine in the standard. If both compounds are active for an organism, abnormally high assay values would not be expected.

* The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Illinois.

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