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Acute Toxicity of Sodium Salts of Sulfapyridine, Sulfathiazole and Sulfamethylthiazole.*

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Comparing the acute toxicity of the sodium salts of sulfapyridine, sulfathiazole and sulfamethylthiazole in mice, Barlow and Homburger[1] found that sulfapyridine administered orally appeared to be considerably more toxic than the thiazole derivatives (3.4 times as toxic as sulfamethylthiazole, and 4.57 times as toxic as sulfathiazole). Long, Haviland and Edwards,[2] using the subcutaneous route for determination of the acute toxicity of the same compounds, reported sodium sulfamethylthiazole (LD_{50}0.86 g/kg) to be more toxic than sodium sulfapyridine (LD_{50}1.0 g/kg), while sodium sulfathiazole was found to be about half as toxic as the latter drug (LD_{50}1.95 g/kg). Van Dyke, Greep, Rake and McKee,[3] in similar experiments placed the LD_{50} for sodium sulfapyridine at about the same level (0.95 g/kg), whereas the LD_{50} of sodium sulfathiazole was found to be at 1.45 g/kg. They made the interesting observation that with similar doses (0.5 g/kg) both compounds were equally toxic, while after administration of large amounts (1.0 g/kg), sodium sulfapyridine appeared to be considerably more toxic. Fifty percent of the mice receiving sulfapyridine died within 3 hours, whereas none of the mice receiving sulfathiazole died spontaneously at the end of 6 hours. Since the blood concentrations of both compounds were found to be about the same within the aforementioned time interval, the early death after sodium sulfapyridine could not be correlated with the level of the drugs in the blood. Long and coworkers[2] stressed the fact that the oral route of administration is not valid for the assay of the acute toxicity of poorly soluble derivatives of sulfanilamide. They pointed out that the solutions of the sodium salts are very alkaline (pH 10 to 11) and the sodium ion therefore is split off in blood and tissues; hence by means of the parenteral

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1 Barlow, O. B., and Homburger, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 317.

2 Long, P. H., Haviland, J. W., and Edwards, L. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 328.

3 van Dyke, H. B., Greep, R. O., Rake, G., and McKee, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 410.

route the true toxicity of the poorly soluble parent drug can be determined with accuracy.

Potentiometric titration of the sodium salts of the 3 compounds, however, showed that precipitation occurs after a slight decrease of pH and is complete at pH 9 for sodium sulfapyridine and at pH 7 for sodium sulfathiazole and sodium sulfamethylthiazole (Lott and Bergheim,[4] Fosbinder[5]). It must be assumed, therefore, that after subcutaneous injection of the sodium salt the parent drug is precipitated out almost completely in the subcutis and then absorbed at a rate depending on the solubility of the free compound. Since sulfathiazole is considerably more soluble, and sulfamethylthiazole somewhat less soluble than sulfapyridine, the different rates of absorption have to be taken into account after subcutaneous administration. (At 28° C 600 mg sulfathiazole, 280 mg sulfapyridine and 200 mg sulfamethylthiazole are soluble in one liter distilled water.)

To eliminate variations due to the different rates of absorption an attempt was made to determine the intravenous toxicity in rats and mice. However, despite very slow injection into the tail vein (10 to 15 minutes), the animals developed severe toxic symptoms; with higher doses death during injection could not be avoided even though the rate of injection was further reduced. The highest single dose tolerated, using a 2.5% solution and 15 minutes as the injection period, was 0.85 g/kg sodium sulfapyridine, 1.0 g/kg sodium sulfathiazole and 0.5 g/kg sodium sulfamethylthiazole.. Some data on the acute intravenous toxicity of sodium sulfapyridine were reported by Marshall and Long[6] for dogs and by Kohn-Richards[7] for rabbits. Kohn-Richards[7] found that 0.5 g/kg killed 25%, 0.8 g/kg approximately 70% of the rabbits, whereas 1.0 g/kg was always fatal.

As a consequence of our experience with intravenous administration, the intraperitoneal route was chosen for a study of the comparative toxicity of the 3 derivatives. Since absorption occurs easily from the large surface of the peritoneal cavity and the extent of absorption can be checked readily at autopsy, this mode of administration was believed to be more advantageous than the subcutaneous route.

Method. 208 male albino rats of uniform age and from a standard strain weighing between 150 and 200 g were used. The animals were kept on a standard diet (Purina Dog Chow Checkers) and were

4 Lott, W. A., and Bergheim, F. H., *J. Am. Chem. Soc.*, 1939, **61**, 3593.

5 Fosbinder, R. J., personal communication.

6 Marshall, E. K., Jr., and Long, P. H., *J. A. M. A.*, 1939, **112**, 1671.

7 Kohn-Richards, R., *Proc. Am. Physiol. Soc.*, 52 Ann. Meeting, 103, March, 1940.

observed for 3 days following the injection. A 2.5 or a 5% solution of sodium sulfapyridine† was used and equimolecular amounts of sodium sulfathiazole and sodium sulfamethylthiazole were administered in the same volume of distilled water. Thus with the different derivatives, the animals received equal quantities of the sulfanilamide portion of the molecules (1.0 g sodium sulfapyridine monohydrate ≏ 1.025 g sodium sulfamethylthiazole ≏ 0.98 g sodium sulfathiazole). Animals sacrificed soon after intraperitoneal injection showed a thin milky layer of fine precipitate covering the entire surface of the abdominal cavity and its contents. Rats that did not die spontaneously within the 3-day period were sacrificed. Complete autopsies were performed on all animals and special attention was given to the presence of drug precipitation in the abdominal cavity, and to concrement formation in the urinary tract. All organs were fixed in 20% formalin for histological examination.

Results. The data dealing with the acute toxicity of the 3 compounds are summarized in Table I.

TABLE I.

Acute Toxicity of Sodium Salts of Sulfapyridine, Sulfathiazole, and Sulfamethylthiazole in Rats.
(Intraperitoneal injection of equimolar amounts.)

Compound	Dose, g/kg	No. of rats	No. dead	% dead (for 10 or more)	Died within hrs (mean value)	No. of dead with precipitation or concrement formation in urinary tract
Sodium	0.5	9	0			
Sulfapyridine	0.75	25	0	0		
	1.00	10	0	0		
	1.12	5	5		8	0
	1.25	15	12	80	9	0
	1.50	10	10	100	3	0
Sodium	0.5	5	0			
Sulfathiazole	0.75	13	1	8		1
(equimolar	1.00	10	1	10	40	1
to sodium	1.12	5	4		26	4
sulfapyridine)	1.25	20	20	100	11	20
	2.00	10	10	100	4	10
Sodium	0.5	16	3	19	58	2
Sulfamethylthiazole	0.6	10	1	10	24	1
(equimolar	0.65	5	5		26	5
to sodium	0.75	20	20	100	20	16
sulfapyridine)	1.00	10	10	100	15	7
	1.5	10	10	100	15	10

† We wish to thank the Maltbie Chemical Company for providing sulfathiazole, sulfamethylthiazole and the sodium salts of these compounds, and Merck & Co. for supplying us with sulfapyridine and sodium sulfapyridine.

It is noteworthy that sodium sulfapyridine appears to have the lowest toxicity, sodium sulfathiazole is slightly more toxic, while sodium sulfamethylthiazole is twice as toxic as sodium sulfapyridine. It may be seen from the table, however, that after higher doses of sodium sulfapyridine (1.12 and 1.25 g/kg) death occurs much earlier than after sodium sulfathiazole. Even sodium sulfamethylthiazole, which is considerably more toxic, requires a much longer time to produce death with the same concentration (*e.g.* 1.50 g/kg). There was a striking difference in the toxic symptoms following injection of sodium sulfapyridine and those resulting from the thiazole derivatives. Sodium sulfapyridine caused an increase in reflex excitability and muscular tone with spastic rigidity of all body muscles which became noticeable within a few minutes after the intraperitoneal injection. Twitching soon followed, which increasing in strength and severity, finally led to attacks of epileptiform convulsions. (A similar picture of intoxication was described for mice and dogs by Marshall, Bratton, and Litchfield,[8] and for mice by Barlow and Homburger.[1]) If death from asphyxia did not occur in this stage the animals recovered gradually.

The thiazole compounds on the other hand produced a state of extreme muscular weakness up to complete flaccidity of all body muscles. While this picture developed with sulfathiazole about 10 to 15 minutes after the administration, it occurred only several hours after the injection of sulfamethylthiazole. All 3 compounds caused severe respiratory disturbances, which may be ascribed, at least partly, to the injection of large amounts of alkali.

It seems important to stress the fact that the alkalinity of the sodium salts of sulfapyridine and the thiazole derivatives is different despite the presence of the same amount of sodium. A 2% solution of sodium sulfapyridine has a pH of 10.7, while both sodium sulfathiazole and sulfamethylthiazole in the same concentration have a pH of 9.4. Thus, "sulfathiazole is more strongly acidic" (Lott and Bergheim[4]). In addition, potentiometric titration reveals that the 3 derivatives possess a very high buffer capacity in the range of pH 9-10 for sodium sulfapyridine and of pH 7-9 for the sodium salts of the thiazole compounds. Thus, considerable amounts of acid are necessary for neutralization. It can, therefore, be expected that the injection of large amounts of the sodium salts will cause disturbances in the acid-base balance of the body, particularly the administration of sodium sulfapyridine with its high alkalinity.

[8] Marshall, E. K., Jr., Bratton, A. C., and Litchfield, J. T., *Science*, 1938, **88**, 597.

Marked peritoneal irritation, with the presence of blood-tinged fluid in the abdominal cavity, was observed after injection of sulfapyridine and sulfamethylthiazole. Sulfathiazole caused less irritation though usually large amounts of clear exudate were found both in the pleural and peritoneal cavities.

Animals that survived after 24 hours showed no precipitation in the abdominal space if sodium sulfathiazole was injected. After sodium sulfapyridine and sulfamethylthiazole small amounts of drug-precipitates were found usually on the liver surface in almost all animals; even when sacrificed after 3 days. This finding is in agreement with the different solubilities of the free compounds and suggests that both sulfapyridine and sulfamethylthiazole are somewhat more toxic than indicated in the table. The small difference in toxicity for mice between sulfapyridine and sulfamethylthiazole as reported by Long and co-workers[2] using subcutaneous injection, can be explained in the light of our own experiments with intraperitoneal injection: in part as a consequence of unfavorable conditions of absorption from the subcutis of relatively insoluble substances, but chiefly from the fact that mice do not acetylate appreciably, and thus do not detoxify sulfapyridine to as great an extent as rats.

The early death after injection of large amounts of sodium sulfapyridine reported by van Dyke and co-workers,[3] and confirmed in our experiments (Table I), seems to be due to the high alkalinity of this compound. Smaller amounts do not exhaust the buffer capacity of the body and, therefore, will not influence the toxicity of the free compound considerably. If, however, the dose is excessive so that neutralization by the available acid reserve is not complete, the toxic picture may be complicated by symptoms of alkalosis. According to the buffer range these symptoms would be expected to be more pronounced after the administration of sodium sulfapyridine than after injection of the sodium salts of the thiazole derivatives.

The assumption is supported by data derived from potentiometric measurements of the pH in peritoneal exudate of rats dying only a few hours after intraperitoneal injection of sodium sulfapyridine and of the pH in blood of rats killed shortly after the intraperitoneal administration of the compound. In both cases definite deviation to the alkaline side could be demonstrated (7.6-7.8 for blood, 7.7 to 8.0 for peritoneal exudate).

The above contention is further strengthened by the fact that intraperitoneal injection of sulfapyridine as a fine suspension even in amounts equivalent to 5 times the absolute lethal dose of sodium sulfapyridine caused but slight depression and none of the symptoms appearing a few minutes after administration of the sodium salt,

whereas sulfathiazole in a dose equimolar to the absolute lethal dose of its sodium salt killed 50% of the animals. The toxic picture caused by sulfathiazole was not unlike that seen after injection of its sodium salt.

The results of van Dyke and associates with oral administration[3] are similar to our findings. They report sodium sulfathiazole to have about 65% of the toxicity of sodium sulfapyridine, whereas upon feeding large amounts of the free compounds, sulfathiazole appeared to be the more toxic.

It is obvious from the data presented that toxicity determinations with the sodium salts of sulfanilamide derivatives having different alkalinities will not give a true picture of the toxicity of the free compounds. At present, therefore, the free compounds injected intraperitoneally as suspensions are being compared.

Conclusions. It has been shown that the acute toxicity of sodium sulfamethylthiazole in rats, as measured by intraperitoneal injection, is almost twice as high as that of sodium sulfathiazole, which in turn is slightly greater than the toxicity of sodium sulfapyridine.

The pH and buffer capacities of solutions of the sodium salts, and differences in solubility of the free compounds as factors influencing the toxicity, are discussed.

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Retention of Water by Pituitary (Posterior Lobe) Extract in Winter Frogs.

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The present report deals with further data bearing upon the mechanism of seasonal variation in the Brunn Reaction. The Brunn Reaction consists of an uptake of water by frogs when injected with suitable doses of pituitary (posterior lobe) extract and it is quantitatively greater in the summer than in the winter.[1] Certain aspects of the seasonal variation have been investigated in this laboratory. The reported increase in the winter of the number of acidophilic cells in the buccal lobe of the frog hypophysis lead us to inject various anterior pituitary and anterior pituitary-like principles along with extract of the posterior lobe but these additions did not depress the

[1] Boyd, E. M., Mack, E. G., and Smith, A. E., *Am. J. Physiol.*, 1939, **127**, 328.