over the culturing area. The preparation was left standing at room temperature for 20 min, after which the cellular suspension was introduced into the culturing chamber.

Method 2: the denaturated collagen was mixed with balanced salt solution (BSS) in various proportions (1 to 50%). The mixture was introduced into the culturing chamber and left standing for at least 12 h, after which it was replaced by the cellular suspension. The presence of a thin layer of collagen was established in some control experiments by staining with anilin blue-orange G-acetic acid according to the method of HEIDENHAIN[8].

Results. The results described in the present communication have first been indicated to us by a previous series of experiments in which we prepared over 100 cultures of dissociated cerebral hemisphere cells in ROSE chambers. For the purpose of the experiments, only a small portion of each coverslip was prepared with reconstituted collagen according to the method of BORNSTEIN[2]. It was noticed, however, that in all the cultures cellular differentiation proceeded on the entire surface of the coverslip. We postulated that some collagen remaining in the denaturated state must have been transferred to the uncovered surface during the washing procedure. Consequently we reasoned that the reconstitution process was not necessary at all, and we performed over 200 experiments to prove it.

We found that both methods of substrate preparation, described above, gave rise to good cellular differentiation, but that the appearance of the cultures during the first 4 days was different and depended on the concentration of the denaturated collagen. With a 100% collagen (method 1) mainly isolated neurons differentiated during the first 4 days (Figure 1) and the glia only started to multiply and to form a monolayer after the 4th day. With a lower percentage of denaturated collagen (1 to 50%, method 2), the flattening of the glia started already after 24 h and the neurons differentiated on the glial layer (Figure 2). This was an improvement in comparison with cultures on plastic alone where the above-mentioned process started only after 3 days. After about 1 week, when the complete glial layer has been formed, there were no discernible differences between the cultures prepared by the 2 methods using the denaturated collagen (Figures 3 and 4).

Discussion. Apart from the simplified procedure for both of the methods described, the second of them (viz, using BBS-denaturated collagen mixture) has a further advantage. As the result of only a thin film of denaturated collagen, the cultures can be used for biochemical investigations equally well as those on plastic substrates. With normal collagen substrate preparations, certain of these investigations have proved to be difficult because of the interference of the excessive amount of collagen with the protein determinations[9].

The method incorporating the simplifications has been equally successful on glass and on plastic surfaces and is therefore applicable to cultures in ROSE chambers, in plastic Petri dishes and in plastic flasks. LUDUENA[10] has cultivated dissociated ganglionic neurons on gelatin substrate with success. It is probable that the denaturated rat tail collagen used in our experiments is structurally very similar to the gelatin used by the above-mentioned author.

Résumé. Nous avons simplifié la méthode de préparation du substrat «collagène» pour la culture de cellules nerveuses dissociées. On obtient une fixation et une différenciation rapide des cellules sur ce substrat. Les cultures conviennent pour des études morphologiques, biochimiques et électrophysiologiques.

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A Simple Method of Studying the Effects of Drugs and Cytotoxic Agents at the Ultrastructural Level

A variety of ways have been described for culturing cells in vitro[1,2]. Before techniques for thin sectioning were developed, several investigators grew cells on formvar-coated cover-slips and examined them after fixation under the electron microscope[3]. A double-embedding procedure was developed by GAY and modified by HOWATSON and ALMEIDA[4]. Many workers now employ standard methods to culture cells and then treat the culture with a chelating agent or with an enzyme in order to disaggregate the cells so that they can be centrifuged down into a pellet for embedding in epoxy resin[1,5]. Such procedures, however, may adversely alter the morphology of the cell. We have found it convenient to grow HeLa cells in 'BEEM' capsules (Ernest F. Fullam, Inc., Schenec-

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tady, New York, USA) which are polythene capsules preshaped to produce specimen blocks with one end pointed like a truncated pyramid. In our studies of cytotoxic substances extracted from sponges, the extract is introduced into the capsules containing the cell culture. The cells may then be exposed to the extract for different lengths of time after which the whole capsule is immersed in fixative to fix the cells. These may then be processed for electron microscopy. This communication describes the method employed by us.

BEEM capsules, sterilized in 70% ethanol, were inoculated with HeLa cells (Strain E, Commonwealth Serum Laboratories, Australia) in McCoy 5a (modified, GIBCO) medium. The cells were allowed to grow for 24 h at 37 °C, after which the medium in the capsules were replaced with fresh medium containing the test substance. After the required time of exposure to the chemical or drug, each capsule was opened and gently immersed in fixative consisting of 4% glutaraldehyde in 0.1 *M* Sorensen's phosphate buffer at pH 7.3 in a glass vial. The fixative was decanted off after 2 min and fresh fixative pipetted into the capsule and left to stand at 4 °C for 1 h. The fixative was decanted and the cells were rinsed gently with 2 changes of phosphate buffer. The tissues were post-fixed in 1% osminum tetroxide[6] for 1 h. Prior to use, the osmium solution was centrifuged at $1500 \times g$ for 5 min as a precaution to remove glass particles which may otherwise be introduced into the culture. The tissue was then

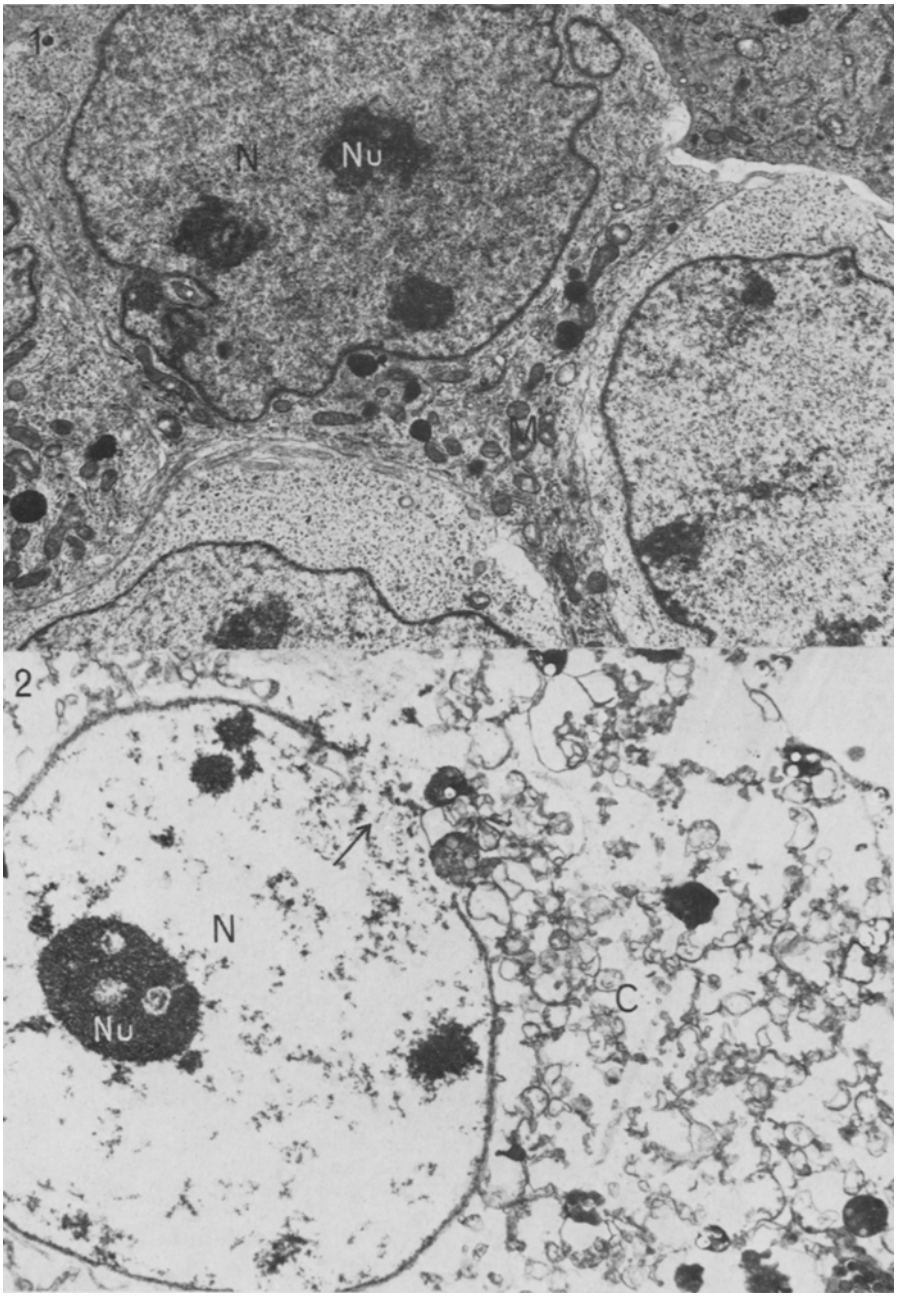

Fig. 1. Control culture of HeLa cells grown in BEEM capsules showing good preservation of the nucleus (N) with prominent nucleoli (Nu), mitochondria (M) and other cytoplasmic organelles. ×8400.

Fig. 2. HeLa cells grown in BEEM capsules treated with an alcoholic extract of a sponge, *Suberites inconstans*[6] showing rupture of the nuclear membrane (arrow) and disruption of cytoplasmic organelles (C) together with loss or nuclear material (N) although the nucleolus is still present (Nu). ×8400.

dehydrated in ascending series of acetone beginning with a 25% solution. This was followed by infiltration with araldite-absolute acetone mixture. The infiltration was allowed to continue overnight in a dessicator because the cover of the BEEM capsule was not completely airtight. Next morning, the araldite-acetone mixture was poured away and pure araldite was gently pipetted into the capsule. After 2 changes of pure araldite, the capsule was placed in an evacuation chamber at 50 °C for 1 h after which time the chamber was gently evacuated for about 10 min. The capsule was then removed and placed in an oven at 60 °C for 24 h for the resin to polymerize. Before sectioning, the blocks were removed from the capsule by making a longitudinal cut along the side of the capsule with a knife. The blocks were then cut, without any trimming, with a diamond knife on a Porter-Blum MT-2B ultramicrotome. The knife and block were aligned at a small angle so that the sections cut would be less than a complete section of the truncated tip of the pyramidal block. The small areas of dense growth can be visualized under the binoculars of the ultramicrotome and the portion to be cut can then be selected. Ultrathin sections were double-stained with uranyl acetate and lead citrate and viewed with a Hitachi HS-8 electron microscope.

Good preservation of cytological details were obtained (Figure 1) by this method. Mitochondria and other cytoplasmic organelles were well-preserved. The morphology of the nucleus and its membrane could be distinctly seen. After exposure to a cytotoxic substance[7], the various effects on the cytoplasmic organelles and nucleus were easily studied (Figure 2). We found this method of preparing HeLa cells for electron microscopy and its application to the study of drugs and cytotoxic substances on cells in culture to be simple and reliable, and the results are reproducible. It does not require the cells to be disaggregated either by chemical or mechanical means in order to be centrifuged down into a pellet prior to embedding in epoxy resin. One disadvantage of this method, however, is that it will be impossible to monitor the action on the drugs or chemical under test. But it has been custommary for us to study the effects of the test substance by phase contrast microscopy on cells which are grown on coverslips before studying them at the ultrastructural level. This should give adequate information regarding the effects of dose and time of exposure in a particular experiment. With this information available, it would be possible to select the desired conditions of the experiment and apply them to the cells grown in BEEM capsules for electron microscopy[8,9].

Zusammenfassung. Angabe einer einfachen Methode zur Untersuchung von cytotoxischen Substanzen an HeLa Zellen und Möglichkeit, HeLa Zellen zu kultivieren.

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Zeitsparende automatische Mitostatikazufuhr bei der Leukozytenkultur zur Chromosomendarstellung

Timesaving Automatic Supply of Mitostatica to Leucocyte Cultures for Chromosome Preparation

In zunehmendem Maße werden zytogenetische Untersuchungen routinemässig bei Mensch und Tier durchgeführt. Nach erfolgter Kultur über 48 bis 72 h muss dem Kulturansatz ein Mitostatikum wie z. B. Colcemid zur Arretierung der Zellteilung in der Metaphase zugeführt werden. Die Colcemideinwirkungszeit beträgt 2 bis 4 h; anschliessend kann mit der Aufarbeitung der Kultur begonnen werden. Zumeist erfolgt der Zusatz von Colcemid zu Arbeitsbeginn am Morgen, und es muss dann die entsprechende Zeit abgewartet werden, um mit der Aufarbeitung beginnen zu können. Diese dauert dann erfahrungsgemäss über den Mittag hinaus. Nach der ersten Fixierung kann aber erst der Arbeitsprozess unterbrochen werden und die Präparate können im Kühlschrank aufbewahrt werden. Um den Nachteil der verzögerten Aufarbeitung zu beheben, haben wir ein kleines und einfaches Gerät gebaut, das die Colcemidzufuhr automatisch in die frühen Morgenstunden zu verlegen erlaubt. Damit kann die Aufarbeitung zeitgerecht begonnen werden und der Arbeitsprozess bis zur Herstellung der Objektträgerpräparate am Vormittag abgeschlossen werden. Dies führt zu einer wesentlichen Vereinfachung des Laborablaufes.

Gerätebeschreibung. Um das Gerät in einem Brutschrank unterbringen zu können, wurde eine möglichst platzsparende Rundform gewählt (Figur 1). Da eine Person in einem Arbeitsgang nicht mehr als 8 bis 10 Kulturen gleichzeitig aufarbeiten kann, wurde das Fassungsvermögen auf 12 Kulturen beschränkt; damit können

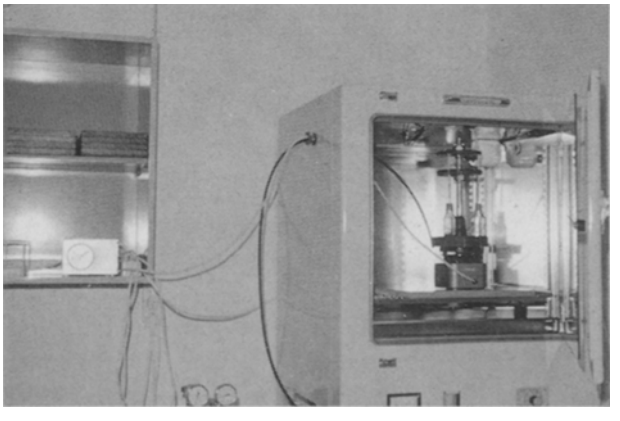

Fig. 1. Photographische Aufnahme des Kultursystems mit dem automatischen Pipettiergerät.