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Antibiotic enactivation by cerebrospinal fluid

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ANTIBIOTIC INACTIVATION BY CEREBROSPINAL FLUID

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PART I

A SURVEY OF THE LITERATURE

INTRODUCTION

In recent years considerable attention has been given to the concentrations of drugs in the various body fluids as a guide to therapy. Although clinical experience has shown that concentrations of antimicrobial substances are only loosely related to therapeutic results, it has also shown that such information is valuable in arriving at dosage schedules (1). The purpose of this thesis is to review the work already done on antibiotics in the cerebrospinal fluid and to point out that because present methods of assay are inadequate, cerebrospinal fluid concentrations of antibiotics are of little use in determining the actual amounts of antibiotic agents reaching the nervous tissues.

Animal experiments have demonstrated that penicillin levels in tissues such as kidney and liver are comparable in magnitude to serum levels (2). Although considerable attention has been given to the study of antibiotic levels in the cerebrospinal fluid, there is no clear evidence that these levels reflect the levels within the nervous or meningeal tissues. Whether or not the presence of an antibiotic in the cerebrospinal fluid is necessary or even helpful in the control of meningeal infection is uncertain. If the infection involves the

meningeal membranes primarily, it would seem that antibiotics would reach the involved tissues through their blood supplies as they do elsewhere in the body. The bacteria present in the spinal fluid may represent only those shed from the inflamed membranes and would disappear when that infection is controlled (3). But, if the bacteria actually grow in the cerebrospinal fluid, antibiotic levels are, at least in cases of meningitis, undoubtedly significant. The success of intrathecal penicillin treatment of experimental meningitis in dogs reported by Pilcher and Meacham (4) is, perhaps, the most valid evidence that cerebrospinal fluid concentrations are important. They found that 500 units a day of penicillin intravenously had little beneficial effect on experimentally induced staphylococcic meningitis but that 50 units a day intrathecally reduced the mortality from 93% to 54%. In the absence of more definite information it is probably safest to agree with the opinion of Boger and Wilson (5) who feel that before antibiotic therapy is instituted in diseases of the central nervous system, there should be reasonable assurance that significant cerebrospinal fluid levels can be obtained in 100% of the cases. Readfearn et. al. (6) justify their investigation of penicillin in the cerebrospinal fluid by saying,

"If penicillin appears in the cerebrospinal fluid, one cannot say that all nervous tissue is being reached, but if penicillin does not appear in the cerebrospinal fluid, one can say that not all nervous tissue is being reached."

LEVELS ACHIEVED DURING THERAPY

In the absence of a more definite index, many investigators have attempted to find means of achieving a therapeutically significant level of penicillin in the spinal fluid. Boger and Wilson (5) point out that no single ideal level has much meaning because it fails to account for defense mechanisms of the individual and the sensitivity of the particular strain. Nevertheless, a concentration of 0.03 units of penicillin per ml. has been considered therapeutically significant because that amount of penicillin is adequate to sterilize actively growing cultures of almost all strains of gonococcus, Group A hemolytic streptococcus, pneumococcus, and about one-half of the strains of meningococcus (5,7).

Intrathecally, rather high concentrations of penicillin can be easily achieved and maintained. Ory et al (7)

reported that in thirteen patients with meningitis, 12 hours after intraspinal injection of 10 - 15,000 units, 10 - 40 units/ml. could be detected. Other investigators (8, 9, 10, 11) have reported detectable amounts of penicillin from 24 to 31 hours after intrathecal injection.

Regardless of therapeutic levels achieved, intrathecal injection has many serious drawbacks. Malaise, headache, vomiting, increased intrathecal pressure, meningeal irritation with pleocytosis, lumbosacral arachnoiditis, and sciatic nerve palsey have all been ascribed to injection by this route (7, 9, 12). Because of secondary irritation it may be difficult to estimate the effect of therapy and how long to continue therapy (12). In cases where a block develops, intracisternal or intraventricular administration may become necessary (12). Because of these disadvantages and because of the difficulties and risks associated with repeated spinal punctures, many investigators have attempted to deliver penicillin into the cerebrospinal fluids by parenteral injection (Table I).

Many of the early investigators were not successful in detecting penicillin in the spinal fluid after intravenous or intramuscular injections (7, 9,15, 16, Table I). These failures may be attributed to the small dosage schedules used. In subjects with uninflamed meninges single

TABLE I

Sampling was by lumbar puncture except where other site is indicated. Aqueous crystalline penicillin was used in all studies and all spinal fluids were assayed by biologic methods. Except in cases with inflamed meninges, it will be noted that early investigators failed to use large enough doses to cause diffusion into the spinal fluid. Even with massive doses very little penicillin reached the spinal fluid. Serum levels were often 100 fold greater than spinal fluid levels.

FLUID FOLLOWING PARENTERAL ADMINISTRATION

Time of Sampling After Administration	Miscellaneous	Conc. of Penicillin in CSF in units/ml
		none
60-140 min.		0.03-0.35
3-4 hrs.		none
30 min.-6 hrs.		0.02 (approx.)
15-60 min.	only 4 out of 9 showed penicillin in CSF	0.1-0.4
2 hrs.	serum levels 0.06-0.9 units/ ml	none
10-150 min.		none
25 min-24 hrs.	only 6 out of 14 showed penicillin. 2 had inflamed meninges	0.09-0.156
immed. after infusion		none
30 min.	77.7% of total group & 100% of group receiving over 20,000,000 had detectable penicillin	none-0.55
5 hrs.		0.04-0.3
	18 of 23 showed detectable penicillin	0.019-0.052
2 hrs. and 3 hrs.	In 2 hr., 14 of 18 showed detectable penicillin. In 3 hrs., 15 of 18 showd detectable penicillin	2hrs-0.031 av. 3hrs-0.026 av.
4-6 hrs by ventricular puncture	73% had assayable penicillin	0.057 (mean)
12 & 84 hrs. after start of RX		0.14 0.04
12 hrs after start RX		

injections of about 100,000 units are necessary before detectable penicillin appears in the spinal fluid in a significant proportion of subjects (19).

By continuous infusion, quantitatively larger amounts of penicillin may be necessary to achieve comparable spinal fluid levels. Neyman (16) could detect no penicillin in the spinal fluid after 1,000,000 units were infused intravenously over a three hour period. Barton et. al. (18) and Schwemlein (17) after delivering 25,000,000 units by continuous drip intravenously for a 24 hour period achieved levels in some cases only slightly higher than those obtained by Boger et al (19) with a single intramuscular injection of 100,000 units. These findings bear out the suggestion of Redfearn et al (6) that intermittent dosage may be more effective than continuous infusion in delivering penicillin into the cerebrospinal fluid.

Boger and Wilson (5) reported that 3 gm. of caronamide orally before injection of penicillin enhances the plasma concentration three to five times and doubles the cerebrospinal fluid concentration. Redfearn et al (6) are in agreement in regard to the effect of caronamide and also find that three doses of 500,000 units at 12, 8, and 4 hours before puncture do not give appreciably higher levels in the spinal fluid than does a single injection

of 500,000 units 4 hours previous to a puncture. Such findings lend credence to the opinion expressed by Boger et al (20) that high plasma concentrations, probably over 10 units/ml., for a short period of time are necessary to cause diffusion of penicillin into the cerebrospinal fluid.

When the subject was suffering from acute meningitis, some of the earlier investigators (13, 11) detected penicillin in the spinal fluid with doses as small as 20,000 units. Intrathecal injections of penicillin absorb more rapidly in patients with inflamed meninges than in other patients (15). By injecting fluorescein dye intravenously Lange et al (2) found that diffusion into the spinal fluid was about three times greater in patients with inflamed meninges. They ascribe this increased diffusion to the increased capillary permiability which accompanies inflamation.

Several investigators (5, 18) have used neurosyphilitic subjects and assumed that there was no alteration in the "barrier" between the blood and the spinal fluid. Redfearn et al (6), in one of the most careful and most recent studies of the subject, have found significantly higher levels in subjects with syphilis of the central nervous system (Table I). These same investigators report an increase in diffusion of penicillin into the spinal

fluid in almost all patients with lesions of the central nervous system and that this increase is roughly parallel to the increase in spinal fluid protein (Table II.). The inference is that pathologic processes damage vascular tissue either locally or generally and cause larger amounts of both protein and penicillin to appear in the spinal fluid.

TABLE II

PENICILLIN AND PROTEIN DIFFUSION INTO CEREBROSPINAL FLUID

After Redfearn et al (6)

Diagnosis	No. of cases	Mean Penicillin units/ml.	Mean Protein mgm %
Neurologically normal (psychiatric)	9	0.04	12
Idiopathic epilepsy	9	0.04	12
Cerebral glioma	14	0.06	20
Cerebral arteriosclerosis	4	0.08	40
Neurosyphilis	10	0.14	80

Penicillin was assayed by a capillary serial dilution method in which penicillin sensitive hemolytic streptococci ferment glucose producing acid detectable by phenol red. Since about 0.037 units of penicillin always inhibited the test strain, that amount and amounts larger could be detected in the spinal fluid. Mean penicillin levels are parallel to mean protein levels, but this relationship did not necessarily hold true in each individual case studied.

Diffusion of penicillin in the elderly tends to be increased even when protein is not increased (16). An increase in spinal fluid pressure does not alter the diffusion of penicillin (16).

In contrast to penicillin, very little study has been made on the diffusion of other antibiotic agents into the cerebrospinal fluids. Streptomycin does not readily diffuse into spinal fluid in normal persons, but, when injected parenterally into patients with meningitis, amounts sufficient to check growth of susceptible organisms may reach the cerebrospinal fluid after repeated injections (22).

Of the three important new antibiotics--chloramphenicol, aureomycin, and terramycin--chloramphenicol accumulates most rapidly in the serum, reaches the highest serum concentrations, and produces the highest cerebrospinal fluid concentrations (1). That aureomycin does diffuse into the spinal fluid has been proven by Lepper et al (23) who detected 0.06 to 0.13 micrograms/ml. in the spinal fluid following doses of 100 mgm intramuscularly and 300 to 700 mgm orally in 6 out of 9 patients. Herrell and Heilman reported similar findings in a series of 8 cases (24). Werner et al detected terramycin in the spinal fluid in 4 out of 8 subjects and concluded that concentrations of terramycin in the spinal fluid after single large daily doses were similar to those obtained for aureomycin (1).. But Herrell et al found a measurable amount of terramycin in only one of six patients and concluded that unlike aureomycin, terramycin does not traverse the "blood-brain barrier" (25).

INHIBITORY EFFECT OF BODY FLUIDS ON ANTIBIOTICS

Table I indicates the only minute quantities of penicillin reaches the cerebrospinal fluid even with massive doses. In attempting to explain why only a small proportion of an antibiotic agent penetrates to the spinal fluid, one is confronted with the problem of the binding of molecules by proteins. Interreactions in protein solutions have long been known, such as the inhibitory effect of milk or serum on many antiseptics. Because sulfadiazine and sulfathiozole were found in spinal fluid in much lower concentration than in serum, they were at one time considered unsuitable for the treatment of meningitis. Dialysis experiments have shown, however, that these drugs were bound to plasma proteins to a degree which accounted for their relative concentrations in the blood and spinal fluid, assuming the spinal fluid to be an ultrafiltrate of blood. Since the unbound drug determines the bacteriostatic level of a given fluid, the effective concentrations of these sulfa drugs were the same in both serum and spinal fluid (26).

The dialysis experiments of Chow and McKee (27) have shown that penicillin combines with serum albumin. But, unlike the sulfonamide-protein complex, the penicillin-albumin complex possesses antibiotic activity. Assuming

spinal fluid to be an ultrafiltrate of blood, none of this penicillin-albumin complex with its antibiotic activity would enter the spinal fluid so that the spinal fluid would be poorer than the serum in antibiotic activity. Since current methods of assay are biologic and measure the antibacterial effect of the unknown, it is conceivable that the binding of antibiotics by plasma proteins accounts for the discrepancy between antibiotic levels in the serum and in the spinal fluid. This explanation could only be valid if the penicillin-protein complex possesses a considerable proportion of the antibacterial activity of the plasma.

That the penicillin-protein complex has antibiotic activity to a significant degree is doubtful in view of the work of Tompsettetal (28). By making series dilutions of penicillin in broth in the presence of 10%, 20%, and 30% human serum and comparing inhibition to growth of a test strain in these series to inhibition in series dilutions in the absence of serum, these investigators found that the effectiveness of penicillin was diminished in proportion to the amount of serum present. They also added bovine albumin to media in concentrations comparable to those attained by adding serum and found a parallel effect but an effect not as great in magnitude. In regard to other antibiotics, it has been shown that aureomycin

is also inactivated by serum but that the effect is much greater. In series dilution of aureomycin, the addition of 50% serum causes a 20 - 50 fold raising of the end point and in series dilution of penicillin, only a four fold increase (29).

McDermott and Nelson (14) have shown that in concentrations ranging from 0.078 to 1.25 micrograms/ml. in serum, penicillin is diffusible through artificial membranes in vitro and into ascitic fluid in vivo. Since at such low serum levels penicillin will not diffuse into the spinal fluid, it is apparent that the binding of penicillin to non-diffusible elements in the serum is not the only reason for the poor diffusion of penicillin into the spinal fluid.

In 1948, Tucker (30) reported that human cerebrospinal fluid, itself, exerts an inhibitory effect on the bactericidal activities of penicillins G, X, and K in vitro as determined by serial dilution bioassay methods. Penicillins G, X, and K proved identical in their susceptibility to the inhibitory effect of spinal fluid. Further, the inhibitory effect resembled that of serum and was proportional to the concentration of spinal fluid present (Table III).

TABLE III

After Tucker (30)

THE INHIBITORY EFFECT OF CEREBROSPINAL FLUID

ON

THE ACTIVITY OF PENICILLIN

% CSF Present in the Culture Media	Activity Index of the Penicillin Based on an Activity of 100 in Plain Broth Culture
59	55 - 56
29.5	66 - 71
14.8	83 - 90
7.4	89 - 90
.6	94 - 98

The activity index represents the percent of activity remaining after inactivation of the penicillin by the varying proportions of spinal fluid.

Heating of the spinal fluid at 100 degrees C for 30 minutes followed by Seitz filtration did not appreciably change the inhibitory effect. Changing the P H within the range of 7.3 to 8.4 did not alter the effect. When various concentrations of penicillin were incubated in 96% cerebrospinal fluid at 37 degrees C, there was progressive loss of penicillin activity.

Although the inhibitory effect of human serum on penicillin has been ascribed in large part to the binding

effect of the plasma proteins, such an effect does not explain the similar property of spinal fluid. For penicillin G and X ~~in~~ the inhibitory effect of cerebrospinal fluid was only 15% to 20% less than that of serum, while the total protein content differed by a factor of 100 - 300 fold. Further, heat coagulation of the protein and its removal by means of Seitz filtration did not appreciably modify the inhibitory effect. Finally, the fact the penicillin incubated in the presence of cerebrospinal fluid progressively lost activity, suggests that there might be actual destruction of penicillin by some unidentified mechanism.

Jordan and Hill (31) report that normal cerebrospinal fluid exerts a similar inhibitory effect on the antibacterial action of streptomycin. This effect is, to a degree, proportional to the concentration of spinal fluid present (Table IV).

TABLE IV
After Jordan and Hill (31)

THE INHIBITORY EFFECT OF CEREBROSPINAL FLUID ON THE ACTIVITY OF STREPTOMYCIN

Amt. CSF Present in Ml.	Conc. at which Growth of Test Strain Was Inhibited in Majority of Cases.
0.5	3.1 micrograms/ml.
0.25	3.1 micrograms/ml.
0.1	0.7 micrograms/ml.
Control no CSF	0.3 micrograms/ml.

Series dilutions of streptomycin were set up in broth and the amount of CSF was added that is indicated in the first column. Adjustments were made so that all tubes had an equal volume and so that corresponding tubes in the various series had equal concentrations of streptomycin.

The determination of streptomycin in spinal fluid by chemical methods as developed by Boxer and Jelinek (32) will detect 100 ± 3% of the streptomycin added to 1 ml. of normal human spinal fluid. These authors report that biological assay of the same samples will detect only 85 ± 11%. This difference substantiates the report of Jordan and Hill (31) that cerebrospinal fluid inhibits the antibacterial activity of streptomycin.

The discoveries of Tucker (30) and Jordan and Hill (31) may very well explain the discrepancy between the apparent failure of antibiotic agents to reach the spinal fluid in anything but minute quantities and the profound therapeutic response frequently obtained in disease, as for example, neurosyphilis. They suggest that effective concentrations of antibiotics may be present, but not demonstrable by current in vitro methods of bioassay because of the inhibitory effect of the spinal fluid itself upon the assay.

PART II

AN ORIGINAL INVESTIGATION

BACKGROUND FOR THE INVESTIGATION

During the summer of 1950 a patient under treatment for congenital syphilitic meningoencephalitis was studied at the Nebraska Psychiatric Unit. As a part of the study, the author was assigned to work with Dr. McFadden of the Department of Bacteriology of the University of Nebraska College of Medicine in determining the amount of terramycin penetrating to the spinal fluid.

With dilution bioassay methods (Appendix I) it was found that although the patient's serum displayed a definite inhibitory effect on the test organism, his spinal fluid had no inhibitory effect. But concurrently, spinal fluid changes indicated a rapid response to treatment (Table V).

TABLE V

SPINAL FLUID FINDINGS IN PATIENT WITH CONGENITAL MENINGO--ENCEPHALITIS UNDER TREATMENT WITH TERRAMYCIN

Date	Eagle	Mazini	Cell Count	Protein in mgm. %	Colloidal Gold
7/11/50	+	+	22	48	5554322111
8/1/50	+	+			2222210000
8/8/50	+	+	1	21	4321100000
8/15/50	+	+	4	36	3321100000
8/22/50	+	+	4	30	3321100000

In neurosyphilis spinal fluid findings are the earliest index to the effect of treatment upon the spriochete. If clinical improvements occur, they are the result of structural changes and do not appear except over long periods of time (33). For dosage of terramycin and method of assay see Appendix I.

Although beneficial effects had been ascribed to penicillin treatment of neurosyphilis without penicillin ever penetrating to the spinal fluid (15,34), no one had ever explained why this apparently anomalous situation should exist. Feeling that the circumstances were similar and that there might be a simple answer to the problem, the author decided to attempt to find out why terramycin was not entering the spinal fluid.

As mentioned in Part I, the studies of Herrell et. al. (25) indicate that terramycin does not readily diffuse into the spinal fluid. In their study, four patients received 1 gm. of terramycin hydrochloride every 6 hours for 24 hours prior to puncture. Serum of these patients contained 8 micrograms/ml. Two additional patients received 1 gm. every 6 hours for 12 hours prior to puncture. Although these doses were twice as large as those received by the patient under treatment at the time that he was showing the greatest improvement in spinal fluid findings, only one out of the six had more than a trace of antibiotic activity in the spinal fluid. Herrell et. al. (25) concluded that terramycin does not readily traverse the "blood-meningeal barrier", yet these same studies indicated that terramycin diffuses into pleural fluid and traverses the placenta. It did not seem likely that terramycin could breach the placental barrier and not the "blood meningeal barrier."

INACTIVATION OF TERRAMYCIN BY CEREBROSPINAL FLUID

Bearing in mind that antibiotic agents are often unstable and easily destroyed, it seemed reasonable that there might be some substance in spinal fluid which inactivates terramycin and, in that way, makes it undetectable. To test out this hypothesis fluid was obtained from a routine pneumoencephalogram and serial dilutions were set up as indicated in Table VI. The cell count, protein, sugar, and chlorides of this fluid were all within normal limits.

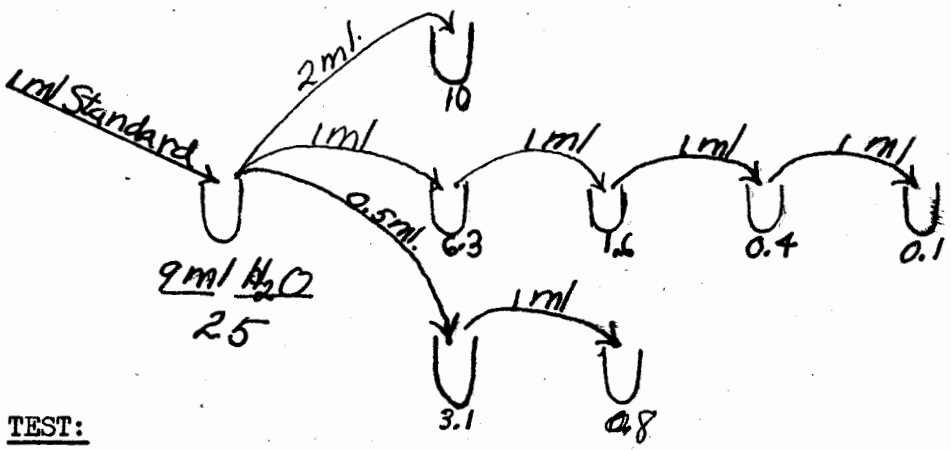
The only difference between the control series and the test series was that in the control the stock terramycin solution was diluted to 25 micrograms/ml with water, and in the test it was diluted to 25 micrograms/ml with spinal fluid. The 25 microgram/ml solutions were diluted into brain heart infusion media in an identical fashion. It was felt that diluting by this method brought all of the terramycin in the test series into contact with spinal fluid.

After 48 hours incubation at 37 degrees C growth had taken place at only 0.1 micrograms/ml in the control but all the way up to and including 6.3 micrograms/ml in the test (Table VII). Blood agar plates were streaked from all tubes of the series and read in 24 hours. The

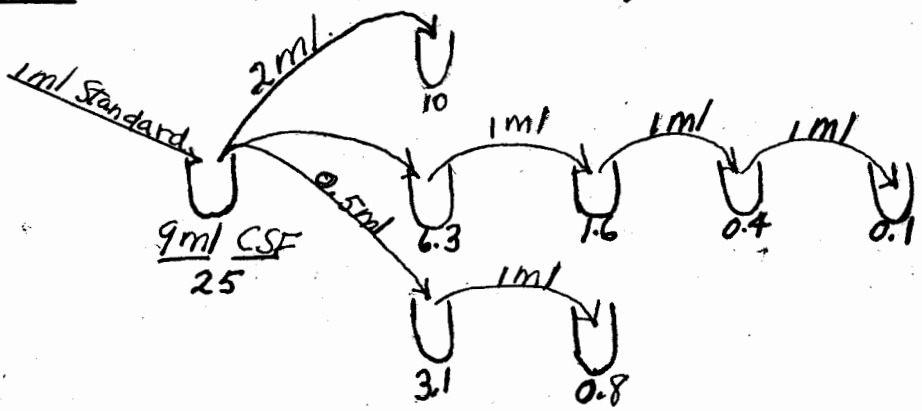
TABLE VI

METHOD OF DETERMINING EFFECT OF SPINAL FLUID ON TERRAMYCIN

CONTROL:



TEST:



Number under each tube indicates the final concentration of terramycin in micrograms/ml. in that tube. In order, the final concentrations were 10, 6.3, 3.1, 1.6, 0.8, 0.4, and 0.1 micrograms/ml.

Each tube with the exception of the 25 microgram/ml. tubes contained 9 ml. of brain heart infusion media before dilution. The two 25 microgram tubes contained 9 ml. of sterile distilled water and 9 ml. of CSF, respectively. The standard was made up by dissolving 250 mgm. of terramycin in 1000 ml. of sterile distilled water. After each transfer the contents of the tube were throughly mixed before transfer to the next tube. Inoculation was accomplished from a 24 hr. blood agar culture of hemolytic streptococcus by loop.

appearance of hemolytic colonies on the blood agar streaked from the tubes 6.3 micrograms/ml to 0.1 micrograms/ml in the test and only from tube 0.1 micrograms/ml in the control verified the tube readings of the previous day.

Similar experiments were repeated with seven other fluids obtained during routine diagnostic lumbar puncture. Of these seven, two had elevated proteins (66 and 100 mgm %), one was the patient who had been treated with terramycin for neurosyphilis, and the other four had no abnormal spinal fluid findings. These trials differed from the original trial only in the concentration of the standard terramycin solution, the quantity of diluent in the first tube, and the use of media instead of water as a diluent in the first tube of the control (Appendix II and Table VII). The substitution of media for water was to eliminate any possible inhibitory effect which might result from water dilution of the media in the control series.

After 24 hours, growth was usually visible at 0.1 microgram/ml in both control and test. In 48 hours the control grew to between 0.4 and 1.6 micrograms/ml and the test to between 3.1 and 6.3 micrograms/ml. The control series remained at the 48 hour level. In 72 hours the test series all showed growth at 10 micrograms/ml, the highest concentration in the series (Table VII).

Subcultures on blood agar similar to those of the first trial were used to verify the results after 72 hours. The use of abnormal spinal fluid did not produce a noticeable difference in effect.

TABLE VII

GROWTH IN DILUTION THROUGH SPINAL FLUID EXPERIMENTS

	Trial I		
	24 hrs.	48 hrs.	72 hrs.
Control		0.1 ug/ml	
Test		6.3 ug/ml	
	Trials 2-7		
Control	0.1 ug/ml	0.4-1.6 ug/ml	0.4-1.6 ug
Test	0.1 ug/ml	3.1-6.3 ug/ml	10 ug

Growth occurred at much higher concentrations when the terramycin had been in contact with spinal fluid (see text).

Two other spinal fluids, both of which had elevated proteins (60 and 42 mgm %) were set up so that the greatest concentration was 33 microgram/ml in each series (Appendix II). The control did not grow beyond 1.9 micrograms/ml. At 48 hours one test series showed no growth in the lowest tube of the series, 4.2. micrograms/ml, or in any higher tube, while the other showed growth at 16.6 micrograms/ml and 20.8 micrograms/ml but not at lower concentrations. In 72 hours the series which had shown growth at 48 hours had growth in all tubes including 33.1 micrograms/ml, and the other series had growth in all but

the two lowest tubes, 8.3 and 4.2 micrograms/ml. These two trials demonstrated that growth would occur at considerably higher concentrations than the top of the first eight trials, 10 micrograms/ml. The beginning of growth at the middle of these series suggested that the phenomenon depended upon the presence of spinal fluid in the culture tube rather than on a passage of terramycin through spinal fluid. For this reason an investigation was undertaken to determine if there was a relationship between the proportion of spinal fluid present in the culture tube and the reduction of antibiotic activity.

EFFECT OF VARYING THE PROPORTION OF SPINAL FLUID

Before an investigation of the effect of varying proportions of spinal fluid on antibiotic activity could be undertaken, it was necessary to know how much spinal fluid could be added to brain heart infusion broth without diluting the broth to a point where it would not support growth of the test strains of hemolytic streptococcus, a very fastidious organism. For this reason several series of varying proportions of spinal fluid in brain heart infusion broth were set up, inoculated with the test strain, and incubated for 72 hours. Diluting brain heart media by 50% with spinal fluid did not cause inhibition to growth in 24 hours, but beyond 50% increasing

periods of time were necessary for visible growth to appear.

Series with a range from 50 to 0.5 micrograms of terramycin per ml., with equal final volumes, but with a different proportion of spinal fluid in each series, were set up as shown in Appendix III. The first trial had 2%, 10%, and 40% spinal fluid present in each of three series. The tubes were inoculated, incubated, and examined for visible growth at 37 hours, sixty hours, and 19 days. The results (Table VIII and accompanying curve) indicated that increasing the percentage of spinal fluid increased terramycin inactivation but that the relationship was not a direct one.

Because the difference in effect was more marked between the 2% and 10% series than between the 10% and 40% series and because spinal fluid in amounts sufficient to prepare the 40% series were difficult to obtain, the next two trials were run at 2%, 5%, and 10%. The results (Table VIII and accompanying curves) were similar to those of the first trial.

The gradual progression of growth up the control series, both in these trials and in the previously described experiments, bears some resemblance to the behavior of aureomycin. Bliss and Chandler (29) found that in series dilution tests of aureomycin the end point moved up day by day. They attributed the progression to the fact that aureomycin was bacteriostatic rather

TABLE VIII

TERRAMYCIN INACTIVATION BY VARYING PROPORTIONS OF CEREBROSPINAL FLUID

Although the methods used were biological in nature and for that reason rather crude, it appears evident that the rate at which the organism overcame terramycin was very slow for the first 24 hrs. Between 24 hrs. and 48 hrs. the rate at which inhibition was overcome greatly accelerated.

In each series there came a concentration which the organism could not overcome in a prolonged period of time. The height of this final inhibiting concentration appeared to be a function of the proportion of spinal fluid present in the culture.

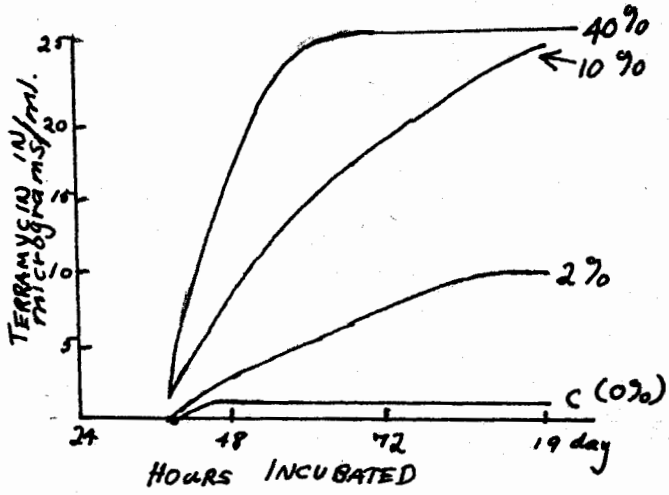
Early, the rate at which the organism was able to overcome inhibition was about the same in all series. But as the final inhibiting concentration was approached in each series, the rate at which inhibition was overcome decreased and for that reason the curves diverge from one another.

Data in trial 2 was insufficient to draw curves, but the data tends to verify the findings in trials 1 and 3.

In trials 1 and 2 spinal fluid examinations were within normal limits. In trial 3 the cell count was 5 mononuclear cells, the protein was 39 mgm%, the serology was positive, and the colloidal gold curve was 5555555421.

Trial 1

CSF	Highest concentration in in micrograms/ml. at which growth appeared in- 37 hrs.	60 hrs.	19 da.
0%	none	1	1
2%	none	5	10
10%	1	15	25
40%	1	25	25

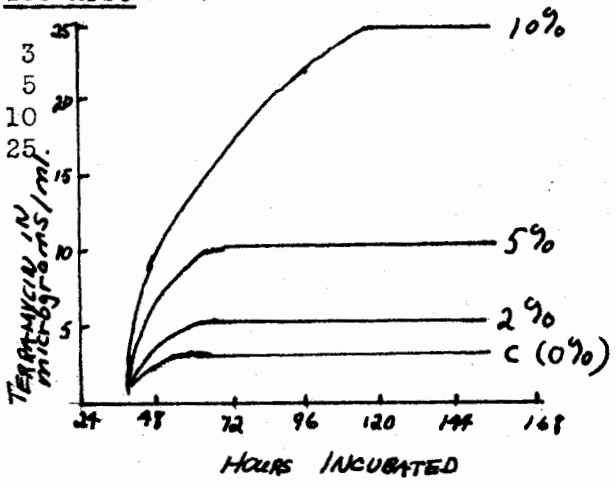


Trial 2

CSF	Highest concentration in micrograms/ml. at which growth appeared in - 62 hrs.	86 hrs.
0%	5	5
2%	20	20
5%	25	25
10%	25	40

Trial 3

CSF	Highest concentration in micrograms/ml. at which growth appeared in- 38 hrs.	65 hrs.	110 hrs.	158 hrs.
0%	1	3	3	3
2%	1	5	5	5
5%	0.5	10	10	10
10%	0.5	15	25	25



than bactericidal in its action and although it arrests growth for the first 24 hours the organisms eventually recover and reach maximum growth.

Whether the recovery of the organisms was a result of destruction of the antibiotic agent or development of resistant strains was not absolutely clear. But the fact that storage of aureomycin in broth at 37 degrees C for 18 hours reduced its activity by 60% (29) lends support to the idea that destruction of the antibiotic is responsible. Because of the similarity in their actions and behavior, it seems probable that the terramycin in the controls in this study, like aureomycin, was being destroyed by incubation in broth.

The similarity in shape between the test curves and the control curve (Table VIII) suggests that the method by which bacteria overcome terramycin is the same in test and control. If this be true, it appears that terramycin incubated in the presence of either broth or spinal fluid is somehow inactivated or destroyed but that the magnitude of this destruction is many times greater in the presence of spinal fluid.

PART III

DISCUSSION

Because of the inactivating effect of spinal fluid on penicillin, streptomycin, and terramycin, the currently used methods of dilution bioassay for determining antibiotic levels in spinal fluid are of doubtful value. Although the work which has been done on spinal fluid concentrations of antibiotics is of significance in that it shows that antibiotics do penetrate to the spinal fluid, such work is quantitatively inaccurate, for much larger amounts of antibiotic agents may be entering the spinal fluid than are detected by present methods.

Theoretically, a bioassay could be corrected for the inactivating effect of spinal fluid by adjusting all tubes in the control and in the test series to the same concentration of spinal fluid with pooled normal spinal fluid. This would equalize the effect in all tubes. But such a procedure would also tend to reduce the sensitivity of the assay making the measurement of therepeutically produced levels impossible.

Tompsett et. al. (28) reported that the effectiveness of penicillin was diminished in proportion to the amount of serum present. The addition of bovine albumin to culture media

in concentrations comparable to those of serum produced a parallel effect but one not as great in magnitude. These findings suggest that the inhibitory effect of serum may not be due entirely to binding by proteins. That protein binding may account for the inactivating effedt of spinal fluid is doubtful. In Tucker's study (30) the inhibitory effect on penicillin was 15% to 20% less in spinal fluid than in serum while the protein content differed by 100 to 300 fold. Further, heat coagulation of the protein and its removal by Seitz filtration did not appreciably modify the inhibitory effect.

It is noteworthy that serum, spinal fluid, and brain heart infusion broth all inactivate certain antibiotics to some degree. The question arises as to whether all animal tissues inactivate atibiotic agents. If such be true, would the difference in magnitude between broth and spinal fluid inactivation be due to a difference in processing or to a difference in the tissues of origin? If the tissue of origin should prove to govern the magnitude of antibiotic inactivation, it may be necessary in the future to choose antibiotics for

a particular patient not only with a view to the organism involved but also with a view to the organ involved. In other words, we may have to consider "tissue spectra" as well as the current "bacterial spectra".

If this inactivating property should turn out to be an attribute of all animal tissue, one might postulate that it is related to the mechanism which renders antibiotic agents relatively non-toxic and protects the animal cell from the fate of the bacterial cell.

SUMMARY

Numerous investigators have reported that on parenteral administration only a very small proportion of most antibiotics penetrate to the cerebrospinal fluid. Pathologic processes, which damage vascular tissue either locally in the nervous system or generally, tend to increase the amount of penicillin penetrating to the spinal fluid, but, even in these cases, levels are not comparable to serum levels. Although antibiotic agents are bound by serum proteins, protein binding does not appear to offer a satisfactory explanation for the failure of antibiotics to reach the spinal fluid.

Since antibiotic concentrations are measured by bioassay, it is evident that a destruction of the bactericidal activity of an antibiotic by spinal fluid would make the antibiotic undetectable and would explain these apparantly low spinal fluid concentrations. Such a destruction of the bactericidal activity of penicillin and of streptomycin by spinal fluid have been reported.

The failure of terramycin to penetrate to the spinal fluid in a case of congenital syphilitic meningoencephalitis led to a study of the in vitro effect of spinal fluid on terramycin. This study was undertaken without preliminary knowledge of previously reported penicillin and streptomycin inactivation. Terramycin incubated in the presence of either broth or spinal fluid appeared to be destroyed, but the magnitude of this destruction was many times greater in the presence of spinal fluid.

The possible implications of antibiotic inactivations by body fluids have been discussed.

CONCLUSIONS

1. No clear evidence exists that spinal fluid concentrations of antibiotics reflect the levels within the nervous tissue.

2. The concentrations of antibiotics in cerebrospinal fluid are probably of significance in the treatment of meningitis.

3. On parenteral administration antibiotics reach the spinal fluid only in very small amounts.

4. Processes which damage vascular tissue and increase protein also increase the amount of antibiotic reaching the spinal fluid.

5. Penicillin and streptomycin are inactivated by spinal fluid.

6. Terramycin incubated in the presence of either broth or spinal fluid is slowly inactivated, but the magnitude of the inactivation is many times greater in the presence of spinal fluid.

7. Current in vitro methods of bioassay of antibiotics in the cerebrospinal fluid are inadequate.

8. Most studies of antibiotic concentrations in the cerebrospinal fluid are probably, at least quantitatively, inaccurate.

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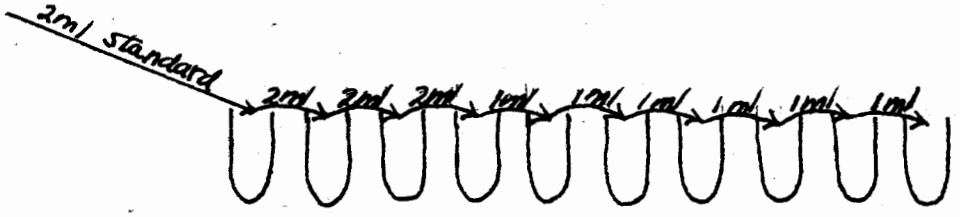
APPENDIX I

METHOD OF ASSAY OF BODY FLUIDS FOR TERRAMYCIN

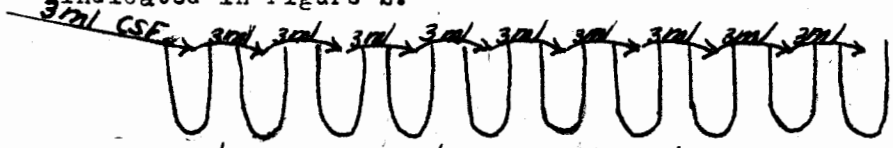
CONTROL: Standard terramycin solution was made up by dissolving 250 mgm in 1000 ml. of sterile distilled water. Two ml. of standard solution was pipetted into the first of a series of 10 tubes containing 3 ml. of media each. The standard was mixed carefully with the media in tube 1 and then 2 ml. were transferred ffom tube 1 to tube 2. Subsequent mixtures and transfers were continued as shown in Figure 1.



Media in each tube	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml
Concentration of terramycin in micrograms/ml.	100	40	16	6.4	1.6	04	0.1	0.25	0.0065	0.0016

Figure 1: Control Series ---Aseptic technique followed throughout. Each tube was inoculated and incubated at 37 degrees C for 24 hours.

SPINAL FLUID: Series dilutions of the spinal fluid to be assayed were set up in a similar fashion adding 3 ml. of unknown to the first tubes and transferring down the series as indicated in Figure 2.



Media in each tube	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml	3ml
Proportion CSF in each tube	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024

Figure 2: Unknown Series----Aseptic technique followed throughout. Each tube was inoculated and incubated at 37 degrees C for 24 hours.

SERUM: Same procedure as for spinal fluid.

MEDIA AND TEST STRAINS: The media for first 3 tests was tryptose broth. The test organism was a strain of staphylococcus aureus. Because of the failure to detect terramycin on previous tests, Richard's strain of hemolytic streptococcus was used on the fourth test. This organism is more sensitive to terramycin than was the original. The use of hemolytic streptococcus required a richer medium, brain heart infusion broth.

COLLECTION OF SAMPLES: Patient was on dose of 500 mgm. terramycin hydrochloride every 6 hours. Spinal fluid and blood were withdrawn 2 hours after 6 a.m. dose, taken immediately to the laboratory, refrigerated, and assayed either the same morning or early the same afternoon. Assays were done after the patient received 2 gm. of terramycin and at weekly intervals for 3 successive weeks. For 3 days before final assay dose was 1.5 gm. every 6 hours.

READING AND CALCULATION: After inoculation by looping or pipette from broth or plate culture and after 24 hours of incubation at 37 degrees C, the tubes were examined for growth in a bright light. Knowing the amount of terramycin in the control which completely inhibited growth in 24 hours and the dilution of the unknown which would completely inhibit growth, it was possible to calculate the amount of terramycin in the unknown. For example, if the control showed complete inhibition of growth at 0.4 micrograms and the spinal fluid series showed complete inhibition at a dilution of $\frac{1}{2}$ but not at $\frac{1}{4}$, there would be 2 X 0.4 micrograms, or 0.8 micrograms of terramycin in the spinal fluid.

APPENDIX II

DETAILS OF DILUTION THROUGH SPINAL FLUID EXPERIMENTS

TRIAL I:
See table VI

TRIALS 2 - 7:
Standard - 50 mgm. terramycin in 400 ml. sterile distilled water = 125 micrograms/ml.

Control:
1 ml. standard in 4 ml. brain heart infusion broth = 25 micrograms/ml.
Dilutions from 25 micrograms/ml. down were 10, 6, 3, 3.1, 1.6, 0.8, 0.4, and 0.1 micrograms diluted as in trial 1 (Table VI)

Test:
1 ml. standard in 4 ml. spinal fluid = 25 micrograms/ml.
Dilutions from 25 micrograms/ml. down were same as control and were made as shown in trial 1 (Table VI)

TRIALS 9 - 10:
Standard - 50 mgm. terramycin in 400 ml. sterile distilled water = 125 micrograms/ml.

Control:

0.3 ml	of	125	micrograms/ml	in	3 ml	brainheart	=	14.7	micrograms/ml
0.2 ml	of	125	micrograms/ml	in	3 ml	brain heart	=	7.8	micrograms/ml
0.1 "	"	"	"	"	"	"	"	"	"	=	4.0	"	"
1.0 "	"	7.8	"	"	"	"	"	"	"	=	1.9	"	"
1.0 "	"	4.0	"	"	"	"	"	"	"	=	1.0	"	"
1.0 "	"	1.0	"	"	"	"	"	"	"	=	0.25	"	"

Test:

2 ml	of	125	micrograms/ml	in	1 ml CSF	=	83.3	micrograms/ml.
2 ml	of	83.3	"	"	"	3 " "	=	33.3	" " .
3 ml	of	38.3	"	"	"	" " "	=	16.6	" " .
1 ml	of	16.6	"	"	"	" " "	=	4.2	" " .
1 ml	of	83.3	"	"	"	" " "	=	20.8	" " .
2 ml	of	20.8	"	"	"	" " "	=	8.3	" " .

APPENDIX III

PREPARATION OF SERIES WITH VARYING PROPORTIONS OF SPINAL FLUID

Stock #1 - 50 mgm. terramycin in 250ml. water = 200 micrograms/ml.
Stock #2 - 3 ml. of stock #1 in 3 ml. B.H. = 100 " "
Stock #3 - 0.5 Ml. of stock #2 in 4.5 ml. B. H.= 10 " "

TEST:

CSF in each series	Final concentration of terramycin in micrograms/ml. in each tube		
	50	40	25
	S , B.H.	S , B.H.	S , B.H.
2% (0.04)	1.0, 0.96	0.8 , 1.16	0.5 , 1.46
5% (0.10)	1.0, 0.90	0.8 , 1.10	0.5 , 1.40
10% (0.20)	1.0, 0.80	0.8 , 1.0	0.5 , 1.30
40% (0.80)	1.0, 0.20	0.8 , 0.4	0.5 , 0.70
	20	15	10
2% (0.04)	0.4, 1.56	0.3 , 1.66	0.2, 1.76
5% (0.10)	0.4, 1.50	0.3 , 1.60	0.2, 1.70
10% (0.20)	0.4, 1.40	0.3 , 1.50	0.2, 1.60
40% (0.80)	0.4, 0.80	0.3 , 0.90	0.2 , 1.00

S above means stock #2 = 100 micrograms/ml.

	5	1	0.5
2% (0.04)	1.0, 0.96	0.2, 1.76	0.1, 1.86
5% (0.10)	1.0, 0.90	0.2, 1.70	0.1, 1.80
10% (0.20)	1.0, 0.80	0.2, 1.60	0.1, 1.70
40% (0.80)	1.0, 0.20	0.2, 1.00	0.1, 1.10

S above means stock #3 - 10 micrograms/ml.

CONTROL:

CSF in each series	Final concentration of terramycin in micrograms/ml. in each tube					
	10		5		3	
	S	B.H.	S	B.H.	S,	B.H.
none	2.0,	0	1.0,	1.0	0.6,	1.4
	1.0		0.5			
none	0.2,	1.8	0.1,	1.9		

S above means stock#3 = 10 micrograms/ml.

S means stock, B.H. means brain heart infusion media, and all figures in the chart refer to ml. except where otherwise indicated. The figure in parenthesis after % CSF is the amount of CSF added to each tube in the series.

All quantities indicated were delivered directly into the culture tube by sterile pipette. There were no transfers as in earlier experiments.

Inoculation was from a 28 hr. culture of Richard's strain of hemolytic streptococcus in brain heart infusion media. The 28 hr. cultures used for all three trials were of the same turbidity as measured by a comparator. The brain heart infusion culture was, itself, inoculated from a 24 hr. blood agar culture. Each tube in the test and in the control was inoculated with one drop of the 28 hr. culture from a tuberculin syringe through a No. 22 needle. The same needle and syringe were used in all trials.

All samples of spinal fluid were negative for occult blood to the benzidine test. Visual readings were verified at the end of the observation period by subculture on blood agar.

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