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THE TOLERANCE OF THE DOG TO INTRATHECAL INJECTIONS OF CRYSTALLINE PENICILLIN

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Although it is known that intrathecal administration of penicillin may have an irritant action on the meninges and on the central nervous system (Walker, 1947 ; Cairns, 1947), no measurements of the degree of tolerance either in the human subject or in the experimental animal are yet available. Moreover penicillin is generally injected into the subarachnoid space dissolved in isotonic sodium chloride solution. Isotonic sodium chloride solution has itself a well-defined irritant action on the meninges (Bedford, 1946, 1948a, 1948b). It is therefore essential that this action should be taken into consideration when determining that of penicillin. In the experiments now reported, a study has been made of the effect of the intracisternal injection of crystalline penicillin in dogs.

Experimental Procedure

The general experimental procedure was similar to that described in earlier publications (Bedford, 1946, 1948a), to which reference for details should be made. In this series of experiments, the dogs were anaesthetized with ether. Crystalline sodium penicillin G(11) ("Avlon," Imperial Chemical Pharmaceuticals, Ltd.) was used throughout the experiments. The penicillin was dissolved in sterile distilled water immediately before injection into the cisterna magna ; in a few experiments it was dissolved in a 0.9 per cent (w/v) solution of sodium chloride in distilled water. Although solutions of penicillin in distilled water were found to have an average *p*H of 5.5, they were in no sense buffered at that *p*H. After the addition of a relatively small volume of cerebrospinal fluid, the solutions rapidly assumed the *p*H of that fluid. A constant volume (1.0 c.c.) of solution was introduced in all experiments. The animals after recovery from the anaesthetic were allowed to survive for six hours ; in a few experiments the survival time was 24 hours. Any animal that displayed symptoms of severe irritation of the nervous system after recovery from the anaesthetic was not allowed to survive.

Results

The effect of the intracisternal administration of 100,000 and 20,000 i.u. penicillin

Two dogs weighing 9.0 kg. and 8.5 kg. respectively were each given 100,000 i.u. penicillin dissolved in 1.0 c.c. distilled water and kept under observation while they recovered from the anaesthetic. As the effects of the ether wore off, the animals were found to be holding the head rigidly retracted ; they repeatedly licked the nose and attempted to scratch the back of the neck with the hind leg. There was a profuse flow of saliva. Generalized tremors heralded the onset of convulsions which speedily passed into status epilepticus. The symptoms displayed by both animals were almost identical. Similar effects were obtained after the administration of 20,000 i.u. to two dogs weighing 8.0 kg. and 8.5 kg. respectively. The animals were destroyed after the onset of general convulsions before complete consciousness had been regained.

The effect of the intracisternal administration of 10,000 i.u. penicillin

The experiments were divided into three groups. In the first group the penicillin was injected dissolved in distilled water and the effects on the pressure and cell content of the cerebrospinal fluid determined at the end of 6 hours. The second group of experiments was similar to the first except that the animals were allowed to survive 24 hours after the injection of the penicillin. In the third group, the penicillin was dissolved in 0.9 per cent (w/v) sodium chloride solution before injection. The effect of the introduction of 10,000 i.u. penicillin in distilled water was studied in twelve dogs: seven were allowed to survive for 6 hours and five for 24 hours. All animals presented evidence of irritation of the

TABLE I

THE EFFECT ON THE CELL CONTENT AND THE PRESSURE OF THE CEREBROSPINAL FLUID OF 10,000 I.U. PENICILLIN AND OF 0.9% NaCl SOLUTION

Penicillin in distilled water				Penicillin in distilled water				0.9% NaCl in distilled water			
Wt. of dog (kg.)	White cells per cu.mm. 6 hr. after injection	Pressure of C.S.F.		Wt. of dog (kg.)	White cells per cu.mm. 24 hr. after injection	Pressure of C.S.F.		Wt. of dog (kg.)	White cells per cu.mm. 6 hr. after injection	Pressure of C.S.F.	
		Before injection	6 hr. later			Before injection	24 hr. later			Before injection	6 hr. later
7.5	4,320	170	130	9.0	340	130	130	9.5	3,250	140	220
12.0	4,230	200	160	11.5	330	140	120	8.5	1,400	140	170
9.5	2,400	130	120	7.5	440	120	90	6.0	3,400	80	220
5.0	1,770	140	160	9.5	170	80	140	10.5	1,070	110	270
8.0	6,790	150	160	10.0	210	100	120	10.0	2,100	170	160
9.0	6,880	110	230					6.0	2,300	125	250
8.5	Destroyed	130	—					10.5	4,500	100	260
Average 8.5	4,398	147	160	9.5	298	114	120	8.7	2,574	123	220

TABLE II

THE EFFECT ON THE CELL CONTENT AND ON THE PRESSURE OF THE CEREBROSPINAL FLUID OF PENICILLIN IN DOSES LESS THAN 10,000 I.U. AND OF DISTILLED WATER

Wt. of dog (kg.)	i.u. penicillin in distilled water	White cells after 6 hr. per cu.mm. C.S.F.	Pressure of C.S.F.		Wt. of dog (kg.)	i.u. penicillin in 0.9% NaCl solution	White cells after 6 hr. per cu.mm. C.S.F.	Pressure of C.S.F.	
			Before injection	6 hr. later				Before injection	6 hr. later
9.4	7,500	340	140	140	9.4	1,000	6,000	80	230
8.2	7,500	415	180	160	12.0	1,000	7,800	130	260
10.0	7,500	600	130	160	10.5	1,000	7,500	120	200
Average 9.2	—	450	150	153	8.6	1,000	4,700	120	170
11.0	5,000	170	180	180	Average 10.1	—	6,500	112	215
9.0	5,000	280	80	80					
10.0	5,000	140	110	120					
						Distilled water			
Average 10.0	—	197	123	127	10.5	—	0	160	180
8.0	2,500	120	80	100	11.5	—	90	150	160
9.0	2,500	50	80	100					
					8.5	—	0	120	110
Average 8.5	—	85	80	100	7.5	—	80	150	170
8.0	1,000	35	120	140	8.6	—	0	130	110
9.5	1,000	40	170	170					
10.0	1,000	20	90	110	Average 9.3	—	34	142	146
7.5	1,000	35	110	85					
Average 8.7	—	32	122	126					

nervous system after recovery from the anaesthetic. The symptoms most commonly encountered were weakness of the forelegs in walking, slight head retraction, chattering of the jaws with salivation, and attempts to scratch the back of the head with the hind leg. Head shaking was a prominent symptom in some animals. With the exception of one dog which passed into status epilepticus after half an hour, convulsions were not observed. These symptoms generally disappeared after one hour and the animals appeared normal at the end of the 6-hour and 24-hour recovery periods. In four experiments the penicillin was dissolved in sodium chloride solution before injection. There was evidence of severe irritation of the central nervous system in all four experiments on recovery from the anaesthetic, and the animals were destroyed after the onset of general convulsions within half to one hour of the administration of the penicillin. The results of the experiments are summarized in Table I, where, for the purpose of comparison, the effect of the injection of 1.0 c.c. of 0.9 per cent (w/v) sodium chloride solution after 6 hours is also indicated. The limited number of experiments in each group did not seem to warrant a statistical investigation of the results.

The effect of the intracisternal injection of crystalline penicillin in quantities less than 10,000 i.u.

Penicillin dissolved in distilled water was injected into the cisterna magna in the following doses: 7,500 i.u. in 3 dogs ; 5,000 i.u. in 3 dogs ; 2,500 i.u. in 2 dogs ; 1,000 i.u. in 4 dogs ; and 1,000 i.u. in sodium chloride solution was injected in 4 dogs. The duration of the experiments was 6 hours. The effect on the cell count and on the pressure of the cerebrospinal fluid is indicated in Table II. There were symptoms of irritation of the nervous system in all experiments except after the injection of 1,000 i.u. ; after 2,500 i.u. these symptoms were barely perceptible. Although the injection of 1,000 i.u. in sodium chloride solution caused the appearance of a considerable number of polymorphonuclear leucocytes and a rise in the pressure of the cerebrospinal fluid, the animals presented no symptoms of irritation of the nervous system. It is evident that the white cell content and the pressure of the cerebrospinal fluid give no indication of irritant action on nerve tissue.

Discussion

In the above experiments, dogs of an average weight of 8.7 kg. were able to tolerate an intracisternal injection of 1,000 i.u. penicillin in 1.0 c.c. distilled water without displaying symptoms of irritation of the nervous system. Although the pressure of the cerebrospinal fluid remained unchanged after 6 hours, polymorphonuclear leucocytes were invariably present, but their number never exceeded fifty per cu.mm. of cerebrospinal fluid (Table II). These findings contrast markedly with the effects which followed the injection of a similar volume of normal saline solution (Table II). Although symptoms of irritation of the nervous system were not observed after the injection of normal saline solution, the pressure of the cerebrospinal fluid was raised and the polymorphonuclear leucocytic content of the cerebrospinal fluid averaged 2,574 cells per cu.mm. at the end of the experiments.

It will be seen from Table II that 1,000 i.u. penicillin dissolved in normal saline solution provoked the appearance of an even greater number of leucocytes and also a rise in the pressure of the cerebrospinal fluid. The unsuitability of normal saline solution as a solvent for penicillin required for introduction into the subarachnoid space is well illustrated by the experiments in which 10,000 i.u. penicillin were administered. In four experiments in which the penicillin was injected dissolved in normal saline solution, the animals developed general convulsions after half to one hour and were destroyed. On the other hand, convulsions occurred in only one animal out of a series of twelve when the penicillin was dissolved in distilled water, although there was evidence of irritation of the nervous system in every instance.

The irritant action of penicillin on the nervous system seems to be of relatively short duration. Marked evidence of irritation may be present on recovery from the anaesthetic, as was observed after the introduction of 10,000 i.u. in distilled water, yet with the exception of one animal that had to be destroyed owing to the onset of general convulsions the remainder appeared normal one hour later. Similarly the number of polymorphonuclear leucocytes in the cerebrospinal fluid 24 hours after the injection of 10,000 i.u. penicillin was of the same order as that found after the injection of distilled water and considerably less than after the injection of normal saline (Bedford, 1948a). These findings suggest that penicillin may disappear relatively rapidly from the nervous system after intracisternal injection.

It is interesting to note that the injection of normal saline causes the appearance of considerably more leucocytes than the injection of crystalline penicillin in a dose capable of exciting

symptoms of irritation of the nervous system. As already stated, a rise in the pressure of the cerebrospinal fluid followed the injection of normal saline which was never found after the injection of penicillin in distilled water. These findings would seem to indicate that the irritant action of sodium chloride solution is restricted mainly to the covering membranes of the nervous system and possibly to the arachnoid villi. Penicillin, on the other hand, rapidly penetrates the membranes, with the production of relatively little irritation. Its main action is on the nerve cells and their processes rather than on the supporting tissue.

Attempts to discover whether degree of dilution or rate of administration have any influence on the effect produced by a given dose of penicillin have so far yielded indeterminate results. Owing to the small size of the cisterna magna of the dog, it would seem probable that better results would be obtained by making observations on the human subject.

Summary

A study has been made of the effect of the injection of crystalline penicillin into the cisterna magna of the dog. The duration of the experiments was 6 hours except in a few instances, where it was 24 hours. The injection of 1,000 i.u. penicillin in distilled water did not produce symptoms of irritation of the nervous system, the number of polymorphonuclear leucocytes in the cerebrospinal fluid after 6 hours was no greater than after the injection of a similar volume of distilled water, and there was no rise in the pressure of the cerebrospinal fluid. No symptoms of irritation of the nervous system followed the injection of a similar dose of penicillin dissolved in 0.9 per cent (w/v) sodium chloride solution, although the cerebrospinal fluid contained a considerable number of polymorphonuclear leucocytes and its pressure was raised. Doses of penicillin greater than 10,000 i.u. invariably caused convulsions, and this also occurred when 10,000 i.u. were injected dissolved in sodium chloride solution. Convulsions, however, occurred in only one of twelve dogs when 10,000 i.u. were injected dissolved in distilled water, although there was evidence of irritation of the nervous system in every instance. A rise in the pressure of the cerebrospinal fluid never followed the injection of penicillin in distilled water.

The significance of these findings is discussed.

References

Bedford, T. H. B. (1946). *J. Physiol.*, **104**, 299.
Bedford, T. H. B. (1948a). *Brit. J. Pharmacol.*, **3**, 80.
Bedford, T. H. B. (1948b). *Brain*, **71**, 403.
Cairns, H. (1947). *Brain*, **70**, 251.
Walker, A. E. (1947). *Arch. Neurol. Psychiat., Chicago*, **58**, 39.