other investigators (Jevons, 1961 ; Knox and Smith, 1961 ; Barber, 1961, 1962 ; Eriksen and Erichsen, 1963 ; Stewart and Holt, 1963 ; Rolinson, 1963). It is possible, therefore, that infections with these strains would respond to treatment with large doses of methicillin. In the presence of quite low concentrations of methicillin (5–10 μg./ml.) they grow poorly ; not all the cells in a given inoculum grow, and on solid medium these tend to be massed together at the site of heavy inoculum, and individual colonies if present tend to be small and atypical. However, in the absence of methicillin these strains appear to have all the characteristics of fully virulent *Staph. aureus in vitro.* Moreover, the strains isolated in this hospital, like those of Stewart and Holt (1963), were able to cause cross-infection in the surgical wards, and in some instances the infection was of a severe generalized nature.

Summary

A simple screening test for penicillinase activity has been used to investigate 432 strains of *Staph. aureus* and its reliability assessed by iodometric estimations with a proportion of strains.

None of 156 penicillin-sensitive strains showed a positive reaction.

The results obtained with 276 penicillin-resistant strains were graded according to the rate of reaction ; 70 (25%) were found to be weak penicillinase-producers, and 125 (45%) were highly active. Out of 120 phage-typable multiple-resistant strains 73 (61%) were strongly positive, only 9 gave weak reactions, and none was negative. On the other hand, of 132 strains resistant to penicillin alone, whether phage-typable or not, only 45 (34%) were strongly positive and 47 gave weak or negative reactions.

Methicillin inactivation by methicillin-sensitive and methicillin-resistant strains has been studied, and it has been shown that the degree of inactivation is related to penicillinase activity rather than methicillin-resistance, unless tests are carried out in such a way that inactivation is dependent on growth in the presence of methicillin.

The clinical application of these findings is discussed.

Our thanks are due to Mrs. June Filbey for technical assistance. We are also indebted to Drs. Y. Chabbert, Nuala Crowley, K. R. Eriksen, Patricia Jevons, R. W. Riddell, and G. T. Stewart for sending us cultures of methicillin-resistant strains and to Dr. M. R. Pollock for strain N.I.524.

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STABILITY OF METHICILLIN AND CLOXACILLIN TO STAPHYLOCOCCAL PENICILLINASE

BY

R. KNOX, M.D., F.R.C.P. *Professor of Bacteriology*

J. T. SMITH, Ph.D. *Lecturer in Bacteriology*

Guy's Hospital Medical School, London

Penicillinase-producing staphylococci are resistant to benzylpenicillin only because large inocula can destroy the drug before it destroys them ; small inocula are almost as sensitive to benzylpenicillin as fully sensitive strains which do not possess penicillinase. This effect of inoculum size is characteristic of penicillinase-producing strains. However, a similar effect can also be observed in populations of bacteria which have a wide range of susceptibility to an antibiotic. Thus the effect of inoculum size must not be taken as prima facie evidence for the penicillinase type of resistance. For example, the 13137 strain of methicillin-resistant staphylococcus (Jevons, 1961 ; Knox, 1961 ; Rolinson, 1961) has been found to contain many organisms which were of the same order of sensitivity as methicillin-sensitive strains of *Staphylococcus aureus,* together with a minority of rather highly resistant organisms (Knox and Smith, 1961).

The resistance of this and other strains to methicillin has been ascribed not to destruction of methicillin but to a mechanism similar to the inherent resistance of penicillinase-negative staphylococci trained towards benzylpenicillin resistance *in vitro.* It has also been suggested that resistance to the newer isoxazolyl penicillins (usually associated with cross-resistance to methicillin) is similarly of the " inherent " type (Knox and Smith, 1963 ; Ayliffe, Barber, and Waterworth, 1963 ; Rolinson, 1963). On the other hand, there have also been suggestions that destruction of these penicillins partly accounts for the resistance of such strains, and it has been implied, though not explicitly stated, that they possess some special ability to destroy methicillin or the isoxazolyl penicillins not possessed by other penicillinase-producing staphylococci (Stewart and Holt, 1963a, 1963b, 1963c ; Eriksen and Erichsen, 1963). This communication shows that the destruction of these penicillins is due to penicillinase, whether the strains are sensitive or resistant to methicillin and the isoxazolyl penicillins.

Materials and Methods

Microbiological estimation of the penicillins was made by the cup-plate assay method with *Sarcina lutea* ATCC 9341 as test organism (Heatley, 1944).

Initial rates of destruction of methicillin, cloxacillin, and benzylpenicillin were estimated by the hydroxylamine method at an initial penicillin concentration of 2 mg./ml. in the presence of 10^{-3}M *p*-chlormercuribenzoate as described by Knox and Smith (1962).

Sensitivity tests were carried out by preparing inocula from overnight broth cultures by dilution in broth, and 0.02-ml. amounts were dropped on to the surface of agar plates containing penicillin. The results were read after nine hours' incubation.

The organisms used were penicillinase-producing coagulase-positive strains of *Staph. aureus.* Strain E3 has been described by Knox (1960) and is methicillin- and cloxacillin-sensitive. The following methicillin- and cloxacillin-resistant strains were used: 13137/1000 (Knox and Smith, 1961); 6367, which was kindly supplied by

Dr. G. T. Stewart; and 5974, which was kindly supplied by Dr. K. R. Eriksen.

Results

The sensitivity to cloxacillin and methicillin of three of the strains of *Staph. aureus* is shown in Table I. It can be seen that the two resistant strains show a greater "inoculum size effect" than the sensitive strain E3.

TABLE I.—*Effect of Inoculum Size on Sensitivity of Three Strains of Staphylococci to Methicillin and Cloxacillin*

Strain of *Staph. aureus*	No. of Cells in Inoculum	Minimum Inhibitory Concentration (μg./ml.)	
		Methicillin	Cloxacillin
E3	4×10^6	6	5
	4×10^4	6	1
	4×10^2	6	1
	4	3	0·5
13137/1000	3×10^6	1,000	250
	3×10^4	1,000	62·5
	3×10^2	100	0·9
	3	25	0·9
6637	9×10^6	>500	1,000
	9×10^4	50	50
	9×10^2	25	0·5
	9	6	0·5

The destruction of cloxacillin was studied by adding 0.5 ml. of a solution containing 100 μg. of cloxacillin/ml. in broth to 4.5-ml. aliquots of overnight broth cultures of the three strains of *Staph. aureus*. These were incubated for a further 24 hours; the organisms were then centrifuged and cloxacillin remaining in the supernatant fluid was estimated by microbiological assay. It can be seen (Table II) that, although the three strains destroyed some cloxacillin, as compared with the uninoculated control, there was no greater destruction by the two methicillin- and cloxacillin-resistant strains than by the fully sensitive strain; it seems probable that this destruction was due to the basal penicillinase level in the three strains. Strains 6637 and 13137/1000 were then grown overnight in 10 μg. of cloxacillin/ml. in broth and the residual cloxacillin was estimated by microbiological assay. It can be seen (Table II) that all the cloxacillin was destroyed. However, when strain E3 was grown in broth with the addition of 0.5 μg. of methicillin/ml., then incubated overnight with 10 μg. of cloxacillin/ml., this pre-induced culture also destroyed all the cloxacillin (Table II).

TABLE II.—*Destruction of Cloxacillin*

Staph. aureus	μg./ml. Cloxacillin (Initial Concentration 10 μg./ml.)	
	Cloxacillin Added Before Growth	Cloxacillin Added After Growth
6637	<0·3	2·50
13137/1000	<0·3	3·25
E3	*	2·55
E3 pre-induced	*	<0·3
Broth control	5·20	5·85

For details see text. * No growth occurred.

The penicillinase activity of all four strains grown in broth, in 0.1 μg. of cloxacillin/ml. and in 0.5 μg. of methicillin/ml., and, in addition, the penicillinase activity of strains 6637 and 13137/1000 grown in 10 μg. of cloxacillin/ml. was determined by the hydroxylamine method using benzylpenicillin (2 μg./ml.) as substrate. Viable counts were made and the results expressed as rates per 10^9 cocci/ml. (Table III). It can be seen that the penicillinase activity of all four strains was greatly increased when grown in the presence of a penicillin. The penicillinase activity of these induced strains against methicillin and cloxacillin was also determined, and it was found that all strains destroyed these penicillins at less than 1% of the rate of hydrolysis of benzylpenicillin. This would seem to provide good evidence that, irrespective of the method of induction used, the penicillinases produced by these four strains were the same as judged by the relative rates of hydrolysis of the three penicillins.

TABLE III.—*Destruction of Benzylpenicillin by Induced and Non-induced Staphylococci*

Staph. aureus	Concentration of Penicillin Used for Induction	Penicillinase Activity (μg. Benzylpenicillin Destroyed/min./10^9 cocci/ml.)
E3	Nil (non-induced)	14
	0·5 μg. methicillin/ml.	633
	0·1 μg. cloxacillin/ml.	1,785
5974	Nil (non-induced)	24
	0·5 μg. methicillin/ml.	1,073
	0·1 μg. cloxacillin/ml.	2,027
6637	Nil (non-induced)	19
	0·5 μg. methicillin/ml.	1,600
	0·1 μg. cloxacillin/ml.	1,700
	10 μg. cloxacillin/ml.	4,229
13137/1000	Nil (non-induced)	12
	0·5 μg. methicillin/ml.	628
	0·1 μg. cloxacillin/ml.	869
	10 μg. cloxacillin/ml.	2,014

Discussion

It has been reported that resistant staphylococci when grown in therapeutic concentrations of cloxacillin (Stewart and Holt, 1963) or methicillin (Eriksen and Erichsen, 1963) destroy the penicillin whereas fully grown cultures do not. But these fully grown cultures were grown in broth alone and hence no control was made for the interaction between the bacteria and penicillin present in the culture medium. Penicillinase is an inducible enzyme in staphylococci, but induction does not occur when the bacteria are not actively dividing (Bondi *et al.*, 1954; Steinman, 1961). The growth of a staphylococcus in a penicillin increases its penicillinase content, whereas when penicillin is added to a fully grown broth culture very little multiplication and hence little or no induction occurs.

Direct controls with methicillin- or cloxacillin-sensitive organisms cannot be made, since they cannot grow in the concentrations of penicillins used for the culture of the resistant strains. However, when the methicillin- and cloxacillin-sensitive staphylococcus E3 is pre-induced by growth overnight in 0.5 μg. of methicillin/ml. it can destroy 10 μg. of cloxacillin/ml. during overnight incubation whereas a culture grown in broth alone will not (see Table II). The Carshalton strain 6637 can also destroy 10 μg. of cloxacillin/ml. if grown in it, but when grown in broth, like the strain E3, it is much less active. Thus when it is cultured overnight in 10 μg. of cloxacillin/ml. it has over 200 times more penicillinase activity than a broth culture (see Table III). This explains why organisms grown in broth alone are not so efficient at destroying penicillins. The total yield of penicillinase produced by different strains varies greatly with the method of induction used; however, Table III shows that, under identical conditions of induction (growth in 0.1 μg. of cloxacillin/ml.), strain E3 can produce as much penicillinase as strain 6637. Finally, as yet there is no evidence of a penicillinase with a special ability to hydrolyse methicillin and cloxacillin, since the strains of *Staph. aureus* used here destroyed the penicillinase-resistant penicillins at similar relatively low rates when compared with their rate of hydrolysis of benzylpenicillin.

Summary

Methicillin and cloxacillin are very slowly destroyed by penicillinase-producing strains of *Staph. aureus* whether or not they possess inherent resistance to these penicillins. Resistant strains when cultured in high concentrations of

methicillin or cloxacillin destroy these more rapidly than when cultured in broth. Sensitive strains cannot, of course, grow in high concentrations of these penicillins and therefore cannot be tested under comparable conditions. However, when such strains are pre-induced they too destroy methicillin and cloxacillin more rapidly than organisms cultured in broth. With all four strains of staphylococci used here, whatever method was used for inducing penicillinase, the rate of hydrolysis of methicillin and cloxacillin was a small fraction (1% or less) of the rate of hydrolysis of benzylpenicillin under comparable conditions. So far there is no evidence in these strains of a specific "methicillinase" or "cloxacillinase."

We are grateful to Dr. G. T. Stewart and Dr. K. R. Eriksen for sending their methicillin- and cloxacillin-resistant strains of *Staph. aureus*, to Beecham Research Laboratories for gifts of methicillin and cloxacillin, and to Miss J. Ramsay for skilled technical assistance.

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MEDICINAL ARSENIC POISONING AND LUNG CANCER

BY

A. O. ROBSON,* M.B., M.R.C.P.
Resident Medical Officer, The Middlesex Hospital, London

A. M. JELLIFFE, M.D., F.F.R., M.R.C.P., D.C.H.
Consultant Radiotherapist, The Middlesex Hospital, London; and Mount Vernon Hospital, Northwood, Middlesex

For many centuries arsenic has been considered to be of medicinal, cosmetic, and forensic value. The first suggestion that the therapeutic administration of arsenic could lead to the development of cancer was made by Sir Jonathan Hutchinson in 1887, when he described five cases of skin cancer following the medicinal use of arsenic, and since then attempts have been made to relate cancer arising in other sites to the previous administration of arsenic. The causal relationship of arsenic to skin cancer is now well established, but the case for arsenic as a visceral carcinogen is unproved.

This report describes six previously unrecorded patients with arsenical skin changes following the therapeutic administration of arsenic, all of whom also developed lung cancers (Table I).

Case Reports

Case 1.—A woman aged 31 was admitted to hospital in September, 1956, with dyspnoea and productive cough for five months. Investigations showed an undifferentiated carcinoma of the left main bronchus, with metastases to the liver, axillary and supraclavicular lymph nodes, skull, pelvis, spine, and right femur. Skin nodules and jaundice appeared, and she died in six weeks. A post-mortem examination confirmed the clinical findings. As a girl in Dublin she was treated for many years with tonics for anaemia. In 1946 she noticed a "wart" below the left nipple. In 1952 "warts" developed on the hands, feet, and trunk. In May, 1956, an intra-epidermal carcinoma below the left nipple (Fig. 1) was subjected to biopsy and treated with a radiostrontium plaque. Typical arsenical keratoses of the hands, legs, and feet were also recorded. The arsenic content of hair was 3.5 p.p.m. (normal 0.3–0.7 p.p.m.). The patient had never smoked at any time.

Case 2.—A woman aged 45 was admitted to hospital in September, 1962, with pleurisy. Investigations showed an inoperable undifferentiated carcinoma of the right main bronchus. She had taken Fowler's solution for psoriasis intermittently over 15 years. At the time of admission arsenical keratoses of the palms and soles were present, and the arsenic content of the hair was 1.5 p.p.m. She had smoked 5 to 10 cigarettes daily for 20 years.

Case 3.—A man aged 54 had a pneumonectomy carried out elsewhere in 1955 for a poorly differentiated carcinoma of the left lung. In 1959 he died of heart failure. At post-mortem examination a localized poorly differentiated squamous-cell carcinoma was found in the right lower lobe, possibly a new primary tumour. In 1920 he was treated for psoriasis for four years with Fowler's solution. In 1933 "warts" on the hands developed, and in 1944 an epithelioma of the left hand was excised. Over 11 years, six courses of superficial *x* rays and an excision were carried out for epitheliomas on the hands, fingers, and thigh. In 1955 three new lesions were excised—an epithelioma, an area of Bowen's disease, and an epithelioma with hypertrophy, pigmentation, and hyperkeratosis of the surrounding skin. Keratoses were present on the palms (Fig. 2) and soles, with pigmentation of the skin. The patient had been a "moderate" smoker for about 30 years.

Case 4.—A man aged 65, after dyspnoea for one month, was found to have a poorly differentiated carcinoma of the right

TABLE I.—*Principal Features of New Cases Described*

Case No.	Sex	Arsenic Administration	Skin Changes	Lung Cancer	Smoking Habits
1	F	Childhood, tonic for debility over many years	Age 21–31, multiple keratoses, intra-epidermal carcinoma	Age 31, undifferentiated carcinoma left main bronchus	Non-smoker
2	F	Age 28–43, Fowler's solution for psoriasis	Age 42–45, multiple keratoses	Age 45, undifferentiated carcinoma right main bronchus	5–10 cigarettes daily for 20 years
3	M	Age 15–19, Fowler's solution for psoriasis	Age 28–54, pigmentation, multiple keratoses, intra-epidermal and infiltrating epitheliomas	(1) Age 50, poorly differentiated carcinoma left main bronchus (2) Age 54, poorly differentiated carcinoma right lower lobe	"Moderate" cigarette smoker for 30 years
4	M	Age 38–41, Fowler's solution for psoriasis	Age 55–65, pigmentation, multiple keratoses, intra-epidermal epithelioma	Age 65, poorly differentiated squamous carcinoma right lung	1–2 pipes daily. No cigarettes
5	F	Age 10–13, tonic after rheumatic fever	Age 16, pigmentation. Age 39–47, multiple keratoses, and infiltrating intra-epidermal epitheliomas	Age 47, carcinoma right upper lobe	Non-smoker
6	F	Age 6–10, Fowler's solution for convulsions	Age 60, keratoses on palms and soles	Age 60, cerebral metastasis, poorly differentiated carcinoma right lung	Non-smoker

*Now at the Royal Victoria Infirmary, Newcastle upon Tyne.