based on the initial nitrogen pressure, and the rate of net loss of nitrogen will decrease as the internal pressure drops, whereas, assuming the oxygen is utilized by the cells, its rate of entry will remain unchanged.

In order to overcome the possibility of uncontrolled leakage of oxygen into culture flasks, T_{15} flasks were modified as shown in the Figure, the ground joints being lubricated with high-vacuum silicone grease. When such flasks were tested by the method described above, no recolorization of the methylene blue was observed, even after eight days, although as soon as the flasks were opened to air the color returned. Less than 2 µl of O_2 therefore had entered such a flask during this time, even after allowance for the reverse (reduction) reaction. A rubber band fastened to the 'rabbit ears' insured a tight seal when the flasks were used at a higher temperature (37°C) in the incubator.

Because we know so little about the respiration and growth of cells cultured at reduced oxygen tensions, it is clear that statements regarding the ability of cells to survive and grow in the absence of oxygen are not valid unless it can be shown that truly anaerobic conditions have been maintained. It is quite possible that there exists a minimum oxygen tension, at which cells may respire submaximally, where growth is not inhibited, and that below this point growth is progressively slowed and eventually stopped, whereupon cell death ensues. In a further paper evidence in support of this view will be presented for cultures of adult mouse fibroblasts[9].

Zusammenfassung. Eine kritische Untersuchung verschiedener Methoden für anaerobe Gewebskultur zeigte, dass in den meisten Einrichtungen die Anwesenheit von Sauerstoff nicht ausgeschlossen werden kann. Eine Modifikation des standardisierten Kulturgefässes wurde entwickelt, die das Halten der Kulturen unter völligem Luftabschluss möglich macht.

MARY E. CLARK[10]

Department of Zoology, University of California, Berkeley (USA), January 6, 1964.

[9] This work was carried out during tenure of a National Science Foundation Predoctoral Fellowship.

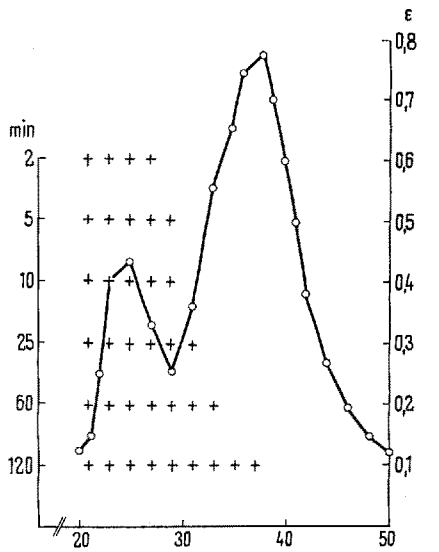
[10] Present address: Department of Zoology, The University, Bristol (England).

Serienbestimmung der Phospholipase-A-Aktivität in chromatographischen Fraktionen

Zur Auswertung chromatographischer Trennungen von tierischen Giften entwickelten wir ein einfaches, für Serienbestimmungen geeignetes Verfahren zum Nachweis von Phospholipase A. In unserer Methode machten wir uns die Entdeckung von HANAHAN[1] zunutze, dass Phospholipase A auch in ätherischer Lösung aus Lecithin eine Fettsäure abspaltet. Das entstehende Lysolecithin ist in Äther schwer löslich und fällt aus.

Von den zu untersuchenden Fraktionen werden 0,01–0,02 ml in Reagenzgläser pipettiert und 1 ml einer ätherischen Lecithinlösung zugefügt. Nach Durchmischen wird die Serie der Röhrchen in bestimmten Zeitabständen beobachtet und verzeichnet, welche Röhrchen eine Trübung durch ausgefallenes Lysolecithin zeigen. Markiert man die positiven Röhrchen auf einem Diagramm mit einem Kreuz und kreuzt man bei jeder neuen Ablesung auch die schon vorher positiv gewesenen Röhrchen wieder an, so erhält man eine Darstellung, die das Maximum und die Verteilung des Fermentes in der Fraktionsserie erkennen lässt (Figur).

Die Lecithinlösung besteht aus Äther (100 vol), Picolin (10 vol) und 4,5 mmolar wässriger Lösung von $CaCl_2$ (1 vol). In 100 ml dieser Mischung werden 1 g gereinigtes Lecithin gelöst. Zur Reinigung wurde Ovo-Lecithin (Merck) in Chloroform-Methanol (1 : 1) gelöst und an Al_2O_3 (Woelm, neutral) chromatographiert[2]. Bei kühler und dunkler Aufbewahrung ist die Lösung praktisch unbegrenzt brauchbar. Schwierigkeiten können dann auftreten, wenn für die Chromatographie der Phospholipase



Abtrennung von Phospholipase A aus einem vorgereinigten Bienengift an Sephadex G 75 (50 × 1,5 cm Säule) in Wasser. Abszisse: Zahl der Fraktionen à 2 ml. Ausgezogene Linie: Proteinmenge nach Folin-Lowry[3]. Kreuze: Positiver Ausfall des Phospholipase-A-Tests. Bei der Ablesung nach 2 min waren positiv Röhrchen 21–27, nach 5 min auch Rohr 29 und schliesslich nach 2 h alle weiteren Röhrchen bis Nr. 37.

[1] D. J. HANAHAN, J. biol. Chem. *195*, 199 (1952).

[2] D. N. RHODES und C. H. LEA, Biochem. J. *65*, 526 (1957).

[3] H. STEGEMANN, HOPPE-SEYLERS Z. *319*, 82 (1960).

A so starke Salzlösungen benutzt wurden, dass die Salze in dem Ätheransatz ausfallen und dadurch eine etwaige Lysolecithinbildung vortäuschen oder verschleiern. In solchen Fällen müssen die Proben durch Ultrafiltration (Kollodiumhülsen) oder Gelfiltration entsalzt werden. Natriumchloridkristalle lassen sich allerdings recht gut von dem amorph aussehenden Lysolecithin differenzieren.

Summary. A simple assay is described for phospholipase A in chromatographic fractions. 0.01 ml of the fraction is added to 1 ml of a 1% solution of purified lecithin in ether (100 vol) – picoline (10 vol) – 4.5 mmol $CaCl_2$ (1 vol). The presence of phospholipase A is indicated by the appearance of a turbidity due to precipitation of lysolecithin. The lag period until precipitation occurs gives an indication of the relative concentration of enzyme present.

W. VOGT und H. STEGEMANN

Medizinische Forschungsanstalt der Max-Planck-Gesellschaft, Göttingen (Deutschland), 16. Dezember 1963.

THEORIA

Comment on the Applicability of Compartment Theory to Interacting Biochemical Systems

During the past two decades considerable progress has been made in biochemistry and in pharmacology due to development of the 'compartment theory'[1–4]. The mathematical discussion of the different phenomena which occur during metabolism of drugs or of biochemical compounds proved to be useful as a tool of analysis.

A fundamental property of living organisms is that there exist chemically defined compartments represented by compounds with 'catalytic' properties, i.e. compounds influencing the reactions taking place in another compartment-system[5], as, for instance, in the case of a given hormone or enzyme which represents the end-compartment of one system of synthesis and simultaneously influences the kinetic reaction(s) between two or more compartments of another system.

If we express this fact in mathematical terms by means of the symbols used in the compartment theory[6], we can designate the compartments of one system Y as $y_1, y_2, \ldots, y_\eta, \ldots, y_m$ and the compartments of a second system X as $x_1, x_2, \ldots, x_\xi, \ldots, x_n$. The functions expressing the amount of metabolites in the x and in the y compartments are $x = x(t)$ and $y = y(t)$ respectively. The reactions schemes are then

$$y_{\eta'} \underset{h_{\eta''\eta'}}{\overset{h_{\eta'\eta''}}{\rightleftharpoons}} y_{\eta''} \quad \text{and} \quad x_{\xi'} \underset{k_{\xi''\xi'}}{\overset{k_{\xi'\xi''}}{\rightleftharpoons}} x_{\xi''}$$

Such systems, as X and Y, can be solved with a system of ordinary linear differential equations with constant coefficients.

In the general case, i.e. if all the compartments of Y influence the kinetic coefficients k of the X system, we may write:

$$k_{\xi'\xi''} = f_{\xi'\xi''}(y_1, y_2, \ldots, y_m)$$

where the function f indicates, in the simplest situation, linear functions of the $y(t)$ functions. Such a case would be for a set of enzymatically controlled reactions whose rates are proportional to the amounts of reacting enzymes.

The solutions of the system Y are represented by multiexponential functions of the time. By substituting for the k the corresponding f functions, we obtain a system of n linear differential equations, but no longer a system with constant coefficients. This system, however, can be solved by the classical analytical methods.

If we consider two compartment-systems between which exist such relations as described above, we may write:

$$Y \Rightarrow X$$

where the double arrow represents the dependence of the transfer rates of the system X upon the functions $y(t)$.

This relation can be extended straightforwardly to the general case of E compartment-systems which interact one with another by a well-known and defined set of functions. If the compartment system X_{ε_0} of the system E possesses n_{ε_0} compartments the total number of k functions of X_{ε_0} will be $n_{\varepsilon_0}(n_{\varepsilon_0} - 1)$.

Because the total number of compartments of E is $\sum_{\varepsilon=1}^{E} n_\varepsilon$, every k of the X_{ε_0} system will be expressed as a function of $\left(\sum_{\varepsilon=1}^{E} n_\varepsilon\right) - n_{\varepsilon_0}$ functions of the time. By substituting for every k of every compartment the corresponding expressions, one obtains a system of $\sum_{\varepsilon=1}^{E} n_\varepsilon$ ordinary non-linear differential equations solved for the derivative.

Such an interpretation may prove to be useful in describing the interaction phenomena between several metabolic chains or between several endocrine glands; or, in general, in the description of every homeostatic mechanism.

Riassunto. Viene tratteggiata riassuntivamente l'impostazione matematica del problema dell'interazione fra più sistemi a compartimenti.

P. F. PERITI[7]

Institut für Strahlenbiologie am Kernforschungszentrum Karlsruhe (Deutschland) 24. Juni 1963.

1 W. GEHLEN, Arch. exp. Path. Pharm. *171*, 541 (1933).
2 E. BECCARI, Arch. int. Pharmacodyn. *58*, 437 (1938).
3 A. RESCIGNO and G. SEGRE, *La Cinetica dei Farmaci e dei Traccianti Radioattivi* (Torino 1962).
4 C. W. SHEPPARD, *Basic Principles of the Tracer Method* (New York and London 1962).
5 H. VON FOERSTER, Brookhaven Symposia on Biology *10*, 216 (1957).
6 A. RESCIGNO, Biochem. biophys. Acta *15*, 340 (1954); *21*, 111 (1956).
7 Present address: Istituto di Radiologia, Università degli Studi, Modena (Italy).