as indicated in Table I, is generally ineffective against fungi, it inhibits a number of bacteria. In these respects it is similar to the antibiotic novobiocin (a 4-hydroxy-coumarin derivative), which is active against bacteria but has no action on fungi[1,6].

The antimicrobial effectiveness of these coumarins was determined by comparison of pour plates containing about 200 bacterial or yeast cells and 500 ppm of the coumarin against control plates without added coumarin, after incubation at 28 °C for 1–4 days. Mold growth inhibition was observed by spot inoculation of the surface of the prepared medium. Bacteria were grown on plate count agar, and yeasts and molds on potato dextrose agar. LEDERBERG's replica plating technique[7] was also employed, master plates of bacteria and yeasts being used to inoculate the velveteen cloth from which the test plates were then prepared[8].

Zusammenfassung. Es wird der Einfluss einer Alkylierung auf die antimikrobielle Wirkung von 7-Hydroxy- und 4-Hydroxy-Cumarin dargestellt.

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PRO EXPERIMENTIS

Tissue Sodium and Potassium: Direct Detection in the Electron Microscope

Previous attempts to localize sodium or potassium in biological tissue have usually depended on electron histochemical techniques[1,2]. These methods are unsatisfactory for two reasons. First it is reasonable to expect that most, if not all, of the sodium and potassium ions would be washed out during the tissue preparation techniques. Secondly there are doubts about the specificity of the histochemical reaction[3].

We have utilized a method which is more direct and should be capable of adaptation to a quantitative technique. We cut ultrathin frozen sections through corneal stroma and stored them freeze dried as described previously[4]. The sections were transported to the high resolution analytical electron microscope, A.E.I. EMMA-4 and analyzed for sodium and potassium. EMMA-4 operates in the following mode. High energy electrons in the microscope beam strike the atoms in the specimen section and a fraction undergo inelastic collisions which excite or ionize the atoms. As electrons drop back into the empty orbitals they emit characteristic X-rays. These emitted X-rays are analyzed in 2 spectrometers mounted on the side of the column. Scanned X-ray spectra for the K lines (quanta emitted due to the relaxation of electrons from the L shell into the K shell) of sodium and potassium emanating from our frozen sections are shown in Figures 1 and 2. A representative area of frozen section through corneal stroma is shown in Figure 3. Similar analysis of ultrathin sections through corneal stroma conventionally processed for electron microscopy[5] and embedded in Araldite showed no detectable quantities of sodium or potassium.

Stromal sodium and potassium concentrations are measured after acid extraction at 172 m*M* and 22 m*M* respectively[6]. It is reasonable to presume that as the

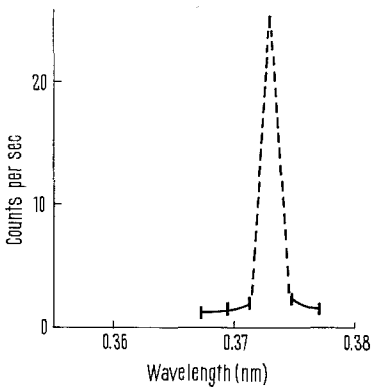

Fig. 1. Emission spectrum of the potassium Kα-line emanating from frozen sections through non-fixed corneal stroma. Section thickness estimated at 150 nm; probable potassium concentration in the stroma is 22 m*M*. Emitted X-rays were analyzed by a lithium fluoride crystal set at the appropriate Bragg angle, through a 3 μm thick Mylar window. Energy of electrons bombarding the section, 40 keV; probe size 0.8 μm. Vertical lines represent twice the standard error on the mean of each reading.

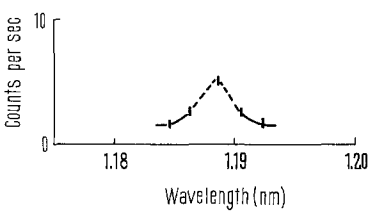

Fig. 2. Emission spectrum of the sodium Kα-line counted simultaneously with that in Figure 1. Probable sodium concentration in corneal stroma is 172 m*M*. Detecting crystal, potassium ammonium phthalate. Vertical lines represent twice the standard error on the mean of each reading.

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physiological bathing medium has the much lower potassium concentration of 4.7 mM[7], under normal conditions most of the stromal potassium would be found in the keratocytes. Although the limit of resolution of analysis is as low as 0.1 μm, the potassium in our sections appeared to be uniformly distributed and not localized in the keratocytes. This strongly suggests that during transfer of the sections they may become rehydrated when the potassium would diffuse through the tissue. It is not possible to know the permeability of the keratocyte cell membrane in an ultrathin section but if one calculates an extreme case when the permeability of the membrane is so high that its presence may be ignored, then it can easily be shown that in a cell 1 μm thick, 50% of the potassium would diffuse out in about 10^{-4} sec.

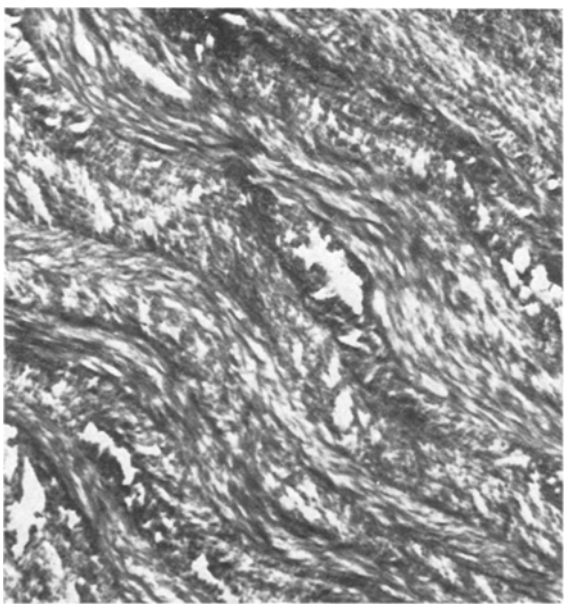

Fig. 3. Ultrathin section through unfixed, frozen corneal stroma showing the lamellae of collagen fibres. × 2500.

Two problems remain to be solved before intracellular distributions of sodium and potassium may be determined. First, the sensitivity of the X-ray activation analyzer to particular atoms must be calibrated and secondly the sections must remain frozen or dessicated throughout the preparation procedure. It is to be hoped that the diffusion of salts through dessicated biological material is negligible[8].

Résumé. Une nouvelle technique pour découvrir sodium et potassium en sections glacés de tissus biologiques est décrivée.

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Die Isolierung von Ribonukleinsäuren aus menschlichen Knochenmarkzellen

Die hohe Aktivität von Ribonukleasen in den Zellen des blutbildenden Systems ist die Ursache dafür, dass unabgebaute Ribonukleinsäuren nach den herkömmlichen Methoden aus diesen Zellen nicht isoliert werden konnten[1]. Erst die Auffindung von Substanzen, die dieses Enzym binden (Bentonit) oder inaktivieren (Natriumdodecylsulfat), hat die Präparation hochmolekularer Ribonukleinsäuren aus menschlichen Leukozyten ermöglicht[2,3]. Nach ähnlichen Verfahren konnten wir nun erstmals weitgehend unabgebaute, biologisch aktive Ribonukleinsäuren aus menschlichen Knochenmarkzellen erhalten.

Die Entnahme des Knochenmarks gesunder Spender erfolgte in Intubationsnarkose aus dem rechten und linken Darmbein und aus dem Sternum. Insgesamt 27 Aspirationen ergaben 420 ml Mark, die in einem eisgekühlten Gemisch aus 200 ml NaCl 0,15 M, 610 ml Phenol 90% und 16 ml Bentonit[4] (10%, gelöst in NaCl 0,15 M) gesammelt wurden. Die Extraktion der cytoplasmatischen RNS erfolgte durch 15 min langes Rühren bei 4 °C. Nach dem Abzentrifugieren der Phenolphase und zweimaligen Enteiweissen des Überstandes (340 ml) mit je 170 ml Phenol und Chloroform fiel bei Zusatz von 340 mg NaCl und 720 ml Äthanol 96 mg RNS aus. Die bei der ersten Zentrifugation erhaltene Interphase (290 ml) wurde dann zur Isolierung der m-RNS mit 218 ml Phenol 90%, 290 ml NaCl 0,15 M und 2,9 g Natriumdodecylsulfat 15 min bei 65 °C nachextrahiert. Die weitere Aufarbeitung erfolgte wie für die cytoplasmatische RNS beschrieben und ergab 26 mg RNS.

Die durch Phenolextraktion in der Kälte erhaltene Ribonukleinsäurefraktion wies bei der Trennung im Saccharosegradienten Gipfel bei etwa 30S, 19S und 5S auf (Figur) und ist somit in ihrem Sedimentationsverhalten weitgehend mit den Präparationen aus anderen Geweben vergleichbar. Das Verhältnis der Extinktionen bei 260 und 280 nm hatte bereits nach der zweiten Enteiweissung mit Phenol-Chloroform einen Wert (E_{280}: E_{260} = 0,48), der auf weitgehende Proteinfreiheit schliessen liess[6].

Die Untersuchung der 65°-Fraktion im Saccharosegradienten ergab das Vorliegen eines polydispersen Produktes, das vorwiegend aus Fraktionen bestand, die Sedimentationskoeffizienten zwischen 2 und 19S hatten

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