

perhaps as far as Angola, and the eastern Mediterranean as far as the Adriatic, between shallow littoral and 1,200 m. These two records, both from littoral waters between L.W.N. and L.W.S., extend the known range of this southern species by about 1° northwards.

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MICROBIOLOGY

Identification of the Bacteriostatic Component of 'Scotch' Brand of Cellulose Adhesive Tape

WE have recently shown that the 'Scotch' brand of cellulose adhesive tape of the Minnesota Mining and Manufacturing (Australia) Pty., Ltd., contains a volatile component the antibacterial activity of which is virtually restricted to coagulase-positive strains of *Staphylococcus aureus*¹. In view of this unusual specificity it seemed important to identify the active agent.

The anti-staphylococcal component was extracted from the tape by a number of organic solvents, but not by water. For chromatography, approximately 30 g of tape was extracted with 100 ml. of boiling alcohol and the extract concentrated to a final volume of about 10 ml. About 0.2 ml. of extract was applied to strips of Whatman 3 MM paper 4 cm wide and developed with a variety of solvents. After drying the position of the active component was detected by cutting the strips into short lengths which were then placed in the lids of nutrient agar plates inoculated with *Staphylococcus aureus* E169¹; the absence of growth on an incubated plate indicated the presence of the active component in that section of the paper strip. With all solvents used, the active component either remained at the origin or moved with the solvent front.

Because of the apparent insolubility of the active component in water and its behaviour on conventional paper chromatograms, separation was attempted by reversed phase chromatography, using Whatman 3 MM paper impregnated with paraffin oil, and methanol/acetone/paraffin oil (3:1:1) as the mobile phase². Under these conditions the active component was confined within R_F values of 0.4 and 0.5. Examination of strips under ultra-violet light revealed a fluorescent area extending for some distance before and behind the area within which the active component was confined. In order to remove this interfering fluorescent material, descending chromatograms were developed overnight with acetic acid/butanol/water (the aqueous phase of a 1:4:5 mixture) in which the active component was immobile. After drying, the strips were impregnated with paraffin oil and then developed with the methanol-acetone-paraffin oil solvent. Under ultra-violet light these strips showed only a slight fluorescence along the length of the paper, with an absorbing spot containing all the anti-staphylococcal activity having an R_F value of 0.46. The active spot gave no reaction with a number of detecting reagents. However, reducing activity was shown with both alkaline silver nitrate and a mixture of ferric chloride and potassium ferri-cyanide; these reagents showed brown and blue colorations respectively, both streaking towards the front. In addition, strong oxidizing activity was shown with alcoholic leuco-methylene blue. These results suggested that the active component could exist in both a reduced and an oxidized state, forming a readily reversible oxidation-reduction system.

Examination of patents³ covering the manufacture of 'Scotch' brand cellulose adhesive tape revealed that the adhesive layer of the tape contains, as an antioxidant, either 2,2'-methylene-bis (4-methyl-6-tert-butylphenol) or 2,5-di-tert-amylhydroquinone. When tested for anti-

staphylococcal activity by direct addition to the lids of inoculated plates, only the latter substance was active.

Reversed phase chromatography of 2,5-di-tert-amylhydroquinone and 2,5-di-tert-amyl-*p*-benzoquinone (prepared by oxidation of the hydroquinone with silver oxide) gave major spots with R_F values of 0.9 and 0.5 respectively; both substances showed minor spots of the corresponding oxidized and reduced forms. These results suggested that the anti-staphylococcal activity of the tape was due to 2,5-di-tert-amyl-*p*-benzoquinone. This was confirmed by tests in which variants of *Staphylococcus aureus* E169 selected for resistance to either the tape or to 2,5-di-tert-amyl-*p*-benzoquinone were found to be completely cross-resistant.

The lowest inhibitory concentration of 2,5-di-tert-amyl-*p*-benzoquinone for *Staphylococcus aureus* E169 was discovered by preparing serial dilutions of the inhibitor in nutrient agar; as little as 3 µg/ml. was sufficient to reduce the colony count to less than 0.01 per cent of that on control plates. In comparison, under similar conditions a concentration of 300 µg/ml. of *p*-benzoquinone was required to produce the same reduction in colony count.

Oxford and Raistrick⁴ investigated the activity of several *p*-benzoquinones against a limited range of bacterial species; they found that strains of *Staphylococcus aureus*, *Bacillus anthracis*, and *Vibrio cholerae* were particularly susceptible. In view of the role of naturally-occurring quinones in biological electron transport⁵ and the inhibitory effect of *p*-benzoquinones on this system⁶, it would be of interest to determine more precisely the 'spectrum' of activity of *p*-benzoquinones in relation to the distribution of ubiquinone and menaquinone in bacteria.

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CYTOLOGY

Chromosome Duplication and the Cell Cycle in Lens Epithelium

PREVIOUS experiments have shown that a penetrating needle injury to the central area of rabbit lens epithelium *in vivo* produces a response in which a large number of cells synthesize DNA and divide¹. This response begins characteristically at the site of injury and progresses farther and farther away from the wound with time. Experiments have indicated that DNA synthesis is initiated in the cells closest to the wound at about 12-14 h after injury². Mitosis is first seen at about 24 h after injury. The rate at which the wave of DNA synthesis travels was calculated to be about 17 µ/h. The typical picture obtained at 48 h after injury shows a band of cells which are synthesizing DNA and completely encircle the wound. Another band of mitotic figures is seen encircling the wound and lying between the DNA synthesis band and the injury but close to the DNA synthesis band. Frequently, when the injuries are larger (these can be obtained by using a larger needle) a 'second wave' of DNA synthesis and mitosis can be seen.