HU, OHA, AOA, and SHA were obtained from Hynes Chemical Research Corporation, Durham, N.C., USA. *E. coli* B and its T_4 bacteriophage were from the American Type Culture Collection. Dose-response relationships of the virus to various concentrations of each compound were assessed following a 10 min absorption interval in synthetic medium[13] with the virus in a 10:1 multiplicity. Upon removal of unabsorbed virus by centrifugation and resuspension 3 times, the infected bacteria were resuspended to the original volume in the synthetic medium containing each filter-sterilized compound. After 90 min at 37 °C, chloroform was added and the tubes were stored overnight at 5 °C. Following centrifugation the amount of infectious virus in the supernatant solution was determined by the double agar layer technique[14]. Plaques were counted independently by 2 operators and the average values were calculated.

Effects of hydroxyurea (HU), oxamyl hydroxamic acid (OHA), salicylhydroxamic acid (SHA), and acetoxyoxamide (AOA) on the yield of infectious T_4 bacteriophage from *Escherichia coli* B.

Compound	Concentration (M)	% inhibition
HU	10^{-5}	3
HU	10^{-4}	13
HU	10^{-3}	63
HU	10^{-2}	71
OHA	10^{-5}	21
OHA	10^{-4}	70
OHA	10^{-3}	93
OHA	10^{-2}	97
SHA	10^{-5}	34
SHA	10^{-4}	49
SHA	10^{-3}	89
SHA	10^{-2}	96
AOA	10^{-5}	24
AOA	10^{-4}	33
AOA	10^{-3}	43
AOA	10^{-2}	95

All values are averages of 3 experiments.

The Table shows that at $10^{-5}M$ and $10^{-4}M$, OHA, SHA, and AOA retarded virus replication and were somewhat more active than HU. At $10^{-3}M$ AOA was less active than HU, but the 3 former compounds induced 95% inhibition or greater at $10^{-2}M$ as compared with 71% inhibition obtained with HU at the same concentration. OHA was the most active compound, yielding 70% inhibition at $10^{-4}M$. Further evaluation of these agents and other hydroxamic acids in a variety of virus systems consequently seems clearly indicated[15].

Résumé. L'acide oxamyl-hydroxamique, l'acide salicylhydroxamique et l'acétoxyoxamide inhibent la reproduction du bactériophage T_4 dans l'*Escherichia coli* B. L'activité de chacun de ces composés était plus grande que celle de l'hydroxyurée dans le même système d'essai. De ce fait, une évaluation plus poussée de ces 3 agents ainsi que d'autres acides hydroxamiques dans une série de systèmes d'essai sur les virus paraît clairement indiquée.

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Some Aspects of Novobiocin Action on *Escherichia coli* and *Staphylococcus aureus*

Novobiocin, an antibiotic originally known as 'streptonivicin', was described in 1956[1]. It is usually employed in the form of its monosodium salt, and is active mainly against Gram-positive bacteria.

In contrast to the many investigations carried out on the mechanisms whereby other antibacterial substances exert their effect, relatively few reports have been made as to the exact nature of the action of novobiocin. The following are among the effects observed in novobiocin-treated bacteria: (a) an inhibition of cell wall, protein and nucleic acid syntheses[2]; (b) induction of spheroplasts[3] (but compare with Wishnow et al.[2]); (c) an increase in the permeability of *Escherichia coli*[4]; (d) an intracellular deficiency of magnesium ions[5], although contrary evidence, viz. that the antibiotic and Mg^{++} do not form a complex, has since been produced[6,7]; (e) an inhibition of deoxyribonucleic acid (DNA) synthesis prior to any inhibition of cell wall, protein and ribonucleic acid (RNA) syntheses[8].

The following experiments describe the effects of novobiocin on viable and total counts and turbidity of growing and non-growing cultures of *E. coli* NCTC 9001 and *Staphylococcus aureus* NCTC 6571. These organisms were grown overnight at 37 °C in 40 ml of nutrient broth No. 2 and nutrient broth (both from Oxoid Laboratories, Ltd., London, England) respectively. The cultures were centrifuged at 2500 rpm for 10 min, the supernatant fluids removed, and the pellets washed twice in sterile water. The suspensions were finally adjusted to contain about 10^8 viable cells/ml. Novobiocin monosodium, B.P., was purchased from Merck, Sharpe and Dohme, Ltd., Hoddes-

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don, Hertfordshire, England. Water, for use in the preparation of media and of washed suspensions, and as a diluent in serial dilution in viable counting, was obtained from an all-glass still.

For the determination of minimum inhibitory concentrations (m.i.c.s) of the antibiotic, a 0.2 ml volume of the washed suspension was included in 10 ml (final volume) of nutrient medium, containing the desired novobiocin concentration, and of final pH 7.2, in McCartney bottles, which were incubated at 37 °C for 2 days. The presence or absence of growth was recorded, and the m.i.c.s determined: these were 4 μg/ml and 500 μg/ml for *S. aureus* and *E. coli*, respectively.

In turbidity measurements, 0.5 ml volumes of the washed suspensions were incorporated in 10 ml (final volumes) of nutrient medium, final pH 7.2, containing the desired final concentration of the antibiotic, in nephelos flasks previously equilibrated at 37 °C, and operating at 100 oscillations/min. Subsequent incubation was at 37 °C, and turbidity was determined with the EEL nephelometer (Evans Electroselenium, Ltd., Harlow, England). With both organisms, there was no increase in turbidity in those flasks containing concentrations of novobiocin at, or above, the m.i.c., whereas in control flasks (novobiocin absent) turbidity increased rapidly. In other experiments, the organisms were pre-incubated into the logarithmic phase before the addition of the antibiotic. Turbidity was measured as before and the results are shown in Figures 1 and 2. In these experiments, also, samples were removed from the nephelos flasks when required for (1) viable counts, using the pour-plate technique, and an incubation period of 48 h at 37 °C, (2) total counts, in duplicate, by means of a Helber slide under the phase-contrast microscope (× 400).

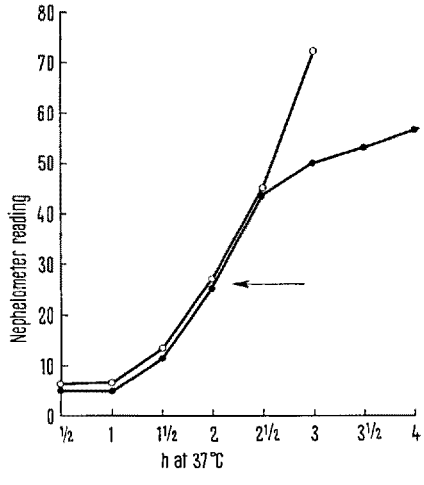

Fig. 1. Effect of novobiocin (added at the time indicated) on logarithmic phase *E. coli* in nutrient broth No. 2 at 37 °C. o——o, novobiocin absent; ●——●, novobiocin 1000 μg/ml.

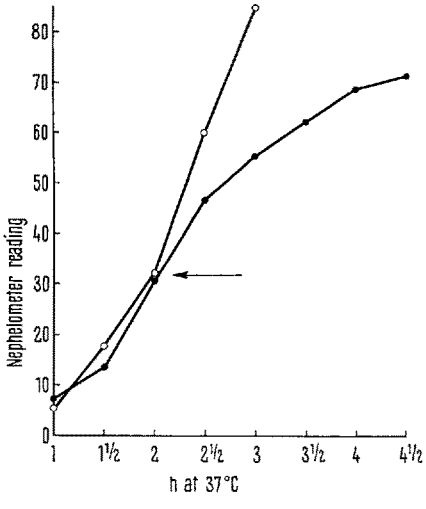

Fig. 2. Effect of novobiocin (added at the time indicated) on logarithmic phase *S. aureus* in nutrient broth at 37 °C. o——o, novobiocin absent; ●——●, novobiocin 10 μg/ml.

When novobiocin was added to logarithmic phase *E. coli* (Figure 1), the turbidity of the suspension continued to increase, although at a somewhat reduced rate than the control (novobiocin absent). The same response was obtained at higher (up to 4000 μg/ml) concentrations of novobiocin. However, this increased turbidity was not accompanied by an increase in viable numbers; on the contrary, novobiocin had a slow, but definite, lethal effect on the cells (Table I). Samples of the culture were removed before and after the addition of the drug, and examined microscopically: under the influence of novobiocin the cells assumed a filamentous shape, and this increase in length would, in the absence of a corresponding increase in viable numbers, be responsible for the increased nephelometer readings.

Essentially the same responses were obtained when the antibiotic was added to logarithmic phase *S. aureus* in nutrient medium (Figure 2, Table I), except that the increase in turbidity of the novobiocin-treated culture would here presumably be correlated with an increase of cell diameter, since filamentous forms were not induced in this organism.

These findings suggest that, in both organisms, novobiocin acts by inhibiting one type of macromolecular synthesis, whilst allowing other such syntheses to proceed. Further evidence was obtained by studying the effect of the drug on non-growing bacteria suspended in 0.05 *M* phosphate buffer, pH 7.2, at 37 °C, where it was found that novobiocin was ineffective against such bacteria (Table II). Although it has been reported[9] that phosphate

Table I. Bactericidal effect of novobiocin (NB) on logarithmic phase *E. coli* and *S. aureus* at 37 °C

Organism	Min after addition of NB	Viable count/ml of suspensions treated with NB concentrations		
		0	10 μg/ml	1000 μg/ml
E. coli	0	2.7×10^8		2.7×10^8
	15	4.2×10^8		2.7×10^8
	30	4.8×10^8		1.8×10^8
	45			1.6×10^8
	60	7.0×10^8		1.3×10^8
S. aureus	0	1.25×10^8	7×10^7	
	15	1.58×10^8	6.5×10^7	
	30	2.3×10^8	5.5×10^7	
	45	2.4×10^8	4.2×10^7	
	60	4.25×10^8	1.67×10^7	

[9] M. L. BUSS, K. A. LEES and V. J. VERGINE, J. Pharm. Pharmac. *11*, 150T (1959).

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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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*

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