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.

14542 P

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

* This work was aided in part by a grant from Smith, Kline, and French laboratories.

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.

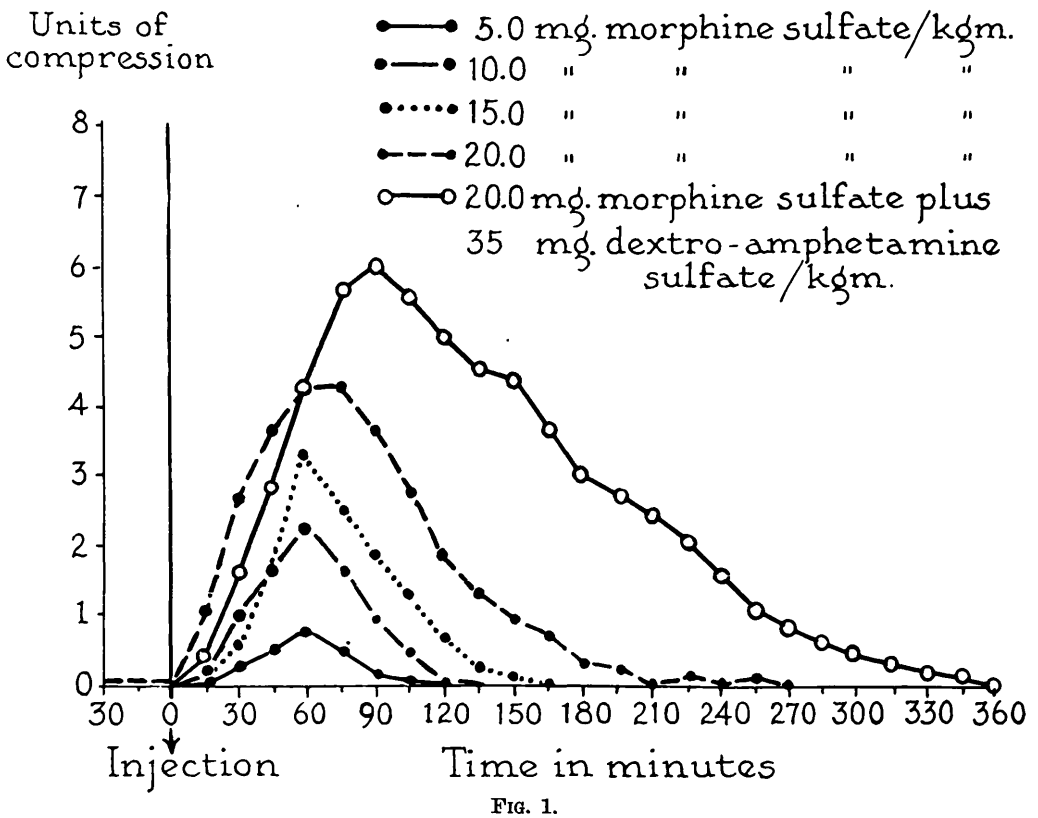

FIG. 1.

no overlapping of results in regard to maxima and durations of the effects. For instance, the least analgesic effect obtained in any animal with 20.0 mg of morphine sulfate plus 35.0 mg of dextro-amphetamine per kilogram was greater than the greatest effect obtained in any animal with 20.0 mg of morphine sulfate per kilogram alone.

In Fig. 1 the units of compression required to produce a squeak are plotted against the times at which the animals were tested. The resulting curves show the changes in sensitivity to the pain stimulus used.

The analgesic effects of 5.0, 10.0, 15.0, and 20.0 mg of morphine sulfate per kilogram are shown in the figure. The smallest doses caused only a reduction of activity in the cages, while the larger doses caused all of the animals to go to sleep. Sleeping apparently did not interfere with the tests for sensitivity, as the sensitivity returned to normal after a time, even though the animals were sleeping between tests. The 20.0 mg dose put the animals to sleep for 4 to 5 hours. After the administration of the 10.0, 15.0, and 20.0 mg doses a positive Straub reaction was seen in all animals. It will be noted from the figure that the pain sensitivity returned to normal within 3½ hours after the administration.

Changes in sensitivity produced by dextro-amphetamine alone could not be demonstrated because of the marked stimulation and increase in activity produced by even small doses. The stimulation and increased activity persisted for some time after the mice were returned to the large cages.

The analgesic effects of a combination of 20.0 mg morphine sulfate with 35.0 mg of dextro-amphetamine sulfate per kilo were readily measurable and were greater than the effects produced by the morphine sulfate alone. The changes in sensitivity are indicated in the figure. It is to be noted that the analgesia was greater and longer lasting than with 20.0 mg of morphine alone. All of the animals remained awake and alert. The Straub reaction

did not develop in any animal. When they were returned to the large cages all of the mice exhibited apparently normal activity. This test continued for 6 hours, the time required for the sensitivity to return to pre-injection level.

While the analgesic effect of dextro-amphetamine alone could not be determined in this experiment it is clear that the drug increased the analgesic effect of morphine while counteracting the narcotic effect of morphine. Antagonism, except with reference to analgesia, was further shown by the inhibition of the Straub reaction.

The method used in this experiment was not entirely satisfactory, but the results obtained are in agreement with those obtained in the dog experiments.

Summary. Experiments have been made on mice which demonstrate that dextro-amphetamine increases the analgesic effects of morphine while counteracting its narcotic action. Although a different method has been used in these studies the results agree with those obtained in dog experiments.

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Specificity of the Epiphyseal Cartilage Test for the Pituitary Growth Hormone.*

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Recently a procedure for the bioassay of the pituitary growth hormone was published in which the width of the uncalcified portion of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats was used as the index of potency.[1] In order to investigate the specificity of this test it was considered necessary to determine the reaction of the epiphyseal cartilage to other hormones. This paper reports the effects of thyroxin, and of purified pituitary thyrotropic, adrenocorticotropic, and lactogenic hormones on the cartilage under the same conditions.

The procedure was exactly as described in the paper on the standardization of growth hormone: Female rats 26 to 28 days of age were hypophysectomized and after a 12- to 13-day post-operative period were injected daily intraperitoneally for 4 days with a particular hormone. Twenty-four hours after the last injection (17 to 18 days post-operative) the rats were autopsied and the proximal end of the tibia was split and fixed in neutral formol. After staining with silver nitrate the uncalcified portion of the epiphysis was measured with microscopic low power using a micrometer eye piece.

The results are summarized in Table I. After injection of thyroxin the cartilage width was slightly greater than in the controls; though the effect was small, it did approach that produced by the growth hormone unit.† No gradation of effect with increasing dosage was noted.

Pituitary thyrotropic hormone had no significant effect on the epiphyseal cartilage at doses as high as 0.6 mg. The preparation was kindly furnished by J. Fraenkel-Conrat.[2] Though not devoid of contaminants the preparation was highly potent having a mini-

* Aided by grants from the Board of Research of the University of California and the National Research Council Committee on Research in Endocrinology.

1 Evans, H. M., Simpson, M. E., Marx, W., and Kibrick, E., *Endocrinology*, 1943, **32**, 13.

† One growth hormone unit is defined as the daily dose of growth hormone which under these conditions produces an increase of 50 micra in the width of the uncalcified proximal epiphyseal cartilage of the tibia.

2 Fraenkel-Conrat, J., Fraenkel-Conrat, H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1940, **135**, 199.