

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 lines⁵.

Zusammenfassung. PPLO-verunreinigte Zellkulturen wurden mit Novobiocin® PPLO-eliminiert behandelt. Nach dem Empfindlichkeitstest folgte die Bestimmung der Toxizität dieses Antibiotikums für Gewebekulturen. Anschliessend wurden maximale, für Zellen nicht-toxische Konzentrationen verwendet. Die entwickelte

Methode ermöglicht, den Wirkungsgrad dieser antibiotischen Behandlung zu bestimmen.

P. BALDUZZI and R. J. CHARBONNEAU

M. Herbert Eisenhart Tissue Culture Laboratory, Department of Microbiology, University of Rochester School of Medicine and Dentistry, Rochester (New York USA), July 30, 1964.

⁵ This work has been supported by grants from the National Cancer Institute, U.S. Public Health Service (CA 05206-04), and from the United Funds of Clayton and Alexandria Bay, New York.

ATP Determination with the Tricarb Scintillation Counter¹

The most sensitive method for the determination of ATP is the measuring of light output in the presence of luciferin-luciferase². 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 prepared³, 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 method³ 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.

E. TAL, S. DIKSTEIN,
and F. G. SULMAN

Department of Applied Pharmacology, School of Pharmacy, Hebrew University, Jerusalem (Israel), June 26, 1964.

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

² B. L. STREHLER and J. R. TOTTER, *Arch. Biochem. Biophys.* **40**, 28 (1952).

³ B. L. STREHLER, in *Methods of Enzymatic Analysis* (Ed. H. V. BERGMAYER, Academic Press, New York 1963), p. 559.

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 drops¹. 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

¹ B. MAKSYMUK, *J. econ. Entomol.* **57**, 16 (1964).

technique of illumination: when a perforated metal sheet was inserted between the light source and the slide, regular patterns appeared within the images of the drops (Figure 1). Drs. M. D. BITRON and G. AILAM of this Institute brought to our attention that this phenomenon might be utilized for the determination of the curvatures of the drops on a slide and hence for the estimation of their volumes. The whole apparatus is schematically described in Figure 2, and ray tracing in Figure 3.

Being of small diameter and significant curvature, any droplet may be considered as a miniature spherical lens of a very short focal length as compared with the focal length of the lens system E. The image of the drop on the photographic plate F, therefore, is composed of the pictures of two separate objects: one object is the periphery of the drop, and the other one is the image of the scattered light source, obtained at about the focus of the drop. The magnitude of the second object is defined by the optical properties of the droplet-lens. Since the distances of both objects from the lens system differ only slightly, their linear magnification at the final image is almost identical. Hence, this image defines not only the diameter but also the optical properties of the droplet. Simple geometrical considerations show that for small drops (up to half the distance between two neighbouring light beams at the grid) the number of light spots which occur within the drop's image is independent of its diameter. The only factor which affects this number is the angle of contact of the drop with the slide. This angle, in combination with the diameter of the drop defines its volume.

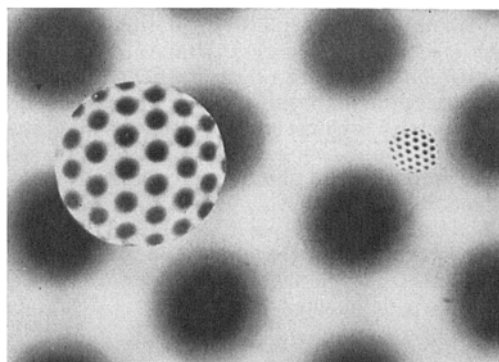


Fig. 1. Enlarged image of the droplets on slide; linear elongation ($\times 10$).

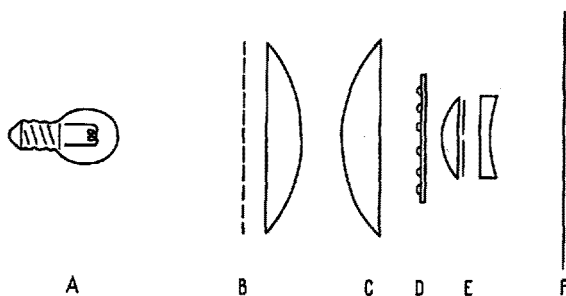


Fig. 2. Schematic description of the photographic system. A - light source; B - perforated metal sheet (grid); C - condensing lenses; D - glass slide with droplets; E - objective lens system; F - photographic plate.

The latter may therefore be calculated from the values of the actual diameter (obtained by dividing the measured diameter in the picture by the linear magnification factor of the enlarging apparatus), and the number of spots. To simplify the calculation, a mathematical relation was developed defining the number of spots as a function of the angle of contact, and the volume as a function of both the spread diameter and the angle.

$$S = \frac{2ac}{p} \left[\frac{\sqrt{\frac{1}{\sin^2 \alpha} - 1} - \sqrt{\frac{1}{n^2 \sin^2 \alpha} - 1}}{\sqrt{\frac{1}{\sin^2 \alpha} - 1} \sqrt{\frac{1}{n^2 \sin^2 \alpha} - 1 + 1}} \right],$$

$$V = \frac{\pi d^3}{3 n^3 \sin^2 \alpha} (2 - 3 \cos \alpha + \cos^3 \alpha),$$

where S = number of spots along the diameter of the image of the drop in the picture, a is the distance of the slide from the grid B , c is the specific factor of the condenser C , p is the separation between two neighboring

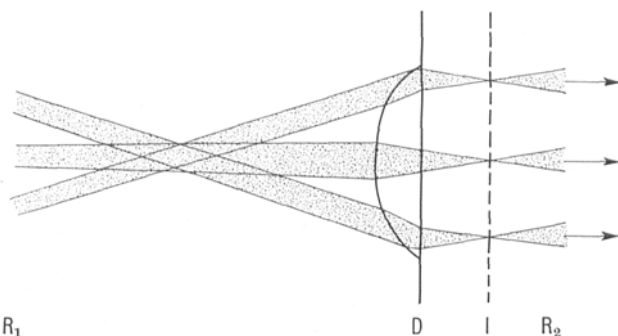


Fig. 3. Light path through a single droplet. R - light beam from the source; I - image of the source; D - droplet; R₂ - light beams leaving the droplet towards lens system E.

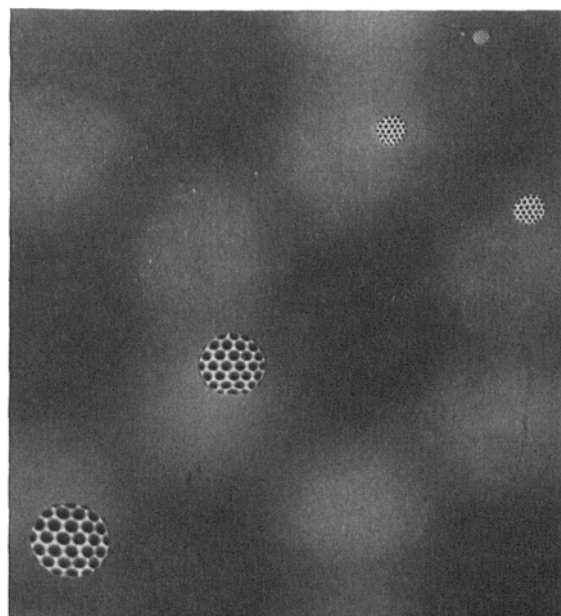


Fig. 4. Enlarged image of the droplets on slide; linear elongation ($\times 20$).

light beams at the grid, α is the angle of contact of the drop with the slide, v is the volume of an individual droplet, d is the diameter of the droplet as measured in the picture, m is the magnification factor of the lens system E , and n is the index of refraction of the liquid of the droplet.

An enlarged picture of the dotted droplets is shown in Figure 4. The droplet sizes and curvatures as measured by this method are well correlated with those obtained by direct microscopic observation, by weighing or by chemical analysis of the droplets.

The significance of this method goes beyond the field for which it was developed: the technique can be applied to analysis of any spray, in food drying, fuel injection, etc. It may also be used for the determination of various values in the state of contact of two transparent bodies, such as the angle of contact between two immiscible liquids, surface tension, etc.

A more detailed paper including the theoretical and mathematical considerations underlying this method is currently in preparation.

Zusammenfassung. Krümmungen und Volumen einzelner Flüssigkeitströpfchen wurden mittels photographischer Methode bestimmt. Das Objekt wurde durch regelmässig streuende Lichtquelle beleuchtet und das Bild auf photographischer Platte festgehalten. Die regelmässigen Muster in den Tröpfchenbildern konnten zur Berechnung des Berührungszustandes der Tröpfchen mit dem Objektträger benutzt werden.

O. NOVICK and E. SHALEM

Israel Institute for Biological Research, Ness Ziona (Israel), July 1, 1964.

Ein Zellhomogenisator nach dem Prinzip von MERKENSCHLAGER und SCHLOSSMANN

Im Zellhomogenisator nach MERKENSCHLAGER et al.¹ werden die Zellen in Suspension mit Glasperlen durch eine sehr schnelle, exzentrische Drehbewegung des Gefässes innerhalb $\frac{1}{4}$ –2 min zerschlagen. Um eine Denaturierung der Eiweisse durch Erwärmung zu vermeiden, wird während des Schüttelprozesses stark gekühlt.

Figur 1 zeigt die Abbildung des neuen Homogenisators. An Hand von Figur 2 wird das Funktionieren der Apparatur erläutert. Der Drehstrommotor (1) (2800 Umdr./min, 50 Watt) treibt das Schwungrad (2), in welchem eine Büchse (3) exzentrisch gelagert ist. Die Büchse dient zur Aufnahme der Flasche mit Zellsuspension und Glasperlen. Sie wird durch den Stift (4) daran gehindert, um ihre eigene Achse zu drehen. Ein Schutzrohr (5), das am Motor befestigt ist, umgibt alle bewegten Teile und umschliesst ein Kugellager (6), in welchem die Schwungmasse (2) abgestützt ist.

Die Motorwelle ist durchbohrt und enthält ein Kapillarrohr (7), das stillsteht und mit Kugellagern in der drehenden Achse gehalten ist. Durch dieses wird zur Kühlung der Zellsuspension flüssige Kohlensäure aus einer Druckflasche mit Steigrohr in die Büchse (2) eingepresst. Der Expansionsraum in der Büchse ist gegen die Motorwelle und die Lager durch die Teflonbuchse (8) und den Ring (9) abgedichtet. Um zu verhindern, dass die Büchse aussen vereist, ist sie innen mit einem längsgerippten Polyäthylenmantel ausgekleidet (12). Die Flasche mit der Zellsuspension kann von vorne in den Mantel eingeschoben und mit gelochtem Deckel (10) gesichert werden. Die elastischen Rippen halten die Flasche fest, bedecken aber nur 20% ihrer Oberfläche, so dass ein guter Wärmeaustausch zwischen Flasche und Kühlgas, das zwischen den Rippen durchströmt, gewährleistet ist.

Der Stift (4) und seine Lagerung sind einer sehr grossen Beanspruchung ausgesetzt. Er ist aus hochlegiertem Konstruktionsstahl hergestellt und gleitet zwischen zwei Kugellagern (11). Die bewegten Massen sind sorgfältig ausbalanciert, so dass die Erschütterungen gering sind.

Das Gerät ist auf einer massiven Grundplatte (13) mit Gummifüssen befestigt. Da die Homogenisierungszeit für reproduzierbare Resultate genau eingehalten werden muss, wird sie automatisch durch einen Impuls gesteuert. Die hierfür erforderlichen elektrischen Elemente sind auf der Unterseite der Grundplatte angeordnet.

Prüfung des Homogenisators. Um Anhaltspunkte über die Leistungsfähigkeit des beschriebenen Homogenisators zu erhalten, wurden Versuche mit Hefe durchgeführt. Hefe gilt als ein verhältnismässig schwer aufschliessbarer Organismus. Den Grad des Zellaufschlusses verfolgten wir mikroskopisch und durch Bestimmung des löslichen Proteingehaltes und der Enzymaktivität. Als Enzymtest dient die Umwandlung von TPN⁺ zu TPNH, die zur Hauptsache, aber nicht ausschliesslich, auf die Tätigkeit

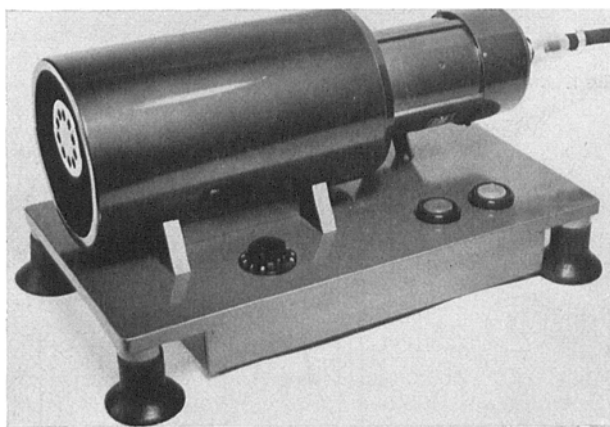


Fig. 1

¹ M. MERKENSCHLAGER, K. SCHLOSSMANN und W. KURZ, *Biochem. Z.* 329, 332 (1957).