Schnecken (z. B. *Cepaea hortensis*) kreuzreagieren. Solche Ergebnisse weisen darauf hin, dass sich im Vorkommen von Agglutininen und Proteinaseinhibitoren in diesen Organen ein allgemeinbiologisches Prinzip der Schutzwirkung manifestiert. Über weitere wichtige Inhaltsstoffe dieser Drüse und der entsprechenden Eier (Enzyme) soll an anderer Stelle berichtet werden. Bemerkenswerterweise kommen diese Schutzprinzipien in der Hämolymphe dieser Tiere nicht vor.

Summary. A strong immunological crossreactivity between the extracts of the albumin gland of snails (*Helix pomatia*) and the content of snail eggs is described, suggesting that the former supplies the eggs with protective substances (agglutinins, protease-inhibitors).

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PRO EXPERIMENTIS

A Rapid Microtechnique for Incorporation Studies in Cell Cultures

In this report we wish to describe a new technique that greatly facilitates study of the uptake of radioactively-labelled precursors by cells cultured as monolayers in vitro. The technique, entailing the use of multi-well plastic trays, has we point out, many advantages over other methods.

The tissue culture trays (Linbro,Model FB-48-TC, with 6×8 wells) are either purchased sterile or are sterilized with 70% ethanol (10 min) followed by UV irradiation for 100 min. The cells under study at the desired concentrations are then pipetted in their medium into the wells (cell suspensions are 200 µl/well).

To ensure that the bottoms of all wells remain horizontal, we inserted the trays against a multi-hole PVC plate designed for the purpose (Figures 1 and 2). A second multi-well tray served as a cover to prevent evaporation, and was held in place by a second multi-hole PVC plate. (The assembly is shown in Figures 1 and 2.) Steel clamps or an iron weight (800 g) were used to hold the two PVC plates firmly together. The assembly, compact and easy to handle, was incubated at 37°C in a CO_2 incubator (Heraeus), 95% air, 5% CO_2.

Before starting an experiment, the cells were allowed to acclimatize for 16–24 h. The assembly enabled a microscopic study of the cells at any time. All manipulations during an experiment (medium change, addition of drugs, addition of precursors) are made under a hood to prevent bacterial contamination. To remove the culture medium, a special pipette is used (Figure 3); for applying new medium, a Eppendorf-micropipette proved useful. To avoid disturbing the monolayer during change of the medium, it is important; 1. to rest the trays on a rack at a tilted (30°) position (Figure 1) and 2. to direct the opening of the special pipette towards the wall of the trough.

To stop incorporation of radioactive precursors, the medium is aspirated and the single wells are filled with 100 µl icecold 10% TCA. The whole trays are then placed in a 5% TCA bath (chromatography tanks, $20 \times 10 \times 20$ cm, refrigerator) and the extraction is repeated twice. To dry the trays we used either 80% ethanol or 5% acetic

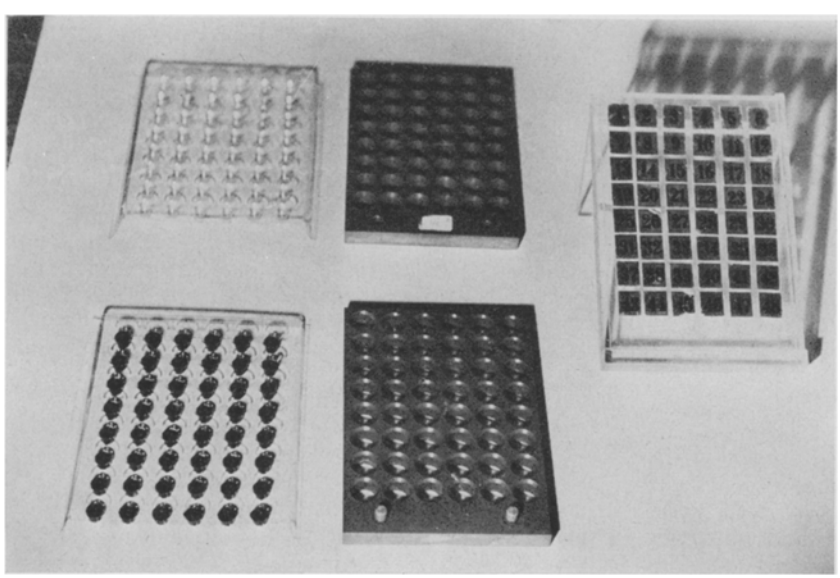

Fig. 1. The whole set consisting of 2 tissue culture trays and 2 PVC plates, together with an oblique rack to handle the trays.

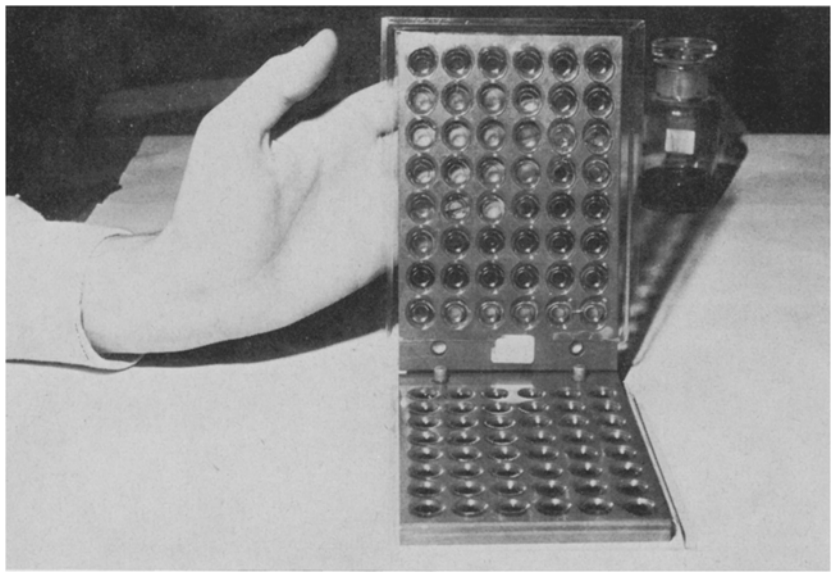

Fig. 2. The whole set before being closed. The upper tray, which is only used as a lid, is not tissue culture quality.

acid (which is necessary if Choline incorporation was studied). The bottoms of the air-dried wells are cut away with scissors (or a special cutting device) and dropped directly into vials for scintillation counting. After adding toluene scintillator (Liquifluor, New England Nuclear), the radioactivity in the vials is counted in a scintillation counter.

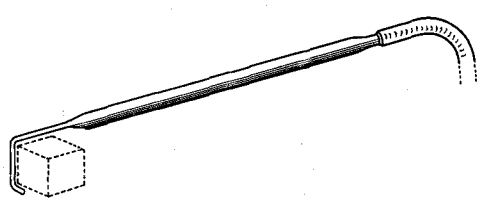

Fig. 3. The special pipette is shown with a hypothetical cube to demonstrate the directions.

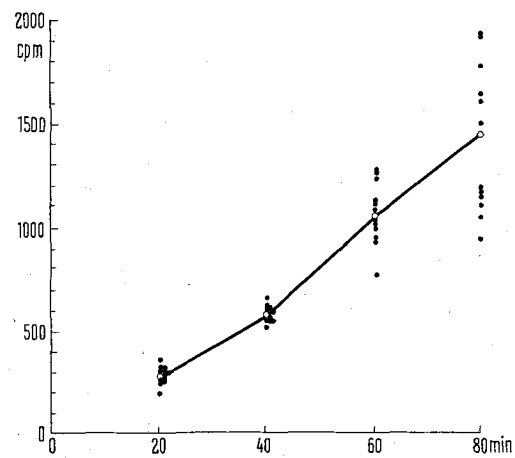

Fig. 4. Time dependent ^{3}H-thymidine incorporation (0.1 μCi) into HeLa-monolayers. The actual counts and mean values are given.

Practical counting yields are 11% of the theoretical amount which may be obtained in a homogeneous, quenchfree scintillator. To determine this yield, the cells were lysed with 10% Sodium-dodecyl-sulfate and counted in a dioxane scintillator. As an example, Figure 4 shows time dependent ^{3}H-thymidine incorporation into HeLa-cells.

Advantages of our procedure are: 1. A more or less unlimited number of single cell monolayer cultures can be processed and measured. 2. The procedure can be carried out quickly (3–5 min. are sufficient to handle 50 single cultures). 3. The amount of radioactive material is small (0.1 μCi thymidine/single culture 'position'). 4. Only small numbers of cells are needed (10,000 cells per single culture), making it possible to carry out a large number of analyses on the same cell culture. 5. Studies with monolayers usually imply lysis of the monolayers and subsequent processing of aliquots of the cell lysates. All these manipulations are unnecessary if the whole culture is measured as described. Especially suitable trays supports and a cutting machine may be obtained from Greiner, Nürtingen (Germany).

Zusammenfassung. Eine Methode zur bequemen Messung radioaktiver Vorläufer in Monolayer-Zellkulturen wird beschrieben. Die auf flexiblen Plastikkulturplatten (z.B. Linbro FB 48-TC) in zahlreichen Einzelkulturen gezüchteten, markierten, extrahierten und getrockneten Monolayer-Kulturen können direkt (beispielsweise mit einer Schere) in Zählgläschen für die Szintillationsmessung eingeschnitten werden.

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