

against glucagon and/or insulin is easily demonstrable. These antibodies can be qualitatively assayed in serum by this method, which can also serve for macro-quantitative assay of the specific antibody. 2. The antibodies produced against glucagon and/or insulin are specific without cross-reaction. They are of the non-flocculating and non-precipitating type.

1. Moloney, P. J., Coval, M., *Biochem. J.*, London,

1955, v59, 179.

2. Courtesy of K. Lange, Bird S. Coler Hospital, Wards Island, N. Y. (Adapted from works of Cohn, E. J., Strong, L. E., Hughes, W. L., Jr., Mulford, D. J., Ashworth, J., Melin, M., Taylor, H. L., *J. Am. Chem. Soc.*, 1946, v69, 459.

3. Gornall, A. G., Bardawill, C. J., David, M. M., *J. Biol. Chem.*, 1949, v177, 751.

4. Wiesel, L. L., Positano, V., Kologlu, J., Anderson, G. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1963, v112, 515.

Received November 26, 1962. P.S.E.B.M., 1963, v112.

High-Powered Phase Contrast Equipment with Long Working Distance for Observation of Culture Flasks.* (28093)

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The widespread use of cell and tissue culture for cytological observations and in studies of virus-cell relationships makes direct, repeated observations of living cells necessary. Usually low power bright field microscopy is the routine method. The low degree of contrast and the limits in magnification due to the thickness of culture containers reduce the usefulness of this method. Finer structural details of individual cells can not be seen.

Phase contrast microscopy has made studies of the structure and behavior of living cells possible without the distortion and artifacts commonly encountered when fixation and staining are used. The advantages of phase contrast microscopy, however, are in many instances limited in tissue culture work unless specially constructed (expensive, fragile, and time consuming) chambers or flasks are used. The walls of such vessels have to be extremely thin and the outside thickness quite small if high magnification and proper phase effect is to be obtained, since normal high power objectives have a short working distance and ordinary phase condensers only a short focal distance. The use of fairly sturdy culture vessels and the benefits of phase contrast at a relatively high magnification was therefore highly desirable.

Such a system has been devised in our laboratory and has been found most useful. We have combined optical equipment from Carl Zeiss, and from Wild Heerbrugg, into a "hybrid" microscope. The objective used, in order to obtain high magnification, was a special water-immersion phase contrast 40 $\times$ objective (Zeiss) with numerical aperture 0.75 and working distance 1.6 mm. It was combined with a Wild special phase condenser with long focal distance. This condenser has a maximal working distance of 20 mm and numerical aperture 0.52 and can be adapted for use on a Zeiss ordinary upright or inverted microscope. The Zeiss objective incidentally can also be used on a Wild microscope by means of an adapting collar.

The system enables us, with phase contrast, to observe and photograph with a 40 $\times$ objective living cells in any flask with parallel roof and floor, when the thickness of the floor does not exceed 1.6 mm and the total thickness of the vessel 20 mm. The optical quality of Earle's T-15 and T-30 flasks(1), (Kontes Glass Co., Vineland, N. J.) has been found satisfactory. Good optical results have also been obtained with 30 ml plastic tissue culture flasks (Falcon Plastics, Los Angeles, Calif.). The latter flasks, however, we do not consider suited for work with infectious

* Supported by a grant from Nat. Cancer Inst.

material; the neck cannot be flamed, and leakage occurs occasionally.

A living culture of primary human amnion cells grown in a T-15 flask is shown in Fig. 1. Micrographs were taken with the Zeiss photomicroscope with 35 mm camera using the described optical combination and an ocular magnification of $4\times$. The outlines of the cells with thin cytoplasmic veils and extensions are easily seen. Conspicuous perinuclear granules, some of which show reversal of phase can be observed. The nucleus has the typical empty look of living nuclei; nucleoli are prominent. The nuclear membrane is clearly seen when not obscured by cytoplasmic granules.

Although more perfect optical conditions may be obtained with thin slides the contrast and the resolution achieved by the above method is satisfactory for most work. For micro-cinematography the water-immersion objective unfortunately is not well suited due to the rather rapid evaporation of the water. The system, of course, can be utilized in phase contrast microscopy with objectives of lower power without any immersion. If phase contrast is not essential the $40\times$ water-immersion objective can be used for flasks with greater outside thickness than 20 mm provided the thickness of the floor does not exceed the working distance of the objective or 1.6 mm.

Summary. Optical equipment permitting the observation of finer structural details of



FIG. 1. Micrograph of primary human amnion cells in T-15 culture flask as observed in the Zeiss photomicroscope with Zeiss water-immersion phase contrast objective $40\times$ combined with the adapted Wild special phase condenser with long focal distance. $560\times$.

living cells cultured in standard containers (T-flasks) has been described.

1. Earle, W. R., Highhouse, F., *J. Nat. Cancer Inst.*, 1954, v14, 841.

Received November 29, 1962. P.S.E.B.M., 1963, v112.

Simultaneous Distillation of Water and Extraction of Fat from Mouse Carcasses and Tissues.* (28094)

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Total fat and water content of mouse livers and carcasses had to be determined for studies to be reported elsewhere. Previous work of this type(1) required drying of the tissue or carcass to constant weight to determine the water content before Soxhlet extrac-

tion of the fat could be undertaken. The method to be described eliminates the time required for this drying process. Distillation of body water and extraction of fat were performed in one simple operation based on the lower specific gravity (0.87) and higher boiling point (110.6°C) of toluene as compared

* This work supported in part by USPHS grant.