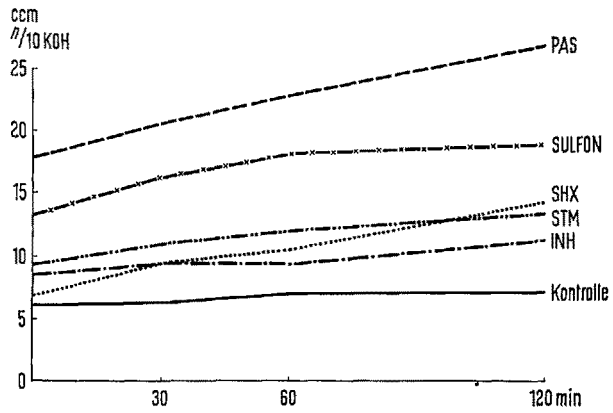

x Zeit in min. y Anzahl ccm n/10 KOH in der Alkohollösung, welche wir für die Titration der Spaltprodukte des Olivenöls (Fettsäuren) gebraucht haben.

30 Versuchen, die bei jedem Antituberkulotikum durchgeführt worden sind. Der Kontrollversuch wurde unter gleichen Bedingungen, jedoch ohne Antituberkulotikum durchgeführt.

Summary. The direct effect of any antituberculotics on pancreatic lipase was studied by the modified method of WILLSTÄTTER. The antituberculotics were found to activate the pancreatic lipase in the presence of Ca^{++}. Any cations, especially copper, decreases the lipase activity. Increasing antituberculotics concentration is accompanied by an increasing effect on the lipolytic action of lipase.

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Decontamination of Pleuropneumonia-Like Organism (PPLO) Infected Tissue Cultures

The problem of decontaminating cell lines infected with pleuropneumonia-like organisms (PPLO) has been approached using treatment with antibiotics[1], heat[2], or immune sera[3]. Results have been successful in the instances recorded, but the routine application of these methods has often failed. In our hands, both the thermal treatment at 41°C for 18 h, repeated several times, or the prolonged use of kanamycin (up to 600 µg/ml) and tetracycline (up to 25 µg/ml) failed to eradicate the contaminants from a line of strain L cells, originally obtained from Dr. W. R. EARLE. We decided, therefore, to test the specific antibiotic sensitivity of the PPLO isolated from this line as a guide to the selection of appropriate antibiotics. Sensitivity discs (Baltimore Biological Laboratory) were placed on plates of Bacto PPLO agar (70 parts), horse serum (20 parts), yeast extract Difco 25% (10 parts), heavily seeded with contaminated tissue culture fluids. Zones of inhibition were recorded after 7 days of incubation at 37°C. Growth of PPLO colonies was inhibited by novobiocin and chloromycetin (5 µg discs), but not by kanamycin, tetracycline, vancomycin (5 µg discs), erythromycin (2 µg discs), bacitracin (10 unit discs) and polymyxin B (50 unit discs).

The maximum non-toxic level of the two active compounds was determined and the following concentrations were eventually chosen for treatment: chloromycetin (diagnostic reagent, Chas. Pfizer & Co., Inc., Brooklyn, N.Y.), 10 µg/ml, and novobiocin, 50 µg/ml. The decontaminating procedure was carried out by establishing three cell lines: one treated with novobiocin, one with chloromycetin, and the third with both antibiotics at the concentration mentioned above.

To assess the efficacy of the decontamination procedures, transfers of the antibiotic-treated lines were carried out and each time sublines were derived to be maintained without antibiotics. When a confluent monolayer had developed, the antibiotic-free cultures were tested for PPLO by the colorimetric method based on the arginine deiminase activity[4], a test which we found to be rapid and sensitive. The PPLO-contaminated cultures were discarded. The ones which showed no evidence of PPLO were further propagated without antibiotics and tested at each transfer. When antibiotic-free sublines were consistently negative for 4 or more transfers, the treated cells were considered decontaminated from the time that the first PPLO-free subline was derived.

The results of this study showed that 8 days of treatment with novobiocin alone was sufficient to eradicate the contaminants. On the contrary, the line treated with chloromycetin was still heavily contaminated after 4 weeks. A disc sensitivity test performed at the end of this period showed that the contaminants were resistant to this antibiotic but not to novobiocin. The line treated with novobiocin and chloromycetin was decontaminated in 10 days.

In summary, the PPLO isolated in our laboratory were resistant to the most widely used antibiotics, namely kanamycin and tetracycline, at the time of the first sensitivity test and became resistant to chloromycetin, one of the two antibiotics used in the decontaminating treatment. The other, novobiocin, was effective in eradicating the contaminants in two different lines in 8 to 10 days.

As a general procedure for decontaminating PPLO-infected tissue cultures, a preliminary antibiotic sensitivity test is recommended. The use of antibiotics to which PPLO strains may easily become resistant, such as chloromycetin, kanamycin and tetracycline, should be avoided. To prevent selection of resistant mutants by the use of low concentrations of antibiotics, a test for the highest non-toxic doses should be performed in cultures different from the ones which will be eventually treated. The use of

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appropriate antibiotic mixtures should further reduce the chances of selecting resistant mutants. A scheme such as the one used in this study seems to be effective and practical to assess the efficacy of a given treatment for the decontamination of infected cell lines[5].

Zusammenfassung. PPLO-verunreinigte Zellkulturen wurden mit Novobiocin® PPLO-eliminierend behandelt. Nach dem Empfindlichkeitstest folgte die Bestimmung der Toxizität dieses Antibiotikums für Gewebekulturzellen. Anschliessend wurden maximale, für Zellen nichttoxische Konzentrationen verwendet. Die entwickelte Methode ermöglicht, den Wirkungsgrad dieser antibiotischen Behandlung zu bestimmen.

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ATP Determination with the Tricarb Scintillation Counter[1]

The most sensitive method for the determination of ATP is the measuring of light output in the presence of luciferin-luciferase[2]. The light emitted can be measured by fluorometer or by quantum counter. We wish to report on the adaptation of a liquid scintillation counter for this purpose. Essentially, this is an extremely sensitive quantum counter method. A Tricarb Automatic Liquid Scintillation counter Model 314EX (Packard) was set for tritium counting at 5 sec counting time. Luciferin-luciferase (0.2 ml) were prepared[2], put into glass counting vials and distilled water added in sufficient quantity to bring the total volume to 2 ml after the addition of ATP solution. Exactly 5 sec after the addition of ATP solution, mixing and putting the vials into the proper compartments of the counter, the Tricarb is switched to 'repeat counting'. Counting begins 20 sec after zero time with 15 sec intermission for print-out. Measuring is concluded after 6 cycles (approximately 2 min) by switching to 'stop'. The result is extrapolated to zero time on graph paper, but since the decay is usually no more than a few % per counting cycle, the first count is accurate enough for most work. Under the above conditions 10^{-10} mol ATP gives 10^3–10^4 counts depending on the enzyme preparation. The background of 2 to 3 counts is negligible. The counts are a direct function of approximately the square of ATP concentration. Thus, a calibration curve has to be prepared for each series on log-log graph paper, usually within the limits of 10^{-11}–10^{-9} mol ATP. By switching the counter from 'coincidence' to 'single channel' the sensitivity can be increased about 300 times. In this case the counts are linearly related to the concentration, but the background is high. However, there is generally no need for such extreme sensitivity.

For extraction of ATP from biological tissues, the hot water method[3] was found to be suitable.

Zusammenfassung. Die Arbeit beschreibt eine neue Technik zur Bestimmung geringster Mengen von ATP (10–11 Mol und weniger) mit der Luciferin-Luciferase-Methode. Die dabei erzeugten Impulse werden mit einem Scintillationszähler gemessen.

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[1] Aided by a generous donation from Mrs. D. TEPERMAN, Canada, in memory of her late husband, JOSEPH TEPERMAN.

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Volume Analysis of Liquid Droplets by a Rapid Photographic Method

In the course of work on a spraying process against agricultural crop pests, difficulties were encountered in measuring diameters of individual drops[1]. This led to an attempt to overcome the discomfort involved in the usual microscopic evaluation by photographing the drops. To begin with, a number of drops from the original spray were sampled on transparent glass slides. The slide, covered with drops, was inserted into a photographic enlarging machine in the place ordinarily occupied by the negative film. Regular illumination of the drops yielded only poor information about the periphery of the spread drops. Changes were therefore introduced into the usual

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