Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1965, 32, 503-513

Determinations of the Sensitivity of *Neisseria gonorrhoeae* to Penicillin Performed on Solid Media of Different Composition

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The increasing frequency of strains of Neisseria gonorrhoeae *with reduced sensitivity to penicillin is making it more and more important to determine the sensitivity of individual strains before and after treatment with this antibiotic. It was recently demonstrated that the concentrations of penicillin inhibitory to* Neisseria gonorrhoea *depend upon the medium employed. This important observation made it desirable to have available for interlaboratory comparisons a non-commercial reproducible medium which would support the growth of the vast majority of the gonococcal strains in circulation. Using a plate dilution procedure, various modifications of the HYL medium described elsewhere were compared with the routine medium for carrying out sensitivity determinations. Both stock and fresh strains of* Neisseria gonorrhoeae *were used in the experiments. The 50% inhibitory concentrations observed on the two media did not differ to any great extent, being only about 40% higher on the routine medium than on the HYL medium. The difference between the HYL medium and the routine medium was somewhat greater for the less sensitive strains than for the more sensitive strains. The variations in the difference from strain to strain were larger for the more sensitive fresh strains than for the less sensitive strains and the stock strains. This observation limits the value of a correction method employing, for example, three reference strains as a means of ensuring comparability between the results of different laboratories.*

INTRODUCTION

In another paper, two of us (Møller & Reyn, 1965)[4] have described the development of a new " chocolate medium " which does not contain any fresh biological substances: a combination of yeast autolysate and liver autolysate is used as a substitute for ascitic fluid and a haemoglobin solution is used in place of heated horse blood. This haemoglobin-yeast-liver (HYL) medium was shown to support the growth of *Neisseria gonorrhoeae* just as well as did the chocolate ascitic-fluid medium previously in routine use for the primary isolation of *N. gonorrhoeae* in the Neisseria Department of the Statens Seruminstitut. Since April 1963, the HYL medium has replaced the ascitic-fluid medium for routine use.

With the increasing frequency of strains of *N. gonorrhoeae* less sensitive to penicillin, it is becoming more important to determine the sensitivity of the individual strains before and after treatment with this antibiotic. At the present time, a great number of sensitivity determinations are carried out in the Neisseria Department of the Statens Seruminstitut, and so far these have been made using chocolate ascitic-fluid agar. However, the production of media would be simplified if the same medium could be used both for diagnostic routine work and for sensitivity determinations. It has recently been demonstrated that the inhibitory concentrations of penicillin depend upon the medium employed (Reyn et al., 1965).[5] Consequently, before switching over to HYL medium for sensitivity determinations, it would be necessary to compare the results of such determinations using HYL medium with those using the ascitic-fluid medium.

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[4] See the article on page 471 of this issue.

[5] See the article on page 477 of this issue.

MATERIAL AND METHODS

Gonococcal strains

The gonococci used in the present study comprised 218 fresh strains from the routine work of the Neisseria Department of the Statens Seruminstitut plus 12 older strains which had been used in previous experiments; two of the latter (50700/1955 and 43562/1944) were control strains and the other ten (I-X) were strains that had been employed in two recent collaborative studies (Reyn et al., 1963 and 1965).[1] These older strains had been kept in a lyophilized state.

Media

The media used were modifications of the chocolate ascitic-fluid-agar medium and the HYL medium (haemoglobin-yeast-liver medium) described by Reyn (1965).[2] The chocolate medium (" routine medium ") used in the present study differed from that used for the primary isolation of *N. gonorrhoeae* in that it contained only 11% of ascitic fluid and no peptone; furthermore the Danish agar was replaced by Japanese agar. The modifications in the formula of the HYL medium are indicated at the appropriate places in the text. In some experiments, the yeast and liver autolysates were replaced either by Bacto-Supplement C [3] or by Lankford's chemical supplement [4] (Lankford, 1950). Both supplements were added in the proportion 1 : 100.

Sensitivity determination

A plate dilution technique, which has been described elsewhere in detail (Reyn at al., 1963), was used for determining the sensitivity of *N. gonorrhoeae* to crystalline penicillin G (Leo). The results were expressed in terms of 50% inhibitory concentrations (IC_{50}) in international units per millilitre (IU/ml).

PRELIMINARY EXPERIMENTS

Preliminary comparative experiments had shown that the IC_{50} values found on the HYL medium were nearly the same as those obtained on the routine medium, which in contrast to the HYL medium contained ascitic fluid and heated horse blood. In these experiments, two different sources of agar (Japanese and Spanish) had been employed. In August 1962, in order to assess the possible influence of the two different agars, four differently-composed media were compared on the same day. The four media were: (1) routine medium with Japanese agar, (2) routine medium with Spanish agar, (3) HYL medium with Spanish agar and 0.45% of haemoglobin, and (4) HYL medium with Spanish agar and 0.9% of haemoglobin. Sensitivity determinations were performed on 24 gonoccocal strains, including the two Copenhagen control strains (50700 and 43562) and the 10 strains (I-X) that had been previously tested in the Scandinavian study.[5] The remainder of the strains were arbitrarily chosen from the daily routine examinations. Unfortunately, this procedure resulted in an uneven distribution as regards sensitivity, too many strains being highly sensitive. In Table 1 and in Fig. 1-3 the results for the four media are compared two by two.

TABLE 1
COMPARISON OF FOUR DIFFERENT MEDIA USING 12 STOCK STRAINS AND 12 FRESH STRAINS OF *N. GONORRHOEAE*

Media [a] compared	Mean differences in log IC_{50} values (IU/ml)	Standard errors
1 and 2	–0.02	0.018
2 and 3	0.13	0.031
2 and 4	0.10	0.031
3 and 4	–0.02	0.018
1 and 3	0.11	0.024

[a] Composition of media:
1: routine medium with Japanese agar.
2: routine medium with Spanish agar.
3: HYL medium with Spanish agar and 0.4% of haemoglobin.
4: HYL medium with Spanish agar and 0.9% of haemoblobin.

It is evident both from the table and from the figures that all four media gave very similar results, the average differences in the log IC_{50} values being

[1] See the article on page 477 of this issue.

[2] See the article on page 449 of this issue.

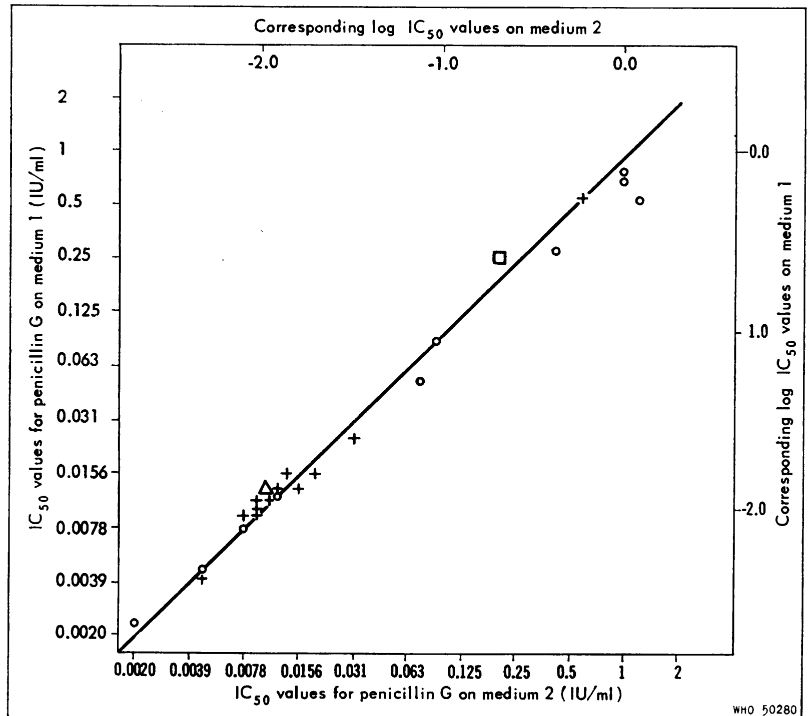
[3] Bacto-Supplement C is the same as Bacto-Supplement B but without glucose; it is dispensed in a freeze-dried state. For further details see: *Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures*, 9th ed., Detroit, Mich., Difco Laboratories, 1953, p. 276.

[4] Formula of Lankford's chemical supplement: glutamine, 0.25%; glucose, 20%; cocarboxylase, 0.0001%.

[5] Collaborative study by Denmark, Finland, Norway and Sweden (Reyn et al., 1963), called, for convenience, " the Scandinavian study ".

FIG. 1

COMPARISON BETWEEN IC_{50} VALUES OF PENICILLIN G OBTAINED ON MEDIUM 1 AND MEDIUM 2[a]

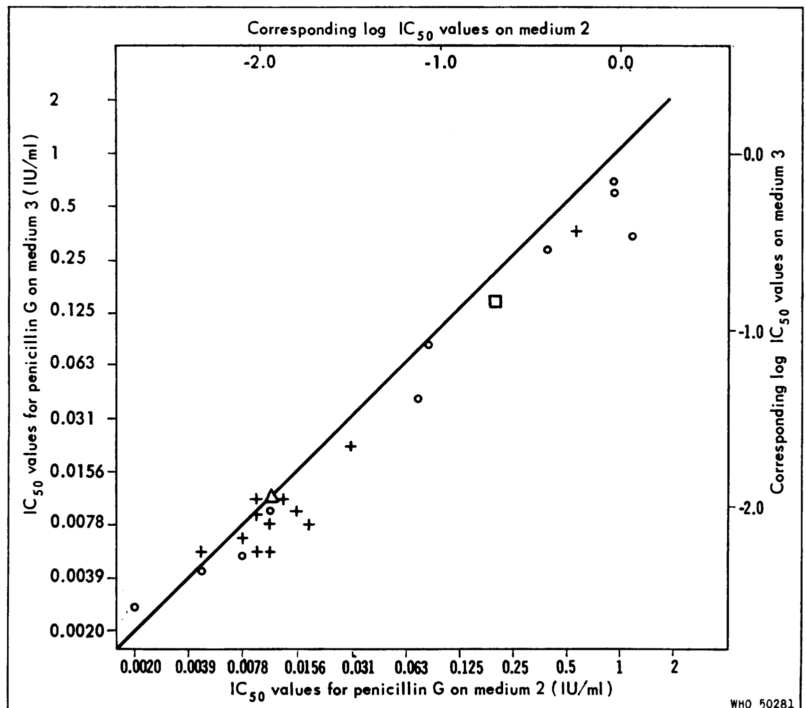


[a] Medium 1 = routine medium with Japanese agar; medium 2 = routine medium with Spanish agar. 24 gonococcal strains were tested on each medium.

+ 12 fresh strains
o strains I to X used in the Scandinavian study (Reyn et al., 1963)
□ control strain 50700/1955
△ control strain 43562/1944

FIG. 2

COMPARISON BETWEEN IC_{50} VALUES OF PENICILLIN G OBTAINED ON MEDIUM 2 AND MEDIUM 3[a]



[a] Medium 2 = routine medium with Spanish agar; medium 3 = HYL medium with Spanish agar and 0.45 % of haemoglobin. 24 gonococcal strains were tested on each medium.

+ 12 fresh strains
o strains I to X used in the Scandinavian study (Reyn et al., 1963)
□ control strain 50700/1955
△ control strain 43562/1944

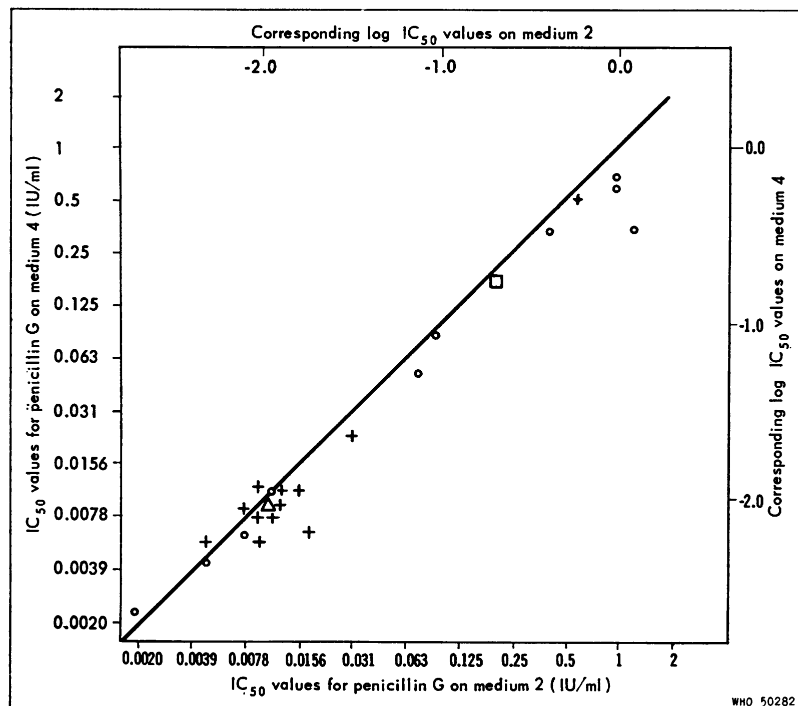


FIG. 3
COMPARISON BETWEEN IC_{50} VALUES OF PENICILLIN G OBTAINED ON MEDIUM 2 AND MEDIUM 4 [a]

[a] Medium 2 = routine medium with Spanish agar; medium 4 = HYL medium with Spanish agar and 0.9 % of haemoglobin. 24 gonococcal strains were tested on each medium.

+ 12 fresh strains

o strains I to X used in the Scandinavian study (Reyn et al., 1963)

□ control strain 50700/1955

△ control strain 43562/1944

very small. The greatest average difference observed (that between medium 2 and medium 3, see Table 1) was 0.13, corresponding to an average ratio of 1.35 (antilog 0.13).

From Fig. 1 and from Table 1 it can be seen that the two different brands of agar did not in any way influence the outcome when the routine medium was used. Fig. 2 and 3 show that the sensitivity values obtained with the HYL media (3 and 4) were only slightly lower than those obtained with the routine medium containing Spanish agar.[1] Medium 4 gave slightly higher values than those obtained with medium 3, the difference in composition between these media being that medium 3 contained 0.45% of haemoglobin and medium 4 contained 0.9% of haemoglobin.

In similar experiments performed in November 1962 using 0.45% of haemoglobin in the HYL medium and comparing this medium with the chocolate medium, the results were not quite as satisfactory as those described above. The average ratio between the IC_{50} values was about 2 and the variation was greater than that found in the August experiments. The greatest deviations were observed among the most sensitive strains. Some of these strains grew in small colonies, and when they were fresh it was often difficult to obtain suitable suspensions for the sensitivity determinations. It was thought that the different batches of yeast and liver autolysates might account for the less satisfactory results and that a final concentration of 0.45% of haemoglobin was perhaps too low to compensate for uncontrollable factors acting during the preparation of the plates.

[1] Statistically, these differences were significant, being more than three times the standard error.

MAIN EXPERIMENTS

In the next experiment, old and new batches of the autolysates were compared and media enriched with 0.45% or 0.9% of haemoglobin were compared with media enriched with heated horse blood, Furthermore, Lankford's chemical supplement was compared with the autolysates and with Bacto-Supplement C. The chocolate medium enriched with ascitic fluid and horse blood was used as a reference. In Table 2 the compositions of 10 different media are given.

It may be seen from the table that it was possible to compare (1) the effects of heated horse blood with

TABLE 2

COMPOSITIONS OF TEN DIFFERENT MEDIA USED IN THE EXPERIMENTS PRESENTED IN TABLES 3 AND 4 AND IN FIG. 4

Medium [a]	Agar base [b] (volume and agar content)	Enrichment				Distilled water (ml)	Total volume (ml)
		Horse blood or haemoglobin			Ascitic fluid or substitute		
		Substance	Volume (ml)	Final concentration (%)			
1	740 ml, Japanese agar, 1.5%	horse blood	60	6.7	ascitic fluid, 100 ml	none	900
2	660 ml, Spanish agar, 1%	haemoglobin, 6%	75	0.45	liver autolysate, 10 ml,[c] plus yeast autolysate, 10 ml [d]	245	1 000
3		haemoglobin, 6%	150	0.9	liver autolysate, 10 ml,[c] plus yeast autolysate, 10 ml [d]	170	1 000
4		haemoglobin, 6%	75	0.45	liver autolysate, 10 ml,[e] plus yeast autolysate, 10 ml [f]	245	1 000
5		haemoglobin, 6%	150	0.9	liver autolysate, 10 ml,[e] plus yeast autolysate, 10 ml [f]	170	1 000
6		horse blood	67	6.7	liver autolysate, 10 ml,[c] plus yeast autolysate, 10 ml [d]	253	1 000
7	660 ml, Spanish agar, 1%	haemoglobin, 6%	150	0.9	Lankford's chemical supplement [g]	159	1 000
8		haemoglobin, 6%	150	0.9	Lankford's chemical supplement [g] without glucose	165	1 000
9		haemoglobin, 6%	150	0.9	Bacto-Supplement C, 10 ml, plus 33% glucose solution, 6 ml	174	1 000
10		haemoglobin, 6%	150	0.9	Bacto-Supplement C, 10 ml	180	1 000

[a] 1 = routine (reference) medium; 2-10 = modified HYL media.
[b] Beef-heart broth without peptone.
[c] Prepared 11 September 1962.
[d] Prepared 28 September 1962.
[e] Prepared 20 March 1962.
[f] Prepared 22 February 1962.
[g] Quantities added: 25 ml of 0.1% glutamine, 0.1 ml of 0.01% cocarboxylase, 6 ml of 33% glucose. Final concentrations: 0.0025% of glutamine, 0.000001% of cocarboxylase, 0.2% of glucose.

those of two different concentrations of haemoglobin and (2) the effects of ascitic fluid with those of (*a*) liver plus yeast autolysates of different ages, (*b*) Lankford's chemical supplement with and without glucose, and (*c*) Bacto-Supplement C with and without glucose. All the media were prepared on the same day and the same batch of beef-heart-agar base was used for the preparation of media 2-10. Within a week after the preparation of a batch, sensitivity determinations to penicillin, colony countings and estimation of colony diameters were performed on three different days for all media. Altogether 10 gonococcal strains were tested, but only 5 or 6 strains were used on any one day. Each day on which sensitivity determinations were made, three older strains were also tested: the two control strains and strain No. VI. The other strains, which were chosen to cover a wide range of sensitivity, were fresh strains from the routine laboratory.

A final concentration of 0.65% of Spanish agar was employed in media 2-10, whereas the reference medium contained 1.3% of Japanese agar. For each strain the differences in the log IC_{50} values between medium 1 and the other media were plotted against the log IC_{50} values for medium 1. The plots showed that media 9 and 10 (containing Bacto-Supplement C) deviated markedly from the other media on the last two days on which determinations were made. The results observed on the first day were about the same as those found on the routine medium and the deviation was ascribed to destruction of the penicillin. Similar results were observed on examination of a new batch of medium 10. The

TABLE 3

DEVIATIONS FROM MEAN LOG IC_{50} VALUES FOR EACH STRAIN ON MEDIA 1 TO 8

	Deviations from mean log IC_{50} values (IU/ml) on medium:								Mean log IC_{50} values (IU/ml)	Range of deviations
	1	2	3	4	5	6	7	8		
Control strains										
50700 [a]	0.08	–0.08	0.00	–0.13	0.11	0.08	0.00	–0.02	–0.75	0.24
43562 [a]	0.01	–0.12	0.01	0.01	0.01	0.03	0.01	0.01	–1.97	0.15
Scandinavian study VI [a]	–0.07	–0.02	0.01	0.20	0.01	0.13	–0.05	–0.20	–2.59	0.40
Mean deviations for three control strains	0.01	–0.07	0.01	0.03	0.04	0.08	–0.01	–0.07		0.15
Other strains										
6798	0.29	–0.08	–0.08	–0.08	–0.08	0.22	–0.08	–0.08	–0.37	0.37
6146	0.26	–0.11	–0.04	–0.19	–0.04	0.11	0.04	–0.04	–0.49	0.45
6550	0.20	–0.11	–0.03	–0.11	–0.03	0.12	–0.03	–0.03	–0.72	0.31
7180	0.09	–0.29	0.02	–0.06	0.17	0.09	0.02	–0.06	–1.97	0.46
5680	0.16	0.01	0.16	0.01	–0.22	–0.07	0.01	–0.07	–2.04	0.38
Scandinavian study X	0.19	–0.11	0.19	–0.11	–0.11	0.04	–0.04	–0.04	–2.14	0.30
7167	–0.15	–0.30	–0.15	0.45	0.15	0.15	0.00	–0.15	–2.56	0.75
Mean deviations for 7 " other strains "	0.15	–0.14	0.01	–0.01	–0.02	0.09	–0.01	–0.07		0.29
Range of deviations	0.44	0.31	0.34	0.64	0.39	0.29	0.12	0.12		
Mean deviations for all 10 strains	0.11	–0.12	0.01	0.00	0.00	0.09	–0.01	–0.07		0.23

[a] Mean of 3 determinations.

results for each medium were compared with the mean log IC_{50} values calculated for each strain, based on the results obtained for media 1 to 8; media 9 and 10, which had shown definite differences from the other media, were omitted.

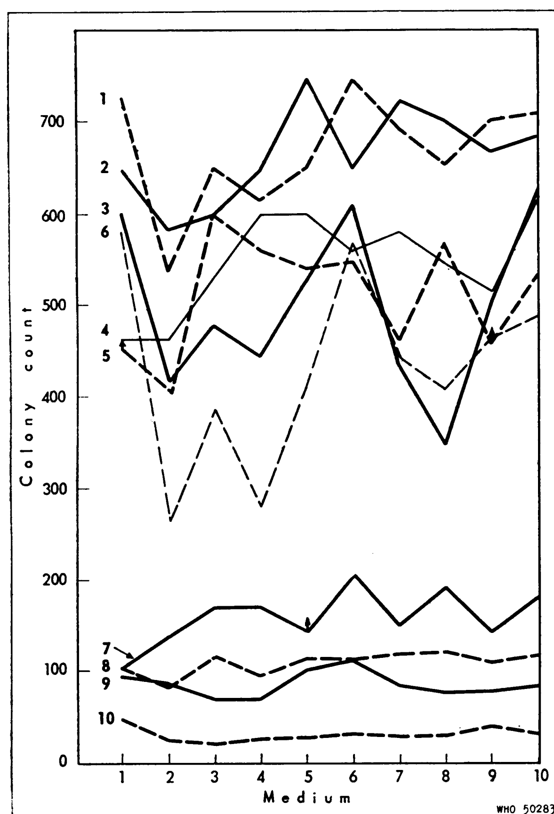
The deviations from these mean values are given in Table 3, in which the strains are arranged according to the mean log IC_{50} values.

The mean deviations for all 10 strains tested demonstrate that only medium 6 reached the same level as that of medium 1, the former showing a mean deviation of +0.09 and the latter a mean deviation of +0.11, corresponding respectively to 23% and 29% above the mean for all media. The structure of the experiments made it possible to estimate the experimental error through an analysis of variance. The residual variance was estimated at about 0.01; this corresponds to a standard deviation of 0.10.

For the control strains alone, no significant differences could be demonstrated between the media; however, when all the strains were taken into account it was found that media 1 and 6 showed significantly higher log IC_{50} values than the mean, while medium 2 showed lower values. Media 1 and 6 contained horse blood and medium 2 contained haemoglobin in a final concentration of only 0.45% and was prepared with autolysates which had been stored for a longer time. However, the deviations are small compared to the range of sensitivity, the highest IC_{50} value being more than 500 times the lowest (Fig. 2).

FIG. 4

COLONY COUNTS OF ELEVEN STRAINS OF *NEISSERIA GONORRHOEAE* GROWN ON TEN DIFFERENT MEDIA



1 = strain 6768
2 = strain 6146
3 = strain 74182
4 = strain 50700 (control)
5 = strain 43562 (control)
6 = strain 6550
7 = strain 5680
8 = strain 7180
9 = strain 7167
10 = strain X

In Table 3, bottom of last column, the range of variation [1] in the mean deviations for all 10 strains on media 1-8 is seen to be 0.23 or 1.7-fold. It is noteworthy that the range of variation is lower for the control strains than for the other strains.

The range of variation per medium was greatest for medium 4 (4-fold) and smallest for media 7 and 8 (about 1.3-fold).

In Fig. 4 and Table 4 the results of the relevant colony counts [2] and the colony diameters are presented for each of the media and for the 10 strains for which the data were complete. Suspensions presumed to contain about 10^8-10^9 organisms per ml were diluted 5×10^4 times or 5×10^5 times and 0.1 ml was plated.

TABLE 4

COLONY DIAMETERS OF ELEVEN STRAINS OF *NEISSERIA GONORRHOEAE* GROWN ON TEN DIFFERENT MEDIA

Medium	Average minimum diameter (mm)	Average maximum diameter (mm)
1	0.5	0.8
2	0.6	1.2
3	0.6	1.0
4	0.5	0.9
5	0.4	1.0
6	0.6	1.4
7	<0.3	0.8
8	<0.3	<0.6
9	<0.4	1.1
10	<0.4	1.0

On the whole, low colony counts were found on medium 2; both this medium and medium 4 contained a final concentration of haemoglobin of only 0.45%.

In order to test whether any significant differences could be demonstrated between the media, an analysis of variance was performed on the square roots (x) of the colony counts (K), i.e., $x = \sqrt{K}$. The use of x instead of K has the advantage that the variance of x is constant, which makes the analysis of variance more simple.

The analysis showed that the values obtained on medium 2 were significantly lower than the average value for all media and the values obtained on medium 6 were significantly higher than the average, although not higher than those obtained on medium 1.

The diameters of the colonies were estimated after incubation for either 24 hours (7 strains) or 40 hours (4 strains), depending on the growth rate. Plates 9-10 cm in diameter and 4-5 mm thick, with

[1] " Range of variation " is used here to mean the ratio of the highest to the lowest IC_{50} value.

[2] Numbers between 50 and 700 were considered useful for our purpose, the principle being to count on plates with as many non-confluent colonies as possible.

TABLE 5

DIFFERENCES IN LOG IC_{50} VALUES DETERMINED ON THREE BATCHES OF TWO DIFFERENT MEDIA USING FIVE DIFFERENT STRAINS

Strain	Differences in log IC_{50} values [a]				Mean values for:	
	A [b]	B [b]	C [b]	Mean	log IC_{50}	IC_{50} (IU/ml)
Scandinavian Study V	0.15	0.23	0.25	0.21	−0.32	0.48
Control 50700	0.08	0.13	0.18	0.13	−0.64	0.23
Scandinavian Study III	0.08	0.18	0.25	0.17	−1.26	0.055
Control 43562	0.00	0.23	0.15	0.13	−0.96	0.0110
Scandinavian Study VII	0.00	0.23	0.20	0.14	−2.17	0.0068
Mean differences in log IC_{50} values for all 5 strains	0.06	0.20	0.21	0.16		

[a] Routine medium minus HYL medium with 1% haemoglobin.
A = batch 1 of each medium; B = batch 2 of each medium; C = batch 3 of each medium. Mean = mean of A, B and C.
[b] Mean of 3 determinations.

from 50 to 700 colonies, were used for the measurements.

The average minimum and maximum colony diameters for 11 strains grown on the 10 media are presented in Table 4; minimum and maximum refer to the smallest and the largest colonies of each strain observed on the plates. The highest values were found on medium 6, but those observed on media 1-5 were nearly as high, whereas the values for media 7-10 were much lower, being lowest on media 7 and 8 (enriched with Lankford's chemical supplement). The addition of glucose to these supplements had no effect, neither on the colony counts nor on the colony diameters. It is possible that the comparatively poor effect of Lankford's chemical supplement was due to the use of a different agar base from that used by Lankford.

In the last series of experiments (March-April 1963) the routine medium was compared with an HYL medium prepared with a final concentration of 1% instead of 0.9% of haemoglobin and with fresh yeast and liver autolysates.

Altogether 200 fresh strains and 5 control strains (50700, 43562, III, V and VII) were employed. The control strains were tested each day on which a determination was made. Three different batches of each medium were prepared and each batch was used on three days and not later than a week after its preparation.

The repeated [1] testing of the 5 control strains made it possible to estimate the experimental error for each of the media separately. The analysis did not disclose any differences between these experimental errors, possibly because some of the sources of error were common to both media. The residual variance of the *difference* (s_d^2) between the log IC_{50} values found on the two media was estimated to be 0.0046. Table 5 shows the mean difference (routine medium minus HYL medium) calculated for each batch of medium and for each strain. The mean difference for all 3 batches and all 5 strains is 0.16. Thus, the IC_{50} values were on the average 45% higher on the reference (routine) medium than on the HYL medium (antilog 0.16 = 1.45). The difference shows some variation from strain to strain; however, this variation mainly concerns the results with batch 1 (column A), the low value of the difference observed with this batch being due to two exceptionally low results obtained on the routine medium.

The *fresh strains* were divided into 2 subgroups (I and II) according to the mean log IC_{50} values for the two media: log $IC_{50} \geq -1.35$ and log $IC_{50} < -1.35$. Table 6 gives a survey of the results. The table shows that for the less sensitive strains the mean difference was in the region of 0.15 (40%),

[1] The strains were tested on each of the three batches of medium on each of the three days it was used.

TABLE 6

MEAN DIFFERENCES IN LOG IC_{50} VALUES DETERMINED ON THREE BATCHES OF TWO DIFFERENT MEDIA ACCORDING TO SENSITIVITY OF STRAIN

Batch	Strain sensitivity [a]	No. of strains	Mean log IC_{50} values			Mean difference A-B	Variance of differences (s_d^2)
			Routine medium (A)	HYL medium (B)	Mean of A and B		
1	I	38	–0.44	–0.58	–0.51	0.14	0.0142
	II	46	–2.00	–2.02	–2.01	0.02	0.0217
2	I	23	–0.44	–0.62	–0.53	0.18	0.0108
	II	21	–1.81	–1.99	–1.90	0.18	0.0192
3	I	39	–0.38	–0.52	–0.45	0.14	0.0112
	II	33	–1.86	–1.97	–1.92	0.11	0.0127
Mean values (all three batches)	I	100	–0.41	–0.56	–0.49	0.15	0.0123
	II	100	–1.91	–1.99	–1.95	0.08	0.0183

[a] The strains were divided into two subgroups:
I = Less sensitive strains (log $IC_{50} \geq -1.35$, $IC_{50} \geq 0.045$ IU/ml);
II = More sensitive strains (log $IC_{50} < -1.35$, $IC_{50} < 0.045$ IU/ml).

as in the case of the control strains. The variance ($s_d^2 = 0.0123$) is much larger than the variance due to experimental error ($s_d^2 = 0.0046$). For the control strains the corresponding value is only 0.0053.[1] This means that the difference between media varies significantly from strain to strain, and that this variation is more pronounced for the fresh strains than for the control strains. For the more sensitive strains the mean differences showed even greater variation; the variance was estimated to be 0.0183. In addition, the mean difference between the media was much lower for batch 1 (0.02) than for either of the other two batches (0.18 and 0.11). The mean difference for all three batches taken together was only half as big with the more sensitive strains as with the less sensitive strains: 0.08 or 20% as compared with 0.15 or 40%, but this difference is due mainly to the results obtained with batch No. 1.

DISCUSSION

Three questions present themselves in relation to the fact that the IC_{50} values obtained depend upon the medium employed, namely (1) why do these values differ, (2) which are the criteria of a " good " medium, and (3) which is the " right " medium ?

(1) The main purpose of the present paper is to compare the IC_{50} values obtained on the HYL medium (and modifications of this) with those obtained on the routine medium. Generally, the results obtained were quite close, the IC_{50} values being only about 40% higher on the routine medium than on the HYL medium.

In the present experiments, using a high number of fresh strains (Table 6) it was demonstrated that the difference between the HYL medium and the routine medium was greater for the less sensitive strains than for the more sensitive strains, but the difference between the two groups was not very pronounced. The variance of the difference between the log IC_{50} values obtained on the two media was found to be greater for the more sensitive strains than for the less sensitive strains (0.0183 as against 0.0123), both variances being greater than that of the control strains.

In previous experiments, using the same method of sensitivity determination on two different media, an American medium (Carpenter et al., 1949) and the Danish routine medium, a similar pattern was observed but the differences observed were greater (Reyn et al., 1965).[2] The routine medium is " enriched " with both horse blood and ascitic

[1] Obtained by adding to the value 0.0046 an estimate (0.0007) of the variance due to the variations between strains.

[2] See the article on page 477 of this issue.

fluid[1] whereas the only protein source in the American medium is 1.0% haemoglobin.

It has been shown that penicillins are readily adsorbed on serum proteins, especially on albumin, presumably resulting in both *in vitro* and *in vivo* inactivation of the drug (Chow & McKee, 1945; Tompsett et al., 1947; Oeff et al., 1955; Marner & Lund, 1957; Gourevitch et al., 1960; Bunn & Knight, 1961; Yakobson & Grigoreva, 1961; Bond et al., 1963). Thus, it would be natural to ascribe the difference in level of sensitivity between the routine medium and the American medium to the fact that in the routine medium part of the penicillin is bound to albumin originating from the horse blood and from the ascitic fluid. On the average, the IC_{50} values obtained on the Danish medium are twice those obtained on the American medium; this may well be explained by assuming that about 50% of the penicillin is adsorbed to protein in the first medium, provided that no adsorption takes place in the American medium.

However, the difference between the more sensitive and the less sensitive strains (both in variance and in sensitivity response to different media) is more difficult to explain. According to Langmuir's theoretical adsorption equation[2] for solutes, the quantity adsorbed by a given mass of adsorbent increases with the concentration of the solute up to the point at which saturation is reached and the fraction of penicillin bound will decrease with increasing concentration. The rate of the decrease will depend on the distance from the saturation point, and in the present case the saturation points of the different media are not known.

It is interesting that the protein content of the HYL medium, which resulted in IC_{50} values closer to those for the routine medium than those obtained with the American medium, is the same as that of the American medium, namely 1% haemoglobin. This observation and the fact that the variance of the difference in the IC_{50} values obtained on the HYL medium and on the routine medium depended upon the age of the strains as well as upon the sensitivity level indicates that growth conditions may also play a role.

(2) All strains of *N. gonorrhoeae* should be able to grow on a medium meant for determination of the sensitivity to penicillin and preferably this medium should not contain substances that destroy (or perhaps adsorb) the antibiotic. It should be easy to prepare, and the ingredients should be cheap and readily available. Furthermore, on storage the medium should keep well and the quality should not vary too much from one batch to another. Most of these requirements are fulfilled by the HYL medium; however, the problem of preventing the destruction or adsorption of penicillin has not yet been completely solved (see above).

(3) The question arises whether the HYL medium is the " right " medium, in the sense that the IC_{50} values obtained on this medium reflect the *in vivo* conditions present in the different infected areas in the best possible way. These conditions vary to a certain extent but generally the penicillin concentrations found in serum or plasma are taken as an indication of the *in vivo* conditions. However, the range of individual variation in serum penicillin titres observed after the administration of a certain dose of penicillin is so great that there is very little possibility of demonstrating a closer correlation between the IC_{50} values obtained for any of the two media employed in the present paper (Lightbown & Sulitzeanu, 1957; Bond et al., 1963).

With a view to the possible use of *reference strains* to ensure comparability between results obtained in different laboratories using the same or different methods for determining the sensitivity of *N. gonorrhoeae* to penicillin, the greater variance found with fresh strains limits the value of such a procedure, even if three reference strains are used (cf. Reyn et al., 1963; Reyn et al., 1965). In its first report, the WHO Expert Committee on Gonococcal Infections (1963) recommended that the Møller-Reyn (HYL) medium be used as a provisional reference medium in a provisional reference method and that international reference strains be made available for this purpose.

[1] The routine medium contains from 0.7% to 0.9% of protein, of which 0.5% to 0.6% is albumin.

[2] Based on Freundlich's empirical adsorption equation (see Crockford & Knight, 1953).

RÉSUMÉ

En raison de la diminution de la sensibilité à la pénicilline qui s'observe chez un nombre croissant de souches de gonocoques, il importe de déterminer cette sensibilité avant et après traitement aux antibiotiques. Or, on a constaté récemment que la concentration de pénicilline qui inhibe la croissance d'une souche donnée varie selon le milieu de culture. La nécessité s'est donc imposée de mettre au point un milieu de culture facile à reproduire, qui permettrait à la fois le diagnostic et la détermination de la résistance.

Le milieu que les auteurs viennent de proposer pour l'isolement primaire du gonocoque (gélose à l'hémoglobine-levure-foie (HYL)), sous diverses de ses modifications, a été comparé au milieu contenant du liquide d'ascite, pour la culture de souches de gonocoques fraîchement isolées et celle de souches de laboratoire. La concentration inhibitrice 50% (CI_{50}) ne différait pas notablement d'un milieu à l'autre. Les valeurs CI_{50} n'étaient supérieures que de 40% sur le milieu de routine, comparé au milieu HYL. La différence était cependant plus accentuée pour les souches les moins sensibles que pour les souches les plus sensibles.

Les variations de la différence d'une souche à l'autre restent un problème à résoudre. Elles étaient plus importantes pour les souches fraîches (plus sensibles à la pénicilline) que pour les souches moins sensibles et les souches de laboratoire. Ce fait limite la valeur de la méthode mise à l'étude dans le but de comparer la sensibilité à la pénicilline des souches fraîches, par rapport à des souches de référence, dans divers laboratoires.

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