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

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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-

venously at six hour intervals). The intravenous route was used invariably for at least the first 48 hours, while after adequate initial clinical response many patients were given the same daily dose by intramuscular injection at four hour intervals during the remainder of the period of treatment.

Chloramphenicol was given by continuous intravenous infusion in a total daily dose of 100 mg./kg. In some patients in whom the diagnosis of *H. influenzae* was not certain on admission, sulfisoxazole and penicillin G (150 mg./kg./day of each drug) were also given until isolation and identification of the organisms were completed.

(3) **Duration of therapy:** Treatment was continued until the patient was afebrile for at least five days, the spinal fluid contained less than 40 cells per cmm. with few or no polymorphonuclear leukocytes, and the C.S.F. sugar was normal.

(4) **Evaluation of response to therapy:** Response to therapy was evaluated by the usual clinical criteria, including duration of fever, improvement in neurological status, decrease in peripheral blood leukocyte count with a return of the differential count toward normal, and improvement in C.S.F. finding, including pressure, cell count, percentage polymorphonuclear leukocytes and glucose and protein content.

Subdural taps were performed whenever indicated (Platou, Rinker and Derrick, 1959). In this report the outcome of therapy was evaluated according to the patient's course in the hospital and status at the time of discharge from the hospital. Untoward findings during the course of treatment in the hospital and any neurological abnormalities were recorded. "Delayed resolution clinically, but no sequelæ at discharge" is a descriptive category which included patients with one or more findings such as: (a) transient subdural effusions, (b) convulsions persisting beyond the first 24 hours in the hospital, (c) persistence of a positive C.S.F. culture the day following admission to the hospital, (d) persistence or recurrence of C.S.F. pleocytosis, (e) documented persistence of nuchal rigidity or neurological findings such as cranial nerve palsies beyond five days even though resolution had occurred by the time of discharge. An additional "slow response" category incorporated the above plus other patients with no abnormality but fever persisting beyond five days of treatment.

(5) **Bacteriological methods:** Isolation and identification of the organisms were accomplished by conventional methods utilizing blood and chocolate agar, serum and thioglycollate broth and both aerobic and CO₂ incubation. Quellung was done in C.S.F. if adequate numbers of organisms were present. Bactericidal levels of ampicillin and chloramphenicol were determined using serial tube dilution techniques in GC medium containing 1% supplement B. (Difco).

Results

Bacteriologic confirmation of the diagnosis of *H. influenzae* type B infection was complete in 68 of the 70 patients in the study group. Blood and C.S.F. cultures were both positive in 34 patients and organisms were cultured from the C.S.F. alone in the remaining 34. Two patients admitted with partially treated purulent meningitis had no positive cultures;

they are included in this series on the basis of organisms seen on Gram's stain of the C.S.F. The cultural recovery of *H. influenzae* from 68/70 patients supported the assumption that antibiotic therapy given prior to admission had little effect on the course of the illness. To date, 164 strains of *H. influenzae* isolated from C.S.F. have been tested for *in vitro* sensitivity to ampicillin. Ninety-one per cent of the strains were killed by 0.4 µg./ml., and only two had bactericidal end points as high as 1.6 and 3.1 µg./ml. respectively.

The age and sex distribution of the patients studied is indicated in Table I. Two of the patients were more than 15 years of age. It should be noted that 14 of 26 patients in the ampicillin group were two years of age or older, and 13 of 44 patients in the control group were in this age group. This was believed to be an unimportant difference, since the distribution of severity of illness, according to the criteria in Table II, was similar within each age group and apparently was not related to age. The expected male predominance was found in both study groups.

The duration of illness prior to admission was similar in both groups. In Table II the patients in each group are recorded according to severity of illness on admission. There was no difference between the two groups.

The duration of fever after therapy was instituted as indicated in Table III. No significant difference is apparent between the two groups. Sixty-four per cent of the chloramphenicol treated patients were afebrile by the fifth hospital day, while 72% of the ampicillin group were in this category.

The C.S.F. was re-examined within one or two days following admission in 24 ampicillin and 38 control patients. Cultures of these follow-up C.S.F. samples were positive in three of 31 specimens from the ampicillin patients and three of 46 samples from controls. These six positive specimens were all obtained less than 18 hours after the start of therapy; no positive C.S.F. cultures were obtained from either treatment group later than 18 hours after admission. The similarity of response in the two treatment groups is further emphasized in Figs. 1 and 2, in which changes in C.S.F. cell count and glucose are shown during the period of therapy. In addition to the data shown, the rate of decline in percentage of polymorphonuclear cells in the C.S.F. during the period of treatment was similar in the two groups.

TABLE I
H. influenzae meningitis
Age and Sex Distribution of Patients by Therapy Group

Therapy Group	Sex	< 1 yr.	1 yr.	2-4 yrs.	5-29 yrs.	Totals
Control	M	10	7	3	3	23
	F	9	5	5	2	21
Total		19	12	8	5	44
Ampicillin	M	7	3	6	0	16
	F	2	0	6	2	10
Total		9	3	12	2	26

TABLE II
H. influenzae meningitis
Clinical Status on Hospital Admission According to Treatment Group

Severity Group	Findings	Control No.	Control (Per cent)	Ampicillin No.	Ampicillin (Per cent)
4+	Coma, shock, semicoma, hypotension*	8	(18)	3	(12)
3+	Convulsions without coma or shock	10	(23)	7	(27)
2+	Temp. >105°R, symptoms >5 days, complicating disease, marked lethargy*	15	(34)	9	(34)
1+	None of above	11	(25)	7	(27)
Total patients		44	(100)	26	(100)

* One or more of the findings listed.

TABLE III
H. influenzae meningitis
Duration of Fever in Hospital by Treatment Group

Number (Per cent distribution) of Patients with Maximum Temperature > 100°F.							
Therapy Group	Days in Hospital						Total
	0-1	2-3	4-5	6-7	8-9	10+	
Control	3	11	11	8	2	4	39*
	(8)	(28)	(28)	(21)	(5)	(10)	(100)
Ampicillin	6	3	9	1	2	4	25**
	24	(12)	(36)	(4)	(8)	(16)	(100)
Total	9	14	20	9	4	8	64

*Four fatalities and one case with incomplete temperature record excluded.

** One fatal case excluded.

TABLE IV
H. influenzae meningitis
 Complications and Sequelæ by Type of Treatment

	Control No. (Per cent)	Ampicillin No. (Per cent)
Death	4 (9)	1 (4)
Neurological Residual*	5 (11)	3 (12)
Severe	1	2
Mild or Questionable	4	1
Subdural Effusions	4 (9)	4 (15)
Required Surgery	0	2
Mild Neurological Residual	1	1
No sequelæ	3	1
Seizures persistent**	1 (2)	2 (8)
Delayed resolution clinically† but no sequelæ at discharge	13 (30)	8 (31)
Temp. >100°F. for 6 days but without any of the above findings	5 (11)	1 (4)
Good response (none of above findings)	18 (41)	13 (54)

* At hospital discharge.

** Some seizures persistent >24 hours after admission.

† Tabulation includes patients with slight subdural effusions which cleared without surgery or neurological sequelæ.

TABLE V
H. influenzae meningitis
 Status on Discharge from the Hospital

Age Group	Treatment Group	No Residual Satisfactory Response	Slight or no Residual with Minor Subdurals or Slow Response	Serious Neurologic Residual or Death	Total
<12 mos.	Control	9	9	1	19
	Ampicillin	0	6	3	9
	Total	9	15	4	28
12-23 mos.	Control	7	3	2	12
	Ampicillin	2	1	0	3
	Total	9	4	2	15
2+ yrs.	Control	6	5	2	13
	Ampicillin	12	2	0	14
	Total	18	7	2	27
All Ages	Control	22	17	5	44
	Ampicillin	14	9	3	26
	Total	36	26	8	70

TABLE VI
H. influenzae meningitis
 Possible Reactions or Complications of Therapy

Type of Reaction	Control (44 patients)		Ampicillin (26 patients)	
	No.	Per cent	No.	Per cent
Any possible reaction	20	(45)	16	(62)
Phlebitis	9	(20)	10	(38)
Skin Rash	4	(9)	5	(19)
Gastrointestinal	3	(7)	1	(4)
Miscellaneous	6	(14)	1	(4)
Eosinophilia ††	3	(7)	1	(4)
Hematologic depression	10	(23)	2+	(8)
Leukopenia†		5		0
Rel. granulocytopenia¶		5		1
Thrombocytopenia††		2		1
Anemia¶¶		3		1

† < 4,000 per cmm. (or < 5,000 if other hematology abnormal).

¶ < 15% polys and bands.

†† marked reduction in platelets on smear.

¶¶ drop in hemoglobin of > 2 g./100 ml.

‡ > 10% eosinophils in peripheral smear.

‡ one patient failed to respond to ampicillin, and developed mild anemia and thrombocytopenia 14 days after the substitution of chloramphenicol.

Complications seen are recorded in Table IV by type of sequelæ and method of therapy. Again, it is apparent that no significant difference is present between the two groups. A strong association was found with coma, shock and/or convulsions on admission and fatal outcome or serious neurological residual. This was the case in both therapeutic groups. None of the 42 patients admitted without the above serious clinical signs had a fatal outcome or neurological sequelæ of consequence. Among the 28 patients admitted with coma, shock or convulsions, four of 18 treated with chloramphenicol died and one had a serious residual. One of 10 in the ampicillin series died, and an additional two patients had severe residual involvement (hydrocephalus).

The status of the patients at the time of discharge by age group and type of therapeutic response is recorded in Table V. Although minor differences were noted among the various age groups, the numbers were small and of doubtful significance. In the total group, the proportion with entirely satisfactory response was 22 of 44 control patients and 14 of 26 ampicillin patients. These proportions may be regarded as nearly identical.

Possible drug reactions or complications of therapy are listed in Table VI. These findings were not proven to be due to the specific antibiotic therapy in any instance. The high incidence of local phlebitis in both groups accompanied the frequent use of polyethylene, indwelling, venous catheters. Twelve patients with some evidence of hematologic depression are also listed in Table VI, although the findings were minimal in most. Eleven cases accompanied the administration of chloramphenicol. One three-month-old patient treated with ampicillin had a peripheral white blood cell differential count of 15% polymorphonuclears and no bands on the seventh day of therapy. This finding was of doubtful significance in view of the age of the patient, lack of accompanying anemia or leukopenia, and a reticulocyte count of 1.6%. The other patient with hematologic depression included in the ampicillin group was treated initially with ampicillin with prompt sterilization of C.S.F. However, the pleocytosis failed to clear, and subdural effusions and hydrocephalus were noted. The marrow depression appeared two weeks after chloramphenicol had been substituted.

FIG. 1.—C.S.F. cell counts during the treatment period.

Discussion

The patients with *H. influenzae* meningitis in this study comprised a group similar to series previously reported (Haggerty and Ziai, 1964). 90% of the patients were between two months and four years old. This age distribution followed the characteristic pattern which, as shown by Fothergill and Wright (1933), reflects inversely the age prevalence of bactericidal serum antibody. Three per cent (two cases) were young adults, a proportion similar to the 2.7% of patients more than 20 years of age recorded in Dolphin and Popham's (1951) review of 915 cases. There was the expected male predominance.

Analysis of the results of therapy with ampicillin and chloramphenicol in these two comparable groups of patients failed to elicit any clear-cut differences. General clinical criteria such as mortality, neurological sequelae, duration of fever, subdural effusions, sterilization of the C.S.F. rates of reduction in the number of cells, per cent polys and protein content in C.S.F. and rise in C.S.F. sugar, were similar in both groups. The C.S.F. cell counts and sugar levels showed no differences on the first and the third days after admission, or afterwards. Among small numbers of observations on the second day, the larger variation in values for the ampicillin patients resulted in differences in distributions of values about the medians for that single day of bordering significance (Figs. 1 and 2). However, in view of the data for the days prior to, and following, this second day of treatment, it is doubtful that these differences were meaningful.

Although all nine ampicillin patients less than one year of age manifested at least some

FIG. 2.—C.S.F. glucose levels during the treatment period.

deficiency in response to therapy, nine of 19 controls in this age group had satisfactory responses (Table V). A trend toward fewer unsatisfactory responses with ampicillin than chloramphenicol was observed in patients two or more years of age. As stated previously, neither therapeutic group was weighted with disproportionate numbers of severely ill or moderately ill patients at any age. Disregarding age, the proportion of satisfactory responses in both therapeutic groups was almost identical. The apparent minor differences in response to ampicillin and chloramphenicol by age group would probably disappear if more patients were tested. Consideration of these data emphasizes the difficulty of being certain that every important variable is known in any clinical study.

The one patient in the ampicillin group who died and the two who survived with major residua were all severely ill on admission. Nevertheless the clinical findings in these two survivors were of interest in that C.S.F. pleocytosis with polymorphonuclear predominance persisted in one, and both developed subdural effusions and hydrocephalus eventually requiring neurosurgical correction. None of the patients in the control group required neurosurgery, although it should be noted that four deaths occurred in this group.

Several studies have demonstrated the *in vitro* effectiveness of ampicillin against *H. influenzae* (Rolinson and Stevens, 1961; Klein and Finland, 1963) including type B strains isolated from C.S.F. in our hospital (Ivler *et al.*, 1963). Although ampicillin displayed significantly more activity *in vitro* than penicillin G against C.S.F. *H. Influenzae*

isolates, the differences were small and of questionable clinical significance (Ivler *et al.*, 1963). Nevertheless, the overall response of patients with *H. influenzae* meningitis treated with ampicillin was comparable to the results observed with chloramphenicol. These favourable results with ampicillin stand in contrast to the reported experience with penicillin G even when patients treated with the latter drug had meningitis due to *H. influenzae* strains sensitive to penicillin G, and C.S.F. penicillin G levels were maintained by intrathecal therapy (Drysdale, McIntosh and Brodie, 1946; Zinnemann, 1946; Thomson, Bruce and Green, 1947). Perhaps qualitative differences in the activity of ampicillin and penicillin G against *H. influenzae* account for the more effective results with ampicillin.

Ampicillin levels in the C.S.F. samples studied to date have been variable, ranging from 0.1 to 1.0 $\mu\text{g./ml.}$ with average C.S.F.-to-serum ratios of 20 to 30%. These C.S.F. levels seem rather low in view of the overall satisfactory clinical response to ampicillin. Indeed, C.S.F. levels of 0.1 to 1.0 $\mu\text{g./ml.}$ barely approximate the *in vitro* minimum bactericidal concentrations (Ivler *et al.*, 1963) and clinical responses have not correlated well with C.S.F. levels. For example, a C.S.F. sample from an eight month old patient who developed subdural effusion and hydrocephalus contained more than 7.0 $\mu\text{g./ml.}$ of ampicillin, one of the highest levels found in any patient.

The peripheral blood differential count of 15% neutrophils noted in a three month old in the ampicillin group on the seventh day of therapy could not be considered definitely abnormal. The anemias, leukopenias, granulocytopenias and thrombocytopenias observed in the control patients on chloramphenicol therapy all subsided after the drug was discontinued. One control patient who developed anemia, thrombocytopenia and leukopenia, concurrently developed staphylococcal pneumonia and extensive phlebitis and cellulitis at the site of an intravenous polyethylene catheter. However, as the patient's differential count remained normal, and the lowest total white blood cell count recorded was 3,100 per cmm. the superinfection was not necessarily related to the mild leukopenia.

Summary

Overall results of treatment of *H. influenzae* meningitis with ampicillin or chloramphenicol appeared comparable, but no definite advantages were demonstrated in the use of ampicillin other than the freedom from hematologic depression. While one death occurred among the ampicillin group and two additional patients required neurosurgical procedures for correction of hydrocephalus, four control patients died and one had severe neurological residual. Further study, perhaps with higher dosage, is indicated.

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