SENSITIVITY OF MENINGOCOCCI TO SODIUM SULPHADIAZINE AND SIX ANTIBIOTICS

J. D. ABBOTT, D. ADAMS AND T. J. COLLINS

Public Health Laboratory, Withington Hospital, Manchester

THERE have been many reports of the emergence of sulphonamide-resistant meningococci in the USA since the outbreak in 1963 of meningitis due to these organisms at a naval training centre in San Diego (Millar *et al.*, 1963). We report the results of sensitivity tests on 170 strains isolated in the UK from 1966 to 1968.

MATERIALS AND METHODS

Eighty-two strains were isolated from cerebrospinal fluid (CSF) and 6 from blood culture. Of these 88 strains, 23 were from the Manchester conurbation and 65 from other areas: 4 from Aberdeen, 20 from Belfast, 6 from Birmingham, 13 from Coventry, 6 from London, 1 from Middlesbrough, 1 from Middlesex, 1 from Plymouth, 5 from Portsmouth, 2 from Southampton, 2 from Stafford, 2 from Sussex and in 2 the source was unknown. There were 72 strains isolated from postnasal swabs, 7 from sputa, 2 from eye swabs and 1 from a vaginal swab. Of these 82 strains, 10 were from Southampton and the rest from the Manchester conurbation.

Confirmation of identity of meningococci

All strains were Gram-negative diplococci, oxidase positive, growing on blood agar at 36°C in a candle jar with typical colonial morphology, and showing no growth, or rarely slight growth, on blood agar after overnight incubation at 22°C. On 10 per cent. hydrocele agar slopes with 1 per cent. added carbohydrate, most strains attacked glucose and maltose, but not sucrose; occasional strains attacked glucose or maltose slowly or not at all. Cultures were preserved for day to day use on Dorset's egg slopes in $\frac{1}{4}$ oz. (5 ml) screw-capped bottles at 30°C, and subcultured about every 3–4 mth. A proportion of strains were preserved by freeze-drying.

Serological typing

All strains were tested as suspensions of organisms heated at 100°C for 30 min. against group A, B and C sera by tube agglutination tests incubated overnight at 50°C.

One group-A serum, two group-B sera and one group-C serum were used. The strains for making the group-A serum, one of the group-B sera, and the group-C serum were obtained from Dr K. W. Slaterus (University of Amsterdam). Another group-B strain obtained from Dr C. M. Patricia Bradstreet (Standards Laboratory for Serological Reagents, Colindale) was used to prepare the second group-B serum.

Sensitivity tests

All drugs were kept at 4°C in a tightly sealed container with a desiccant. Dilutions were made in sterile, distilled water from the freshly weighed out powder and used on the day of the test. The drugs used were sodium sulphadiazine (May and Baker), benzylpenicillin (Glaxo Laboratories, 1665 IU per mg), ampicillin (Beecham Research Laboratories,

Received 28 May 1969; revised MS accepted 23 Sept. 1969.

840 μg per mg), erythromycin (Eli Lilly "Ilotycin"), tetracycline hydrochloride (Pfizer, 984 μg per mg), chloramphenicol (Parke Davis), and cephaloridine (Glaxo Laboratories). Because of the low solubility of erythromycin in water the initial solution was made in methanol and not in distilled water.

Sulphonamide sensitivity

One millilitre of the appropriate dilution of sodium sulphadiazine was added to 19 ml of Bacto Mueller-Hinton medium (Difco Laboratories) at 50°C and plates were poured in plastic petri dishes.

Antibiotic sensitivity

One millilitre of the appropriate dilution of antibiotic and 1 ml of defibrinated horse blood were added to 18 ml of Oxoid blood agar base no. 2 CM271 at 50°C and plates were poured as above.

Cultures for testing were grown for 1 day on blood agar plates in a candle jar and then inoculated into 2 ml of 10 per cent. horse serum in Oxoid nutrient broth no. 2 CM67 in

TABLE I
Sensitivity of 170 strains of meningococci to sodium sulphadiazine

Source	Number of strains inhibited by sodium sulphadiazine (μg per ml)										Total number of strains	
	<0.1	0.1	0.2	0.4	0.8	1.6	3.2	6.4	10	50		100
CSF	1	0	8	38	21	14	1	2	1	1	1	88
Postnasal swabs and other	0	4	12	28	23	12	1	0	1	1	0	82

$\frac{1}{4}$ oz. (5 ml) screw-capped bottles; these were incubated with caps loose at 36°C in a candle jar overnight. The following morning they were removed from the candle jar, but kept at 36°C until ready for use. The serum-broth cultures usually showed uniform turbidity on gentle shaking, though occasional strains, particularly those from postnasal swabs, yielded granular growth. Before use, the serum-broth culture was diluted about 1 in 150 in Oxoid nutrient broth no. 2 CM67 and this dilution was inoculated on the dried plates containing sulphadiazine or antibiotics with a phage applicator (Tarr, 1958). The inoculum contained between 1000 and 10,000 organisms. The plates were incubated at 36°C in candle jars and the results were recorded after 1 and 2 days' incubation. The plates were examined by naked eye, and inhibition was recorded where there was no growth, or fewer than 10 colonies; faint growth seen with a $\times 8$ lens but not by naked eye was ignored. When sulphonamide-resistant strains were found the sensitivity tests to sulphadiazine were repeated with an inoculum of about 500–2000 organisms. The inoculum was put on by hand with a nichrome wire loop of about 1.0–1.5 mm internal diameter. This facilitated the reading by yielding a clear-cut end-point.

RESULTS

We found in general that it was preferable to read the plates after 2 days' incubation, as occasional strains grow poorly in 1 day; the minimum inhibitory

TABLE II
Sensitivity of nine sulphadiazine-resistant strains of meningococci to six antibiotics

Laboratory no.	Source	Area	Serological type	MIC (μ g per ml) of							Degree of resistance to sodium sulphadiazine
				sodium sulphadiazine	benzylpenicillin	ampicillin	erythromycin	tetracycline	chloramphenicol	cephaloridine	
46	PN	Manchester	Untypable	50	0.08	0.16	3.2	0.4	0.8	3.2	Moderate
93	CSF	Manchester	Untypable	50	0.04	0.04	0.4	0.8	1.6	3.2	Moderate
111*	CSF	Birmingham	A	6.4	0.08	0.08	3.2	0.8	1.6	3.2	Slight
142	PN	Manchester	Untypable	10	0.04	0.04	0.8	0.8	3.2	3.2	Slight
150	CSF	Southampton	B	100	0.08	0.08	0.4	0.8	1.6	3.2	High
180	CSF	Belfast	A	10	0.02	0.04	0.4	1.6	3.2	1.6	Slight
182	CSF	Manchester	B	6.4	0.08	0.08	1.6	1.6	3.2	3.2	Slight
1105	...	USA	B	100	0.08	0.16	0.8	0.8	1.6	6.4	High
1079	...	USA	B	50	0.04	0.08	0.8	0.8	0.8	3.2	Moderate

* Some agglutination also with group-C serum.

PN = Postnasal swabs.

concentrations (MIC) given in the text and tables therefore refer to results after 2 days' incubation.

All 170 strains were tested with sodium sulphadiazine (table I). One hundred and sixty-one out of 170 (95 per cent.) were inhibited by 1.6 μ g per ml,

TABLE III
Sensitivity of 116 strains of meningococci to 6 antibiotics

Source	Antibiotic	Number of strains inhibited by antibiotic at concentration (μ g per ml)				
		<0.02	0.02	0.04	0.08	0.16
CSF . Postnasal swabs and other }	Benzyl-penicillin	{ 2 0	{ 6 2	{ 26 32	{ 21 24	{ 2 1
CSF Postnasal swabs and other }	Ampicillin	{ 0 0	{ 2 1	{ 20 12	{ 31 31	{ 4 15

Source	Antibiotic	Number of strains inhibited by antibiotic at concentration (μ g per ml)						
		<0.2	0.2	0.4	0.8	1.6	3.2	6.4
CSF . Postnasal swabs and other }	Erythromycin	{ 4 0	{ 0 0	{ 19 11	{ 26 29	{ 6 5	{ 2 14	{ 0 0
CSF . Postnasal swabs and other }	Tetracycline	{ 0 0	{ 0 0	{ 7 13	{ 39 28	{ 10 18	{ 1 0	{ 0 0
CSF . Postnasal swabs and other }	Chloramphenicol	{ 0 0	{ 0 0	{ 0 0	{ 9 14	{ 43 45	{ 5 0	{ 0 0
CSF . Postnasal swabs and other }	Cephaloridine	{ 0 0	{ 0 0	{ 0 0	{ 0 1	{ 10 7	{ 46 45	{ 1 6

but two strains required 3.2 μ g per ml; all these strains have been classed as sensitive. Seven strains—no. 46, 93, 111, 142, 150, 180 and 182—showed increased resistance to sodium sulphadiazine. We have arbitrarily classed as “slightly resistant” strains with MIC of 6.4 and 10 μ g per ml, “moderately resistant” with MIC of 50 μ g per ml, and “highly resistant” with MIC of 100 μ g per ml or more. Further details on these seven strains are given in table II, together with the results of two strains—no. 1105 and 1079—kindly supplied by Dr Harry A. Feldman of the State University of New York. Five of the seven resistant strains were grown from cerebrospinal fluid (CSF) and two from postnasal swabs. Four of the strains showed slight resistance, two

were moderately resistant, and one strain from CSF was highly resistant. Two of the resistant strains, including the one highly resistant strain, were group B and so were the two resistant strains from the USA. One strain showing slight resistance was group A and another slightly resistant strain agglutinated with both the type-A and type-C sera; the remaining three strains were untypable. Resistant strains were not confined to cultures from one area.

Tests for sensitivity to antibiotics were carried out on the first 112 strains investigated, which included three sulphadiazine-resistant strains (no. 46, 93 and 111). Since there was no evidence of resistance to the antibiotics tested, further tests were carried out only on the sulphadiazine-resistant strains. The results of the sensitivity tests to six antibiotics are shown in table III.

Over half of the strains were inhibited by 0.04 μ g benzyl-penicillin per ml, and over 97 per cent. by 0.08 μ g per ml; a third of the strains were inhibited by 0.04 μ g of ampicillin per ml, 84 per cent. by 0.08 μ g per ml and the remainder by

TABLE IV
Serological typing of 170 strains of meningococci

Source	Group A	Group B	Untypable	Not tested	Total
Cases of clinical infection*	10	32	44	2	88
Postnasal swabs and other	5	19	50	8	82

* Six strains from blood culture and 82 from CSF.

0.16 μ g per ml. Three-quarters of the strains were inhibited by 0.8 μ g of erythromycin or tetracycline per ml, the remainder by 3.2 μ g of erythromycin per ml, and all but one by 1.6 μ g of tetracycline per ml. About 20 per cent. of the strains were inhibited by 0.8 μ g chloramphenicol per ml, 94 per cent. by 1.6 μ g per ml and the rest by 3.2 μ g per ml; about 15 per cent. were inhibited by 1.6 μ g cephaloridine per ml, over 90 per cent. by 3.2 μ g per ml and the remainder by 6.4 μ g per ml. The sensitivity to antibiotics of the seven sulphadiazine-resistant strains and of the two strains from the USA are shown in table II. There is no evidence of increased resistance to any of the antibiotics tested.

The results of serological typing are shown in table IV: 9 per cent. of the strains were group A, 32 per cent. were group B, and 59 per cent. were untypable with group A and B sera. A rather higher proportion of strains from CSF or blood were typable (42 out of 86 = 49 per cent.), compared with strains from nasopharynx and other sites (24 out of 74 = 32 per cent.).

DISCUSSION

Interest in the clinical aspects of sulphadiazine-resistant strains followed the report in 1963 of the outbreak of meningococcal infection amongst recruits at

the San Diego Naval Training Centre (Millar *et al.*, 1963), in which sulphonamide prophylaxis failed. In another report from California, Leedom *et al.* (1965) found that 35 of 106 strains (33 per cent.) of meningococci isolated from civilians with meningococcal disease had an MIC of 100 μg or more per ml to sulphadiazine. Feldman (1965) tested over 500 strains from the USA, Western Europe and Africa, from both civilian and military sources: resistance was detected amongst group-B strains and to a lesser extent in group-C strains submitted from both the USA and Europe. Wiggins and Schubert (1967) at the Communicable Disease Center, Atlanta, Georgia, examined over 1600 cultures isolated from 1964 to 1966, using as their criterion of resistance growth in a medium containing 10 μg of sulphadiazine per ml. The resistance rate ranged from 28 per cent. in 1964, to 31 per cent. in 1965 and 39 per cent. in 1966. There have been many other reports of sulphonamide-resistant meningococci from the USA (Gauld *et al.*, 1965; Eickhoff and Finland, 1965; Ivler *et al.*, 1965; Farrell and Dahl, 1966). In Great Britain, Fallon and Brown (1966) examined ten cultures of which nine were fully sensitive to sulphadiazine and one showed scanty growth at a level of 10 μg per ml.

In the present survey of 170 strains isolated in the United Kingdom one was highly resistant (MIC 100 μg per ml) to sulphadiazine, two were moderately resistant (MIC 50 μg per ml) and four slightly resistant (MIC 6.4–10 μg per ml). Any classification of resistance, particularly to sulphonamides, depends upon the technique used, e.g., the medium, the time of incubation and the size of the inoculum. Leedom *et al.* classified strains of meningococci inhibited by 1–5 μg sulphadiazine per ml as sensitive, those with MIC of 10, 20 and 50 μg per ml as intermediate, and those with MIC of 100 μg per ml or more as resistant. These workers, however, used an inoculum of 10^5 – 10^6 organisms, whilst the end-point of our resistant strains is based upon an inoculum of about 500–2000 organisms.

Certain implications arise from the presence of sulphonamide-resistant meningococci. According to reports from the US Army, quoted by Brown and Condit (1965), organisms resistant to 1 μg per ml or more cannot be eradicated from the nasopharynx of carriers with standard courses of sulphadiazine. Although other drugs—penicillins and tetracyclines—suppress the carriage of meningococci during treatment, it is the experience of several workers (Bristow, Van Peenan and Volk, 1965; Leedom *et al.*; Dowd *et al.*, 1966) that carrier rates revert to normal when the drugs are stopped. Ivler *et al.* point out that there is an immediate need for a prophylactic agent for use in treating nasopharyngeal carriers in high-risk groups, such as military recruits or household contacts of patients. According to Leedom *et al.*, meningococci with an MIC of more than 50 μg per ml could be expected to produce disease that is clinically resistant to sulphonamides. Maximum serum levels of sulphadiazine with normal doses of sulphadiazine are about 100 μg per ml and CSF levels are about 67–80 per cent. of the serum levels. The outstanding advantage of sulphonamides for treating meningococcal disease is the free diffusibility into cerebrospinal fluid, but benzyl-penicillin or ampicillin given in large enough doses also penetrate in adequate concentration when the meninges are acutely inflamed (*Br. Med. J.*,

1966). Workers in the USA agree that at the present time sulphonamide-resistant meningococci are sensitive to other antibiotics—benzyl-penicillin, ampicillin, chloramphenicol and tetracycline (Ivler *et al.*). In our own series, of 116 strains tested all were sensitive *in vitro* to these antibiotics. Feldman advises treatment with massive doses of penicillin G, and if sulphonamides are used he recommends that they should be a secondary rather than the primary drug. Mathies *et al.* (1965) had good results in the treatment of meningococcal meningitis with ampicillin: of 56 patients treated with ampicillin three died (5 per cent.), and of 77 patients treated with penicillin seven died (9 per cent.). Until recently sulphadiazine was the drug of choice in the UK, but according to Garrod and O'Grady (1968) treatment with penicillin in addition to sulphonamides is now the practice. Benzyl-penicillin or ampicillin must be given in large doses parenterally—by the intravenous or intramuscular route—and there is still a reasonable case for giving sulphonamides as well, since in our present series of 88 strains from CSF and blood, only one strain showed a high level of resistance to sulphonamides.

McKenzie *et al.* (1967) comment on ten patients with meningococcal meningitis treated with cephaloridine by intramuscular and intrathecal injection that it was their “strong clinical impression that patients with meningococcal meningitis were slower to respond than patients treated conventionally”. The majority of our strains required 3.2 µg of cephaloridine per ml to inhibit growth compared with an MIC of 0.12–1.0 µg per ml quoted by Garrod and O'Grady. In the series reported by McKenzie *et al.* the levels of cephaloridine in the cerebrospinal fluid 24 hr after intrathecal injection were below our MIC in over half the cases. By contrast, Dr A. G. Ironside tells us that he found that in a series of cases treated with the standard dosage of 2 mega units of benzyl-penicillin, irrespective of age, 4 hourly, by intramuscular injection, the penicillin level in the cerebrospinal fluid exceeded the MIC in 17 of 18 cases.

Chloramphenicol has been used effectively in the treatment of meningococcal meningitis (Florey, 1957) and is an alternative treatment for patients allergic to penicillin.

Erythromycin and tetracycline have been used in attempts to reduce the carrier state of sulphadiazine-resistant meningococci (Dowd *et al.*).

The distribution of serological types appears to be different in this country from that in the USA. In a series from California examined by Ivler *et al.* there were 126 strains from the CSF and blood, and 188 from the pharynx: of these 0.9 per cent. were group A, 70.4 per cent. group B, 13.4 per cent. group C and 15.3 per cent. untypable. By comparison in our series of 160 cultures tested, 9 per cent. were group A, 32 per cent. group B and 59 per cent. untypable. Most of the sulphonamide-resistant strains isolated in the USA and Europe were group B, although some were group C (Feldman). The one highly resistant strain in our series was group B and the two strains agglutinating with group-A sera showed low levels of resistance only.

It would be prudent to continue to monitor strains of meningococci isolated in this country for sensitivity to sulphonamides and antibiotics in order to detect changes in sensitivity.

SUMMARY

One hundred and seventy strains of meningococci isolated in the UK from 1966 to 1968 were tested for sensitivity to sodium sulphadiazine. Eighty-eight strains were isolated from CSF or blood and 82 strains from postnasal swabs and other sources. Seven strains were resistant to sulphonamides: one was highly resistant (MIC 100 μg per ml), two were moderately resistant (MIC 50 μg per ml) and four were slightly resistant (MIC 6.4 or 10 μg per ml). One hundred and sixteen strains, including the seven sulphonamide-resistant strains, were tested for sensitivity to six antibiotics: benzyl-penicillin, ampicillin, erythromycin, tetracycline, chloramphenicol and cephaloridine. None of the strains showed any increase in resistance to these antibiotics.

One hundred and sixty strains were typed serologically: 15 (9 per cent.) were group A, 51 (32 per cent.) were group B and 94 (59 per cent.) were untypable.

We thank Dr K. W. Slaterus for providing us with prototype strains of group A, B and C; Dr Harry A. Feldman for the two sulphonamide-resistant strains; and the many pathologists in the UK who so kindly provided current strains of meningococci. We are most grateful to Dr C. M. Patricia Bradstreet and Miss A. J. Tannahill, Standards Laboratory for Serological Reagents, Colindale, for advice in the preparation and use of meningococcal sera and for generous supplies of their own sera; also to Dr M. T. Parker, Chairman of the Public Health Laboratory Service Steering Committee on Antibiotic Resistance of Pathogens, and Dr A. G. Ironside, Clinical Director, Department of Infectious Diseases, Monsall Hospital, for their help and encouragement.

REFERENCES

- BRISTOW, W. M., VAN PEENAN, P. F. D., 1965. *Amer. J. Publ. Hlth*, **55**, 1039.
AND VOLK, R.
BRITISH MEDICAL JOURNAL (LEADING ARTICLE) 1966. *Br. Med. J.*, **1**, 1253.
BROWN, J. W., AND CONDIT, P. K. . . . 1965. *California Med.*, **102**, 171.
DOWD, J. M., BLINK, D., MILLER, C. H., 1966. *J. Infect. Dis.*, **116**, 473.
FRANK, P. F., AND PIERCE, W. E.
EICKHOFF, T. C., AND FINLAND, M. . . . 1965. *New Engl. J. Med.*, **272**, 395.
FALLON, R. J., AND BROWN, W. . . . 1966. *Br. Med. J.*, **1**, 1598.
FARRELL, D. G., AND DAHL, E. V. . . . 1966. *J. Amer. Med. Assoc.*, **198**, 1189.
FELDMAN, H. A. 1965. *Br. Med. J.*, **1**, 436.
FLOREY, M. ETHEL 1957. The clinical application of antibiotics 3, Chloramphenicol and the tetracyclines, Oxford, pp. 80-81.
GARROD, L. P., AND O'GRADY, F. . . . 1968. Antibiotic and chemotherapy, *Edinburgh and London*, pp. 258 and 315.
GAULD, J. R., NITZ, R. E., HUNTER, D. H. 1965. *Amer. J. Epidem.*, **82**, 56.
RUST, J. H., AND GAULD, R. L.
IVLER, D., LEEDOM, J. M., MATHIES, A. W., 1965. In Antimicrobial agents and chemotherapy, 1964, ed. by Gladys L. Hobby, Ann Arbor, p. 358.
JR, FREMONT, J. C., THRUPP, L. D.,
PORTNOY, B., AND WEHRLE, P. F.
LEEDOM, J. M., IVLER, D., MATHIES, A. W., 1965. *New Engl. J. Med.*, **273**, 1395.
THRUPP, L. D., PORTNOY, B., AND
WEHRLE, P. F.

- MCKENZIE, P., LOVE, W. C., LAWSON, J. H., PINKERTON, I. W., JAMIESON, W. M., AND STEVENSON, J. 1967. *Postgrad. Med. J.*, **43**, Suppl. on cephaloridine, p. 142.
- MATHIES, A. W., JR, LEEDOM, J. M., THRUPP, L. D., IVLER, D., PORTNOY, B., AND WEHRLE, P. F. 1965. In Antimicrobial agents and chemotherapy, 1964, ed. by Gladys L. Hobby, Ann Arbor, p. 610.
- MILLAR, J. W., SIESS, E. E., FELDMAN, H. A., SILVERMAN, C., AND FRANK, P. 1963. *J. Amer. Med. Assoc.*, **186**, 139.
- TARR, H. A. 1958. *Mon. Bull. Minist. Hlth*, **17**, 64.
- WIGGINS, G. L., AND SCHUBERT, J. H. . 1967. *Bact. Proc.*, p. 96.