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Experiments on the Toxicity of the Calcium Salt of Penicillin.

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The physical properties of the calcium salt of penicillin make it easier to handle than the deliquescent sodium salt. However, owing to its supposed toxicity,[1] its use is not advised.[2] In the experiments demonstrating its toxicity[1] a crude salt containing 50 units/mg was used. Ten mg given intravenously and 20 mg given subcutaneously caused severe but not fatal reactions in 20 g mice. In previous experiments[3] it had been shown that the crude sodium salt containing 40 to 50 units/mg caused "serious embarrassment" when injected into mice. With purification the sodium salt becomes relatively less toxic;[1] 20 mg of the salt containing 250 or 325 units/mg produced no effect in 20 g mice. There is reason to believe that the calcium salt might undergo a similar detoxification and these experiments were done to verify this.

In the early experiments of the Oxford group[1] the calcium content of the preparation has not been determined. The fact, however, that even 10 mg of crude salt given to mice intravenously proved to be toxic, makes a direct correlation with the calcium content of the salt very improbable. It is a safe assumption that the calcium content of such a crude preparation must have been under 10%, or, in other words, less than 1 mg in 10 mg of the salt used.

Method. Two samples of freeze-dried calcium salt were used,† A contained 400 units/mg and B 230 units/mg. The calcium content of salt B was 1 mg per 5,000 units. The salts were dissolved in sterile distilled water and injected into mice intramuscularly and intraperitoneally. The intramuscular injections were made into the left scapula region. Each salt was dissolved in sufficient water that the required dose was contained in 0.01 cc per gram of mouse.

The mice used weighed between 13 and 28 g. They were observed frequently within the first 3 hours of injection and again approximately 12 and 24 hours after injection. Those mice which were injected intraperitoneally in the first experiment were injected intramuscularly in the second and vice versa.

Results. Experiment 1. Salt A was used. Dose: 50 units per gram of mouse. Ten mice were injected intraperitoneally and 10 were injected intramuscularly. The mice showed no reaction within 24 hours of injection

Experiment 2. Salt B was used. Dose: 100 units and 0.02 mg calcium per gram of mouse. Ten mice were injected intraperitoneally and 10 intramuscularly. The mice showed no reaction within 24 hours of injection.

The observations were continued for several more days, but no signs of local irritation or other toxic signs were noticed. More concentrated solutions of the salts could not be produced and therefore higher doses could not be given. The dose employed in the second experiment is approximately 50 times that at present used in human therapeutics as the daily dose of the sodium salt on a weight for weight basis.

No toxic effects were noted in the mice after intramuscular injection in spite of the excessive doses used.

The salts A and B and another sample containing 280 units/mg were used in humans without ill effect. Aqueous solutions of the

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† Preparations obtained from Reichel Laboratories, Kimberton, Penn.

salts were given to 9 patients in intravenous, intramuscular, and even subcutaneous application. In 2 cases 80,000-100,000 Oxford units were administered in constant intravenous drip for 2 x 24 hours. The doses given intramuscularly (6 to 8 times within 24 hours) ranged from 5,000-20,000 units (1-3 cc) and were equally well tolerated without local or systemic reaction.

Conclusion. Satisfactorily purified Ca-salt of penicillin is not more toxic for man and mice than its Na-salt.

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Impairment of Growth and Myelinization in Regenerating Nerve Fibers Subject to Constriction.*

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A nerve scar is known to impede the passage of regenerating nerve fibers. However, whether or not fibers that succeed in getting through a constriction remain handicapped in their further development has never been determined. The experiments to be described in the following prove that they do.

Material and Method. In 11 white rats (120-230 g) the sciatic nerve was crushed *cca.* 5 mm above its bifurcation. The tibial and peroneal nerves were cut at the knee, and a segment of artery, small enough to produce local nerve constriction, was slipped over one of the proximal stumps, as described previously.[1,2] Fig. 1 illustrates the operation (C, crush; S, constriction sleeve). Nerve regeneration is thus started simultaneously in both nerves at level C. Fibers of the constricted nerve must grow through the bottleneck, while fibers growing through the unconstricted stump serve as controls. After regeneration periods of from 4 to 15 weeks, the nerves were fixed and stained in osmic acid or in Protargol (Bodian) and Mallory Azan. Fiber diameters were measured and compared at levels both proximal (P) and distal (D) to the constriction sleeve.

Results. In 3 cases the sleeves had failed to constrict. No difference between test and control nerves was noticed; hence, mere presence of a sleeve does not affect regeneration. In the remaining 8 cases, constrictions of varying degrees had persisted, associated as usual with edema and with damming of axoplasm.[2,3] Numerically, fibers have regenerated as extensively through the constricted as through the unconstricted nerves. They contrasted sharply, however, with regard to caliber and myelinization. The fibers that had passed through the constriction were markedly thinner and delayed in myelinization as compared with the regenerated fibers of the control nerve at the same level.

Photographs of representative samples from cross-section D of nerve pairs regenerated with (A) and without (B) constriction, taken at identical magnifications 8 weeks (Fig. 2) and 10 weeks (Fig. 3) after the operation, illustrate the differences. A lower power view (Fig. 4) of both the test (tibial; bottom) and control (peroneal; top) nerves shows the contrast in myelinization (osmic acid stain).

In one case (R 188; 6 weeks regeneration time), all regenerated fibers of the constricted tibial and the unconstricted peroneal (total: 5997 fibers) were measured. Their size distribution is given in Table I. While 50%

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