

Neisseria gonorrhoeae isolated from disseminated and localised infections in pre-penicillin era

Auxotypes and antibacterial drug resistances

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SUMMARY Interest in the evolution of gonococcal auxotrophy led to a study of 72 strains isolated between 1935 and 1948 from the urogenital tract (57 patients), the eye (two patients), and from disseminated gonococcal infections (11 patients and probably two others). Two cervical isolates with nutritional requirements for proline, arginine, histidine, and biotin were oxidase-positive, Gram-negative diplococci, but their identity as *Neisseria gonorrhoeae* was uncertain because they were atypically susceptible to colistin and did not produce acid in glucose media. The *N gonorrhoeae* strains were highly susceptible to 11 other antibacterial drugs but not to sulphadiazine. Defects of one or more pathways for the biosynthesis of methionine, proline, arginine, threonine, lysine, the branched-chain amino acids, hypoxanthine, and thiamine pyrophosphate were found in 39 of the 70 strains, including four isolated in the pre-sulphanilamide era. Unexpectedly, methionine was required for the growth of 11 (21%) of the 52 Danish strains and for 13 (72%) of 18 strains isolated in the USA. The Danish strains included 28 (54%) that did not require any of the compounds used for differentiating auxotypes, whereas this type was represented by only three (17%) of the USA strains. None of the gonococci required uracil or other pyrimidines. This suggests that the requirements for arginine, hypoxanthine, and uracil commonly found in recent isolates from disseminated gonococcal infections probably evolved after treatment with sulphonamide was replaced by penicillin.

Introduction

The development of gonococcal resistances to antibacterial drugs over the past four decades is well known.¹⁻⁴ In contrast, little is known about the evolution of the nutritional requirements of *Neisseria gonorrhoeae*. Gonococci isolated in the 1970s require a variety of compounds for growth in chemically defined media.⁵ Some components, such as cysteine or cystine, are required by all members of the species. Various other compounds are required by some gonococcal isolates but not by all. This diversity of nutritional requirements differentiates between *N gonorrhoeae* isolates by a system of auxotyping.⁶ The accompanying study⁷ characterises strains isolated in 1975 from patients in one community and cites other auxotyping studies.

N gonorrhoeae strains with requirements for compounds that include arginine, hypoxanthine, and uracil have attracted special attention because of their propensity for invading the bloodstream.⁸⁻¹⁰ These gonococci, called AHU strains by Knapp and Holmes,⁸ are widely prevalent¹¹⁻¹³ and increasing in some communities.¹⁰ A peculiar feature of AHU strains is their uniform susceptibility to penicillins, streptomycin, and most other antibiotics; this occurs even in areas where many other isolates of *N gonorrhoeae* are relatively resistant. Further studies of gonococci from disseminated infections indicated that penicillin susceptibility was more notably related to dissemination than was the AHU pattern of nutritional requirements.^{10, 14} Present information suggests that the AHU pattern is best regarded as an indicator of gonococci which may possess one or more properties conducive to dissemination.

Treatment of patients with antibiotics in doses that are only partially effective is recognised as a form of natural selection which has resulted in the decreased susceptibility of gonococci to various drugs. Never-

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theless, the possible effects of selection on auxotrophy remain obscure. An important question concerns the prevalence of AHU strains and other auxotrophic gonococci before the introduction of penicillin in the mid-1940s. Since *N gonorrhoeae* strains isolated between 1935 and 1948 from patients with disseminated or localised infections are available, we have been able to examine the nutritional requirements of gonococci isolated before the penicillin era and to obtain baseline data on their susceptibilities to various antibacterial drugs with differing mechanisms of action.

Materials and methods

The methods described in the accompanying study⁷ were used to determine the nutritional requirements and responses to 13 antibacterial drugs of 72 strains of *N gonorrhoeae* isolated more than 30 years ago. These bacteria were preserved by freeze-drying in glass ampoules.

COLLECTIONS OF GONOCOCCI

Eighteen strains of *N gonorrhoeae* from the collection of Sara Branham¹⁵ were obtained through the kind co-operation of Carl E Frascch (Bureau of Biologics, Food and Drug Administration, Bethesda, Md, USA; table I).

Strains from Danish patients were obtained from the collection held by the *Neisseria* Department of the Statens Seruminstitut, Copenhagen, Denmark. Detailed examinations were made of 97 isolates from urogenital specimens. Some isolates, however, were related, being from two anatomical sites, or repeated cultures from a given patient, or cultures from

marital partners. The related isolates are regarded as representatives of one strain since they resemble one another in all cases except two, which are described below.

The Statens Seruminstitut designations of the 54 strains of this study group are listed below with the total number of related isolates where more than one representative of a given strain was studied (in parentheses). Eight strains were isolated in 1940: 17792, 17941 (2), 17932 (2), 18000 (2), 18218 (2), 18887 (2), 19832, and 20514 (2). Four strains were isolated in 1941: 23363 (2), 31956, 32328 (2), and 32423 (3). Ten strains date from 1942: 50004, 50601, 51768 (2), 65171 (2), 65983 (5), 65988 (4), 66179 (3), 66375, 68808, and 69016 (2). The following 27 strains were isolated in 1943: 23157, 23232, 25590, 26509, 26876 (2), 28466, 29098, 29654, 30517 (2), 40635 (3), 40914 (2), 41835 (3), 41842, 42809 (2), 43467, 44353 (2), 45045, 45689, 46269 (2), 46473 (2), 46615 (2), 47750 (3), 50123, 50602 (2), 58449 (2), 61350 (2), and 64683. Strains 65479 (3) and 65485 (3) were isolated in 1944. Sulphadiazine-resistant strains 7764/45, W-18/46, and W-58/46, isolated in Copenhagen in 1945 and 1946, were described earlier^{5,6} and studied in more detail here.

The lyophilised cultures were revived on multiple plates of GCMBS agar⁷ supplemented with rabbit blood or sheep blood. Subcultures were made routinely on GCMBS agar. The identification methods given earlier^{16,17} were used to determine the species.

NUTRITIONAL REQUIREMENTS

The standard NEDA media were used for auxotyping.^{7,17} All strains required cysteine or cystine for growth. A strain which grew on all other auxotyping

TABLE I *Origins and auxotypes of 18 strains of Neisseria gonorrhoeae isolated in the USA, 1935-48*

Patient					
Site	Sex	Locality	Month/year	Strain	Auxotype
CSF	F	Hartford, CT	7/35	G-2	Met-Lys
Unknown		York, PA	6/36	G-4	Pro-Met
CSF		Memphis, TN	10/36	G-5	Met*
Eye		Rochester, NY	12/36	G-7	Zero
Urine		Rochester, NY	1/37	G-9	Zero
Eye		Rochester, NY	1/37	G-10	Pro
CSF	F	Washington, DC	9/37	G-11	Pro-Arg-Met* †
CSF	F	Washington, DC	11/37	G-12	Arg-Met*
CSF	M	Washington, DC	11/37	G-14	Arg-Met*-Ile
Blood	M	Washington, DC	3/38	G-15	Met
CSF		Valhalla, NY	10/38	G-17	Met*
Blood		Baltimore, MD	5/39	G-20	Pro
Urethra	M	Baltimore, MD	5/39	G-22	Pro-Met
Urethra	M	Baltimore, MD	10/37	G-23	Zero
Blood	M	Washington, DC	6/39	G-24	Met*
Unknown		Fort McPherson, GA	4/45	G-26	Pro-Met-Leu-Hyx
CSF	M	Washington, DC		G-27	Met*
Blood	F	Washington, DC	4/48	G-28	Arg-Met*-Thr

*Requirement for methionine is satisfied by homocysteine but not cystathionine

†Auxotype of G11-1 and seven other spontaneous mutants independently derived from the parent strain G-11 which does not grow on the auxotyping media

media was classified in the Zero auxotype. The designations for other auxotypes include one or more of the following: Pro (proline), Arg (arginine), Met (methionine), Hyx (hypoxanthine), Thp (thiamine pyrophosphate), Lys (lysine), Leu (leucine), Ile (isoleucine), Thr (threonine), His (histidine), and Bio (biotin).

An auxotype is a category in which gonococci are classified according to their pattern of nutritional requirements. Auxotypes do not carry superscripts¹⁷; however, a superscript is used when referring to a particular nutritional trait displayed by bacteria, such as an arginine requirement (Arg⁻) or non-requirement (Arg⁺).

DETERMINATION OF RESISTANCE

GCMBS agar was the basal medium used for tests of the antibacterial activities of various concentrations of 13 drugs. The benzylpenicillin used in this study was potassium penicillin G (control No W653905, potency 1580 IU/mg, generously supplied by Wyeth Laboratories Inc, West Chester, Pennsylvania). Spectinomycin sulphate was U-18409E (potency 641 µg/ml, generously supplied by the Upjohn Company, Kalamazoo, Michigan). Other antibiotics, the test method, and the end-point assigned for resistance were the same as before.⁷

Results

The 18 strains listed in table I and 50 Danish strains (table II) were typical representatives of *N gonorrhoeae*. Negative reactions in glucose tests were

TABLE II *Auxotypes of the 72 N gonorrhoeae strains isolated, 1935-48*

Auxotype	No of strains	
	Denmark	USA
Zero	28	3
Pro	3	2
Arg	3	0
Met	3	5
Hyx	1	0
Thp	2	0
Pro-Arg	1	0
Pro-Met	3	2
Pro-Thp	3	0
Arg-Met	2	1
Met-Thp	2	0
Met-Lys	0	1
Pro-Arg-Met	0	1
Arg-Met-Ile	0	1
Arg-Met-Thr	0	1
Pro-Arg-His-Bio	2*	0
Pro-Met-Ile-Thp	1	0
Pro-Met-Leu-Hyx	0	1

*Identity as *N gonorrhoeae* uncertain

found, however, for the two Pro-Arg-His-Bio strains and for two Zero auxotype strains, SS50004/42 and SS51768/42 (and the related isolate SS51769/42). Acidity was not detected in repeated tests performed either on a growth medium or in Elrod-Braun buffered salts solution containing 1% or 10% glucose.¹⁶ All strains, including these four, were oxidase-positive and required cysteine or cystine. The Zero auxotype strains appeared to be gonococci which were atypical only with respect to the glucose reaction; their antibiotic resistances were similar to those of the typical gonococci. The identification of the Pro-Arg-His-Bio strains was less certain and most of their resistances differed from the mean values for the 70 strains; consequently, the resistance levels for these two were not included in table III. It is not known whether the Pro-Arg-His-Bio strains, SS68808/42 and SS25590/43, came from the same or different patients, but each was regarded as a case of gonorrhoea. Two different municipal outpatient clinics obtained the cultures, one in October 1942 from a patient described as "female, 23 years" and the other in June 1943 from a "female, 24 years." Both strains were oxidase-positive, Gram-negative diplococci and their inability to acidify glucose, maltose, sucrose, or lactose might allow them to be classified as *Branhamella catarrhalis*¹⁸ or *Neisseria flavescens*¹⁹ rather than as atypical *N gonorrhoeae*. Studies of the genetic relationship to these species are needed for accurate identification.

NUTRITIONAL REQUIREMENTS

The most notable findings were the large number of strains (33%) which required methionine (table II) and the absence of AHU strains even among the gonococci isolated from disseminated infections (table I). Pyrimidine biosynthesis was normal in all strains as shown by their growth on media lacking uracil or other pyrimidines. Also, arginine biosynthesis was normal in the two strains which required hypoxanthine, G-26 and SS40914/43 (together with the related isolate SS39781/43).

The diversity of auxotypes was unexpectedly large; 18 auxotypes were found (table II) compared with 14 in the first study.⁷ Furthermore, additional types could be obtained by classifying Thp⁻, Met⁻, and Arg⁻ strains in auxotypes subdivided to indicate the ability or inability of the gonococci to utilise particular precursor compounds for growth. Thus, appreciable growth of seven Thp⁻ strains occurred only on a medium containing thiamine pyrophosphate, whereas thiamine partially satisfied the requirement of one strain listed as Thp (SS30517/43 and the related isolate SS30778/43). Of the 24 Met⁻ strains (table II), eight listed in table I multiplied on a medium in which methionine was replaced by homo-

cysteine, the immediate precursor in this biosynthetic pathway.²⁰

The 12 Arg⁻ strains bore one or more lesions of three different gene loci (*argA*, *argE*, and *argG*) that encode enzymes needed for arginine biosynthesis. (a) The Pro-Arg strain (SS23157/43) and one Arg-Met strain (represented by isolates SS46269/43 and SS46459/43) could grow if arginine was replaced by any one of the following intermediate compounds: N-acetylglutamate, N-acetylornithine, ornithine, citrulline, or argininosuccinate. This indicated that most of the arginine biosynthetic steps were operative and suggested that the enzyme which synthesises N-acetylglutamate (encoded by *argA*) was defective.^{20, 21} (b) Nine of the other Arg⁻ strains could utilise ornithine, citrulline, or argininosuccinate for growth but not the acetylated precursors (*argE* lesions).^{21, 22} (c) The arginine requirement of *G11-1* was satisfied by argininosuccinate but not by any of the other precursor compounds listed (*argG* lesion). Strain *G11-1* also bore a mutant *argE* gene locus, which was detected accidentally when the locus was segregated by genetic transformation. Treatment of an Arg⁺ recipient with *G11-1* DNA produced a transformant which was Arg⁻ and able to utilise citrulline and ornithine but not N-acetylornithine.

Strain *G-11* (table I) was an isolate from cerebrospinal fluid from a patient (BM) described by Branham and colleagues.¹⁵ Growth of the parent strain *G-11* was fastidious on complex media and extremely limited on the defined media. The nature of this limitation, whether due to an unsatisfied nutritional requirement or to inhibition,¹⁷ is not yet known. Every large population of *G-11* cells, however, contained a few mutants, which formed colonies on NEDA media. *G11-1* and seven other spontaneous mutants have been examined and found to be identical in their nutritional requirements, and their resistance profiles were similar to one another and to the parent *G-11*. In addition to strict requirements for proline, arginine, and methionine, the mutants were stimulated by thiamine pyrophosphate. Growth does not occur on NEDA lacking leucine, isoleucine, and valine or on media containing any one or two of these amino acids. Because the biosynthetic pathways of the branched-chain amino acids are complex and interrelated,²⁰ the precise requirements remain to be determined.

Thirty-one of the strains were represented by two or more isolates, all but one having been cultured from sexual partners or from different specimens from the same patient. For 29 strains similar auxotypes and resistances showed that the processes of isolation, lyophilisation, storage for 35 years, reconstitution, and further subculturing had not produced notable differences between the related isolates. In

two instances concerning the Met-Thp auxotype, however, a nutritional requirement was lost, presumably owing to genetic instability; only the double auxotrophs were listed. The primary culture of the Met⁻ Thp⁻ strain SS65171/42 produced "fine growth", but after two subcultures larger colonies were observed among the small ones. A large-colony isolate which was preserved in 1942 was Met⁻ Thp⁺ and represented a mutant selected for improved growth on a medium presumably containing a suboptimal concentration of thiamine pyrophosphate. The second instance of deviation concerned cultures from two different urethral specimens from one man: SS17941/40 was Met⁻ Thp⁻ and the other isolate was Thp⁻ but Met⁺.

ANTIBACTERIAL DRUG RESISTANCES

The levels of resistance to 12 drugs for 70 of the pre-1950 strains are given in table III. All 70 were resistant to colistin at a concentration ≥ 25 $\mu\text{g/ml}$, 25 strains were resistant to 50 $\mu\text{g/ml}$ and 23 to at least 75 $\mu\text{g/ml}$. The drug responses of the two Pro-Arg-His-Bio strains were omitted from table III. Their colistin resistance was limited to 0.1 $\mu\text{g/ml}$ in contrast to the resistance of typical gonococci.²³ Also, these two strains were more resistant than most of the 70 strains to benzylpenicillin (0.05 U/ml), oxacillin (0.5 $\mu\text{g/ml}$), erythromycin (0.5 and 1.5 $\mu\text{g/ml}$), spectinomycin (15 and 20 $\mu\text{g/ml}$), vancomycin (40 and 30 $\mu\text{g/ml}$), and lincomycin (80 and 50 $\mu\text{g/ml}$). Their resistances to the remaining six drugs fell within the ranges given in table III.

The wide range of resistance to sulphadiazine (table III) was similar to the response to sulphathiazole reported for strains isolated in 1960.²⁴ On the other hand, the high susceptibilities of the 70 strains to benzylpenicillin and most of the other drugs was typical of bacteria with no previous exposure to these selective agents.^{1, 24} Compared with the Danish strains, however, the gonococci isolated in the USA showed greater diversity of drug responses. Thus, the five strains resistant to oxacillin at a concentration of 0.5 $\mu\text{g/ml}$ were *G-5*, *G-15*, *G-17*, *G-24*, and *G-26*. *G-15* and *G-26* were also resistant to erythromycin at 1.0 $\mu\text{g/ml}$ and *G-26* to rifampin at 0.5 $\mu\text{g/ml}$. The gonococci that were most susceptible to antibiotics included *G-11*, *G-23*, and SS50602/43 with resistance to benzylpenicillin limited to 0.005 U/ml and oxacillin to 0.05 $\mu\text{g/ml}$. The resistance to tetracycline of *G-17* and *G-23* was 0.02 $\mu\text{g/ml}$ and the chloramphenicol resistance of *G-11* was 0.1 $\mu\text{g/ml}$. The resistance of 69 of the 70 strains (table III) was distinctly greater to oxacillin than to benzylpenicillin as expected.⁷ The exception was the Zero auxotype strain SS45689/43 which was resistant to oxacillin at a concentration of 0.05 $\mu\text{g/ml}$ and to benzyl-

TABLE III Responses of 70 *N gonorrhoeae* strains to the antibacterial actions of 12 drugs

Antibacterial drug	No of strains with indicated resistance*:									
	0.005	0.01	0.02	0.05	0.1	0.2	0.5	1.0	1.5	2.0
Benzylpenicillin	4	59	7	0	0	0				
Oxacillin		0	0	4	18	43	5	0		
Tetracycline		0	2	19	48	1	0			
Chloramphenicol			0	0	2	68	0			
Erythromycin		0	0	1	30	36	1	2	0	0
Rifampin		0	20	47	2	0	1	0		
Nalidixic acid					0.1	0.2	0.4	0.6		
					0	39	31	0		
Streptomycin	0.5	1.0	2.0	5	10	15	20	30		
Spectinomycin	0	2	18	50	0	0				
		0	1	12	51	6	0	0		
Vancomycin	1.0	2.0	3.0	5	10	20	30	40		
Lincomycin	0	1	5	20	31	12	1	0		
			0	2	19	39	10	0		
Sulphadiazine	0.2	0.5	1.0	2.0	5	10	20	35	50	≥100
	5	14	9	11	3	15	5	3	3	2

*Expressed as the highest concentration of drug permitting growth; concentrations of benzylpenicillin in IU/ml all other drugs in µg/ml.

penicillin at 0.01 U/ml. This strain was inhibited by vancomycin at 3 µg/ml in the standard test, and smaller inocula were more susceptible, which suggests that these gonococci would not have been recovered on the vancomycin-containing media now used for primary isolation.⁷

Discussion

Disseminated gonococcal infections were caused by 11 of the 18 strains listed in table I and probably also by strains G-4 (referred from a sanatorium) and G-26 (from a US army medical laboratory). It is remarkable that although all 13 strains possessed one or more auxotrophic traits, none required uracil. The Zero auxotype accounted for only one-sixth of these 18 isolates compared with more than half of the Danish strains (table II), all of which were from urogenital sites. Defects of one or more pathways for biosynthesis of proline, arginine, methionine, threonine, lysine, the branched-chain amino acids, hypoxanthine, and thiamine pyrophosphate were found in 39 of the 70 *N gonorrhoeae* strains. A nutritional requirement for glutamine was not detected, although it was common at that time in gonococci isolated in Texas²⁵ where thiamine pyrophosphate-requiring strains were also found.²⁶ The unexpected variety of biosynthetic defects indicated that these nutritional traits have evolved for a very long time.

Successful treatment of gonorrhoea with penicillin was reported in 1943,²⁷ but Fleming²⁸ and Harkness²⁹ noted in 1944 that the limited supply prohibited its use for treating venereal diseases. Perdrup³⁰ gave

1947 as the year penicillin was becoming widely used in Denmark. Since more than 60 of the *N gonorrhoeae* strains listed in tables II and III were isolated before 1944, we must look to other agents as potential sources of selection of auxotrophic mutants.

Gonococci have been exposed to many compounds because patients often had both syphilis and gonorrhoea. Urethral irrigations with potassium permanganate,²⁹ organic preparations of silver,^{31 32} or proflavin³² were commonly prescribed for gonorrhoea. The therapeutic value of arsphenamine in syphilis was described by Ehrlich in 1910.³³ This compound and other organic arsenicals, together with bismuth, were in use for more than 30 years.³¹⁻³³ Older remedies for syphilis listed by Magian³² were mercury compounds, iodides, and preparations of gold, silver, platinum, antimony, zinc, copper, thallium, iron, and manganese. The bacteriostatic action of the salts of heavy metals is well established and ferrous and manganese compounds are known bacterial mutagens.³⁴ Nevertheless, beyond recognising the potential effects of these many chemicals on gonococci in patients' tissues, it is not possible now to assess their contribution to the evolution of auxotrophy. On the other hand, a causal relation can be shown between sulphonamide resistance and a requirement for methionine.

The first use of sulphanilamide for treating gonorrhoea, according to Harkness,²⁹ was by Dees and Colston in 1937.³⁵ Other more active sulphonamides were soon synthesised, but within 10 years treatment failed in most cases.²⁴ The rapid development of resistance was aided by challenging the gonococci

with subcurative doses of sulphonamides, for example, by self-treatment,³⁶ by prophylactic use to control respiratory disease,³⁷ or during successful treatment of streptococcal disease. Furthermore, there was no consensus on many important features of sulphonamide treatment even by 1939.³³

Twenty per cent of *N gonorrhoeae* isolates from Danish patients in 1940 were sulphonamide-resistant and approximately 40% were resistant in 1944.²⁴ All 11 of the Danish Met⁻ strains (table II) showed sulphadiazine resistance of $\geq 10 \mu\text{g/ml}$ and all but one of the eight strains with a resistance of $\geq 35 \mu\text{g/ml}$ (table III) required methionine. The Zero auxotype strain SS50602/43 was resistant to $35 \mu\text{g/ml}$, as were strains SS7764/45 (Pro-Met-Thp) and G-11 (table I). Resistance to a sulphadiazine concentration of $50 \mu\text{g/ml}$ was shown by strains SS58449/43 (Met), G-22, and G-26. Resistance of $\geq 100 \mu\text{g/ml}$ characterised strains SSW-18/46 (Pro-Met) and SSW-58/46 (Met). It is important to point out, however, that the sulphadiazine resistance of the Met⁻ strains G-2 and G-4 was limited to $1 \mu\text{g/ml}$. Both G-2 and G-4 were isolated in the presulphonamide era. Their defects, as well as those of the 11 Danish Met⁻ strains, however, apparently involved the final step of methionine biosynthesis.

A further study has examined spontaneous sulphadiazine-resistant (Sul-r) mutants selected for increased resistance by growth on sulphadiazine-containing GCMBS agar. Strain SS41835/43 and several other Met⁺ sulphadiazine-susceptible (Sul-s) *N gonorrhoeae* strains gave rise to Sul-r colonies which were picked, purified, and auxotyped. Nearly half of the Sul-r mutants were Met⁻; the others remained Met⁺. Homocysteine did not satisfy the requirement of any of these Met⁻ mutants, indicating that the terminal step in methionine biosynthesis was affected. Many of the mutations were, however, distinguishable by genetic transformation.

Transformation mediated by deoxyribonucleic acid (DNA) had earlier shown the non-identity of the mutations borne by several Sul-r isolates from patients and suggested the relationship between sulphonamide resistance and methionine auxotrophy.³⁸ Separate exposures of a Sul-s Met⁺ recipient to DNA preparations from two Sul-r Met⁻ strains (SS7764/45 and SSW-18/46, cited above) yielded Sul-r transformants which were selected on sulphadiazine-containing medium. Cultures on defined media later showed that the Sul-r transformants were Met⁻. These transformants were used in a second round of transformation in which a recipient population bearing the defective *met* determinant from SSW-18/46 was transformed to Met⁺ by treatment with DNA from the transformant

bearing the non-identical *met* lesion of SS7764/45. The Met⁺ transformants selected on methionine-free defined medium were found subsequently to have regained susceptibility to sulphadiazine.³⁸ Because of the known antagonism between *p*-aminobenzoic acid and sulphonamides and the reports that higher concentrations of *p*-aminobenzoic acid were produced by Sul-r than by Sul-s gonococci,³⁹ the levels of this metabolic product were examined. No significant differences were found in the amounts of *p*-aminobenzoic acid produced by the Met⁻ and Met⁺ transformants.³⁸

The genetic findings support the hypothesis that sulphonamide treatment of gonorrhoea was responsible for selecting Met⁻ mutants as well as sulphonamide-resistant mutants. Another effect of the drug was to eliminate many gonococci which were highly susceptible to sulphadiazine. Resistance of $\leq 1 \mu\text{g/ml}$ characterised 40% of the gonococci listed in table III. In contrast, none of the 97 *N gonorrhoeae* isolates in 1975 were so susceptible,⁷ although similar percentages of the two groups of gonococci showed resistance of $\geq 35 \mu\text{g/ml}$ (11% and 9% respectively).

The advent of penicillin led to a reduction of the prevalence of sulphonamide-resistant gonococci from a peak of 85% in 1949-1950 to 5% of strains examined 10 years later.²⁴ Also, the decline in prevalence of Met⁻ gonococci testifies to a loss of the competitive advantage which methionine auxotrophy must have provided in the presence of a sulphonamide. The percentages of Met⁻ strains among North American isolates in the 1970s are reported to be 2.5% (15 of 600 isolates) in Rochester, New York⁴⁰; 2.4% (6 of 251)⁶ and 1% (1 of 97)⁷ in Milwaukee, Wisconsin; 1.8% (2 of 114) in Seattle, Washington⁸; and 7.8% (19 of 243) in Hamilton, Ontario, Canada.¹³ Three of 166 (1.8%) isolates in Stockholm, Sweden,¹¹ were Met⁻ as was one of 341 (0.3%) isolates in Strasbourg, France.⁴¹ It will be of interest to monitor the prevalence of Met⁻ gonococci in communities where sulphamethoxazole-trime-thoprim treatment is common.

Sulphonamides inhibit the biosynthesis of folic acid by their action on dihydropterotic acid synthetase, the enzyme responsible for incorporating *p*-aminobenzoic acid. Tetrahydrofolic acid coenzymes perform essential transfers of one-carbon units required for biosynthesis of various compounds, including methionine. Methionine biosynthesis in *Escherichia coli* is very sensitive to inhibition by sulphanilamide.⁴² Recently, methionine (as a specific methionyl-tRNA) has been found to play a key role in the initiation of polypeptide synthesis.⁴³ The development of gonococcal resistance to sulphonamides involves at least two independent mechanisms. (a) Mutations leading to

structural alterations of dihydropteroic acid synthetase selectively reduce its affinity for sulphamamide compared with *p*-aminobenzoic acid.⁴⁴ (b) Mutations affecting one enzyme required in methionine biosynthesis secondarily enhance gonococcal resistance to sulphonamides; as yet, there is only circumstantial evidence for this mechanism and it has not been recognised in other genera. In enteric bacteria, the methylation of homocysteine to yield methionine may involve three enzymes specific to the methionine pathway, which are encoded by *metF*, *metE*, and *metH*.⁴⁵ A mutation affecting a gene homologous to one of these three genes is probably responsible for the Met⁻ Sul-s phenotype of strains *G*-2 and *G*-4, whereas a mutation of a different *met* gene produced the phenotype of *G*-22 and other Met⁻ Sul-r *N gonorrhoeae* strains. The observation that some Met⁻ gonococci are Sul-s indicates that the requirement for an exogenous source of this critical end-product of folate metabolism does not of itself generate resistance to sulphonamides.

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