with the temperature and duration of heating (Halvorson and Church, 1957; Murrell, 1961).

TABLE II

VARIATION IN RECOVERY OF *B. subtilis* AFTER POST-IRRADIATION HEAT-SHOCK

Dose (rad.)	Percentage increase over unheated recovery		
	3 min./60°	15 min./60°	5 min./90°
0	59	64	82
4×10^5	37	27	-25
6×10^5	13	17	-34

The results indicate that pre-irradiation sensitises *B. subtilis* spores to heating normally sub-lethal and used to activate dormant spore suspensions.

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The Antibacterial Action of Glycine

SIR,—It is known that some D-amino-acids exert an inhibitory effect on certain Gram-negative bacteria, and that in the presence of sucrose as a stabilising agent, morphological variants can be preserved (Jeynes, 1957; Welsch, 1958; Lark and Lark, 1959; 1961).

The term "spheroplasts" has been suggested for these variants which might retain at least a portion of the original cell wall (Brenner and others, 1958) and which have also been induced by treating *Escherichia coli* with various penicillins (Russell 1962; Turner and Russell 1962).

In the present preliminary report, an investigation has been made into the quantitive aspects of the effect of glycine on *E. coli* in a nutrient medium in the presence or absence of sucrose and Mg^{++} ions. These ions have previously been found to be essential in stabilising penicillin-induced spheroplasts (Lederberg 1956; Hugo and Russell 1960).

In our experiments 0·1 ml. of an overnight 37° broth culture of the organism was added to 10 ml. tubes of nutrient broth containing 0·33 M sucrose and 0·25 per cent w/v $MgSO_4 \cdot 7H_2O$, and varying concentrations of glycine. After incubation of all tubes at 37° for 4 hr., three samples were examined.

(1) 1 ml. was added to 9 ml. of sterile water to lyse any spheroplasts present. Further serial dilutions were made if necessary, 1 ml. samples being finally plated into 10 ml. of nutrient agar.

(2) 1 ml. was added to 9 ml. of 0·33 M sucrose: Mg^{++} broth with the aim of protecting any spheroplasts from osmotic shock, and after further serial dilution if necessary in this medium, 1 ml. samples were plated into 10 ml. of 0·33 M sucrose: Mg^{++} agar.

(3) Aliquots were examined by phase-contrast microscopy.

The results of a typical experiment are shown in Table I.

TABLE I

THE EFFECT OF GLYCINE ON *E. coli*

Glycine concentration mg./ml.	Count of survivors in agar per ml.	Count of survivors in 0·33 M sucrose Mg^{++} agar per ml.	Presence of spheroplasts
0	$>10^7$	$>10^7$	−
20	$1{\cdot}9 \times 10^4$	$4{\cdot}3 \times 10^4$	++
25	$2{\cdot}4 \times 10^3$	1×10^4	+
30	$8{\cdot}5 \times 10^2$	$2{\cdot}1 \times 10^3$	+

++ Optimum number of spheroplasts.
+ Few spheroplasts.
− No spheroplasts.

The optimum concentration of glycine to induce spheroplasts, was 20 mg./ml. At 15 mg./ml. of glycine, spheroplasts, bizarre forms and rods could be observed microscopically. Above 25 mg./ml. the number of spheroplasts dropped sharply, although the bacteria were still killed, as indicated by the survivor counts. In agar containing sucrose and Mg^{++} ions, survivor counts were slightly higher than those obtained in ordinary nutrient agar alone. This suggests that some spheroplasts, at least, were able to survive in the presence of the stabilisers, and produce colonies.

In nutrient broth, to which sucrose and $MgSO_4 \cdot 7H_2O$ had not been added, no spheroplasts were induced by any concentration of glycine.

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