THE PASSAGE OF PENICILLINS INTO THE CEREBRO-SPINAL FLUID AND BRAIN IN EXPERIMENTAL MENINGITIS—EXPERIMENTAL INVESTIGATIONS ON RABBITS

ARNE LITHANDER

*Bacteriological Department of Serafimerlasarettet,
Stockholm.*

THE majority of the experiments were made on adult rabbits. Young animals were used in one series. Live bacteria, dead bacteria or bacterial toxins were injected in a volume of 0.5 ml. into the cisterna magna with the animals under intravenous nembutal narcosis. The controls were given an injection of 0.5 ml. physiological saline solution in the cisterna. A day or so later, the rabbits were examined with special reference to signs of meningitis. The animals were classified by group on the basis of the intensity of the signs of meningitis. The classification is shown in Table I. The control rabbits exhibited no signs of meningitis or other conditions in any one of the experiments. Benzylpenicillin* was used in some of the experiments, ampicillin in others.

Sensitivity tests in agar plates were used for the determination of the concentration of penicillin and ampicillin. The preparation under test was filled in holes in the agar plates. Only 0.06 ml. of the preparation was required in each hole. The concentrations of penicillin in plasma, cerebrospinal fluid (C.S.F.) and brain will be expressed in the following as log (mean concentration $\times$ 100).

*The benzyl penicillin in the present investigation was kindly supplied by AB KABI, Stockholm, Sweden, the ampicillin by AB Astra, Södertälje, Sweden.—Subsequently the term "benzyl penicillin" will be abbreviated to "penicillin".

The rabbits were anesthetized with nembutal before the tests were made. C.S.F. was removed by puncture of the cisterna magna. The animal was killed immediately thereafter and a blood sample was taken from the jugular vein with a small admixture of heparin to prevent coagulation. The brain was removed and freed of blood and C.S.F.

Experiments with Penicillin

These experiments were made with live and dead bacteria and with bacterial toxins. *Staphylococcus aureus*, α -, β -, and γ -streptococci, pneumococci and meningococci were used in the experiments with live bacteria while *staphylococcus aureus*, β -streptococci and meningococci were used in the experiments with dead bacteria. The toxins were scarlatinal and staphylococcic toxin and endotoxin¹.

An intravenous injection of penicillin was given about once a day after the injection of bacteria or toxins in the cisterna. In most series the dose was 20,000 units per kilogram body weight. The controls were given the same dose of penicillin as the experimental rabbits.

Samples of C.S.F., blood and brain were

¹The endotoxin used in the experiments was Bactera lipopolysaccharide (*Esch. Coli* 0 111:4 from the Difco Laboratories, Detroit, Mich., U.S.A.).

TABLE I

Groups of Rabbits, Classified According to Intensity of Signs, which had Received Injections of Live Bacteria, Dead Bacteria or Bacterial Toxins in the Cisterna Magna.

Signs of meningitis	Symbols representing the intensity of signs
No signs of meningitis (but slightly impaired condition)	—
Suggested signs of meningitis (suggested nuchal rigidity)	(+)
Moderate signs of meningitis (moderate opisthotonus)	+
Mild signs of meningitis (slight opisthotonus)	++
Pronounced signs of meningitis (pronounced opisthotonus)	+++

FIG. 1.—Penicillin concentration in C.S.F. at different intervals after intravenous penicillin injection of 20,000 units per kg. The broken line denotes controls, the unbroken line rabbits with meningitis.

removed 30 minutes later in most of the experiments. In one experiment with live staphylococci, samples were taken after 15, 30, 60, 90, 120 and 150 minutes.

Experiments with Live Bacteria

Experiments with staphylococci. The concentration of penicillin in plasma was high 15 minutes after the injection, but dropped rapidly thereafter. There was no difference in this respect between controls and experimental animals.

It appears from Fig. 1 that the penicillin concentration in C.S.F. was high after 15 minutes but later decreased. It was higher at all intervals in the experimental animals than in the controls. The difference was significant at all intervals.

The examinations 30 minutes after the injection of penicillin comprised more animals than at other intervals, and the animals were therefore studied more thoroughly. At this interval we also used young animals which were given doses of 20,000 units of penicillin/kg. body weight.

The concentration of penicillin in C.S.F. was consistently higher in experimental animals than in controls 30 minutes after the injection of penicillin. It increased with a certain concentration in plasma in relation to the signs of meningitis in the experimental animals. The correlation between the concentration of penicillin in C.S.F. and in plasma was high both in controls and experimental animals. There was no difference in this respect between adult and young rabbits.

With the interval 30 minutes we also gave penicillin doses of 5,000 and 80,000 units/kg. to adult rabbits. The penicillin concentration in C.S.F. was approximately five times greater in the experimental animals than in the controls with a dose of 20,000 units/kg. body weight, 11 times greater with a dose of 80,000 and four times greater with a dose of 5,000. The concentration of penicillin in C.S.F. in relation to that in plasma was relatively speaking highest with the dose of 5,000 units, and lowest with the dose of 80,000 units.

The penicillin concentration in the brain was low at all intervals and in all symptom groups. It was consistently higher, however, in rabbits with meningitis than in the controls. This was the case regardless of the size of the dose of penicillin and of the age of the animal.

FIG. 2.—Penicillin concentration in C.S.F. in different symptom groups in meningitis produced by varying bacterial species.

Microscopic examination revealed that the damage to the meninges usually corresponded with the clinical signs of meningitis. This indicates that the passage of penicillin into the cerebrospinal fluid was related to the pathological changes in the meninges.

Studies with other species of bacteria were made in the same manner as with staphylococci, one series for each species. For the injection in the cisterna magna we used $0.2-2 \times 10^9$ bacteria of α -streptococci,

$0.25-4 \times 10^9$ β -streptococci (group A), $0.5-6 \times 10^9$ γ -streptococci, 0.05×10^9 pneumococci (type III and 22 A) and $2-10 \times 10^9$ meningococci.

Examination 24 hours after the intracranial injection of bacteria showed wide variations with regard to signs of meningitis in the case of all the species of bacteria. The penicillin dose was 20,000 units/kg. The interval between penicillin injection and sampling was 30 minutes.

FIG. 3.—Penicillin concentration of C.S.F. in rabbits after intracisternal injection of dead bacteria.

Figure 2 shows that penicillin concentration in C.S.F. increased significantly with the degree of meningitis with all species of bacteria. In meningitis caused by β -streptococci, it was higher than with the other bacterial species. Even before the clinical signs of meningitis had appeared, the concentration was at the same level as in symptom group +++ with staphylococcal meningitis. In meningitis produced by meningococci, α - and γ -streptococci, the penicillin concentration in C.S.F. was higher in almost all symptom groups than in meningitis caused by staphylococci.

The ratio $\frac{\text{penicillin concentration in plasma}}{\text{penicillin concentration in C.S.F.}}$ also increased with the intensity of the signs of meningitis. It was highest in infections with β -streptococci, after which came pneumococci. In infections with β -streptococci, this ratio was high even before the clinical signs of meningitis were discernible.

The penicillin concentration in the brain was low in all symptom groups. When the meningitis was caused by β -streptococci, meningococci or pneumococci there was significant tendency of the penicillin concentration in the brain to increase with the degree of meningitis.

The pathological changes in the meninges were usually in proportion to the intensity of the signs of meningitis. There was no difference between the different species of bacteria in this respect.

Experiments with Dead Bacteria and Bacterial Toxins

Dead β -streptococci (group A), *staphylococcus aureus* or meningococci, staphylococcal toxin, scarlatinal toxin or endotoxin were used for the injections in the cisterna in different series. The day after the injection, each animal was given an intravenous injection of 20,000 units of penicillin/kg. The interval between the injection of penicillin and the removal of samples was 30 minutes. Most rabbits showed no signs of meningitis whatever. There was a suggestion of nuchal rigidity in a few. The penicillin concentration in plasma was approximately the same in all the series.

The penicillin concentration in C.S.F. (Fig. 3 and 4) was considerably higher in the experiments with dead β -streptococci than in the other five series. In the series with dead staphylococci and with staphylococcal toxin, the penicillin concentration in C.S.F. was only slightly higher than in the controls which had been given intracisternal injections of physiological saline solution. The remaining series with scarlatinal toxin, endotoxin, and dead

FIG. 4.—Penicillin concentration in C.S.F. in rabbits after intracisternal injection of bacterial toxins.

meningococci occupied an intermediate position.

The penicillin concentration in C.S.F. was related to the concentration in plasma. The ratio $\frac{\text{C.S.F.}}{\text{P}}$ was the highest in the experiments with dead β -streptococci. With these dead bacteria it was the same as in the experiments with live staphylococci, α - and γ -streptococci and meningococci when clinical signs of meningitis were either absent or very mild. It was second highest with dead meningococci and scarlatinal toxin.

The penicillin concentration in the brain was almost invariably low in all the series.

The brain showed only slight anatomical changes and scarcely any signs of meningitis. There was no difference in this respect between the different species of bacteria.

Experiments with Ampicillin

The experiments with ampicillin were organized in the same way as those with benzyl penicillin. Only adult rabbits were used. *Hæmophilus influenzae* bacteria (six strains), which had recently been isolated from clinical cases, were used to infect the meninges. Between two and five billion

bacteria were injected in the cisterna in a volume of 0.5 ml. The minimal inhibitory concentration (measured in paper disc sensitivity tests) varied between 0.05 and 0.5 $\mu\text{g./ml}$. The strain with the greatest sensitivity was used in approximately half the therapeutic experiments.

Investigations on the Passage of Ampicillin into C.S.F.

The day after the intracisternal injection both experimental rabbits and controls were examined with special reference to signs of meningitis. The classification by symptom group was the same as in the previous experiments. After this examination the rabbits, both controls and experimental animals, were given an intravenous injection of 12,000 $\mu\text{g.}$ ampicillin per kg. Samples were taken 30 minutes after intravenous injection of ampicillin. Determination of the ampicillin concentration in the various substances was made on agar plates with hole tests according to the same technique used for benzyl penicillin.

The ampicillin concentration in plasma was higher in symptom group +++ than in the others.

FIG. 5.—Correlation between C.S.F. and plasma concentration of ampicillin at various degrees of meningitis.

The ampicillin concentration in C.S.F. was consistently higher in infected rabbits than in controls. In symptom group +++ the concentration was much higher (mean 2 $\mu\text{g./ml.}$) than in the other groups. There was a relatively high concentration of ampicillin (mean 0.40 $\mu\text{g./ml.}$) in C.S.F. even in experimental animals which lacked signs of meningitis (group -). The ampicillin concentration in C.S.F. from controls was very low (mean 0.05 $\mu\text{g./ml.}$).

TABLE II

The Ratio
Ampicillin Concentration in C.S.F. $\left(\frac{\text{C.S.F.}}{\text{P}}\right)$
Ampicillin Concentration in Plasma
in Rabbits Following Intravenous Injection of 12,000
Units Ampicillin kg. weight.

	N	$\frac{\text{C.S.F.}}{\text{P}}$
Symptom groups		
Controls	10	1.3
Infected rabbits:		
—	13	14.0
(+)	11	19.1
+	12	21.9
++	12	22.9
+++	11	27.6

The ratio $\frac{\text{C.S.F.}}{\text{P}}$ (Table II, Fig. 5) was considerably higher in experimental animals than in controls. The concentration of ampicillin in C.S.F. was 14% of that in plasma even before signs of meningitis had appeared. The correlation grew more pronounced as the clinical signs of meningitis increased in intensity.

Ampicillin could be found in the brains of most of the rabbits in symptom group +++, but only rarely in the animals in the other groups. The concentration in group +++ varied between 0.09 and 0.9 $\mu\text{g./ml.}$ brain substance.

Microscopic examination of the meninges of the experimental rabbits revealed that, generally speaking, there was good correspondence between pathological changes and degree of signs of meningitis. At the same time, the meninges showed mild inflammatory changes even in animals which lacked clinical signs of meningitis.

Therapeutic Experiments with Ampicillin

Rabbits were given intracisternal injections of *Hæmophilus influenza* bacteria. One or two days later the rabbits in each symptom group were divided into three series by drawing lots. The animals in one of the series were given no treatment and were used

TABLE III

Results of Treatment of Rabbits with *Hæmophilus Influenzæ* Meningitis with Intravenous Injections of Ampicillin. Dosage: 48,000 and 12,000 $\mu\text{g.}/\text{kg.}$ body weight, respectively.

Symptom group at the first dose of ampicillin	48,000 $\mu\text{g.}/\text{kg.}$		12,000 $\mu\text{g.}/\text{kg.}$		Controls	
	N	% dead	N	% dead	N	% dead
(+)	24	8.3	17	11.8	35	22.9
+ and ++	22	18.2	10	40.0	27	70.4
Total	46	13.4	35	22.2	62	43.5

as controls. In the other two series the animals were given four daily intravenous injections of 48,000 and 12,000 $\mu\text{g.}$ ampicillin per kg. body weight respectively. The rabbits were examined daily 10 days after the injection of bacteria. The injections of ampicillin were continued under the rest of the observation period, i.e. eight to nine days.

A relatively large number of rabbits which had received the 48,000 $\mu\text{g.}$ dose and a small number given the 12,000 $\mu\text{g.}$ dose had diarrhea five to eight days after the first ampicillin injections. Cultures of the fæces of these animals produced *Esch. coli* only. Diarrhoea was not observed in any of the controls.

The results of the therapeutic experiments are shown in Table III. (No rabbits with diarrhoea are included in Table III).

The table reveals that the mortality was lower for treated animals than for controls. The difference was greatest when the signs of meningitis were distinct at the outset (symptom groups + and ++). The dose of 48,000 $\mu\text{g.}/\text{kg.}$ gave better results than 12,000 $\mu\text{g.}/\text{kg.}$ This difference also was greatest when the meningitis was pronounced at the outset.

Among the rabbits which survived, there was a great difference between controls and those given ampicillin. In the latter the signs of meningitis disappeared in most cases three to six days after the beginning of the treatment. This was true regardless of the size of the ampicillin dose. The signs of meningitis were still present at the end of the observation period in the majority of the controls and increased in intensity in many cases.

The signs of meningitis increased in intensity in most of the rabbits which died

during the observation period. This was true of both controls and experimental animals. The length of survival varied considerably but was shorter for the controls.

The ampicillin concentration in C.S.F. varied considerably, but was generally high, even in cases in which the interval between the injection of ampicillin and the removal of samples was long.

Culture of C.S.F. gave bacteria in practically all the controls but in only two of the treated animals. Four or five injections of ampicillin were sufficient to rid the C.S.F. of bacteria.

Summary

Rabbits with experimental meningitis produced with different species of bacteria were given intravenous injections of benzyl penicillin or ampicillin.

The concentration of these drugs in C.S.F. was much higher in infected animals than in controls. It increased with the intensity of the signs of meningitis. In meningitis produced by β -streptococci and by *Hæmophilus influenzae bacteria*, the concentration of benzyl penicillin and of ampicillin, respectively, in C.S.F. was high even before the clinical signs of meningitis had appeared. Relatively high concentrations of benzyl penicillin were found in C.S.F. following the injection of dead β -streptococci into the cisterna magna, despite the fact that the animals showed no signs of meningitis.

The concentration of benzyl penicillin in brain was somewhat higher in rabbits with meningitis than in controls. It was usually unrelated to the intensity of clinical signs of meningitis. After injection of ampicillin in rabbits with *Hæmophilus influenzae* menin-

gitis, however, the drug was found in brain almost exclusively in animals with intensive signs of meningitis.

Ampicillin, particularly in large doses, had a distinct therapeutic effect in experimental *Hemophilus meningitis*

The pathological changes in the meninges corresponded to the clinical picture. In experiments with β -streptococci and *Hemophilus influenzae*, however, mild changes were discernible even before signs of meningitis had appeared.

H. INFLUENZÆ MENINGITIS: A CONTROLLED STUDY OF TREATMENT WITH AMPICILLIN

LAURI D. THRUPP, JOHN M. LEEDOM, DANIEL IVLER, PAUL F. WEHRLE,
JOHN F. BROWN, ALLEN W. MATHIES and BERNARD PORTNOY.

From the Communicable Disease Service, Los Angeles County General Hospital, and the Infectious Disease Laboratory, the Departments of Medicine and Pediatrics, University of Southern California School of Medicine.

THE case fatality rate in *Hemophilus influenzae* meningitis has been reduced to 5-10% by present methods of anti-bacterial and supportive therapy. As this rate is still far from optimal, the incidence of both early and late neurological sequelæ is appreciable, and the antibiotics presently employed have definite, and occasionally serious toxicity, new approaches to therapy must be evaluated.

Preliminary *in vitro* studies performed in our laboratory (Ivler, Thrupp, Leedom, Wehrle and Portnoy, 1963) indicated that 126 strains (118 type B) of *H. influenzae* isolated from cerebrospinal fluid (C.S.F.) were sensitive to ampicillin. Bacteriostatic and bactericidal levels did not differ significantly, and 91% were killed by 0.4 µg./ml. Only one had a bactericidal level as high as 1.6 µg./ml. In addition, sensitivities of meningococci and pneumococci to ampicillin were similar to those to penicillin G. These data, in conjunction with the apparent low toxicity of ampicillin suggested the trial of ampicillin as a single drug treatment for bacterial meningitis, in contrast to control groups treated with conventional therapy. The present report summarizes the experience to date with *H. influenzae* meningitis. Eighteen of these 70 patients were included in a preliminary report (Ivler *et al.*, 1963).

Materials and Methods

(1) **Selection of Patients:** All patients more than two months of age with bacterial meningitis admitted to the Communicable Disease Service of the Los Angeles County General Hospital were included in the ampicillin study. Chart numbers assigned at the hospital central admitting office, and not subject to control of the ward physicians, were used to insure proper randomization. Patients with even (2, 4, 6, etc.) numbers were assigned to the ampicillin group while patients with chart numbers ending 1, 3, 5, etc., were given conventional therapy. During the period considered (June, 1963 to March, 1964) a total of 72 patients were admitted with *H. influenzae* meningitis. Of these, 44 with odd chart numbers were assigned to the control group, and 28 with even numbers were eligible for ampicillin therapy. The difference in the size of the two groups was unexpected, although within the limits of chance variation. Two of the 28 patients were excluded from the ampicillin group, one by error in assignment and the other because of concomitant severe facial cellulitis following a dog bite where additional therapy was indicated. These two patients were not tabulated with either group. Most of the patients had received some antibacterial therapy prior to admission, usually subtherapeutic dosages, ineffective drugs, or both. No attempt was made to evaluate this, since it was assumed to provide an equal, and probably negligible, effect in both treatment groups.

(2) **Administration of antibacterial therapy:** Sodium ampicillin was reconstituted in 0.85 per cent NaCl and administered rapidly (15-20 minutes) at four hour intervals in a total daily dose of 150 mg./kg. of body weight. (The first seven patients treated received 100 mg./kg. per day intra-