To date there is no record of methicillin being given directly into the C.S.F. Douthwaite and Trafford (1960) have given it systemically to treat a staphylococcal meningitis and have used irrigations of it to treat an extradural abscess. We have given 3 mg. daily into the ventricle for 50 days in three cases. There has been no rise in the C.S.F. cell count or protein content, apart from that associated with a small ventricular haemorrhage in one of the cases.

Despite the unusually long periods of treatment with methicillin and the unique circumstances favouring persistence of the infecting organisms (Stewart, 1961), there was no evidence in the two cases of the *Staph. aureus* infection of the organisms developing any drug resistance. The reisolated organisms, however, in the three cases infected with *Staph. albus* showed a slight but definite trend towards resistance.

Summary

Four cases are described in which septicaemia developed as the result of colonization of Spitz–Holter valves by staphylococci.

Systemic drugs, including methicillin, failed to control the infection. The source of the infection could be reached by a combination of intraventricular (or local) and systemic administration of the drug. Thus the septicaemia could be controlled during the therapy, but recurred when it was discontinued. The complete cure necessitated removal of the valve.

Methicillin was given for long periods intraventricularly and systemically without signs of toxicity.

In the three cases of infection due to *Staph. albus* the organisms gradually acquired a degree of resistance to methicillin during the therapy; in the two cases infected with *Staph. aureus* the organisms showed no change in sensitivity to this drug.

We are indebted to Mr. G. H. Macnab for his advice in preparing this paper and for permission to treat these cases; to Mr. H. H. Nixon for the details of Case 5; to Beecham Research Laboratories for the supply of "celbenin"; to Sister Allen and staff for the extra work involved in treating these cases; to the pathological services of the Hospital for Sick Children, Great Ormond Street, for the many laboratory procedures undertaken; to Miss Jean White and Mr. R. J. Holt for technical assistance to Dr. Stewart; and to Mr. Martin for preparing copies of the graphs.

REFERENCES

Cohen, S. J., and Callaghan, R. P. (1961). Unpublished.
Douthwaite, A. H., and Trafford, J. A. P. (1960). *Brit. med. J.*, 2, 687.
Rolinson, G. N., Stevens, S., Batchelor, F. R., Cameron Wood, J., and Chain, E. B. (1960). *Lancet*, 2, 564.
Stewart, G. T. (1961). *Brit. med. J.*, 1, 863.
—— Nixon, H. H., and Coles, H. M. T. (1960). Ibid., 2, 703.

At a February meeting of London County Council it was said that the total annual mileage travelled by the Council's ambulances in the last three years for which figures are available had risen from 4,173,784 miles in 1957, and 4,478,287 miles in 1958, to 4,504,336 miles in 1959. The percentage of accident and other emergency calls had remained around 13% during the three years. Between 1957 and 1959 the average time taken from receipt of the call to reach the scene of the accident had increased from 6.4 to 6.6 minutes, and the overall time from receipt of the call to reaching hospital with the patient had increased from 20 to 20.9 minutes, mainly owing to the increasingly heavy road traffic. The percentages of delays of more than one hour of the total numbers of non-priority cases removed increased from 2.6 in 1957 to 12.5 in January, 1961. These figures covered collection from home and return from hospital.

CHANGES IN SENSITIVITY OF STAPHYLOCOCCI TO METHICILLIN

BY

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Reports from a variety of sources (Garrod, 1960; Branch *et al.*, 1960) have confirmed the preliminary findings of Knox (1960), Thompson *et al.* (1960), and Stewart (1960a) that the sodium salt of methicillin (BRL 1241; "celbenin"; "staphcillin") is uniformly active against *Staphylococcus aureus*, irrespectively of the resistance of this organism to penicillin G. Resistance to methicillin can, however, be produced artificially *in vitro* (Rolinson *et al.*, 1960; Stewart, 1960a; Barber, 1960), and it is obvious that the usefulness of the drug will be governed largely by the prevalence of comparable resistance *in vivo*.

The object of the present study is to examine some clinical and microbiological aspects of this problem, with regard not only to *Staph. aureus* but also to *Staph. albus*, which appears to be able to acquire resistance more readily (Stewart, 1960b).

Methods

The bacteriological methods employed were similar to those already described (Stewart, 1960a), with the following modifications:

Investigation of Resistance.—Induction of resistance *in vitro* was attempted by serial passage of the organisms, at intervals of 24–48 hours, (*a*) by the gradient-plate technique (Szybalski, 1953), and (*b*) by repeated subcultures in liquid media containing increasing concentrations of drug. Before each successive transfer the organisms were subcultured into drug-free broth for four hours to put them into the freely growing logarithmic phase. Assays of organisms thus treated, and of reisolated strains from patients and carriers, were performed by titrations in broth. The bactericidal effect was estimated from plate counts made after four hours at 37° C. and the overall bacteriostatic effect by reading the turbidity of the tubes after overnight incubation. In the case of an organism showing apparent resistance the assay was repeated in parallel with an assay of the parent strain, in comparable inoculum. By these criteria, resistance was identifiable when (1) there was no bactericidal action at the originally effective concentration, and (2) when the minimal inhibitory (bacteriostatic) concentration rose at least twofold. Tests for inactivation of methicillin and penicillin G were also carried out at various stages, as described below.

Spraying Experiments.—A small pavilion-type ward, in which staphylococcal cross-infection had already occurred, was selected for investigation of the consequences of spraying methicillin according to the technique of Elek and Fleming (1960).

Resistance to Methicillin in vitro

A number of strains of *Staph. aureus* and *Staph. albus*, freshly isolated from various routine specimens sent to the laboratory, were passaged repeatedly in liquid and solid media containing rising concentrations of methicillin. Tests on solid media were made by inoculating the organisms serially on to gradient plates containing

increasing concentrations (0→10, 10→20, 20→40, etc.) μg. of drug per ml. By this means 2 out of 13 strains of *Staph. aureus* acquired resistance in 13 passages to 10 μg. of methicillin per ml. In contrast, five out of seven strains of *Staph. albus*, similarly tested, acquired resistance to over 40 μg./ml., including two strains which in the same period developed resistance to over 1,000 μg./ml. (Table I).

TABLE I.—*Effect of Passage in vitro on Resistance of Staphylococci to Methicillin*

Organism	No. of Strains	Medium*	Minimal Inhibitory Concentration (μg./ml.)		No. of Passages
			Original	Final	
Staph. aureus ..	11	Solid	2–4	2–4	13
,, ,, ..	2	,,	2–4	10	13
,, ,, ..	3	Liquid	2–4	10–20	13
Staph. albus ..	2	Solid	5	2,000	11
,, ,, ..	3	,,	2–4	40	13
,, ,, ..	2	,,	2–4	20	13
,, ,, ..	1	Liquid	2–4	80	13

* Containing rising concentrations of methicillin.

Tests were then conducted in liquid media: the organisms were grown for 24 hours in nutrient broth containing a subinhibitory concentration of drug (0.5–2.5 μg./ ml.). Subcultures were then made into progressively higher concentrations of drug in broth every 24 to 48 hours, the inoculum at each serial subculture being taken from the highest of a range of concentrations, doubling upwards from the previous range, showing visible growth. For assay at any particular level, the organisms were grown for 24 hours in drug-free broth and tested against an appropriate range of concentrations of the drug. By this technique *Staph. aureus* acquired resistance up to 20 μg./ml., but not further, in 12–13 passages, whereas *Staph. albus* acquired resistance to 80 μg./ml. (Table I).

The strains of staphylococci already rendered resistant in solid media were also assayed independently in liquid media, and showed similar levels of resistance in both media.

Further tests were performed upon the two strains of *Staph. albus* which had shown a propensity to develop high resistance very rapidly. This resistance developed continuously, in geometric fashion, as far as 2,000 μg/ml. at which point passage was terminated (Fig. 1). Subcultures made from plate to plate by the velvet-pad replica technique showed that 50% or more of the colonies at any given stage acquired sufficient resistance to grow each higher concentration. Inoculum-size was therefore of relatively little importance in determining ability of a culture to show resistance, and, when the fully resistant organisms were assayed in varying inocula, loss of resistance was not apparent until the standard inoculum was diluted to 10^{-5} or further. Resistance was retained by cultures stored for four weeks at −20°, 4°, 20°, and also through five subcultures at 37° C. in drug-free media. The resistant organisms showed no change in colonial appearance, cell-morphology, staining properties, or in their biochemical reactions in mannitol, plasma, urea, and trehalose.

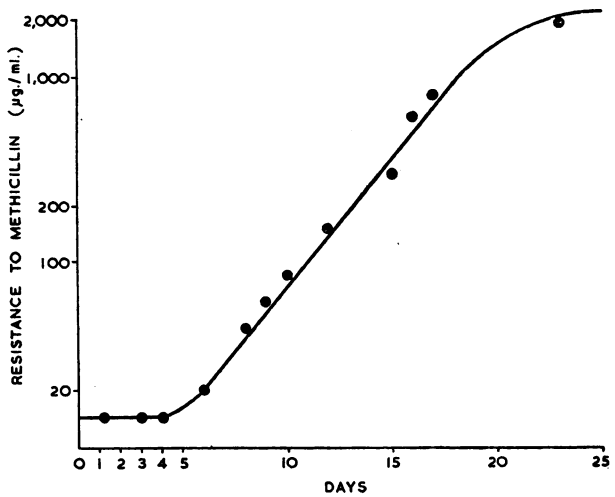

FIG. 1.—Resistance to methicillin of a strain of *Staph. albus* after repeated passage in media containing the drug.

When mixed cultures of *Staph. aureus* and *Staph. albus* were inoculated serially on to gradient plates containing rising concentrations of methicillin, resistance to more than 20 μg./ml. was again rapidly acquired by *Staph. albus* but not by *Staph. aureus*. Growth of *Staph. aureus* in a culture mixed with a highly resistant strain of *Staph. albus* produced no change in the sensitivity of either organism when re-isolated.

Strains of *Staph. albus* with acquired resistance to methicillin were assayed at various stages against penicillin G. The end-points of inhibition varied with inoculum-size to a greater extent than in the case of methicillin, but experiments performed with comparable inocula (Table II) showed that two strains, rendered resistant to 2,000 μg. of methicillin per ml., acquired some cross-resistance *pari passu* to penicillin G. One strain displayed fiftyfold resistance to penicillin G, compared with nearly 1,000-fold resistance to methicillin; the other strain showed a lower order of cross-resistance (tenfold) to penicillin G, despite equal induced resistance to methicillin.

TABLE II.—*Cross-resistance to Penicillin G of Two Strains of Staph. albus Rendered Resistant in vitro to Methicillin*

Strain	Inoculum	Resistant to	
		Methicillin (μg./ml.)	Penicillin G (μg./ml.)
J original	Standard*	2·5	10
	1 : 1,000	2·5	2·5
J resistant	Standard	2,000	500
	1 : 1,000	1,500	10
S. original	Standard	1·0	10
	1 : 1,000	1·0	1
S. resistant	Standard	2,000	100
	1 : 1,000	1,000	10

* Approximately 10^6 cells/ml.

Methicillin and penicillin G were to some extent (10–50%) inactivated by cultures of resistant *Staph. albus*. Such inactivation was demonstrable, however, only by assaying the Seitz-filtrates of broth cultures containing the drugs; specific inactivating substances were not demonstrable when filtrates or whole-cell suspensions from drug-free cultures were added to the penicillins, assayed against controls, or when filtrates or whole-cell suspensions from cultures containing subinhibitory concentrations of the drugs were similarly treated.

Resistance to Methicillin in vivo

Tests on Reisolated Staphylococci from Cases and Carriers

Attempts were made to reisolate *Staph. aureus* from 50 cases of active infection with this organism treated with methicillin. In 12 cases the original strain was reisolated on one or more occasions from the primary lesion, prior to cure, and in 20 cases the infecting strain was isolated from the usual carrier site in the anterior

nares during or after treatment. The organisms thus reisolated showed no alteration in their original sensitivity level of 2–4 μg./ml.

Most patients with *Staph. aureus* infection responded to treatment rapidly, and reisolation of the organism from the lesions—though not from the nares—was practicable only for a few days. In two cases, however, there was an opportunity for more prolonged study of the reisolated organism. Case 3 in the series of Callaghan *et al.* (1961) had a Spitz–Holter valve septicaemia (*Staph. aureus* type 80) treated for about eight weeks with methicillin. The organism was reisolated six times during and after this treatment and showed no change in sensitivity. Case 5 of the same series had a similar septicaemia due to *Staph. aureus* type 47/75, which was reisolated, with unchanged sensitivity, over a period of five weeks and also from the valve itself, which was then excised and found to be heavily colonized within by the organism. In these patients, therefore, foci of organisms outside the main blood-stream had been continuously exposed to the drug in low concentrations for weeks. This would usually be regarded as a favourable condition for the development of drug resistance *in vivo* by the organism, but none developed (Fig. 2).

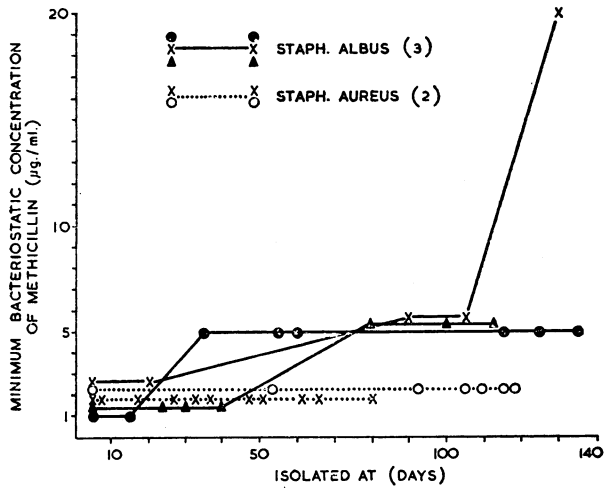

FIG. 2.—Sensitivity to methicillin of three strains of *Staph. albus* and two strains of *Staph aureus* repeatedly reisolated from patients receiving treatment with this drug.

In some of these patients, *Staph. albus* was also present passively in the anterior nares or elsewhere on the skin, but isolates or reisolates of these organisms again showed no acquisition of resistance. There were, however, some differences in the minimum inhibitory concentrations of methicillin effective against the two types of staphylococci: strains of *Staph. aureus* were, without exception, sensitive to 1–4 μg./ml., whereas *Staph. albus* required a wider range (1–10 μg./ml.) for comparable degrees of inhibition, though the majority of strains were inhibited within the range 1–5 μg./ml. irrespectively of whether or not the patients carrying the strains had received methicillin. In three unusual cases of active (septicaemic) infection due to *Staph. albus* treated with methicillin and described by Callaghan *et al.* (1961) the reisolated organisms showed some resistance during and after treatments (Fig. 2).

Tests in a Ward Sprayed with Methicillin

During a period of 12 weeks one ward was sprayed at regular intervals daily with 4 g. of methicillin according to the method of Elek and Fleming (1960). Nasal swabs were taken from all patients and staff in the ward before and (weekly) during this procedure. All strains of *Staph. aureus* and *Staph. albus* thus isolated or reisolated were assayed against methicillin. The results (Table III) showed that no resistant strains appeared. No signs of active staphylococcal infection or cross-infection were noticed during this period.

TABLE III.—*Nasal Carriage of Staphylococci in Patients and Staff in a Ward Sprayed with Methicillin*

No. of Individuals	*Staph. aureus*	*Staph. albus*
Originally positive	23	25
Became negative	10	5
Remained ,,	1	1
Became negative, then positive	3	3
Changed from *aureus* to *albus* or vice versa	1	2
Not followed-up	8	7
Negative throughout	1	1
Resistant strains (No. found/No. isolated)	0/29	0/66

Discussion

These results show that representative strains of *Staph. albus* can be readily and rapidly rendered resistant to methicillin by serial passage in media containing the drug. This resistance develops in a continuous geometric fashion; it may be tenfold within a few days and can reach levels (2,000 μg./ml.) which for all practical purposes are absolute. When developed, resistance is relatively stable and carries cross-resistance to penicillin G.

Tests on strains of *Staph. albus* reisolated from a minority of children under treatment with methicillin provide evidence that resistance also occurs *in vivo*. Such resistance is of a much lower order, but it can exceed 10 μg./ml., which might for the present be regarded as the extreme upper limit of the therapeutic range of the drug. The majority of strains reisolated from patients retain unchanged sensitivity, as do the strains serially reisolated from the ward in which methicillin was sprayed intensively for a period of 12 weeks. Resistance *in vivo* is therefore uncommon as yet, but it can occur in the case of infections with *Staph. albus*.

The behaviour of *Staph. aureus* towards methicillin is different. Some strains can be made resistant *in vitro*, but the process is slow and the level of resistance (20–50 μg./ml.) usually much lower. Other strains show no ready capacity to develop resistance, even under favourable conditions, *in vitro*. Tests made upon a larger number of strains, isolated and reisolated from the nares and from lesions in patients under treatment with the drug, show no evidence as yet of any resistance *in vivo*; strains isolated and reisolated from nasal carriers in the ward sprayed with methicillin also show unchanged sensitivity. In this respect there is therefore little reason for changing the view previously expressed (Stewart, 1960a, 1960b) that *Staph. aureus* shows no sign to date of developing resistance *in vivo* to methicillin, even when conditions are favourable (Elek and Fleming 1960; Callaghan *et al.*, 1961), and only a limited capacity to develop resistance *in vitro*. If allowance is made for differences in technique, this conclusion is similar, so far as the results *in vitro* are concerned, to those of Rolinson *et al.* (1960), Knox (1960), and Barber (1960).

Since this paper was submitted letters from Jevons (1961), Rolinson (1961), and Knox (1961) have been published. These draw attention to a strain (type 7/47/53/54/75/77) isolated from a patient with an infected wound and from two nasal carriers in the same ward.

This strain has two populations of *Staph. aureus,* one of which shows standard sensitivity to methicillin while the other, minority, population of cells shows resistance to 25 μg./ml. Through the kindness of Dr. Jevons I had an early opportunity of examining this strain, with results in no way different from her own or those of Rolinson and Knox. The minority population of resistant cells has colonial and other properties different from those of typical *Staph. aureus,* but, in so far as it was isolated from two individuals, neither of whom had received methicillin, in an environment in which the drug was not being used, it must clearly be accepted as the first naturally resistant strain. Since then I have learned of one other similar strain (Elek, 1961, personal communication). It is clear, therefore, that natural resistance can occur, but the data at present available suggest that this is of a very low order of frequency—on Jevons's figures alone, one out of 5,440 strains examined. This is probably much lower than the original frequency of natural resistance to penicillin G, but time alone will tell whether or not such strains are the progenitors of an increasing resistant population.

The results reported here also reveal further differences between the action of methicillin and penicillin G, as well as between *Staph. albus* and *Staph. aureus.* With the reservation quoted above, there is ample evidence that all typical strains of *Staph. aureus* are fully sensitive to methicillin, irrespectively of any resistance which they might possess to penicillin G (Thompson *et al.,* 1960; Rolinson *et al.,* 1960; Stewart, 1960a; Garrod, 1960; Jevons, 1961). The strains of *Staph. albus* described here with resistance to methicillin, show cross-resistance to penicillin G. Resistance of *Staph. aureus* to penicillin G is often associated with penicillinase-production, but the resistant strains of *Staph. albus* studied here produce no substances capable of completely inactivating methicillin. The resistant mutants of *Staph. aureus* described by Barber (1960) equally failed to inactivate methicillin. It is clear, therefore, that the mechanisms associated with staphylococcal resistance to the natural benzylpenicillins do not account for acquisition of resistance to the synthetic derivative methicillin.

Largely as a result of studies *in vitro,* there has been a tendency recently (*British Medical Journal,* 1961) to regard methicillin as a drug which should be kept in reserve for difficult staphylococcal infections lest more widespread use should lead to acquisition of resistance on the part of the organisms. The present studies do not support this view: naturally resistant strains of *Staph. aureus* are very rare indeed; resistance develops with difficulty *in vitro,* and, on present evidence, not at all *in vivo*; and the drug is not destroyed by penicillinase-forming staphylococci. If these properties are considered in relation to its powerful bactericidal action, lack of toxicity, and undoubted therapeutic effect even in severe infections (Douthwaite and Trafford, 1960; Stewart *et al.,* 1960), methicillin would appear to be the most effective antistaphylococcal agent available. Its disadvantages—narrow spectrum and parenteral administration—might even serve a purpose by restricting its use to precisely those infections—that is, those due to *Staph. aureus*—for which it is specifically indicated and in which it has a unique life-saving effect.

Summary

Freshly isolated strains of staphylococci were investigated for evidence of resistance to methicillin.

Some strains of *Staphylococcus aureus* showed a limited capacity to develop resistance artificially *in vitro*; there was no evidence of resistance *in vivo* in strains reisolated from patients and carriers treated with methicillin, even under conditions favouring the emergence of resistant strains.

Staph. albus acquired a high degree of drug-resistance readily *in vitro*; a lesser degree of resistance developed in strains reisolated from patients during prolonged therapy with methicillin.

Resistant strains of *Staph. albus* showed cross-resistance to penicillin G but did not form penicillinase.

Resistance to this form of synthetic penicillin appeared to depend on mechanisms different from those involved in resistance to penicillin G.

Grateful acknowledgment is made to Mr. H. H. Nixon and to other colleagues for their co-operation and interest; and to Beecham Research Laboratories for supplies of "celbenin."

REFERENCES

Barber, Mary (1960). *Brit. med. J.,* **2**, 939.
Branch, A., Rodger, K. C., Lee, R. W., and Power, Edna E. (1960). *Canad. med. Ass. J.,* **83**, 991.
Brit. med. J., 1961, **1**, 113.
Callaghan, R. P., Cohen, S. J., and Stewart, G. T. (1961). *Brit. med. J.,* **1**, 860.
Douthwaite, A. H., and Trafford, J. A. P. (1960). Ibid., **2**, 687.
Elek, S. D., and Fleming, P C. (1960). *Lancet,* **2**, 569.
Garrod, L. P. (1960). *Brit. med. J.,* **2**, 1695.
Jevons, M. Patricia (1961). Ibid., **1**, 124.
Knox, R. (1960). *Brit. med. J.,* **2**, 690.
—— (1961). Ibid., **1**, 126.
Rolinson, G. N. (1961). Ibid., **1**, 125.
—— Stevens, Shirley, Batchelor, F. R., Cameron Wood, J., and Chain, E. B. (1960). *Lancet,* **2**, 564.
Stewart, G. T. (1960a). *Brit. med. J.,* **2**, 694.
—— (1960b). Ibid., **2**, 1085.
—— Nixon, H. H., and Coles, H. M. T. (1960). Ibid., **2**, 703.
Szybalski, W. (1953). *Antibiot. Chemother.,* **3**, 915.
Thompson, R. E. M., Harding, J. W., and Simon, Rosemary D. (1960). *Brit. med. J.,* **2**, 708.

ELECTROENCEPHALOGRAPHIC CHANGES IN CEYLONESE BOXERS

BY

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The recent publications in the local press and the articles in foreign periodicals about the fatalities and hazards of boxing prompted us to carry out a study of the electroencephalographic changes (if any) in Ceylonese boxers. We were especially interested in this study, as we noticed that some of our university boxers showed definite changes in their attitude to work and also significant E.E.G. changes when we were carrying out our routine medical tests.

Several workers have described the changes that could take place during either amateur or professional boxing. Busse and Silverman (1952), studying the E.E.G. changes in 24 professional boxers, found dysrhythmic E.E.G.s in 37.5% of these boxers. There was some evidence that men who had been knocked out showed severer E.E.G. disturbances than those who had