JCI The Journal of Clinical Investigation

STUDIES ON BACTERIA DEVELOPING RESISTANCE TO PENICILLIN FRACTIONS X AND G *IN VITRO* AND IN PATIENTS UNDER TREATMENT FOR BACTERIAL ENDOCARDITIS

Harry F. Dowling, Harold L. Hirsh, C. Barbara O'Neil

J Clin Invest. 1946;25(5):665-672. https://doi.org/10.1172/JCI101747.

Research Article

STUDIES ON BACTERIA DEVELOPING RESISTANCE TO PENICILLIN FRACTIONS X AND G *IN VITRO* AND IN PATIENTS UNDER TREATMENT FOR BACTERIAL ENDOCARDITIS

By HARRY F. DOWLING, HAROLD L. HIRSH, AND C. BARBARA O'NEIL

(From the George Washington Medical Division, Gallinger Municipal Hospital and the Department of Medicine, George Washington University, Washington)

(Received for publication January 25, 1946)

Although penicillin G is the fraction of penicillin which is best known and most used, 2 other known fractions of this antibiotic have been isolated in crystalline form, penicillin F and penicillin X. Since the latter fraction is now being manufactured in appreciable quantities, it is important to determine whether it is superior in any way to penicillin G. We have attacked this problem by attempting to answer the following questions:

(*1*) What are the relative sensitivities of different bacteria to penicillins X and G?

(*2*) If the resistances of bacteria are raised *in vitro* by the use of penicillin X or penicillin G, what effect would this have upon the ultimate sensitivities of the organisms to the 2 fractions?

(*3*) If the resistance of an organism should be raised during the course of treatment of a patient with 1 of these fractions of penicillin, would there be any change in the relative sensitivity of the organism to the 2 fractions?

(*4*) Is there any difference in the absorption, distribution and excretion of penicillin X as compared with penicillin G?

(*5*) Is there any difference in the clinical results obtained with the use of the 2 fractions?

The last 2 questions have been discussed in another publication (1). In the present paper we shall give the results which we have obtained in seeking answers to the first 3 questions.

MATERIALS AND METHODS

Several preparations of penicillin X were used. One commercial preparation contained 75 per cent of sodium penicillin X; others, 90 and 95 per cent.[1] Several brands of commercial sodium penicillin G, made by various manufacturers, were used. Crystalline calcium penicillin G, obtained from the Food and Drug Administration, was also employed. In a few of the tests, a preparation of crystalline sodium penicillin G[2] was compared with the crystalline calcium penicillin G, and found to give identical results.

The tests for penicillin sensitivity combined in each tube 2 reagents: (*a*) 0.2 from a series of 2-fold dilutions of penicillin, and (*b*) 0.5 ml. of a standard dilution of each micro-organism in a medium containing 0.2 per cent of horse blood. A 24-hour culture of staphylococci (or other bacteria which grew readily) in tryptose phosphate blood broth was diluted in the same medium to the 10^{-6} dilution; streptococci, to 10^{-4}; pneumococci and meningococci, to 10^{-3}; influenza bacilli, to 10^{-2}. To the final dilution to be used, 0.2 per cent of the horse blood was added, and 0.5 ml. aliquots transferred to tubes already containing the required dilutions of penicillin. After 18 hours incubation the end-point tube showing no hemolysis or turbidity was recorded as containing the minimal effective concentration of penicillin. The end-points were checked by streaking the last negative tube on a blood-agar plate.

To increase the resistance of the bacterial strains to penicillins X and G, each was first tested for sensitivity and then transferred serially through: (*1*) a tube of an appropriate medium which contained the greatest amount of penicillin X which would allow growth; (*2*) a tube of same medium containing the greatest amount of penicillin G which would allow growth; and (*3*) a control tube of the medium containing no penicillin. The media employed were tryptose phosphate broth in the case of staphylococci, brain-heart infusion broth for streptococci and meningococci, and the same medium plus 1 per cent agar for pneumococci. At 24-hour intervals (or, in the case of meningococci, at 48-hour intervals), 0.2 ml. of the control culture was transferred to another tube of control medium. If the organisms in the tubes containing penicillin had grown well, 0.2 ml. of the culture was transferred from each tube to another tube which contained double the concentration of the same fraction of penicillin. If they had not grown well, the transfer was made to a tube containing the same concentration as on the previous day. This procedure was continued until the organisms persisted in growing poorly when transferred in the same concentration of penicillin for 3 successive days. When this plateau had been reached in each of the fractions of penicillin, the experiment was terminated as far as that particular strain was concerned, and the sensitivities of

[1] Supplied by the Lederle Laboratories, Inc.

[2] Supplied by Merck and Company

the organisms which had been grown in the 2 fractions of penicillin, and of the control organisms, were tested.

The bacteria used in determining sensitivities and for the elevation of resistance were obtained from patients with various kinds of infections, with the exception of a few of the staphylococci and non-hemolytic streptococci. These were cultured from the blood of healthy persons with a transient bacteremia following the extraction of teeth.

RESULTS OF STUDIES ON COMPARATIVE RESISTANCE

Several investigations have been reported in which the comparative resistance of certain organisms to penicillins X and G were determined. Welch and his associates (2) stated that the X fraction was more effective than commercial penicillin against 1 strain each of *Klebsiella pneumoniae, Bacillus cereus* and pneumococcus Type I. Libby and Holmberg (3) tested 1 strain each of 10 different bacteria, and found only slight differences in their relative sensitivities to the 2 fractions. Ory and his co-workers (4) studied 209 strains, and found that the streptococci, pneumococci, gonococci and meningococci were generally from 2 to 8 times as sensitive to penicillin X as to penicillin G, on a unit-for-unit basis, while the staphylococci, 3 strains of Friedlander's bacillus, and 1 of *Hemophilus influenzae,* showed essentially the same resistance to the 2 fractions.

We have placed our results in the same form (Table I) as was used by Ory, in order to make comparison easy. One hundred and one strains were tested for resistance to penicillins X and G. Commercial penicillin preparations were used in the case of 56; crystalline penicillin preparations, in the case of 14; and in the case of 31 strains all 4 of these preparations were employed. Inasmuch as no significant difference could be observed in the results obtained with the crystalline and commercial preparations of either fraction, no distinction has been made between them in the table.

When a comparison of sensitivity to penicillin X and penicillin G is made on the basis of units, most of the organisms were more sensitive to penicillin X. Only 4 strains were more sensitive to the G fraction: 1 strain each of staphylococcus and *Hemophilus influenzae* and 2 strains of *Streptococcus viridans.* Seventeen strains (including the single strain of *C. diphtheriae* tested) were equally sensitive to the 2 penicillin fractions, while 80 strains were from 2 to 32 times more sensitive to penicillin X than to penicillin G.

The antibacterial effects of the 2 fractions may be better compared, however, on the basis of weight. Each mgm. of crystalline penicillin G has a potency of about 1,650 units; each mgm. of crystalline penicillin X, approximately 900 units (2).

TABLE I

Comparative sensitivity of various bacteria to penicillin X and penicillin G

Sensitivity, arranged according to units	Staphylococcus	Beta-hemolytic streptococcus	*Streptococcus viridans*	Pneumococcus	Gonococcus	Meningococcus	*E. typhi*	*H. influenzae*	*C. diphtheriae*	All bacteria	Sensitivity, arranged according to weight
More sensitive to X											
32-fold		1				1	1			3	16-fold
16-fold		2	2			2				6	8-fold
8-fold		1		2		5	4			12	4-fold
4-fold	6	7	5	6		8		2		34	2-fold
2-fold	4	8	6		2	2	1	2		25	Same sensitivity to X and G
Same sensitivity to X and G	4	6	2	1	2	1			1	17	More sensitive to G 2-fold
More sensitive to G 2-fold	1		2					1		4	4-fold
Total	15	25	17	9	4	19	6	5	1	101	

TABLE II

Range of sensitivity of 101 strains of bacteria to penicillin X and penicillin G

Organism	Amount of penicillin required to inhibit growth of least susceptible strain and most susceptible strain	
	Penicillin X	Penicillin G
	units per ml.	*units per ml.*
Staphylococcus	3.6 to 0.006	14.3 to 0.01
Beta-hemolytic streptococcus Group A	0.09 to 0.0007	0.4 to 0.0007
Streptococcus viridans	0.7 to 0.0007	1.4 to 0.0004
Pneumococcus Types I, III, IV, VII, and XIV	0.1 to 0.001	0.09 to 0.006
Gonococcus	0.006 to 0.0007	0.01 to 0.0007
Meningococcus Groups I, II and II alpha	0.04 to 0.006	0.4 to 0.01
E. typhi	1.4 to 0.6	9.0 to 2.2
H. influenzae Type B	1.1 to 0.1	9.0 to 0.6
C. diphtheriae (one strain tested)	0.02	0.09

Accordingly, a given weight of penicillin G is approximately twice as potent in terms of units as the same weight of penicillin X. In the extreme right-hand column of Table I we have arranged the legend so as to compare the relative sensitivities of the strains according to the weights of the 2 fractions used in the tests. Measured in this way, the 2 fractions showed less difference in their effectiveness upon the various bacteria. Twenty-five per cent of the strains showed equal sensitiveness to the 2 fractions, 54 per cent of the strains were from 2 to 16 times as sensitive to X as to G, and 21 per cent were from 2 to 4 times as sensitive to G as to X.

The range of the sensitivities of the various organisms to the 2 penicillin fractions is shown in Table II. All strains of beta hemolytic streptococci, gonococci and meningococci were very sensitive to penicillin X, and usually to penicillin G also. Staphylococcus and streptococcus strains varied from resistant to very sensitive. The few strains of *Hemophilus influenzae* and *Eberthella typhi* investigated were moderately resistant to penicillin X, and even more resistant to the G fraction. The 1 strain of *C. diphtheriae* encountered was moderately sensitive to both fractions.

RESULTS OF RAISING THE RESISTANCE OF BACTERIA *in vitro*

Ten strains of staphylococci, 4 of pneumococci, 2 of meningococci and 1 each of alpha-hemolytic and beta-hemolytic streptococci were made more resistant to both fractions of penicillin. Table III shows the original sensitivities of these

TABLE III

Development of resistance to penicillin X and G in vitro

Organism	Source		Number of transfers made	Original sensitivity		Sensitivity after transfers					
						In penicillin X		In penicillin G		In control medium	
	Material	Disease		Sensitivity tested by using penicillin:							
				X	G	X	G	X	G	X	G
				units per ml.		*units per ml.*		*units per ml.*		*units per ml.*	
Staphylococcus	Pus	Abscess of leg	43	0.2	0.4	28.6	28.6	3.6	3.6	1.43	1.43
Staphylococcus	Blood	Transient bacteremia following tooth extraction	47	0.01	0.02	0.2	0.5	0.2	0.9	0.02	0.04
Staphylococcus	Blood	Transient bacteremia following tooth extraction	42	0.04	0.09	7.2	3.6	0.2	0.08	0.04	0.04
Staphylococcus	Pus	Abscess of skin	14	0.02	0.02	1.4	1.4	1.4	1.4	0.09	0.09
Staphylococcus	Blood	Endocarditis	18	0.02	0.02	0.9	0.9	3.6	3.6	0.18	0.36
Staphylococcus	Urine	Pyelitis	28	0.9	1.8	14.3	28.6	7.2	14.3	1.79	1.79
Staphylococcus	Blood	Transient bacteremia following tooth extraction	23	0.01	0.02	0.2	0.5	0.2	0.9	0.02	0.04
Staphylococcus	Throat	Acute pharyngitis	44	0.2	0.2	1.8	0.9	7.2	14.3	0.71	1.43
Staphylococcus	Throat	Acute pharyngitis	18	0.04	0.04	0.3	0.3	1.8	3.6	0.09	0.09
Staphylococcus	Sputum	Bronchiectasis	19	0.01	0.01	1.8	1.8	28.6	28.6	0.04	0.04
Pneumococcus Type I	Blood	Pneumonia	50	0.003	0.01	1.4	0.7	0.09	0.4	0.01	0.04
Pneumococcus Type III	Spinal fluid	Meningitis	50	0.01	0.04	0.7	0.7	0.09	0.4	0.02	0.04
Pneumococcus Type XII	Spinal fluid	Meningitis	36	0.01	0.09	0.2	0.2	1.4	1.4	0.01	0.04
Pneumococcus Type XII	Blood	Pneumonia	26	0.01	0.01	0.06	0.06	0.1	0.1	0.01	0.02
Beta-hemolytic streptococcus, Group A, Type II	Throat	Scarlet fever	37	0.001	0.01	0.7	1.4	0.4	0.7	0.006	0.04
Alpha-hemolytic streptococcus	Urine	Pyelitis	30	0.02	0.04	0.2	0.2	0.4	0.4	0.04	0.09
Meningococcus Group I	Spinal fluid	Meningitis	13	0.02	0.09	Not done	1.8	3.6	3.6	0.02	0.09
Meningococcus Group II	Spinal fluid	Meningitis	28	0.04	0.2	2.9	2.9	0.2	1.4	0.02	0.04

TABLE IV

Comparative sensitivity to penicillin X and penicillin G of bacteria after resistance was raised in vitro

Sensitivity arranged according to units	Before resistance raised to penicillin					After resistance raised to penicillin X					After resistance raised to penicillin G					Sensitivity arranged according to weight
	Staphylococcus	Pneumococcus	Streptococcus	Meningococcus	Total	Staphylococcus	Pneumococcus	Streptococcus	Meningococcus*	Total	Staphylococcus	Pneumococcus	Streptococcus	Meningococcus	Total	
More sensitive to X 16-fold			1		1					0					0	More sensitive to X 8-fold
8-fold		1			1					0	1			1	2	4-fold
4-fold		2		2	4	1				1	1	2			3	2-fold
2-fold	4		1		5	2		1		3	3		1		4	Same sensitivity to X and G
Same sensitivity to X and G	6	1			7	5	3	1	1	10	4	2	1	1	8	More sensitive to G 2-fold
More sensitive to G 2-fold					0	2	1			3	1				1	4-fold

* This determination was not made in the case of one strain.

strains to penicillins X and G, and the sensitivities after they had been made resistant. The control cultures which were passed along in broth without penicillin often showed changes in sensitivity to penicillin when tested at the end of the experiment. Usually these were slight, there being a 2- or 4-fold increase or decrease, as compared with the original sensitivity. In the case of 3 of the staphylococcus strains, the sensitivity of the control cultures increased 4- to 18-fold. No explanation can be given for this phenomenon. In every instance, except 1, the resistance of the strains transferred in penicillin was raised above that of the control cultures.

The degree of resistance which could be induced varied greatly from strain to strain and also in the same strain, depending upon which fraction was used. The resistance of some strains was raised higher by means of penicillin X, and that of others was raised higher with the use of penicillin G. When the resistance of a strain had been raised by employing 1 fraction, there was little difference in the sensitivity of that strain to the 2 different fractions. For instance, the sensitivity of 1 staphylococcus strain was 28.6 units per ml. of either penicillin X or penicillin G when its resistance had been raised by means of penicillin X, and 3.6 units per ml. of each penicillin fraction when its resistance had been raised by the employment of penicillin G.

In Table IV we have compared the relative sensitivity of the organisms to the 2 fractions of penicillin before and after the induction of resistance. When the comparison is made on a unit basis, 11 of these strains were more sensitive to penicillin X than to penicillin G before their resistance was raised, and 7 were equally sensitive to the 2 fractions. After the resistance of these strains was raised to penicillin X, only 4 were more sensitive to that fraction than to penicillin G, while 10 were equally sensitive to the 2 fractions, and 3 were more sensitive to penicillin G. On the other hand, after the resistance of the strains had been raised to penicillin G, 9 were more sensitive to penicillin X, 8 were equally resistant to the 2 fractions, and 1 was more sensitive to G than to X.

As has been stated before, the weight of each fraction of penicillin used in testing the strains is a truer method of comparison. When the comparison is made in this way (the extreme right-hand column in Table IV) it is seen that the number of strains possessing greater sensitivity to penicillin X before their resistance was raised, was approximately the same as the number of those possessing greater sensitivity to penicillin G. After their resistance had been raised to penicillin X, 1 was more sensitive to penicillin X, 13 were more sensitive to penicillin G, and 3 were equally sensitive to the 2 fractions; whereas after their resistance had been raised to penicillin G, 5 were more sensitive to penicillin X, 9 to penicillin G and 4 were equally sensitive to the 2 fractions. It is evident from these figures that raising the resistance of these strains to penicillin X caused them to be relatively more sensitive to penicillin G than to penicillin X, whereas raising their resistance to penicillin G left the proportions of those resistant to the 2 fractions about the same as before.

Studies on strains which became resistant in patients under treatment with penicillin

While we were investigating the relative sensitivity of bacteria to penicillin X and penicillin G, we encountered a patient, J. F., with a bacterial endocarditis caused by a *Streptococcus viridans* which developed increasing resistance to penicillin while the patient was under treatment with this antibiotic.

This patient was a 22-year old colored male with a questionable history of syphilis, and no history of rheumatic fever. Three weeks before admission he developed headache, followed by pain and stiffness in the cervical spine and swelling of both feet and ankles. He was acutely ill on admission to the hospital, with a temperature of 102° F, a pulse rate of 112, and a blood pressure of 140 systolic and 60 diastolic. There was a systolic thrill in the 3rd and 4th interspaces to the left of the sternum, and systolic and diastolic murmurs, maximal in the same area and transmitted over the entire precordium. The liver edge was 4 cm. below the right costal margin. The spleen was not felt. Roentgenogram of the chest revealed slight enlargement of the left ventricle and of the pulmonary conus area, and a moderate increase in the pulmonary vascular shadows.

When 6 blood cultures, taken on different days, had grown the *Streptococcus viridans*, as shown in Figure 1, the patient was started on penicillin G by mouth. For the first 24 hours the dose was 100,000 units in amphojel every 4 hours; and for the next 24 hours, 200,000 units every 4 hours. Since this did not seem to be influencing the course of the disease, the dose was changed to 25,000 units of penicillin G intramuscularly every 2 hours. A test of the etiologic organism at this time for sensitivity

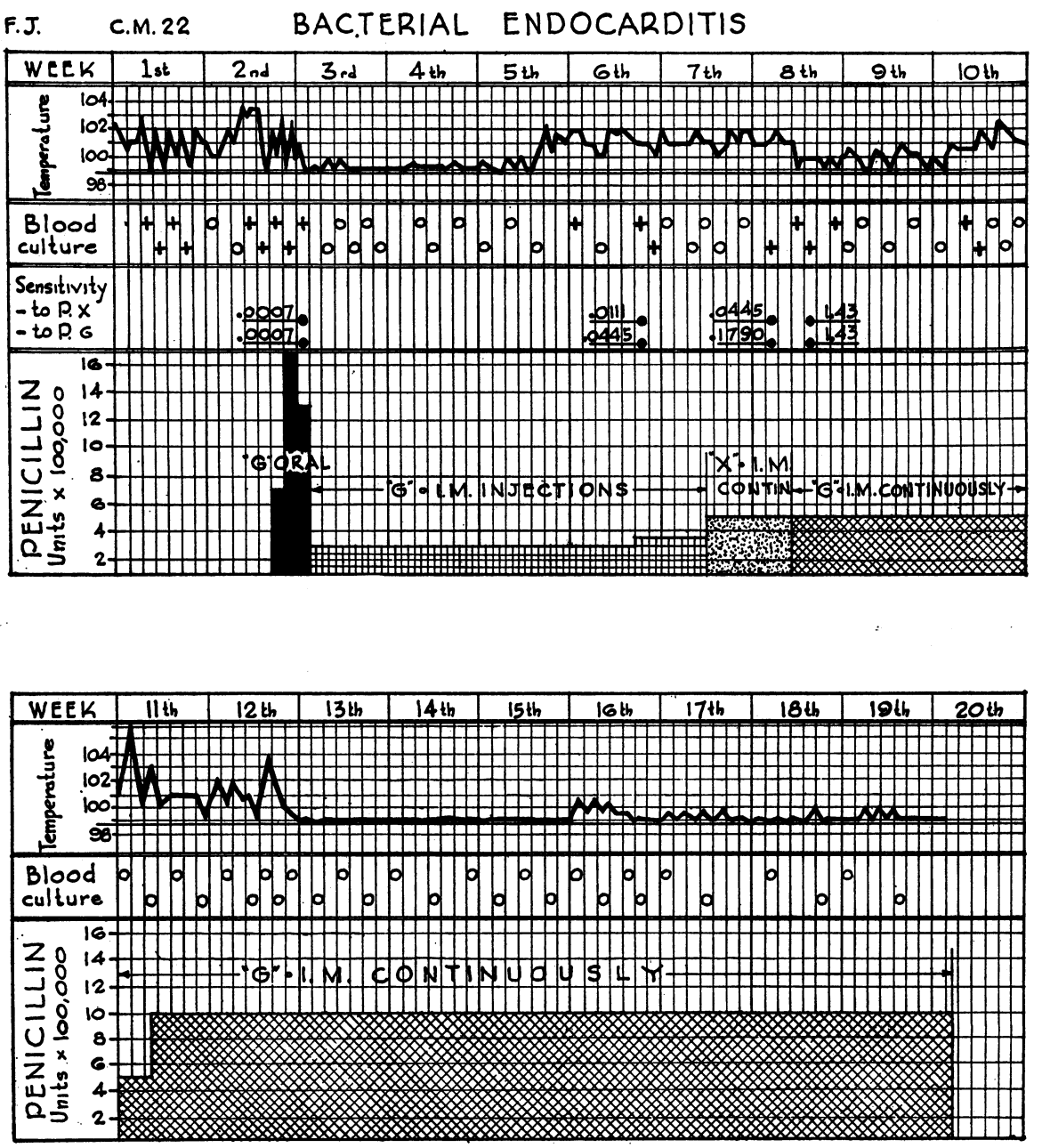

FIGURE 1.

to penicillin revealed that 0.0007 unit per ml. of either the X or G fraction was sufficient to inhibit its growth.

The temperature fell and the blood cultures became negative on the intermittent intramuscular injections, but after a week fever and bacteremia reappeared. The sensitivity of the organism cultured at this time had risen to 0.01 unit per ml. in the case of penicillin X, and 0.04 unit in the case of penicillin G. Penicillin X was tried in doses of 500,000 units per day by continuous intramuscular infusion without success. Positive blood cultures continued to make their appearance, and the sensitivity of the organisms reached 0.04 unit per ml. and 0.18 unit per ml. for the X and G fractions, respectively. Due to the scarcity of penicillin X at that time, treatment was resumed with penicillin G in doses of 500,000 units per day by continuous intramuscular infusion. The sensitivity of the organism was again tested and found to be 1.4 units per ml. for both the X and G fractions. As a result of this, the dose was doubled. After a few days on 1,000,000 units per day by intramuscular infusion, the patient im-

TABLE V

Relative sensitivities to penicillin X and penicillin G of a strain of Staphylococcus aureus isolated from a patient with endocarditis

Day of disease	Sensitivity of organism: Sensitivity to penicillin X	Sensitivity of organism: Sensitivity to penicillin G
	units per ml.	*units per ml.*
15	0.01	0.04
29	0.02	0.09
34	0.09	0.2
36	0.09	0.2
41	0.2	0.4

proved considerably, the temperature fell, and the blood culture became and remained sterile. After 9 weeks of this regime, the patient left the hospital against advice. He was examined 4 months later and found to be free of symptoms and of all evidence of infection.

With the exception of the first few days, when the patient was being treated orally, the concentrations of penicillin in the blood were always higher than the amount of penicillin required to inhibit the growth of the organism *in vitro*. In spite of this, the infection remained active until a dose of 1,000,000 units per day was given.

Another patient, L. M., a 34-year old colored female, had an endocarditis caused by *Staphylococcus aureus*, an organism which became more resistant to penicillin during the course of treatment. When this organism was first cultured from the blood, on the 15th day of the disease, it was sensitive to 0.01 unit per ml. of penicillin X and to 0.04 unit per ml. of penicillin G (Table V). On the 29th day, its resistance to each fraction had increased 2-fold. Organisms cultured from the blood at intervals during the next 12 days showed gradually increasing resistance to both the X and G fractions until 0.2 and 0.4 units per ml. respectively, were required to inhibit the growth of the organisms.

During the period in which these cultures were obtained, the patient was being treated with increasingly larger doses of penicillin G, starting with 15,000 units every 3 hours by intramuscular injection on the 15th day of the disease. Before the organisms were cultured from the blood for the last time (on the 41st day), the patient had been receiving 1,000,000 units per day by continuous intramuscular infusion. She was continued on penicillin treatment for 8 more weeks, during which she became afebrile and symptom-free, and her blood cultures remained negative. She has recently been discharged from the hospital, apparently infection-free.

DISCUSSION

The excellent results obtained with the commonly used G fraction of penicillin do not preclude the possibility that some other fraction may be still more effective. It is necessary, therefore, to determine whether bacteria causing human infections are more sensitive to the X fraction than to penicillin G, or whether they develop resistance against 1 fraction without developing it against the other. Upon comparing the relative sensitivity of various bacteria from human sources to approximately equal amounts of penicillin X and penicillin G by weight, we found that slightly more than ½ of the strains were more sensitive to X than to G, ¼ were equally sensitive to both fractions, and slightly less than ¼ were more sensitive to G than to X. When our figures are compared with Ory's (4), using the weight of the penicillin fraction as a basis of measurement in both instances, the results are very similar. Libby and Holmberg (3) compared the sensitivity of a few organisms to known weights of penicillin X and G, and found that the ratio of the number of μg. of penicillin G to the number of micrograms of penicillin X required to inhibit growth of the organisms varied from 0.6 to 2.0. It is evident from these 3 investigations that, while some strains of bacteria tested showed considerably more sensitivity to penicillin X than to penicillin G, and some showed more sensitivity to G than to X, nevertheless most strains showed very little difference in sensitivity to the 2 fractions.

Since most of the bacteria which are causing human infections at the present time have presumably not yet come in contact with penicillin, it is possible that great differences in their sensitivities to the various fractions of penicillin will develop only after prolonged contact with 1 of these fractions. We might expect the organism to become relatively more resistant to the fraction with which it had been in contact. Actually, as shown in Table III, when a strain's resistance to 1 fraction was increased, its resistance to the other fraction increased also. Further evidence is provided by the cases of the 2 patients with bacterial endocarditis: the organisms developed resistance to both penicillin X and G while the patients were being treated with the G fraction. Flippin and his associates (5) reported the development of resistance to penicillin G by the organism of a patient under treatment with this fraction. Before treatment, 0.025 unit of penicillin G per ml. was sufficient to inhibit its growth. Six months later 0.75 unit per ml. was required. At the same time, however, growth of the organism was inhibited by

only 0.05 unit per ml. of penicillin X. Treatment was changed from penicillin G to penicillin X, and soon after this the patient improved and apparently recovered completely. Since no determination of sensitivity to penicillin X was made on the organism recovered before penicillin was administered, it is impossible to tell whether the organism developed any resistance to this fraction during the course of treatment. In the case of our patient, with *Streptococcus viridans* endocarditis, the organism was found to develop resistance to the 2 fractions simultaneously, the sensitivity finally reaching 1.43 units per ml. of either fraction. Fortunately, the patient recovered after prolonged treatment with 1,000,000 units of penicillin G per day. The organism in the case of our patient with *Staphylococcus aureus* endocarditis also developed increasingly greater resistance to both the X and G fractions simultaneously.

It has already been pointed out that, following passage of the organisms through penicillin X, resistance to penicillin X was developed to a measurably greater degree than resistance to penicillin G. Similar results were obtained when strains were passed through penicillin G. In every instance, however, there was a simultaneous increase in resistance to both fractions, and this increase was greater than the difference between the fractions.

SUMMARY AND CONCLUSIONS

1. One hundred and one strains of bacteria from human sources were tested for sensitivity to the 2 penicillin fractions X and G. When the weights of the penicillin fractions employed were used as the basis for comparison, 55 strains were from 2 to 16 times more sensitive to penicillin X than to penicillin G, 21 were 2 to 4 times more sensitive to penicillin G, and 25 were equally sensitive to each fraction.

2. Sixteen strains were made resistant to penicillin X and penicillin G by serial passage in media containing increasingly larger amounts of these fractions. At the termination of the experiments, some of the strains whose resistance had been raised to penicillin X had become relatively more sensitive to penicillin G than to penicillin X. Among the strains made resistant to penicillin G, there was no significant difference between the number which were more sensitive to penicillin X than to penicillin G at the beginning of the experiments, and the number which were more sensitive to penicillin X at the end of the experiments. In every instance when the resistance to 1 fraction was raised, the resistance to the other fraction followed along, to the same or nearly the same extent.

3. The sensitivity of the etiologic organisms to penicillins X and G were studied in a patient with *Streptococcus viridans* endocarditis and in a patient with *Staphylococcus aureus* endocarditis. In each instance, while the patient was receiving penicillin, the organism responsible for the infection developed resistance to both penicillin fractions simultaneously.

4. It is concluded that organisms which cause human infections usually do not show great differences in their relative sensitivity to penicillin G and penicillin X. In most instances, when resistance to 1 of these fractions increases, resistance to the other fraction will also increase.

We wish to thank the members of the Georgetown Medical Division for the opportunity of studying one of the cases of endocarditis.

BIBLIOGRAPHY

1. Hirsh, H. L., Dowling, H. F., and Sweet, L. K., Treatment of various infections with penicillin X, with a preliminary note on the value of penicillin X in scarlet fever. Ann. Int. Med., 1946, 25, 78.
2. Welch, H., Putnam, L. E., Randall, W. A., and Herwick, R. P., Penicillin X. Successful treatment of gonorrhea with a single intramuscular injection. J. A. M. A., 1944, 126, 1024.
3. Libby, R. L., and Holmberg, N. L., The activity of penicillins G and X in vitro. Science, 1945, 102, 303.
4. Ory, E. M., Meads, M., and Finland, M., Penicillin X. Comparison with penicillin G with respect to sensitivity of pathogenic organisms and serum levels. J. A. M. A., 1945, 129, 257.
5. Flippin, H. F., Mayock, R. L., Murphy, F. D., and Wolferth, C. C., Penicillin in the treatment of subacute bacterial endocarditis. A preliminary report on twenty cases treated over one year ago. J. A. M. A., 1945, 129, 841.