ANTIBIOTICS WHICH AFFECT PROTEIN SYNTHESIS: THE UPTAKE OF ^{14}C-CHLORAMPHENICOL BY BACTERIA

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Chloramphenicol (CAP) inhibits the growth of Staphylococcus aureus by blocking protein synthesis (1). This result has been confirmed by many workers using various bacterial species and it appears that CAP blocks bacterial protein synthesis at some stage in the transfer of amino acids from transfer-RNA into protein (2).

A study has been made of the uptake and localization of D-threo-(^{14}C methylene)-chloramphenicol (specific activity 9.90 mC/mM) (^{14}C-CAP) in bacteria. Experiments were carried out with S. aureus strain Duncan and Bacillus megaterium strain KM (minimum growth inhibitory concentration 10 µg. CAP/ml. in each case). ^{14}C-CAP was not firmly bound to the bacteria and could be removed by washing with saline (Fig.1), water, buffered salts solution (3) or a solution of 10 µg CAP/ml. This finding is consistent with the known reversibility of the bacteriostatic activity of CAP.

Although prolonged washing caused a marked reduction in the radioactivity present in the bacteria it was hoped that by using defined conditions, specific binding sites for CAP could be found even though the association was reversible. The uptake of ^{14}C-CAP by S. aureus approached the maximum at a concentration of 5 µg.CAP/ml.; increasing the concentration to 80 µg./ml. resulted in a two fold increase in uptake (Fig.2a). The rate of uptake was initially high but fell rapidly and after 2 min. a slower linear rate was established (Fig.2b). The uptake of ^{14}C-CAP was energy

dependent, maximum uptake occurring during incubation of S. aureus in a complex growth medium at 37°C. In a defined medium containing salts

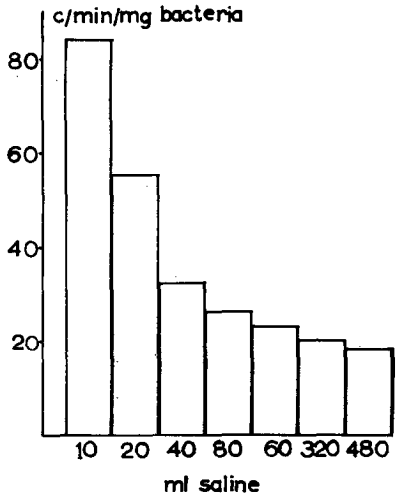

Fig.1. The effect of washing on the retention of ^{14}C-CAP by S. aureus.

Suspensions of S. aureus were incubated in a defined medium (salts solution, glucose, amino acids, pyrimidines and purines) + 5 µg. of ^{14}C-CAP/ml. for 10 min. at 37°C. The bacteria were removed by filtration through membrane filters, washed on the filter with saline and the radioactivity determined.

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solution, glucose, amino acids, pyrimidines and purines (1) the uptake was reduced by 15%. Omission of glucose or amino acids from this medium reduced the uptake by 30%. The uptake of ^{14}C-CAP after exponential growth has ceased, was only 20% of the uptake by bacteria harvested during the exponential growth phase.

The ^{14}C-CAP uptake reported above does not represent the maximum values since some CAP would be removed by washing. CAP uptake was also measured without washing the bacteria and corrections made for the ^{14}C-CAP present in the intercellular fluid.

When S. aureus was broken ultrasonically after incubation in the presence of ^{14}C-CAP and fractionated by centrifugation, all the radioactivity was associated with the 105,000g. pellet ("ribosomes") and supernatant fraction (Table 1). Although the uptake of ^{14}C-CAP by S. aureus increases with

Table 1

Fractionation of *S. aureus* after the uptake of ^{14}C-CAP.

Fraction	c/min. / mg.bacteria	^{14}C-CAP uptake: µg.CAP / mg.bacteria	^{14}C-CAP uptake: molecules CAP / bacteria
1,000 g pellet "whole cells"	(discarded)	-	-
40,000 g pellet "cell walls"	0	0	0
105,000 g pellet "ribosomes"	9	2.25×10^{-3}	6×10^{3}
Soluble fraction	106	2.65×10^{-2}	6×10^{4}
Total	115	2.90×10^{-2}	7×10^{4}
Soluble fraction "Ribosomes"	12	-	-

^{14} *S. aureus* (3 mg./ml.) was incubated in defined medium containing 10 µg. ^{14}C-CAP/ml. The bacteria were removed by centrifugation and the pellet was rinsed 3 times with salts solution, resuspended and the bacteria ultrasonically disintegrated. The broken bacteria were fractionated by centrifugation. A control experiment was carried out in which the bacteria were incubated in the presence of 200 µg. ^{12}C-CAP/ml. and 10 µg. ^{14}C-CAP/ml. were added at the end of the incubation period.

time of incubation, the relative proportions of the radioactivity in these fractions remained constant. However the radioactivity associated with the soluble fraction decreased considerably compared with that in the ribosomal fraction when the bacteria were washed.

The uptake of ^{14}C-CAP was markedly reduced by erythromycin, ostreogrycins A, B and G and streptogramin (a mixture of ostreogrycins A, B and G), but was unaffected by terramycin, aureomycin and puromycin.

In a cell-free system consisting of a suspension of "ribosomes" in

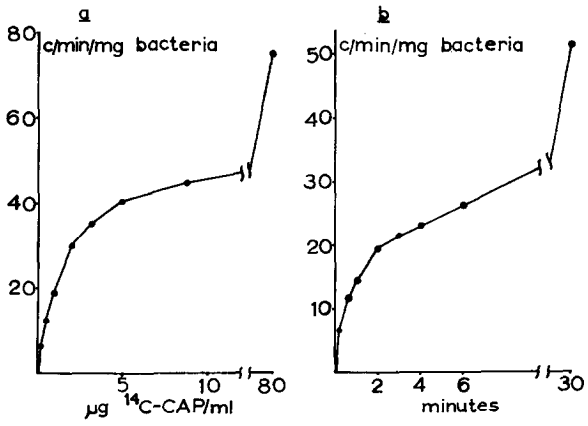

Fig.2a. The effect of ^{14}C-CAP concentration on the uptake by S. aureus.

Fig.2b. Time course of the uptake of 10 μg. ^{14}C-CAP/ml. by S. aureus.

Suspensions of 3 mg. dry wt. S. aureus/ml. in the defined medium were incubated in the presence of ^{14}C-CAP. The uptake of the antibiotic was stopped by adding 200 μg. ^{12}C-CAP and immediately cooling. The bacteria were removed by centrifugation, washed 3 times with salts solution containing 3×10^{-3}M Mg^{++} (3) resuspended in water and radioactivity assayed.

salts solution, the "ribosomes" were found to be saturated at a concentration of 30 μg. ^{14}C-CAP/ml. (Fig.3a). The association was immediate and not energy dependent and was unaffected by the incubation temperature. This association was reversible. The uptake of ^{14}C-CAP into the "ribosomes" fraction was prevented by ^{12}C-CAP, ostreogrycin A or B and erythromycin but was unaffected by terramycin, aureomycin and puromycin. (Fig.3b). Essentially similar results were obtained with B. megaterium.

Specific irreversible binding of ^{14}C-CAP by sensitive strains of S. aureus and B. megaterium has not been found but the results suggest that there is an association of CAP with ribosomes. Erythromycin (4) and streptogramin (5), inhibitors of protein synthesis, prevent this association: on the other hand terramycin, aureomycin and puromycin do not. A close relationship between the binding site and the primary point of action of an antibiotic seems probable. Thus antibiotics which reduce CAP uptake may inhibit the same reaction whereas others, e.g. aureomycin and terramycin, although having a

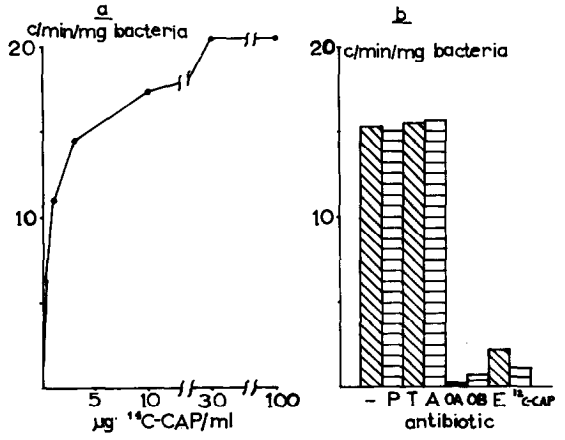

Fig.3a. The effect of ^{14}C-CAP concentration on the uptake by the "ribosomes" of S. aureus.

Fig.3b. The effect of some antibiotics on the uptake of 10 µg. ^{14}C-CAP/ml. by the "ribosomes". Preincubation for 1 min. with 100 µg. antibiotic/ml. T, Terramycin; A, Aureomycin; OA, Ostreogrycin A; OB, Ostreogrycin B; E, Erythromycin; P, Puromycin; ^{12}C-CAP, ^{12}C-chloramphenicol.

A suspension of S. aureus in salts solution was disrupted ultrasonically and centrifuged at 40,000 g. for 20 min. The supernatant fluid containing the ribosomes and soluble fraction was incubated with ^{14}C-CAP. After 5 min. at 4°C the suspension was centrifuged at 105,000 g. for 4 hr. and the radioactivity in the pellet determined. In a control experiment 200 µg. ^{12}C-CAP were present during the incubation period and ^{14}C-CAP was added at the end.

similar overall effect on protein and nucleic acid synthesis may act at a different locus.

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