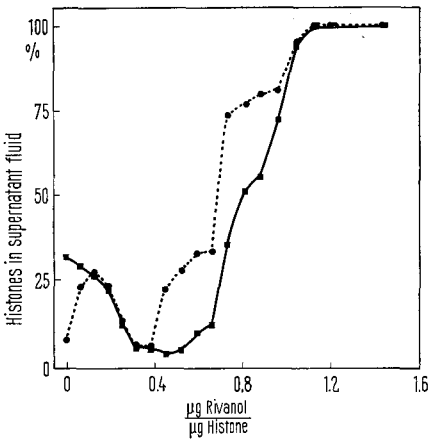

Fig. 7. Competition between rivanol and histones in the reaction with RNA (■) and DNA (●). Equal volumes of nucleic acid (highly polymerized RNA from yeast or calf thymus DNA, 500 µg/ml, both from Calbiochem) and histone (from calf thymus, 250 µg/ml) solutions were mixed and increasing amounts (0–600 µg/ml) of rivanol in 0.03 *M* acetate buffer pH 4.7 were added. The precipitate which formed was removed by centrifugation, rivanol was subsequently removed from the supernatant fluids by precipitation with KBr and the concentration of histones was determined spectrophotometrically[17].

Zusammenfassung. Die Anwendbarkeit von Rivanol als Protein-Fraktionierungsmittel wurde geprüft und an den folgenden Beispielen bewiesen: 1. durch gemeinsame Trennung und teilweise Reinigung folgender Enzyme aus geeigneten Rohmaterialien: Peroxidase und Xanthinoxidase; Desoxyribonuklease I, Ribonuklease, Trypsin und Elastase; Amylase, Cholinesterase und Muramidase; 2. durch Extraktion von Histonen aus Nukleoproteinen.

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PRO EXPERIMENTIS

A Very Simple Trick to Produce Controlled CO_2 Concentrations in the Gas Phase Overlying Cell Cultures

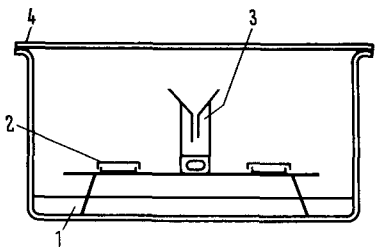
There is more and more need for precise pH control of mammalian cell culture meida. This is usually done by adding carbon dioxide to the air overlying the culture dishes. As experienced by many culturists and emphasized again recently by FERENCZ and NARDONE[1], this requires gas flow incubators and a high rate of gas consumption, which is costly. In addition, these systems do not allow easy adjustments of concentrations of the gas mixture according to needs.

In this laboratory, we are using with success a very simple trick, commonly used by chemists, that obviates all this. In large glass vessels that can be made air-tight, the required amount of CO_2 is produced *after* closing the lid.

In practice, we use large dessicators or aquaria with ground edges and lids. Water is put on the bottom to maintain water vapor saturation. On a tray above the water are placed first the Petri dish cultures, then a narrow high beaker containing normal phosphoric acid. The required amount of sodium bicarbonate (p.a.) is weighed and put in an old-fashioned pharmaceutical *cachet* envelope; just before closing the lid, the cachet is dropped in the acid and a little funnel added to prevent projections. About 1 min later, the cachet envelope is partly dissolved and bubbles of CO_2 appear. Equilibrium is reached within less than $^1/_2$ h and maintained for days.

The CO_2 appears near the ceiling of the sealed chamber; it mixes well with the air and no difference in pH of the medium (as checked by the indicator color) is observed in replicate dishes placed at different levels (up to 10 cm). There is only a very temporary rise of pressure (0.02 Atmosphere in several tests), as the vessels, very tight when the pressure gradient is from outside to inside, allow microbubbles of the gas mixture to escape between lid and vessel even for small pressure differences when the gradient is reversed.

The amount of bicarbonate can be determined from the volume of the vessel. Theoretically a partial pressure of 0.01 Atmosphere at 37 °C will be obtained in a volume



Schematic view of one of the possible arrangements. 1, water; 2, Petri dishes containing the cultures on a tray; 3, vessel containing phosphoric acid and bicarbonate *cachet* with funnel to prevent projections; 4, ground glass lid and edge.

[1] N. FERENCZ and R. M. NARDONE, Expl Cell Res. *53*, 139 (1968).

of 5 l by the CO_2 produced by the decomposition of 168 mg of $NaHCO_3$. In practice it will be found that about twice this quantity is necessary to maintain a pH of 7 in medium 199 for instance. Preliminary trials will determine the required amounts for the type of culture and medium used. This will then be extremely reproducible. The amount of H_3PO_4 is to be calculated so that there remains an excess after the reaction.

We have used this system regularly during the last year. 2 strains, HeLa cells and Chinese hamster fibroblasts, grow at least as efficiently as in closed bottles. Cells from mouse embryos and clones from bone marrow[2] are also very successfully cultured in these conditions.

Résumé. Du $NaHCO_3$ protégé par une enveloppe de cachet pharmaceutique ne commence à être décomposé par H_3PO_4 qu'après environ une minute. Cela permet de produire un taux déterminé de CO_2 dans l'atmosphère d'un récipient clos où sont placées les cultures de cellules (souches, embryonnaires, moëlle osseuse, etc.).

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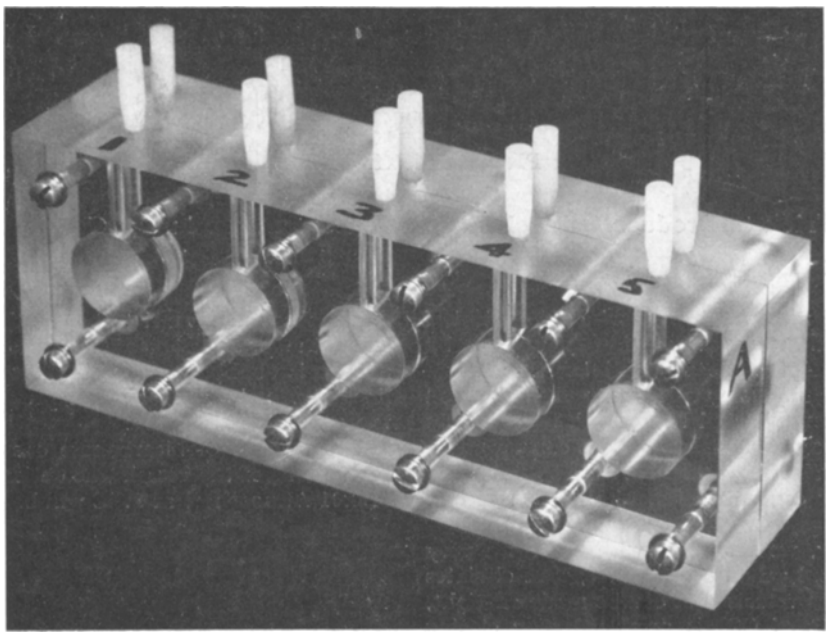
[2] T. R. Bradley and D. Metcalf, Aust. J. exp. Biol. med. Sci. *44*, 287 (1966).

An Improved Device for Equilibrium Dialysis

The technique for equilibrium dialysis[1] is useful in studying the interactions between compounds of high molecular weight and small dialyzable chemical species. Several investigators[2,3] have employed this technique to measure the binding of inorganic ions with soluble protein.



The device shown in the Figure was fabricated from 2 pieces of $8 \times 3 \times 1$ inch plastic. Five 1 inch diameter wells were 'end-milled' into each piece to a depth of $^1/_2$ inch. The dialysis membrane was placed between the 2 pieces, and the device was assembled with 12 bolts and wing nuts.

Protein solution can be introduced to one side of the membrane through a $^3/_{16}$ inch diameter hole with a pipette. Solutions containing the dialyzable species can similarly be introduced to the other side of the membrane. The holes are closed with plastic plugs, and the device can be immersed in a constant temperature bath. Aliquots from each side of the membrane can be removed with a pipette for analysis.

The device shown in the Figure requires less material than required when conventional dialysis tubing is used and the entire system is much more compact. Equilibrium is established somewhat more slowly with this device than with conventional dialysis tubing because of the smaller area of the membrane. Radiotracer experiments show that 18 h is sufficient time to equilibrate mono-, di- or trivalent metal ions at concentrations up to 0.005 *M*.

Zusammenfassung. Ein einfaches Gerät für gewisse Dialyseprobleme wird beschrieben.

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[1] T. R. Hughes and I. M. Klotz, *Methods of Biochemical Analysis* (Interscience, New York 1956), vol. 3.
[2] D. W. K. Cotton, Br. J. Dermat. *76*, 99 (1964).
[3] J. O. Pierce and K. L. Stemmer, Archs envir. Hlth *12*, 190 (1966).