I would suggest that, in future, more attention be given to the correlation of surface pressures with field observations of dipterous species, particularly in those problems related to variations in the activity of Diptera attacking man and animals. By a consideration of the surface pressure distribution, it may be possible to forecast changes in the activities of adults in the field, provided the temperature and other factors are favourable to activity. In this regard, the effect of the winds around areas of low pressure should be considered.

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Phase Difference Microscopy

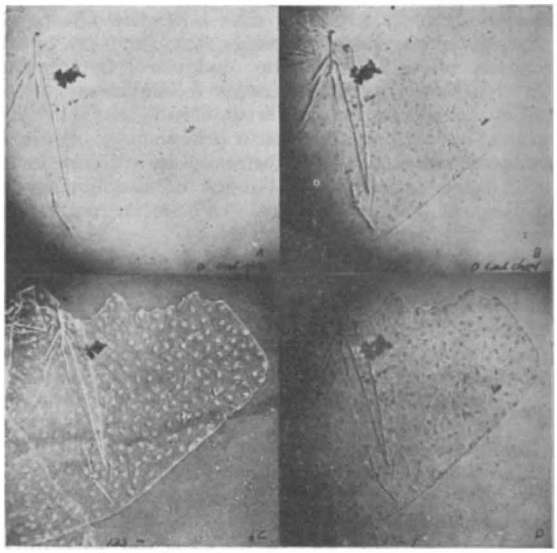
THE microscope does not reveal detail in uncoloured, transparent specimens even though it is known to be present and of sufficient size to be resolvable. When the detail includes regions of different refractive index, phase differences will be involved in light passing through the specimen, and if these phase differences are changed into intensity differences, the detail may be seen since the eye is sensitive to the latter although not to the former.

This change can be accomplished by inserting an annular stop into the condenser of the microscope and inserting a phase plate into the objective at its back focal plane. The phase plate consists of an annulus having a different transparency from the rest of the plate and of proper size to intercept the light coming directly from the opening in the condenser. The plates can be made so that the detail in the specimen may be seen either brighter or darker than its surroundings.

Abbe considered phase differences, but made little practical use of them. Conrady and Rheinberg[1] used phase difference microscopy to show and photograph a grating. Zernike[2] extended the treatment of these differences and urged their use in microscopy. Köhler and Loos[3] used his method with an annular plate and described some of the advantages of this kind of microscopy. The general theory and optical design have been extended in our Research Division by Dr. Harold Osterberg and Dr. R. K. Luneberg, coating methods for the phase plates have been developed by Dr. Helen Jupnik and practical tests and applications have been made by me, all working under Mr. A. H. Bennett, director of research.

The Spencer equipment includes a variety of phase plates having *both* retarding and absorbing properties of improved thin-film coatings. Both positive and negative plates are available for a range of 0–0·4 λ retardation and 0–100 per cent transmission. *Absorption differences* have been found by us to be as important as retardation differences in making some specimens visible under the microscope. The microscope exhibited at the Cleveland meetings of the American Association for the Advancement of Science had the phase plates mounted in a disk so that they could be rotated successively into place within the objective.

Phase difference microscopy has been found useful with unstained, transparent tissues, both plant and animal. Fig. 1 shows the appearance of epithelial tissue, living and unstained, from the nictitating membrane of the frog eye. The fine detail in fibroblasts from a chick embryo should be seen, but is not seen with the usual microscope objective. (Stopping the condenser down to give a narrow illuminating cone loses the fine detail in a lot of diffraction patterns.) Bacteria, blood cells, mould and Protozoa can be made clearly visible against their background; this facilitates study and counting. The resolution of the lenses appears not to be reduced, and considerable time and material are saved by not staining the specimens. The 'visualization' of transparent cells will give an interesting check on previous information obtained from killed and stained cells and tissues.



FROG NICTITATING MEMBRANE EPITHELIUM. × 40 APPROX. *A*, ORDINARY MICROSCOPE, APERTURE FILLED. *B*, SAME, APERTURE HALF FILLED, DETAIL LARGELY DIFFRACTION PATTERNS. *C*, BRIGHT-, AND *D*, DARK-PHASE WITH PHASE-DIFFERENCE MICROSCOPE.

Industrial applications may include the examination of transparent fibres, as glass and plastics, and surface detail on materials embedded in media of slightly different index. Small particles, within the limit of resolution of light microscopy, may be counted and measured, as in homogenized milk and in mayonnaise. The microscopic polishing marks on a transparent glass surface have been demonstrated and photographed in our laboratory.

The positive phase difference giving dark detail is more useful for measurement, and the negative phase showing bright particles is preferable for counting. Either appearance is possible with no damage to the specimen. When the particles are of differing size, as within a large *Paramecium*, they may selectively be made lighter or darker with respect to those of different size. Magnifications used range from 100 to 2,000 diameters and include dry and homogeneous immersion objectives. This development extends greatly the usefulness of the light microscope to include transparent materials of importance to several branches of science, medicine and industry.

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