THE EXCRETION OF PENICILLIN IN THE SPINAL FLUID IN MENINGITIS[1]

RAMMELKAMP and Keefer[2] found that penicillin injected intravenously is not excreted into the spinal fluid. Similar observations have not been conducted on patients with meningitis. The present study was undertaken to determine whether significant amounts of penicillin are excreted into the subarachnoid space in patients with meningitis following intravenous or intramuscular administration.

MATERIAL AND METHODS

Eight patients with meningitis were chosen for this study. In 6 of these, meningococci were recovered from the spinal fluid. None of the subjects had received any form of specific therapy prior to this study. Penicillin,[3] in a dilution of 5,000 Oxford units per cc of isotonic saline solution, was administered intramuscularly to 2 subjects and intravenously to 6 subjects, taking one minute for each injection. Three of the subjects received 20,000 units, and the other 5 were given 40,000 units. The diagnostic lumbar puncture was performed 60 to 140 minutes later and the spinal fluid thus obtained was assayed for its penicillin content, using a modification of Foster's turbidometric method.[4] On the basis of their estimated potency, the samples of spinal fluid were diluted in sterile water to give a solution containing approximately 0.1 Oxford unit per ml. Varying amounts of the dilutions were then added to tubes containing 10 ml of nutrient broth, inoculated with *Staphylococcus aureus,* and incubated 16 hours at 37° C. Turbidometric measurements of the amount of growth were made with a photoelectric colorimeter. The potency of the samples was calculated from a standard curve run at the same time as the test samples.

As controls, spinal fluid was obtained from 3 of these subjects ten days after discontinuing penicillin and was assayed for its antibacterial effect.

RESULTS

In all 8 subjects, penicillin was excreted in the spinal fluid (Table 1). Subject 1, who received 20,000 units of penicillin intravenously, showed a concentration of 0.35 unit of penicillin per cc of spinal fluid 60 minutes after injection. One hundred and twenty minutes after a similar injection, 0.03 unit of penicillin per cc of spinal fluid was found in Subject 2. When 20,000 units were administered intramuscularly to Subject 3, 0.05 unit per cc of spinal fluid was found 120 minutes later.

TABLE 1

SHOWING THE AMOUNT OF PENICILLIN IN THE SPINAL FLUID AFTER INTRAVENOUS AND INTRAMUSCULAR ADMINISTRATION

Subject	Amount of penicillin administered (units)	Route of administration	Interval after administration (minutes)	Amount of penicillin in spinal fluid (units/cc)
1	20,000	Intravenous	60	0.35
2	20,000	Intravenous	120	0.03
3	20,000	Intramuscular	120	0.05
4	40,000	Intravenous	90	0.32
5	40,000	Intravenous	125	0.26
6	40,000	Intravenous	125	0.09
7	40,000	Intravenous	75	0.04
			135	0.12
8	40,000	Intramuscular	140	0.26

Ninety minutes after the intravenous injection of 40,000 units of penicillin, 0.32 unit per cc of spinal fluid was found in Subject 4. One hundred and twenty-five minutes after the same dose, 0.26 unit per cc of spinal fluid was detected in Subject 5, and 0.09 unit per cc in Subject 6. In Subject 7, who likewise received 40,000 units intravenously, the spinal fluid showed 0.04 unit per cc in 75 minutes and 0.12 unit per cc in 135 minutes. Subject 8 was given 40,000 units intramuscularly, and 140 minutes later 0.26 unit per cc was found in the spinal fluid.

It is evident from these data that the amounts of penicillin excreted in the spinal fluid in meningitis vary with different subjects. Nevertheless, the concentrations found in these studies, particularly after the administration of 40,000 units, are sufficient to produce a marked bacteriostatic effect.

None of the control samples of spinal fluid showed any antibacterial effect.

COMMENT

Rammelkamp and Keefer[5] found that penicillin in concentrations of 0.019 to 0.156 unit per cc of serum produced maximum bactericidal effects against the *Streptococcus hemolyticus,* and at least 0.156 unit per cc was necessary for maximum bacteriostatic action against *Staphylococcus aureus.* Further, they observed that the antistreptococcal action of whole blood containing 0.007 unit of penicillin per cc is much greater than that of whole blood containing 5.1 mg of sulfadiazine per 100 cc of blood. When these data are correlated with our findings, the concentrations of penicillin in the spinal fluid of our subjects may be regarded as adequate for the control of men-

[1] This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the U. S. Navy. The opinions and views set forth are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

[2] C. H. Rammelkamp and C. S. Keefer, *Am. Jour. Med. Sci.,* 205: 342, 1943.

[3] The sodium salt of penicillin was used in this study.

[4] J. W. Foster, *Jour. Biol. Chem.,* 144: 285, 1942.

[5] C. H. Rammelkamp and C. S. Keefer, *Jour. Clin. Invest.,* 22: 425, 1943.

ingeal infections produced by susceptible organisms, without the need of supplementary intrathecal therapy. Inasmuch as the concentration of penicillin at comparable periods varies with different subjects, it would appear, however, that larger and/or more frequent doses than have generally been administered intravenously or intramuscularly may be required to obtain the maximum bacteriostatic effects and to preclude the development of penicillin resistance. Whether this method of therapy will be as effective or produce clinical responses as promptly as that observed when combined with intrathecal administration (Rosenberg and Arling)[6,7] must await clinical trial. Further studies along this line are indicated.

SUMMARY AND CONCLUSIONS

(1) Penicillin was administered in doses of 20,000–40,000 Oxford units intravenously or intramuscularly to 8 subjects with meningitis. Sixty to 140 minutes later penicillin was found in the spinal fluid in concentrations of 0.03 to 0.35 unit per cc.

(2) These data suggest that penicillin administered intravenously or intramuscularly in adequate dosages may be effective in the treatment of meningitis without supplementary intrathecal therapy.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE GOLDEN HAMSTER (CRICETUS AURATUS) AS A TEST ANIMAL FOR THE DIAGNOSIS OF LEPTOSPIROSIS

THE investigation of leptospirosis due to infection with *Leptospira canicola* has been seriously handicapped by the lack of a readily susceptible laboratory host, whereas numerous species are known to be susceptible to *L. icterohaemorrhagiae*.[1]

Meyer, Stewart-Anderson and Eddie[2] chose young guinea pigs weighing 50–100 grams for their studies on canine leptospirosis. They state that the spirochetes associated with dog infections are of a low pathogenicity for rodents and that in young guinea pigs weight loss was a better criterion of infection than a febrile reaction. However, three to five passages were required to produce a definite weight loss and an occasional fatal infection.

The purpose of this paper is to report the use of hamsters for the isolation of both *L. canicola* and *L. icterohaemorrhagiae* from naturally infected dogs, and further, to report the first instance in which the classical strain, *L. icterohaemorrhagiae,* has been isolated from the dog in the United States.

SUSCEPTIBILITY OF HAMSTERS TO VIRULENT STRAINS OF LEPTOSPIRA

Our early attempts to isolate Leptospira from the blood and urine of dogs following the injection of suitable material into young guinea pigs were as unsatisfactory as those reported by Meyer and his co-workers.[2] The experimental data on the use of hamsters reported by Morton[1] and the suggestion of Dr. Carl L. Larson, of the U. S. Public Health Service, led to the choice of hamsters three to four weeks old and weighing 25 to 30 grams for experimental work.

Leptospira canicola was first isolated in young hamsters after injection of the urine of an acutely ill dog whose serum on the day the urine sample was collected had a positive agglutination titer for *L. canicola* in a dilution of 1–2,000 and a cross titer for *L. icterohaemorrhagiae* in a 1–10 dilution. The urine specimen was obtained by catheter on February 15, 1943, and centrifuged in an angle centrifuge at 3,500 RPM for one-half hour. The sediment was suspended in sterile saline solution and injected intraperitoneally into four young hamsters and four young guinea pigs. Within 9 to 10 days the hamsters died of leptospirosis. *L. canicola* was demonstrated by dark-field examination of portions of the kidney and liver, in sections stained by a silver staining method and cultured in Fletcher's broth. The injected guinea pigs remained normal in appearance.

This strain of *L. canicola* isolated from dog urine injected in young hamsters has been labelled Strain "A" and the confirmation of its pathogenicity for hamsters has been reported by Larson[3] in his paper on "Experimental Leptospirosis in Hamsters." On the second passage of this strain in hamsters marked icterus appeared three to four days after injection of the animals, death following within 5 to 6 days after inoculation.

Later the owner of the dog from which Strain "A" was isolated became seriously ill with canicola fever, the diagnosis being based on the serologic findings and the demonstration of leptospira in his urine by dark-

[6] D. H. Rosenberg and P. A. Arling, *U. S. Naval Med. Bull.* In press.
[7] *Ibid.: Jour. Am. Med. Assoc.* In press.
[1] H. E. Morton, *Proc. Soc. Exp. Biol. and Med.*, 49: 566, 1942.
[2] K. F. Meyer, B. Stewart-Anderson and B. Eddie, *Jour. Am. Vet. Med. Asn.*, 95: 710, 1939.
[3] C. L. Larson, Public Health Reports, 59: 522, 1944.