buffer has some effect on the potency of novobiocin over extended periods of storage, it is unlikely that there is any decrease in novobiocin concentration during the short test period (2 h) employed here.

The total counts (Table III) of logarithmic phase cultures of both *E. coli* and *S. aureus* treated with novobiocin remained virtually constant over a period of 2 h, indicating that the antibiotic does not induce lysis in either organism. It is difficult to correlate this finding with that of HAHN[3] who showed that novobiocin induced spheroplast formation in *E. coli*. Novobiocin did not induce spheroplasts in the present strain of *E. coli*, and this result agrees with other findings[2].

BROCK[10] found that Mg^{++} ions overcame the inhibitory effects of novobiocin against *E. coli* but not against *S. aureus*. We have found, in preliminary experiments, that high concentrations of Mg^{++} have a slight alleviating effect on novobiocin action against *E. coli*, but not *S. aureus*. However, these experiments also demonstrated that filamentous forms were still induced in *E. coli* by novobiocin when Mg^{++} ions were present. The influence of Mg^{++} on novobiocin action could well, therefore, be re-investigated.

Thus, the close similarity in response (Figures 1, 2; Tables I–III) of *E. coli* and *S. aureus* to concentrations of novobiocin of the order of twice the m.i.c., suggests that this substance has the same action against both organisms. The wide difference in sensitivity of the 2 organisms to the antibiotic could be explained in terms of differences in permeability of the bacterial cells to novobiocin, although evidence for this contention must await the results of further experiments.

Table II. Effect of novobiocin (NB) on the viability of washed suspensions of *E. coli* and *S. aureus* at 37 °C

Organism	Time (min)	Viable count/ml of washed suspensions treated with NB concentrations		
		0	10 µg/ml	1000 µg/ml
E. coli	0	1.3×10^7		1.3×10^7
	120	1.3×10^7		1.0×10^7
S. aureus	0	1.3×10^7	1.3×10^7	
	120	1.3×10^7	1.0×10^7	

Table III. Effect of novobiocin (NB) on total counts of logarithmic phase *E. coli* and *S. aureus* at 37 °C

Organism	Min after addition of NB	Total count/ml of suspensions treated with NB concentrations		
		0	10 µg/ml	1000 µg/ml
E. coli	0	3.1×10^8		3.16×10^8
	120	2.74×10^9		2.68×10^8
S. aureus	0	1.17×10^8	1.30×10^8	
	120	9.1×10^8	1.37×10^8	

Résumé. La novobiocine a rendu impossible la croissance des organismes *Escherichia coli* et *Staphylococcus aureus*, mais n'a pas produit de lyse. Le taux de mortalité de ces 2 organismes a été lent. La novobiocine n'agit guère sur les bactéries en suspension dans l'eau. Nous en concluons que dans ces 2 organismes la novobiocine empêche la synthèse macromoléculaire.

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[10] T. D. BROCK, J. Bact. *72*, 320 (1956).

Action of Cloxacillin and Nafcillin on *Escherichia coli*

Cloxacillin (sodium 3-*o*-chlorophenyl-5-methyl-4-isoxazolyl penicillin) and nafcillin (sodium 2-ethoxy-1-naphthyl penicillin) are 2 of the newer penicillins; both are penicillinase-stable, are more active than methicillin (sodium 2,6-dimethoxyphenyl penicillin) against benzylpenicillin-resistant staphylococci, and may be administered orally as well as by injection[1].

The mode of action of other new penicillins is similar to that of benzylpenicillin[2–5]. Differences in sensitivity of an organism to various penicillins might represent differences between the penicillin molecule and some component of the bacterial cell[6]. Recently, WARREN and colleagues[7–10] have found that there was a quantitative difference between nafcillin and the isoxazolyl penicillins, oxacillin (sodium 5-methyl-3-phenyl-4-isoxazolyl penicillin) and cloxacillin in the disorganization of the cell wall structure of *Staphylococcus aureus*. These and other findings led WARREN and GRAY[11] to propose that oxacillin and cloxacillin had a different mode of action from that of nafcillin against *S. aureus*.

In view of these latter reports, it was decided to compare the effects of one isoxazolyl penicillin (cloxacillin) and nafcillin on a Gram-negative organism, *Escherichia*

[1] B. LYNN, Antibiotica Chemother. *13*, 125 (1965).
[2] A. D. RUSSELL, J. Pharm. Pharmac. *14*, 390 (1962).
[3] H. J. ROGERS and J. MANDELSTAM, Biochem. J. *84*, 299 (1962).
[4] H. BOMANN and K. G. ERIKSSON, J. gen. Microbiol. *31*, 339 (1963).
[5] M. M. GRULA and E. A. GRULA, J. gen. Microbiol. *41*, 155 (1965).
[6] G. N. ROLINSON, Proc. R. Soc. [B] *163*, 417 (1965).
[7] G. H. WARREN and J. GRAY, Proc. Soc. exp. Biol. Med. *113*, 251 (1963).
[8] G. H. WARREN and J. GRAY, Proc. Soc. exp. Biol. Med. *114*, 439 (1963).
[9] G. H. WARREN and J. GRAY, Proc. Soc. exp. Biol. Med. *116*, 317 (1964).
[10] G. H. WARREN, S. B. ROSENMAN and C. HORWITZ, Proc. Soc. exp. Biol. Med. *117*, 730 (1964).
[11] G. H. WARREN and J. GRAY, Proc. Soc. exp. Biol. Med. *120*, 504 (1965).

coli in nutrient broth and in broth containing 0.33 *M* sucrose and 0.01 *M* magnesium sulphate, $MgSO_4 \cdot 7\,H_2O$. The final pH of all media was 7.4. Cloxacillin and nafcillin were gifts from Beecham Research Laboratories, Ltd., Brentford, England and Wyeth Laboratories, Inc., Philadelphia, USA, respectively.

The organism, a laboratory strain of *E. coli* type 1, was grown overnight at 37 °C in 20 ml nutrient broth. The culture was centrifuged at 2000 rpm for 30 min, the pellet washed twice with 10 ml of sterile water, and eventually resuspended in 10 ml sterile water. A 0.5 ml volume of this washed suspension was included in 10 ml (final volume) of nutrient broth, containing the required penicillin concentration, in McCartney bottles which were incubated at 37 °C. Presence or absence of growth was noted after incubation for 48 h, and the minimum inhibitory concentration (m.i.c.) determined. The m.i.c.s for cloxacillin and nafcillin were 7.5 and > 20 μmoles/ml, respectively.

To measure cell lysis, 0.5 ml of a washed suspension was added to nutrient broth in nephelos flasks, previously equilibrated at 37 °C, which were operated at 100 oscillations/min in a thermostatically controlled water-bath 37° ± 0.1 °C). When the cultures had reached the logarithmic phase of growth, 0.1 ml of a penicillin solution was added to give a final volume of 10 ml and the desired concentration of the antibiotic. Subsequent incubation was at 37 °C; turbidity measurements before and after the addition of the requisite penicillin were determined with the EEL nephelometer (Evans Electroselenium, Ltd., Harlow, England). The effects of different concentrations of cloxacillin and nafcillin on logarithmic phase cells are shown in Figures 1 and 2.

To detect spheroplast induction, a 0.5 ml volume of a washed suspension was incorporated into 10 ml (final volume) of the nutrient broth containing sucrose, magnesium sulphate and the desired penicillin concentration, in McCartney bottles which were incubated at 37 °C. Samples were removed after 4–5 h, and examined under the phase-contrast microscope (× 400). The results of such experiments are shown in the Table.

The results of the m.i.c. experiments showed that cloxacillin was considerably more active than nafcillin against this strain of *E. coli*, and this is confirmed by the results of experiments made in hypertonic media (Table). A concentration of cloxacillin below 7.5 μmoles/ml induced mainly morphologically bizarre forms, of the type described by HUGO and RUSSELL[12], and these are indicative of the effects of subinhibitory concentrations of a penicillin on a Gram-negative organism. Similar bizarre forms, without spheroplasts, were induced by nafcillin at concentrations of 10–20 μmoles/ml. Thus, nafcillin itself also possesses a typical (but weak) penicillin-like action on this organism.

Figure 1 shows that cloxacillin induced lysis of *E. coli* in nutrient broth at a concentration (7.5 μmoles/ml) which was inhibitory to growth and which was also the minimum concentration needed to induce true spheroplasts. However, even the highest concentration (15 μmoles/ml) of nafcillin used did not induce lysis, and this finding substantiates that in the Table, since the morphological variants induced by this penicillin are not osmotically fragile.

Spheroplast induction in *Escherichia coli* at 37 °C

Antibiotic	Concentration (μmoles/ml)	Microscopical appearance of bacteria[a]
Absent	–	Rods, no morphological variants
Cloxacillin	5.0	Mostly partial spheroplasts. Few true spheroplasts.
	7.5	Mostly true spheroplasts. Few partial spheroplasts.
	10.0	True spheroplasts.
Nafcillin	10.0	Mainly long rods.
	15.0	Partial spheroplasts, no true spheroplasts.
	20.0	Partial spheroplasts, no true spheroplasts.

[a] The term 'partial spheroplast' includes long and bulbous forms, as described in detail by HUGO and RUSSELL[12]. 'True spheroplast' denotes a spherical form free from any attachment.

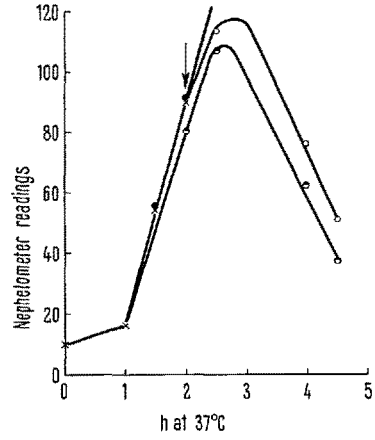

Fig. 1. Effect of concentration of cloxacillin (added at ↓) on the growth of *Escherichia coli* in nutrient broth at 37 °C. Concentrations of cloxacillin (μmoles/ml): x——x, 0; •——•, 5.0; o——o, 7.5; ⊖——⊖, 10.0.

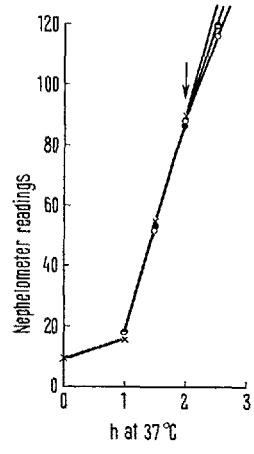

Fig. 2. Effect of concentration of nafcillin (added at ↓) on the growth of *Escherichia coli* in nutrient broth at 37 °C. Concentrations of nafcillin (μmoles/ml): x——x, 0; •——•, 5.0; o——o, 7.5; ⊖——⊖, 10.0. There was no decrease in nephelometer readings on prolonged incubation. Other experiments indicated that 15 μmoles/ml nafcillin did not induce lysis.

[12] W. B. HUGO and A. D. RUSSELL, J. Bacteriol. *82*, 411 (1961).

A comparison of the effects of these 2 penicillins with other penicillins[2,13] and with 6-aminopenicillanic acid (6-APA)[14] on this strain of *E. coli* is of interest. Thus, both cloxacillin and nafcillin are considerably less effective in inhibiting growth and inducing lysis and spheroplast formation than are benzylpenicillin, phenoxymethylpenicillin, propicillin, phenethicillin, phenbenicillin[2], ampicillin[13] and 6-APA[14]. It has recently been observed[5] that, in common with other penicillins, cloxacillin inhibited bacterial cell division (resulting in the formation of filaments) in an *Erwinia* species at concentrations far below those needed by nafcillin. According to ROLINSON[6], differences in sensitivity of an organism to various penicillins could be related to the relative affinity of each antibiotic for a component of the bacterial cell wall.

The overall conclusion of these findings is to demonstrate that each penicillin has a similar mode of action against *E. coli*.

Résumé. Divers aspects de l'action de la cloxacilline et de la nafcilline sur *Escherichia coli* ont été étudiés. La cloxacilline s'est montrée plus efficace que la nafcilline quand il s'agissait d'arrêter la croissance et d'amener la lyse et la formation de sphéroplastes; par contre, elle s'est montrée moins efficace que plusieurs autres pénicillines (y compris l'acide 6-aminopénicillanique) à ces égards. Ces résultats tendent à démontrer que la cloxacilline et la nafcilline possèdent un mode d'action qui est typique des pénicillines sur cet organisme.

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8 September 1967.

[13] T. D. TURNER and A. D. RUSSELL, J. Pharm. Pharmac. *14*, 395 (1962).
[14] A. D. RUSSELL, unpublished data.

Pregnancy Blocking Capacity and Inbreeding in Laboratory Mice

Blocking of luteal function and, hence, implantation, following a discrimination of pheromones in the urine of stud and strange males is a phenomenon that has been documented for a variety of types of laboratory mice[1–4], wild house mice[5], and, a non-murid rodent, the deermouse[6]. MARSDEN and BRONSON[7] were unable to detect any effect of exposure to strange males in any of the 5 highly inbred strains of mice they tested. Two experiments dealing with genetics and the blocking phenomenon are reported here: (1) an evaluation of blocking capacity in hybrid mice obtained from crossing 8 inbred lines and (2) separately testing males and females of one non-blocking, inbred strain to determine if the loss in blocking capacity in that strain could be traced exclusively to either sex.

Eight-way cross hybrids used in the first experiment were obtained from crossing the following lines at The Jackson Laboratory: SJL/J, BALB/cJ, C57BL/6J, CBA/J, 129/J, BDP/J, LP/J, and RF/J. The first 5 strains have been subjected to intra-strain, and some degree of inter-strain, testing for pregnancy blocking responses; all with negative results. Sixth generation hybrid offspring were evaluated for blocking capacity at 75–90 days of age. Females were paired with non-littermate males for 4 days. Stud males were removed when an insemination was detected and either the female remained isolated in her home cage as a control or a strange (8-way cross) male was put into her home cage for 2 days in an attempt to block her implantation. Autopsy on day 7 post insemination revealed the following pregnancy rates: control, 75% of 24; strange male exposed, 92% of 25. The strange male effect, therefore, could not be demonstrated.

The second experiment substituted C57BL/6J males and females separately into a proven blocking system to determine if the loss in blocking capacity previously reported for this strain[7] could be exclusively traced to either sex. All C57BL/6J mice were reared in Bar Harbor and shipped to Burlington where the senior author tested them in a system known to result in a strange male blocking response: insemination of Swiss-ICR albino females by males of the same strain, removal of the studs, and exposure for 7 days to a wild house mouse male in the female's home cage[5,8]. Averaging several experiments for appropriately aged females under these conditions shows a reduction in implantation rate from 92% for control (isolated) ICR females to 49% following exposure to one strange wild male (Table). A lower pregnancy rate (as low as 15%) is possible by simultaneous exposure to more than 1 male[8]. Substituting C57BL/6J females into this system revealed a complete lack of response to the strange wild male; pregnancy rate being lowered by only

Pregnancy blocking attempts in ICR or C57BL/6J females (ICR studs used throughout)

Inseminated female	Test exposure	No.	% Pregnant
ICR	no male[a]	225	92
ICR	wild male[a]	218	49
C57BL/6J	no male	27	74
C57BL/6J	wild male	51	69
ICR	C57BL/6J male	60	80
ICR	ICR male	60	75

[a] Data in part from CHIPMAN and FOX[8] to illustrate the normal degree of blocking found in the standard test system.

[1] H. M. BRUCE, J. Reprod. Fert. *1*, 96 (1960).
[2] H. M. BRUCE, J. Anim. Sci. *25* (Suppl.), 83 (1966).
[3] A. S. PARKES and H. M. BRUCE, Science *134*, 1049 (1961).
[4] R. K. CHIPMAN, J. A. HOLT and K. A. FOX, Nature *210*, 653 (1966).
[5] R. K. CHIPMAN and K. A. FOX, J. Reprod. Fert. *12*, 233 (1966).
[6] F. H. BRONSON and B. E. ELEFTHERIOU, Gen. comp. Endocr. *3*, 515 (1963).
[7] H. M. MARSDEN and F. H. BRONSON, Nature *207*, 878 (1965).
[8] R. K. CHIPMAN and K. A. FOX, J. Reprod. Fert. *12*, 399 (1966).