

## MODE OF ACTION OF A NEW COLICIN

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### 1. Introduction

Colicin adsorption to sensitive bacteria leads to cell death. At present, three major types of colicin action are recognized based on the biochemical disfunctions induced by the colicin; one, exemplified by colicin E2, leads to DNA degradation [1]; another, represented by colicins E3 and D, inhibits protein synthesis [1, 2]; and the third type, which includes the majority of the colicins studied (E1, K, I and A) and a bacteriocin (JF 246), affects all macromolecular synthesis and the accumulation of amino acids and  $\beta$ -galactosides [3–7].

In this paper we report the studies on the biochemical effect induced by a bacteriocin synthesized by the strain, *Shigella* AD03-8. This bacteriocin, designated colicin S.8 following Fredericq's nomenclature [8], can be identified as different from any of the other well characterized colicins by the type of resistant mutant it selects [9]. Like with colicin K, strains resistant to colicin S.8 are also resistant to phage T6 [9].

Colicin S.8 affects all macromolecular synthesis and the accumulation of several substrates. Action of the colicin is drastically reduced at 10°; loss of sensitivity is expressed immediately after transfer to the lower temperature.

### 2. Materials and methods

*Escherichia coli* K12, strain 3000 Y14, HfrH *thi*<sup>-</sup> *lac*<sup>-</sup> (*i*<sup>+</sup>*z*<sup>-</sup>*y*<sup>+</sup>) was used for these studies.

Colicin S.8 was prepared from strain *Shigella* AD03-8 by the same procedure used for colicins E1,

K or A [6, 10]. The number of killing units of colicin was calculated from the survival in mixtures of bacteria and colicin, from the equation  $B/B_0 = e^{-m}$ , where  $B/B_0$  is the survival ratio and  $m$ , the multiplicity of killing units.

Strain 3000 Y14 was grown aerobically at 37° in minimal-glucose medium [11] supplemented with thiamine, to  $2 \times 10^8$  cells/ml. The culture was centrifuged, resuspended in fresh medium and equilibrated for 5 min at the desired temperature. Unless otherwise specified, colicin was added 5 min before the addition of the radioactive label; survival was determined 4.5 min after addition of the colicin.

For incorporation experiments, samples taken at different times after addition of the radioactive substrate were mixed with equal volumes of 10% cold trichloroacetic acid (TCA); after 30 min the precipitates were collected on Millipore filters (0.45  $\mu$ m pore size), washed with 5% cold TCA and dried. For uptake experiments, cells were resuspended in minimal medium with chloramphenicol (50  $\mu$ g/ml) and equilibrated at the corresponding temperature; after addition of the radioactive substrate samples were taken at different times, filtered on Millipore filters, washed with minimal medium and dried.

Radioactivity was measured in an automatic scintillator spectrometer (Model 720, Nuclear Chicago Corp.).

### 3. Results and discussion

Addition of colicin S.8 to a log culture of strain 3000 Y14 inhibits the incorporation of labeled iso-

leucine, uracil or thymidine into acid-insoluble material. The effect of colicin S.8 on uracil or isoleucine incorporation at 30° or 37°, is illustrated in figs. 1 and 3. Colicin S.8 also affects the accumulation of isoleucine, lactose or methyl-thio- $\beta$ -D-galactoside (TMG), and induces efflux of the accumulated substrate, as shown in fig. 2A for isoleucine. The accumulation of  $\alpha$ -methyl-D-glucoside ( $\alpha$ MG), which is mediated by a phosphoenolpyruvate-dependent phosphorylation system, is not inhibited by colicin S.8 (fig. 2B).

The effect of colicin S.8 on protein synthesis might be secondary to the effect on uptake, since depletion of the pool of isoleucine (measured as the difference between total uptake and radioactivity in the acid-insoluble fraction) parallels the halt of incorporation of this amino acid into protein (fig. 3).

Colicin S.8 does not induce DNA degradation, or induction of prophage  $\lambda$  in a strain lysogenic for this phage.

The action of colicin S.8 on the biochemical target is very slow, even at rather high multiplicities of colicin (fig. 4A). The comparison of the velocity of the effect of colicin S.8 on uptake of isoleucine with that of other colicins with similar mode of action, and with multiplicities giving similar survival values, permits the ordering of these colicins with regards to the velocity of effect (figs. 4A and 4B). Colicins S.8 and K appear as the slowest acting of the colicins tested and colicin E1 as the fastest. Similar conclusions were reached for colicins S.8, K and E1, by measuring the velocity of loss of accumulated TMG.

Cells can be rescued from the lethal damage induced by certain colicins by trypsin treatment [12–14]. Also, lethal action of colicin S.8 can be prevented by addition of trypsin. A large fraction of the cells (about 10–20%) can be rescued by trypsin at 20 min after addition of colicin S.8 at a multiplicity of about 50 killing units. The degree of rescue by trypsin of the different colicins

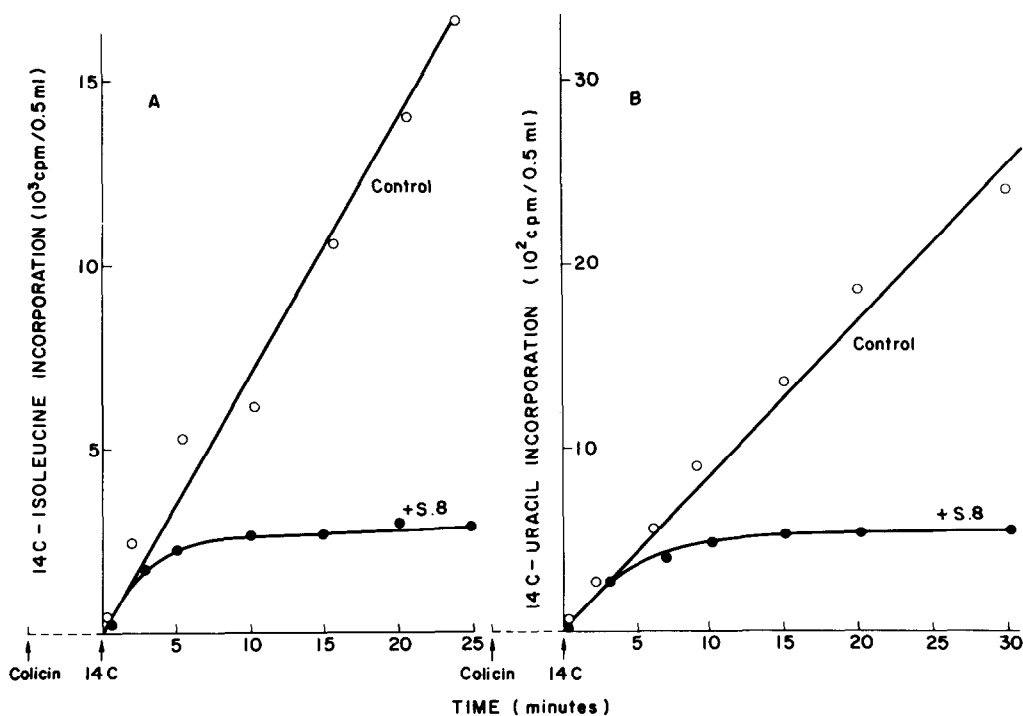


Fig. 1. Effect of colicin S.8 on isoleucine and uracil incorporation. A) Incorporation of [<sup>14</sup>C]isoleucine at 37°. [<sup>14</sup>C]Isoleucine ( $2 \times 10^{-5}$  M; 0.1  $\mu$ Ci/ml) was added at 0 min. Survival: 0.12%; B) Incorporation of [<sup>14</sup>C]uracil at 30°. [<sup>14</sup>C]Uracil ( $2 \times 10^{-5}$  M; 0.1  $\mu$ Ci/ml) was added at 0 min. Survival: 0.17%.

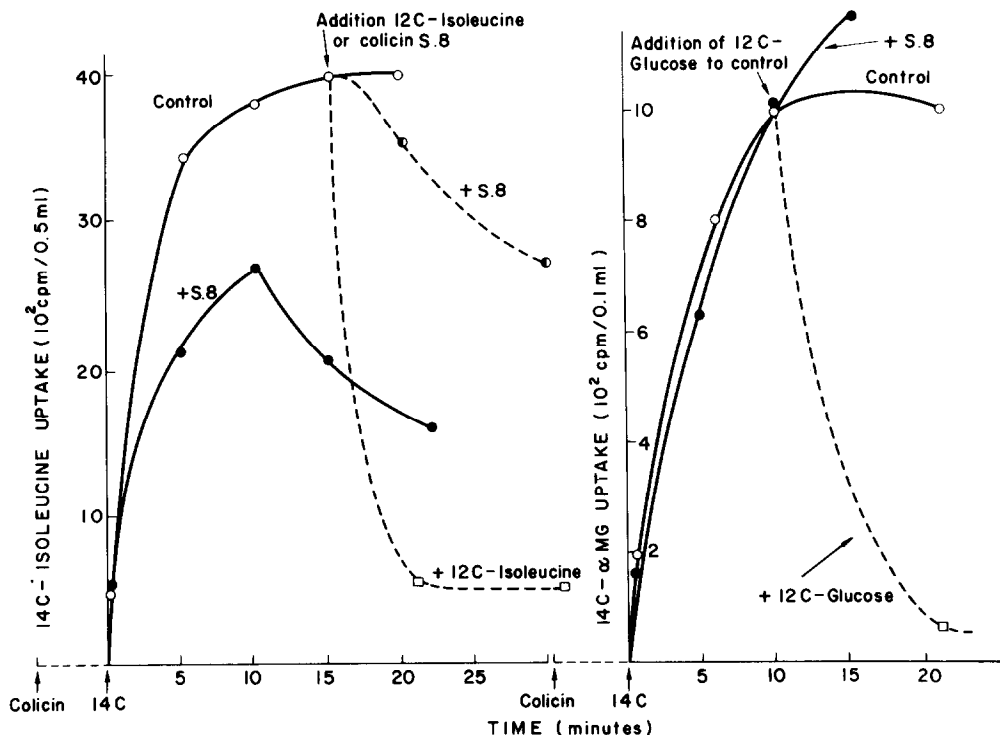
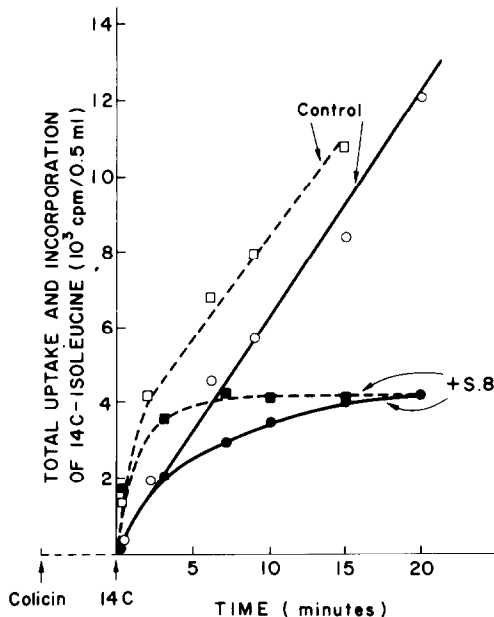


Fig. 2. Effect of colicin S.8 on accumulation of isoleucine and  $\alpha$ MG and efflux of isoleucine. A) Effect on accumulation and efflux of  $[^{14}\text{C}]$ isoleucine.  $[^{14}\text{C}]$ isoleucine was added at 0 min ( $2 \times 10^{-6}$  M;  $0.05 \mu\text{Ci/ml}$ ). Survival: 0.12%. Colicin S.8 or  $^{12}\text{C}$ -isoleucine ( $4 \times 10^{-4}$  M) were added to aliquots of the control culture at 15 min. B) Effect on  $\alpha$ MG accumulation.  $[^{14}\text{C}]\alpha\text{MG}$  ( $2.6 \times 10^{-5}$  M;  $0.1 \mu\text{Ci/ml}$ ) was added at 0 min. Survival: 0.1%. At 10 min,  $^{12}\text{C}$ -glucose ( $2 \times 10^{-3}$  M) was added to an aliquot of the control culture.



(S.8, K, A or E1) at 10 to 20 min after addition of comparable multiplicities of colicin (ranging from 20 to 80) appears to be related to the velocity of the effect observed for each colicin: larger rescue values are observed for the slower acting colicins (Nagel de Zwaig and Vitelli-Flores, unpublished results).

The biochemical effect of colicin K is greatly reduced at lower temperatures [15]. As shown in fig. 5A, the effect of colicin S.8 on isoleucine uptake or incorporation is notably reduced at  $20^\circ$ ; at  $10^\circ$ , addition of

Fig. 3. Correlation between the effect of colicin S.8 on total uptake and incorporation of isoleucine.  $[^{14}\text{C}]$ isoleucine was used at a concentration of  $2 \times 10^{-5}$  M ( $0.1 \mu\text{Ci/ml}$ ). Samples were taken at different times and either a) filtered and washed with minimal medium, for determination of total uptake, or, b) mixed with an equal volume of cold 10% TCA, for measurement of the TCA insoluble radioactivity. Survival: 0.17%.  $\square, \blacksquare$ , total radioactivity;  $\circ, \bullet$ , TCA insoluble radioactivity.

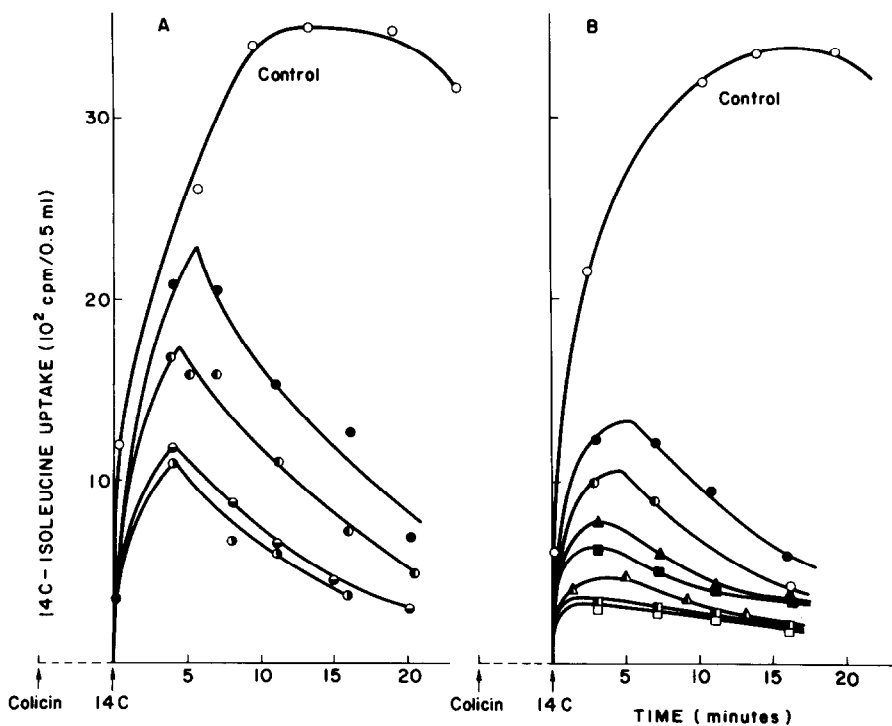


Fig. 4. Effect of colicins S.8, K, A and E1 on the uptake of isoleucine at 30°. A) Effect of colicin S.8. Survival: ●, 0.41%; ○, 0.12%; ◐, 0.016%; ◑, 0.014%. B) Effect of colicin K. Survival: ●, 0.4%; ○, 0.065%. Effect of colicin A. Survival: ▲, 0.26%; ◀, 0.05%. Effect of colicin E1. Survival: ■, 5%; ◼, 0.2%; ◑, 0.05%.

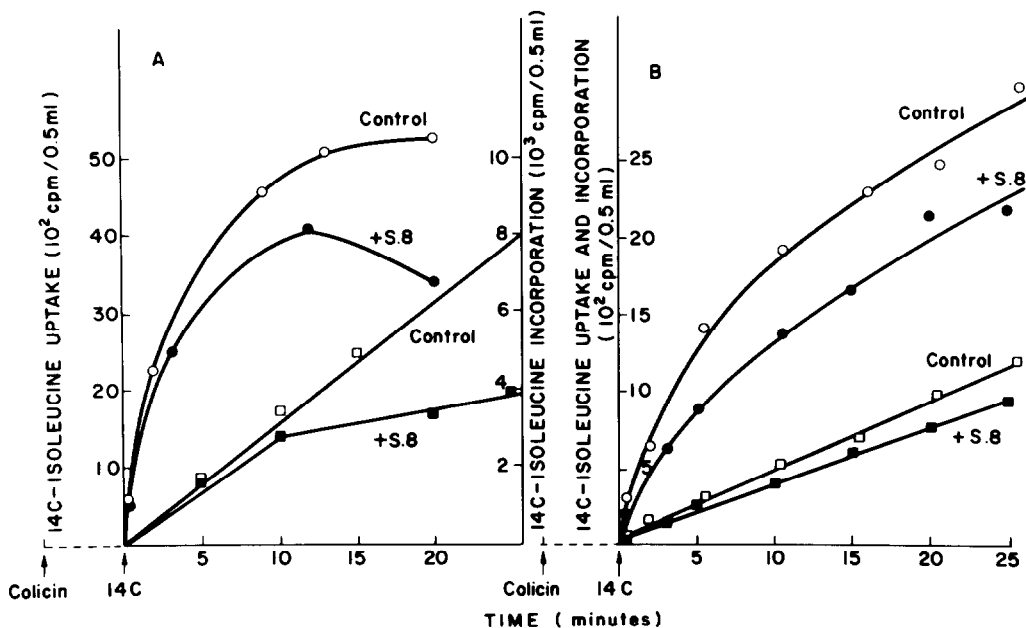


Fig. 5. Effect of colicin S.8 on uptake and incorporation of isoleucine at lower temperatures. ○, ●, Uptake; ◻, ■, incorporation. A) Effect at 20°. Survival: 0.02%. B) Effect at 10°. Survival: 0.03%.

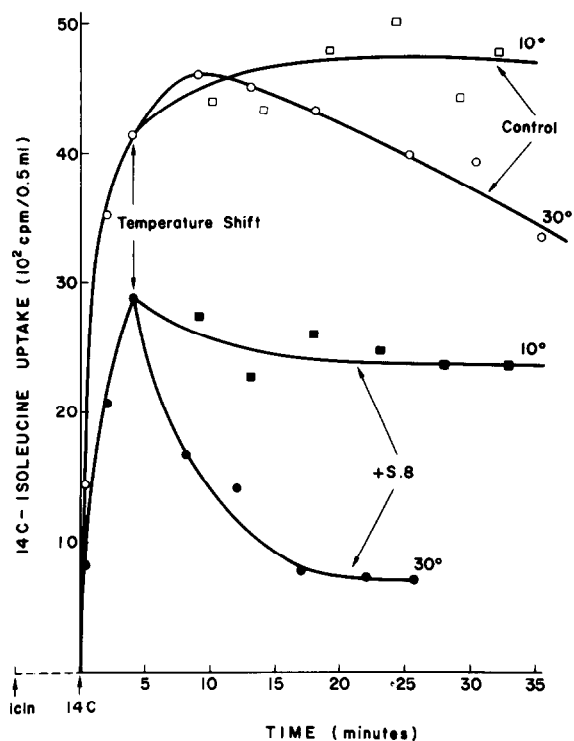


Fig. 6. Effect of the transfer to low temperature on the action of colicin S.8 on the uptake of isoleucine. Cells were incubated at 30°. Survival: 0.036%. Aliquots of the control culture and of the culture with colicin were transferred to 10°, 5 min after addition of [<sup>14</sup>C]isoleucine. Samples from the cultures at the two temperatures were taken at different times.

colicin S.8 at multiplicities of colicin that give very low survival values (0.03%) determines very little inhibition on uptake or incorporation, at least during the first 30 min after addition of the colicin (fig. 5B). The change of response of the sensitive cell with the change in temperature is manifested almost instantaneously, since as seen in fig. 6, the efflux of [<sup>14</sup>C]isoleucine induced by the action of colicin S.8 at 30° is immediately halted when the culture is transferred to 10°.

The temperature dependence on colicin action may

reflect: i) a requirement for a temperature dependent reaction, ii) a change in the structural state of the membrane, resulting from a change in the conformation of the phospholipids, or iii) a combination of these two types of effect.

Cells of an unsaturated fatty acid auxotroph [16] of *E. coli* grown on elaidate are less sensitive to the biochemical effect of colicin S.8 than oleate grown cells (Nagel de Zwaig and D. Rekarte, unpublished results); this suggests that the fatty acid composition, or state of membrane phospholipids, can affect the response to the action of colicin S.8.

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