Presence of Carbonate in a Low-density Fraction of Diaphyseal Bone

USING a method of centrifugation in liquids progressively increasing in specific gravity¹, it is possible to separate, from a sample of bone powder, fractions at various degrees of mineralization². These fractions, which can be recovered quantitatively, can then be analysed by appropriate chemical and biophysical techniques. We used infra-red spectroscopy for the examination of the carbonate present in bone, comparatively for a low specific gravity fraction (1.7 g/c.c.) and a pool of the high specific gravity fractions (1.8–2.3 g/c.c.) derived from diaphyseal bone samples of normal rats. It must be pointed out that less than 0.5 per cent of the samples belonged to the 1.7 gravity fraction. We used a Perkin-Elmer 221 infra-red spectrophotometer equipped with a sodium chloride prism. Potassium bromide pellets were prepared with various concentrations of whole unashed diaphyseal bone after separation.

Fig. 1

Of the three regions of infra-red absorption by carbonate groups³, two were satisfactorily demonstrated (860–875 cm⁻¹ and 1,400–1,500 cm⁻¹) in both the low and the high specific gravity fractions. No absorption was observed at 710 cm⁻¹. Table 1 gives the location and intensity of all the absorption bands and Fig. 1 shows the spectra recorded between 1,300 and 1,700 cm⁻¹. In that region, three carbonate bands can be seen located at 1,506, 1,433, 1,401 cm⁻¹ and 1,501, 1,433, 1,408 cm⁻¹ respectively for the low and the high specific gravity fractions; but their relative intensity differs: in the low specific gravity fraction, the band located at 1,506 cm⁻¹ is more intense than the bands found at 1,433 and 1,401 cm⁻¹, whereas the reverse is true in the high specific gravity pool. In both spectra, the band located at 1,639 cm⁻¹ can be attributed to adsorbed water. The band located at 866 cm⁻¹ was poorly resolved and showed no apparent difference from one spectrum to the other.

Two facts emerged from these results: (a) carbonate is present in both the early calcifying and the fully mineral-

Origin of the band	ν_2 CO ₃ ⁼	ν_2 CO ₃ ⁼	PO ₄ ⁼	H ₂ O
Material				
Low density	1,506 m 1,433 w 1,401 w	868 w	ca 1,050 sb	1,634 s
High density	1,501 vw 1,433 w 1,408 w	868 w	ca 1,050 sb	1,639

ized parts of bone; (b) the change in the relative intensities of the three carbonate bands found in the 1,400–1,500 cm⁻¹ region indicates that carbonate is present in more than one chemical form and/or environment, the proportion of which differs in the low and the high specific gravity fractions⁴⁻⁶.

Chemical analysis for carbon dioxide could not be carried out because only very small amounts of the low density material were available; this, however, appears to be critical before trying to propose any further interpretation of the infra-red data.

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Action of Rifamycin on Incorporation of Amino-acids into Protein in Cell-free Systems from *B. subtilis*

RIFAMYCINS are new antibiotics^{1,2} having a marked inhibitory effect on the growth of many Gram-positive bacteria, including staphylococci^{3,4}, and have already been shown to affect bacterial respiration⁵. In this communication some results are reported concerning the effect of rifamycin SV and rifamycin M₁₄ on the incorporation of amino-acids into protein by cell-free extracts of *B. subtilis* strain A.T.C.C. 6633.

Rifamycins have no effect on amino acyl-adenylate formation as studied by measuring the ATP-³²P pyrophosphate exchange or on amino acyl-RNA formation. Results of these experiments are shown in Tables 1 and 2. Fig. 1 shows that rifamycin does not affect pyruvate kinase activity as measured by the method reported by Bücher *et al.*⁶.

For the examination of the incorporation of L-isoleucine labelled with carbon-14 into proteins by cell-free extracts, *B. subtilis* strain A.T.C.C. 6633 was cultivated in a medium containing 1.2 per cent casamino-acid, 0.3 per cent glucose, 0.1 per cent yeast extract, 0.45 per cent KH₂PO₄, 0.12 per cent MgSO₄, 0.81 per cent NaHPO₄. Cells were collected during logarithmic growth phase and disrupted by grinding with alumina Norton.

Results obtained using crude extracts prepared according to the method of Tissières⁷, and by using washed ribosomes and 105,000g supernatant (S-105) prepared

Complete mixture	Additions	Percentage exchange
"	- amino-acids	55
"	+ 0.0015 μ mole/ml. rifamycin SV	25.5
"	+ 0.015 μ mole/ml. rifamycin SV	56
"	+ 0.015 μ mole/ml. rifamycin SV	55.4
"	+ 0.03 μ mole/ml. rifamycin SV	50.4

The reaction mixture contained the following in μ mole/ml.: 50 *tris*-HCl pH 7.4; 10 MgCl₂; 10 ATP; 10 each of 20 L-amino-acids; 10 ³²P-pyrophosphate (specific activity 14,000 c.p.m./ μ mole); 1.5 mg/ml. homogenate protein. ATP-³²P pyrophosphate exchange was measured as described by Demoss *et al.* (ref. 9). Radioactivity was determined by a windowless gas-flow counter.