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Reduction of 2-(p-Nitrobenzenesulfonamido)-Pyridine to Sulfapyridine in the Animal Body.

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The substitution of a nitro for the amino group in sulfonamides, produces compounds with bactericidal activity *in vivo*[1, 2] and *in vitro*.[3] Flynn and Kohl[4] have shown that p-nitrobenzenesulfonamide is capable of reduction in the body. We became interested in 2-(p-nitrobenzenesulfonamido)-pyridine† because of its apparent lack of toxicity on oral administration in spite of its antistreptococcal activity *in vivo*.[5] This finding was of sufficient interest to warrant a study of the fate of 2-(p-nitrobenzenesulfonamido)-pyridine after oral and intravenous administration in the dog, and following oral administration in man, rabbit, and the rat.

2-(p-Nitrobenzenesulfonamido)-pyridine is an insoluble, almost colorless compound which melts with decomposition at 192-3°C (corrected). On reduction with tin and HCl, sulfapyridine is obtained. The solubility in water is less than 1 mg % at 20°C.‡ This compound has also been described by Burton, *et al.*, but they did not give any specific data except to state that due to its insolubility *in vitro* studies were difficult.

In order to study the fate of 2-(p-nitrobenzenesulfonamido)-pyridine in the animal body, the following procedure was used in determining this compound in the blood. Two cc of blood were diluted to 40 cc with distilled water and placed in a boiling water bath for 3 minutes. Seven drops of 1% acetic acid were added, and the tube was heated in the bath for another 3 minutes. After cooling, it

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1 Mayer, R. L., and Oechslin, C., *Compt. Rend. Acad. Sc.*, 1937, **205**, 181.

2 Buttle, G. H., Foster, T. D. G. E., Gray, W. H., Smith, S., and Stephenson, D., *Biochem. J.*, 1938, **32**, 1101.

3 Burton, H., McLeod, J. W., McLeod, T. S., and Mayr-Harting, A., *Brit. J. Exp. Path.*, 1940, **5**, 288.

4 Flynn, L. M., and Kohl, M. F. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 466.

† These nitro compounds have been kindly supplied by George A. Breon and Co.

5 Unpublished data.

‡ Personal communication with Doctor H. P. Brown (1940).

was diluted to 50 cc, mixed, and filtered. Bratton and Marshall's method[6] was applied, using 9 cc filtrate and 1 cc 4 N HCl. Another portion of filtrate (18 cc plus 2 cc 4 N HCl) was shaken for 5 minutes with 0.5 g zinc dust to effect a reduction, filtered, and 10 cc of this filtrate was used for the color determination. The difference between these two values gave a very good index of the quantity of 2-(p-nitrobenzenesulfonamido)-pyridine present. Determinations in the urine were made by employing appropriate dilutions of urine and treating the diluted urine in the same manner. After adding 2-(p-nitrobenzenesulfonamido)-pyridine to blood, it was possible by this method to account for 85-90% of the compound present.

The toxicity of orally ingested 2-(p-nitrobenzenesulfonamido)-pyridine in the dog, rat, or mouse is practically *nil*. This low toxicity is apparently due to poor absorption as shown by the low blood level. After administering 0.5 g/kg suspended in 5% acacia in a single dose, the highest blood level reached in 3 hours was 1.1 mg %. Following repeated doses of 0.2 g/kg at 2-hour intervals for 10 hours, we succeeded in raising the blood level to 4.0 mg %. Slow intravenous injection of a 0.1% solution of 2-(p-nitrobenzenesulfonamido)-pyridine in alkaline saline (.005 N NaOH) produced toxic reactions manifested in the form of vomiting and salivation, when the blood level reached 6-7 mg %. At a level of 10-11 mg % defecation and violent retching became evident. These symptoms subsided in the course of 3-4 hours without any apparent harm to the animal.

A striking difference was shown in toxicity of p-nitrobenzenesulfonamide and 2-(p-nitrobenzenesulfonamido)-pyridine following oral administration. This difference is due to two factors: more rapid absorption of p-nitrobenzenesulfonamide, and an apparent inability of the kidney to eliminate this compound. The blood levels at which toxicity became apparent were essentially the same for both drugs. Following oral doses of 0.1 g/kg of p-nitrobenzenesulfonamide the dogs manifested toxic symptoms and showed high blood levels over a period of 24-48 hours due to the slow excretion from the kidney. Even though 2-(p-nitrobenzenesulfonamido)-pyridine was poorly absorbed we found that Marshall's method showed a considerable amount of the reduced compound in the blood. The concentration of the reduced compound did not reach its highest peak until 12 hours after the oral administration of 2-(p-nitrobenzenesulfonamido)-pyridine. Following intravenous administration, although the level of 2-(p-nitrobenzenesulfonamido)-pyridine was

[6] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

TABLE 1.
Comparison of Blood and Urine Levels in the Dog Following Administration of p-Nitrobenzenesulfonamide and 2-(p-Nitrobenzenesulfonamido)-pyridine.

Time in hr	2	4	8	12	24	48	72
p-Nitrobenzenesulfonamide							
Intravenous							
.06 g/kg 4-hr period							
		Blood mg%					
N	4.0	5.8	4.7	4.1	2.8	1.4	0.6
R	0.2	0.4	1.0	1.2	1.4	1.1	0.4
		Urine mg total excreted.					
N	10.2	27.4		45	54	66	74
R	2.0	8.5		74	162	287	375
Oral 0.1 g/kg in 2 doses							
		Blood.					
N		5.5*	6.0*		4.0	2.0	1.0
R		1.0	1.0		1.5	1.3	1.0
		Urine.					
N		20	22		22	22	22
R		4.0	8.0		186	372	489
2-(p-Nitrobenzenesulfonamido)-pyridine							
Intravenous							
0.1 g/kg 4 hr period							
		Blood.					
N	1.7	3.3	0.8	0.5	0.4	0.4	
R	0.15	0.15	0.5	0.4	0.3	0.0	
		Urine.					
N	152	297			798	815	
R	3.7	9.9			370	483	
Oral 0.5 g/kg single dose							
		Blood.					
N		0.5	0.5	0.3	0.4		
R		1.0	2.0	3.2	2.8		
		Urine.					
N			27		27		
R			114		514		

N—Nitro compound, R—Reduced compound. *Retching and Vomiting.

much higher than after oral administration the amount of reduced compound was much lower, suggesting that reduction occurs before the drug reaches the blood. Urinary excretion also reflected the greater reduction after oral administration.

We believe this difference can best be explained by assuming that much of the reduction of 2-(p-nitrobenzenesulfonamido)-pyridine takes place in the large intestine. To substantiate this assumption, incubation of dog's ileal and colonic contents mixed with 2-(p-nitrobenzenesulfonamido)-pyridine gave high values for reduced compound, while the contents of the stomach and duodenum showed only traces. From tryptose phosphate broth containing 2-(p-nitrobenzenesulfonamido)-pyridine inoculated with *B. coli communior* and incubated at 37.5°C for 5 days, we were able to isolate a compound having a melting point 190-191°C and a diazo-color value of sulfapyridine, hence one reduction product of 2-(p-nitrobenzenesul-

fonamido)-pyridine is sulfapyridine. Undoubtedly the greater part of the reduction takes place in the intestinal tract. However, macerated liver or kidney incubated at 37.5°C in a phosphate buffered solution (pH 7.4) also caused a reduction of 2-(p-nitrobenzenesulfonamido)-pyridine.

2-(p-nitrobenzenesulfonamido)-pyridine fed to rabbits was isolated from urine in the form of acetylsulfapyridine. Following oral administration of 7-8 g of 2-(p-nitrobenzenesulfonamido)-pyridine daily to man, free sulfapyridine was isolated from the urine.

We wish to point out that 2-(p-nitrobenzenesulfonamido)-pyridine may have a therapeutic application in the treatment of lower intestinal infection, since the reduction of 2-(p-nitrobenzenesulfonamido)-pyridine in the intestinal tract by *B. coli* would undoubtedly give rise to significant concentrations of sulfapyridine and probably to other reduction compounds which might be therapeutically effective.

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Injection of Heavy Water into the Cerebro-Spinal Fluid Space.*

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After Barbour[1] and his associates had demonstrated the sympathicomimetic action of heavy water in mice when 1/5 of the body water was replaced by heavy water it seemed interesting to study the effect of partial replacement of cerebrospinal fluid by normal saline made up of heavy water. The experiments were carried out in 2 akinetic catatonic patients (male, age 35, female, age 30). A certain amount (see below) of cerebrospinal fluid was withdrawn and replaced by a physiological saline solution made up in 99.5% deuterium oxide instead of water. This was done by lumbar puncture in the recumbent position and the head lowered during the injection. In the first case 2 cc and 3.8 cc of heavy water saline were thus injected at an interval of 6 days. In the second case 10 cc were injected first, 22 cc and 14.2 cc later with an interval of 14 days between each injection. The cerebrospinal fluid was examined 8 hours after

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[1] Barbour, H. G., and Herrmann, J. B., *J. Pharm. and Exp. Ther.*, 1938, **62**, 158.