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The Relative Toxicity of Six Salts of Penicillin.

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(Introduced by R. P. Herwick.)
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The hygroscopic nature of commercial penicillin sodium is a definite deterrent to efficient and economic handling of this material from a production standpoint, and for this reason alone there is a definite trend towards the development of a penicillin salt which is less hygroscopic, safe, and possessing the same therapeutic powers as those shown for the sodium salt. The calcium salt of penicillin which has been produced by a number of manufacturers is less hygroscopic and more stable than the sodium salt of this compound and at the present time is being studied to determine its therapeutic value in comparison with penicillin sodium. Other salts of penicillin (magnesium, ammonium, and barium) have been commercially prepared either for clinical trial or for use as standards, but few data have been accumulated as to their safety or efficiency as therapeutic agents.

In the present study 6 salts of penicillin have been investigated for their relative toxicity in mice. Five of these salts, sodium, ammonium, strontium, potassium, and magnesium, were prepared from a single master lot of penicillin. The sixth salt studied, calcium, was a commercial preparation. The latter salt is to be used as a reference standard and is to be distributed monthly by the Food and Drug Administration to all manufacturers of penicillin.

A pooled lot of penicillin sodium was used in the preparation of the 5 salts listed above. This pooled material was made up of the products of several manufacturers and contained as well portions of several lots of penicillin prepared in our pilot plant. The pooled material, containing approximately 6,000,000 units of penicillin, was first adsorbed on charcoal. It was then eluted with 75% acetone, following which the acetone was removed by vacuum distillation. The aqueous solution remaining was acidified and extracted with chloroform. The chloroform was then divided into 5 equal portions and shaken out with the calculated amounts of the proper carbonates or hydroxides to produce the sodium, ammonium, strontium, potassium, and magnesium salts of penicillin. As soon as preparation of the various salts had been completed, each was diluted to the same volume with distilled water, ampuled, shell frozen and dried under vacuum on a Cryochem apparatus (50 microns of mercury) for a 22-hour cycle. During the last four hours of drying all ampuls were heated to a temperature of 37°-39°C.

To conserve material, and since all salts were dried at the same time in the same volume on a single apparatus, moisture determinations were not made. A portion of each salt was assayed to determine potency in units per milligram and a second portion was assayed chemically to determine percent of cation present. The remainder of each salt of penicillin was then used to determine by intravenous injection the LD_{50} in 20-g mice. The LD_{50} in 20-g mice of the cations of solutions of the acetates of sodium, ammonium, strontium, calcium, potassium, and magnesium was also determined. From 6 to 12 mice were injected at each dose level and repeat tests were made with the same numbers of animals at the critical concentrations of all the preparations studied (Table I).

Even though 5 of the 6 penicillin preparations were produced from a single master lot of penicillin, there is a marked difference in the potencies of the various salts. The lowest potency material, penicillin strontium, was found to assay 285 units per milligram, while the penicillin magnesium, which had the highest potency, assayed 1028 units per milligram. It is extremely important when discussing the relative toxicity of a group of salts of penicillin such as those described in Table I

TABLE I.
Relative Toxicity of 6 Salts of Penicillin in Mice.

Salt of Penicillin	LD_{50} in units	LD_{50} in mg of Penicillin	Units per mg	% of cation	LD_{50} in mg of cations	Milliequivalents of cations of penicillin at LD_{50}	Acetates	LD_{50} in mg of cations	Milliequivalents of cations of acetates at LD_{50}
Na	21,000	32.0	656	7.72	2.47	0.11	Na	7.8	0.34
NH_4	6,600	19.9	332	6.96	1.38	0.08	NH_4	1.9	0.11
Sr	4,850	17.0	285	13.53	2.3	0.05	Sr	3.75	0.09
*Ca	3,700	11.9	310	8.16	0.97	0.05	Ca	1.03	0.05
Mg	7,600	7.4	1028	4.23	0.31	0.03	Mg	0.38	0.03
K	2,850	5.9	479	12.45	0.73	0.02	K	1.09	0.03

* Commercial lot; all other salts prepared from a single master lot.
Relative toxicity based on milligrams of cation at LD_{50} dose of salts of penicillin:
$Na < Sr < NH_4 < Ca < K < Mg$
Relative toxicity based on milligrams of cation at LD_{50} dose of acetates:
$Na < Sr < NH_4 < K < Ca < Mg$

to give consideration to the potencies of the individual salts. If the salts of penicillin are tabulated in the order of their toxicity based on *units* at the LD_{50} dose the order would be (increasing toxicity): Na, Mg, NH_4, Sr, Ca, and K. It is obvious that magnesium, which is known to be quite toxic on intravenous injection, is not in the proper position in this series. The apparent lower toxicity of penicillin magnesium in the series is due to its high potency (1028 units per milligram). Studies have been made[1] of another lot of penicillin magnesium (commercial preparation) which had a potency of only 70 units per milligram. The LD_{50} in this case proved to be 710 units, and this dose contained 0.325 mg of Mg^{++}. It will be noted (Table I) that the LD_{50} of Mg^{++} is 0.31 mg on the above high potency material (1028 units per mg) and 0.38 mg for the magnesium ion of magnesium acetate. Obviously, therefore, with both these high and low potency samples of penicillin magnesium the toxicity is due to the cation.

If one considers the milliequivalents of the 6 cations of the penicillin salts present at the LD_{50}, then the relative toxicity would result in the following series (increasing toxicity): Na, NH_4, Sr, Ca, Mg, and K. The standard error at the LD_{50} was found to vary from a low of ± 215 units with penicillin calcium to a high of ± 400 units with penicillin ammonium. When the milliequivalents are computed for the cations of the acetates, the cations align themselves in the same order as did the cations of the salts of penicillin. In the case of the acetates the standard error at the LD_{50} was found to vary from a low of ± 0.02 mg with magnesium acetate to a high of ± 0.30 mg with calcium acetate.

The order of relative toxicity (increasing toxicity), based on milligrams of cation at the LD_{50} dose of the salts of penicillin, is Na, Sr, NH_4, Ca, K, and Mg, while the relative toxicity based on milligrams of cation at the LD_{50} dose of the acetates is Na, Sr, NH_4, K, Ca, and Mg. If the number of milligrams of the cation at the LD_{50} of the penicillin preparations is compared with the number of milligrams of the cation at the LD_{50} of the acetates, with 4 of these salts of penicillin there is little difference in the weights of the cations at the LD_{50} doses, potassium and ammonium showing a difference of 0.36 and 0.52 mg, respectively, while the difference observed with calcium is only 0.05 mg, and that of magnesium only 0.07 mg. It would appear then that so far as these 4 penicillin preparations are concerned the toxicity is primarily due to the cation. In the case of sodium and strontium, on the other hand, the conclusion is drawn that the toxicity of the penicillin preparations made from these cations is only partially due to the cation present and that some of the toxicity must be assigned to either the penicillin itself or, perhaps more likely, to organic contaminants resulting from the process of extraction. But even with these

[1] Welch, H., Price, C. W., Neilsen, J. K, and Hunter, A. C., in press.

2 salts of penicillin it would appear from these studies that, with more potent (purer) preparations, their toxicities also would be due primarily to the concentration of the cation present.

Summary and Conclusions. Five salts of penicillin prepared from a single master lot were investigated for their acute intravenous toxicity in mice. A sixth salt, a commercial preparation of penicillin calcium, was studied.

Based on milliequivalents of the cation used in the preparation of these salts their relative toxicity was found to be (increasing toxicity): Na, NH_4, Sr, Ca, Mg, and K. The same relative toxicity was found for the acetates of these cations.

The relative toxicity based on milligrams of the cation at the LD_{50} dose of the salts of penicillin is (increasing toxicity): Na, Sr, NH_4, Ca, K, and Mg, while the relative toxicity based on milligrams of the cation at the LD_{50} dose of the acetates is (increasing toxicity): Na, Sr, NH_4, K, Ca, and Mg.

The toxicity of salts of penicillin is primarily due to the cations used in their preparation.

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The Analgesic Effect of Morphine Alone and in Combination with Dextro-Amphetamine.*

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Synergism of morphine and dextro-amphetamine in the production of analgesia has been demonstrated in dog experiments.[1] Investigations of a similar nature, though with a different method, have been carried out on mice. The results of these experiments are here reported.

The pain thresholds were determined in the mice by pinching the tails, as described by Haffner[2] and Molitor and Robinson.[3] A pair of dissecting forceps was modified by the placement of a rotating wedge between the blades in such a way that the approximation of the blades was limited by the thickness of the wedge at the selected setting. The rotating wedge was calibrated to indicate successive 0.5 mm closures between the tips of the blades. The forceps were applied to pinch the base of the tail and the wedge rotated to allow increasing amounts of pressure to be applied. The value indicated on the disc, referring to the spacing between the blades when the mouse indicated pain by squeaking, was interpreted as the threshold of pain. The forceps were always applied to the same part of the tail and care was taken not to cause tissue injury.

For each experiment each animal was kept in an individual cage which allowed some movement but kept the tail in proper position for the test. The mice used were all of one strain and each animal was used only once.

At the beginning of each test, before the administration of any drug, the normal sensitivity of each animal to the stimulus described was tested 3 times at 15-minute intervals. Animals which did not give uniform responses in these preliminary tests were discarded. Then the drug or drug combination was injected subcutaneously and the tests repeated at intervals of 15 minutes for 2¼ to 6 hours to follow changes in sensitivity.

Each drug and combination of drugs was tested 4 times on groups of 12 animals each. The results are given in terms of mean values for all animals in each dosage group. The mean values are accurate because there was

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1 Goetzl, F. R., Burrill, D. Y., and Ivy, A. C., *Fed. Proc. Am. Soc. Exp. Biol.*, 1943, **2**, 1, 16.

2 Haffner, E., *Deutsche med. Wchnschr.*, 1929, **251**, 731.

3 Molitor, H., and Robinson, H., *Anesth. and Analg.*, 1938, **17**, 188.