binding protein. This agreement confirms the reality of the binding data.

Binding studies are performed to obtain quantitative and qualitative information about the mechanisms of ligand biopolymer interactions. With our flow dialysis method, the utmost information is gained with only a few individual experiments. Using the Scatchard plot to evaluate binding parameters, the whole saturation range has to be covered as shown earlier[16]. One single flow dialysis experiment leads to the complete Scatchard plot over the covered saturation range (1% of the initial concentration can be reached within 200 min depending on the experimental conditions). In contrast to this, all conventional methods require a set of individual binding experiments, each representing one isolated point of the Scatchard plot which has to be evaluated by a fitting procedure. Further advantages of our method result from the fact that only one experiment has to be performed. The small volume required for this experiment allows one to perform binding studies even with a very small amount of the biopolymer, which is often only available in limited quantities. In order to avoid a possible denaturation or degradation of molecules used, a small series of very short experiments at different initial ligand concentrations is required. The addition of the resulting individual Scatchard plots should give a continuous plot, proving the stability of the system studied under the experimental conditions. Continuous monitoring of the released ligand in the flow compartment allows detection of sensitively small disturbances during the experiment, such as membrane polarization, denaturation and degradation of the binding system and changes of the flow rates. Monitoring the releasing compartment is less sensitive in this respect.

The accuracy of the binding parameters evaluated depends on the accuracy of the diffusion constant used. Consequently the properties of the membrane used have to be held within narrow limits. If a good membrane quality is used and the preparation standardized, reproduceable results can be obtained.

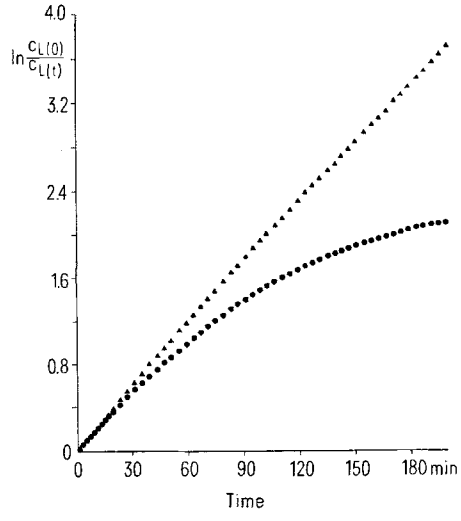

Fig. 4. Release of biotin in the presence of the binding protein. ●, total concentration; ▲, free concentration.

[16] H. G. Weder, J. Schildknecht, R. A. Lutz and P. Kesselring, Eur. J. Biochem. *42*, 475 (1974).

Contribution to the Primary Tissue Culture Technique. A New Method for Mechanical Cell Dispersion[1]

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Summary. A new technique for preparing primary tissue cultures by mechanical cell dispersion was idealized. The time required to prepare a culture by this procedure was greatly reduced and the cultures obtained are morphologically and physiologically of better quality than that obtained by the classical methods of enzymatic cell dispersion.

A new technique for mechanical cell dispersion was developed which was of special value for primary tissue cultures, in contradistinction to the classical methods of enzymatic cell dispersion.

Proteolytic and chelating agents, among which trypsin and Versene, respectively, are the most commonly used, seem to present certain incompatibilities with perfect success of cell cultures. The action of these agents is based on the removal of Ca^{++} and Mg^{++} ions, the elements mainly responsible for the integrity of the cell matrix[3].

The consecutive posterior contacts between the cell layer in growth and these enzymatic agents seem to produce biochemical intra- and intercellular disturbances, since it becomes more and more difficult to obtain cell lines in vitro from primary cultures.

Concerned with the prevention, or at least a reduction of a close contact between the tissue and such agents, a method has been developed by us for a mechanical cell dispersion suitable to supply viable cells, producing cultures of a much better quality as well as a reduction of

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[2] The authors wish to thank Mrs. Sibylle Heller for the translation.

[3] I. Zeidman, Cancer Research *9*, 386 (1947).

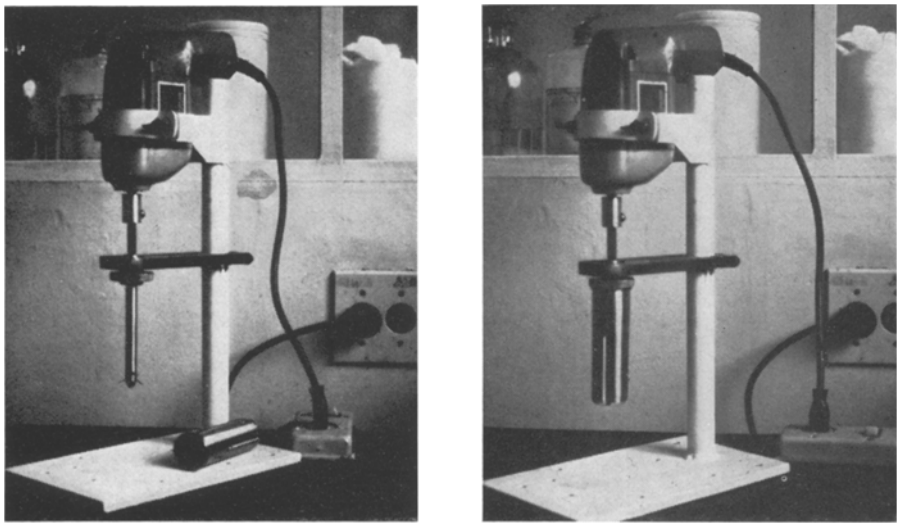

Fig. 1. The equipment idealized for the practice of this new method for primary tissue culture.
A) The vertical rotatory shaft is adapted to the electric drill and the propeller-like system of sharp blades adapted at its distal portion is seen. B) The whole set with the container adapted.

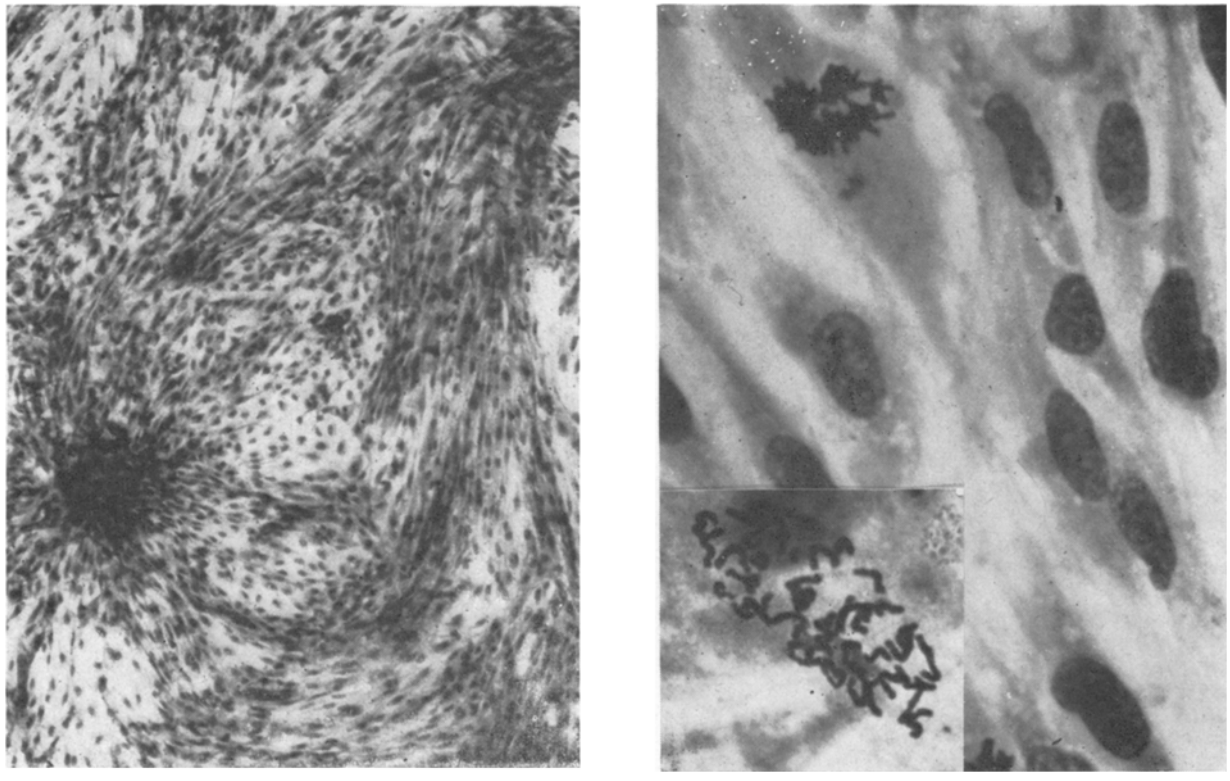

Fig. 2. A) Cell monolayer, after 5 days in culture. Giemsa. × 200. B) Metaphase plates at different augmentations, without any artefact for chromosome preparation. Giemsa. × 700 and × 1000.

about $^2/_3$ of the time necesary to prepare a similar culture by routine methods of enzymatic cell dispersion.

As biological material, we used the cortex renal of 1–8-day-old rabbits, but this method may be extended to other animal tissues.

The equipment necessary for the development of this technique consists of an electromotor with a 2,500–2,800 rpm capacity (electric drill) to which a vertical rotatory shaft is adapted, equipped with a propeller-like system of sharp blades at its distal portion (Figure 1a). To this set, a container also of sterilizable material of about 50 ml capacity is connected to receive, besides the material to be triturated, 20 ml of the culture medium (Figure 1b). In operating this system for 3–4 min, the tissue is transformed into a 'pulp' of cells suspended in the medium, and is then centrifuged at 900 rpm/5 min. The sediment obtained is resuspended in a fresh medium supplemented with bovine serum and is then inoculated in culture bottles.

About 6 days later, the wall of the bottle is coated with a cell layer (Figures 2a and b) both morphologically and physiologically better than that obtained by the enzymatic digestion procedures.