suggest that all the half-cystine of the enzyme can be accounted for as thiol groups, and that there is no disulphide bond in the molecule. Recent centrifugation studies showing that the enzyme dissociates into sub-units of molecular weight 40000 on alkylation (Thomson, Eveleigh, Laws & Miles, 1967) and in the presence of high concentrations of denaturant (Yue, Palmieri, Olsen & Kuby, 1967), even in the absence of reducing agent, also strongly support this view.

Creatine kinase from bovine skeletal muscle and bovine brain also consists of two sub-units, which are dissociated by full alkylation, and these enzymes give thiol values very similar to those reported in Table 1 for the rabbit muscle enzyme (Thomson *et al.* 1967). It seems likely therefore that these features are common to all mammalian creatine kinases (Thomson *et al.* 1967; Eppenberger, Dawson & Kaplan, 1967).

The involvement of unreactive thiol groups in maintaining the conformation of proteins has been discussed by Cecil (1963). The possibility that the unreactive thiol groups of creatine kinase are involved in such non-covalent interactions would rationalize the low reactivity of at least some of the six unreactive groups.

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Menaquinone-4 and -5 in a Bacterium

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Menaquinones having side chains containing 7, 8 and 9 isoprene units have been found in bacteria (Pennock, 1966). In a study of the distribution of menaquinones in Micrococcaceae, Jeffries *et al.* (1967) found menaquinones in all strains examined; several distinct patterns, formed by the relative percentages of individual isoprenologues (menaquinones-6, -7, -8 and -9) were revealed. Whereas menaquinone-6 was found in most of the non-pigmented species of *Micrococcus* examined, this isoprenologue was absent from *Staphylococcus* species. The subject of this communication is a strain of *Staphylococcus aureus* (N.C.T.C. 8511, propagating strain 53 of the International Phage Typing series) supplied by Dr Ruth Z. Korman (Cornell University), who described the production of pleiotropic coagulase-negative mutants from it (Korman, 1963). Two other cultures of strain N.C.T.C. 8511, received from different Laboratories

in the United Kingdom, were also examined and found to be of bacteriophage type identical with that of the Korman strain.

Staphylococci were grown on the surface of nutrient agar (blood-agar base; Oxoid Ltd., London, E.C. 4) at 37° and harvested after 48 hr. incubation, to give at least 1.6 g. wet wt. of cells. After extraction into ethanol, the lipids were chromatographed on chromatoplates [50% (v/v) benzene in light petroleum against silica gel G], as described by Jeffries *et al.* (1967), to separate the combined isoprenologue fraction. This was eluted with ether, and the isoprenologues were separated by reverse-phase partition paper chromatography [aq. 95% (v/v) ethanol against liquid paraffin] as described by Horth *et al.* (1966). The developed chromatograms were examined under u.v. light and the individual menaquinone bands were eluted separately into ether. They were

Table 1. *Menaquinone patterns of Staphylococcus aureus*

Values for total menaquinone are given as means $\pm$ S.D., with the numbers of assays in parentheses. Although the variation found in total menaquinone was often considerable, there was little variation in the percentages of the individual isoprenologues. MK, Menaquinone; N.D., not detected. N.C.T.C., National Collection of Type Cultures, London.

Strain and source	Total MK ($\mu\text{g./g. wet wt.}$)	Mean percentage of individual isoprenologues					
		MK-9	MK-8	MK-7	MK-6	MK-5	MK-4
Variety of bacteriophage types, antibiotic-sensitivity patterns and sources (18 strains examined)	256 $\pm$ 83 (1)	13	73	14	N.D.	N.D.	N.D.
N.C.T.C. 8511 (Korman)	98 $\pm$ 24 (5)	N.D.	17	48	11	15	9
N.C.T.C. 8511 (Baird-Parker)	233 $\pm$ 148 (3)	7	77	15	1	N.D.	N.D.
N.C.T.C. 8511 (Asheshov)	257 $\pm$ 140 (3)	9	72	18	1	N.D.	N.D.

characterized by co-chromatography with authentic menaquinones-6, -7, -8 and -9 as markers. R_M values were determined for the R_F values of the isoprenologues, and ΔR_M values were calculated for the individual members of the isoprenologues series by the method of Green, Marcinkiewicz & McHale (1963). The quantity of each menaquinone found was measured as described by Jeffries *et al.* (1967).

The menaquinone fraction of strain N.C.T.C. 8511 (Korman culture) was separated into five substances, three of which (R_F values 0.342, 0.223 and 0.152) were chromatographically identical with menaquinones-6, -7 and -8, found previously in Micrococcaceae, and two of which (R_F values 0.614 and 0.480) were substances not previously observed in this Family. From their calculated R_M values and mean ΔR_M value for the isoprenologous series (0.250 in this system), the two new substances were assigned the probable structures of menaquinone-4 and menaquinone-5 respectively. When the total menaquinone fraction was chromatographed, together with authentic markers, in a second system [aq. 95% (v/v) acetone against petroleum jelly on Whatman no. 1 paper] and the R_M and ΔR_M values were calculated for the five compounds, these assignments were confirmed (R_F values in this system of menaquinones-4, -5, -6, -7 and -8 respectively: 0.643, 0.533, 0.426, 0.316 and 0.231; ΔR_M 0.190). The menaquinones-4 and -5 were purified further by adsorption paper chromatography on ZnCO_3 -impregnated paper and their u.v. spectra were then typical of menaquinones (λ_{max} 243, 249, 262, 271 and 330 m μ ; λ_{min} 246, 255, 267 and 295 m μ in cyclohexane). Each substance, on reduction with KBH_4 in ethanol, gave a typical menaquinol spectrum (λ_{max} 246 m μ).

The atypical menaquinone pattern given by the Korman culture of strain N.C.T.C. 8511 (the presence of menaquinones-4 and -5, the absence of menaquinone-9, and the altered menaquinone-7/menaquinone-8 ratio) are shown in Table 1. The two other cultures of strain N.C.T.C. 8511 showed

patterns similar to each other, not markedly different from that typical of most strains of *Staph. aureus* and strikingly different from that of the Korman culture. The isolation, from micro-organisms, of menaquinone isoprenologues with C_{20} and C_{25} side chains has not, as far as we are aware, been recorded previously, although Lester, White & Smith (1964) described demethyl-menaquinone-5. It has been suggested that menaquinone-4 is the physiological form of vitamin K in mammals and birds (Billeter & Martius, 1960; Billeter, Bollinger & Martius, 1964). Martius & Esser (1959) gave menadione to vitamin K-deficient chicks and isolated menaquinone-4 from the liver; in germ-free rats, however, menaquinone-4 was not synthesized from menadione, suggesting that the intestinal micro-flora may play a part in this synthesis (Gustafsson, Daft, McDaniel, Smith & Fitzgerald, 1962).

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