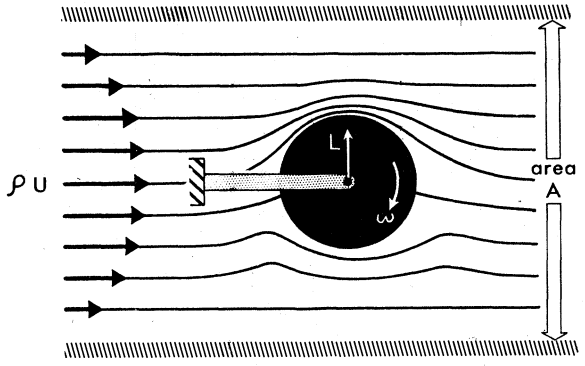

Fig. 15. Schematic drawing of mass flowmeter using Magnus effect.

pneumatic force-balance arrangement or by the calibrated deflection of a cantilever beam lying on the axis of fluid flow.

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Irradiation of Parts of Individual Cells II. Effects of an Ultraviolet Microbeam Focused on Parts of Chromosomes

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In about half of the dividing cells of our cultures of newt heart (*1*), one or more chromosomes tarry near the centrosome(s) during part of metakinesis and metaphase. For periods ranging up to 30 min at 22°C, these "centrophilic" chromosomes lie with their kinetochores presented to the centrosome(s), but they regularly migrate, kinetochore foremost, to the metaphase plate before anaphase. With phase-contrast microscopy, the kinetochore is clearly distinguishable as a constricted region (Fig. 1*A*).

With a proton microbeam (*1*), preliminary experiments indicated that irradiation of a chromosome segment containing the kinetochore resulted in the chromosome drifting about the cell instead of migrating straightway, kinetochore foremost, from the vicinity of the centrosome to the metaphase plate. We have investigated this effect further by means of a newly developed *ultraviolet* microbeam (*2*).

As early as 1912, Tschachotin (*3, 4*) obtained a microbeam by using refracting lenses to reduce the image of an ultraviolet source to microscopic dimensions, but the difficulties associated with aiming have limited the usefulness of his devices (*5*), especially for irradiation of very small regions in preparations as complex as tissue cultures. By using a *reflecting* objective and the principle of incident illumination, we have constructed a simple apparatus in which the same lens is used simultaneously for observation and for ultraviolet microbeam bombardment (Fig. 2).

The aiming and viewing portion of the device is a microscope with a reflecting objective. The magnifying system is fixed, focusing being accomplished by adjusting the height of the stage. The image of the target is brought into focus in the plane of a set of aiming cross hairs in the ocular. An adjustable telescope, focused on these cross hairs, permits compensation for variations in eyesight and, thus, insures that a target under observation can always be brought into focus at exactly the same point below the objective.

For ultraviolet bombardment, a quartz lens focuses

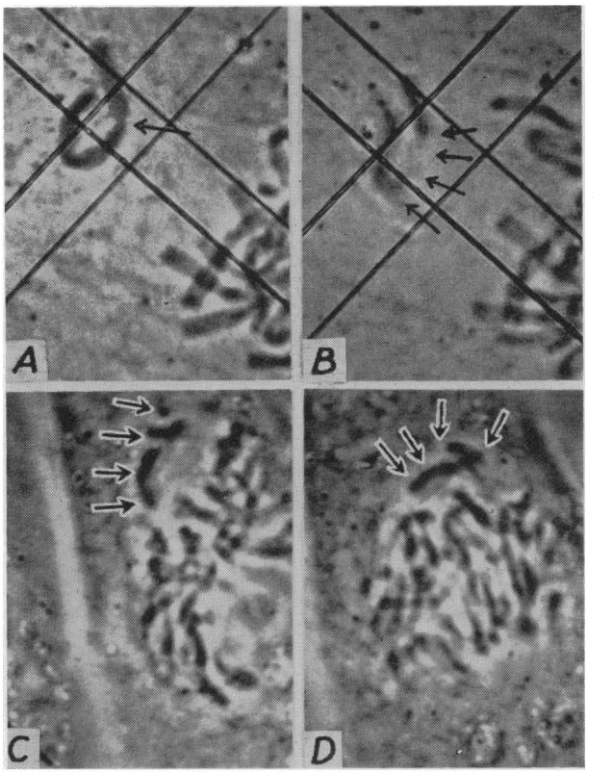

Fig. 1. Effect of irradiating a chromosome in the kinetochore region. (*A*) Before irradiation during metaphase (kinetochore at arrow). (*B*) Immediately after irradiation; irradiated segment "pale" (arrows). (*C*) Irradiated chromosome (arrows) drifting. (*D*) Anaphase. Irradiated chromosome (arrows) still drifting; never joins metaphase plate. (Phase-contrast microscopy. Prints from 16-mm motion-picture film. Each print represents an actual area 30 by 40 μ.)

the image of an ultraviolet source onto a small "primary" aperture. An appreciable fraction of the light passing this aperture is reflected by a partially aluminized glass plate onto the objective. A greatly reduced image of the primary aperture is thus formed below the objective. By adjusting the optical path length between primary aperture and reflecting objective, the microbeam is focused onto the same plane for which the viewing microscope is in focus.

The objective we are currently using (American Optical #1200, with quartz dust cover removed) contains only reflecting surfaces and, therefore, in the absence of any intervening refracting material, brings visible and ultraviolet light to a common focus. With our tissue cultures, the thickness of such material (a mica cover slip 5 μ thick plus a few microns of culture medium) introduces negligible chromatic aberration (*6*). Accordingly, the primary aperture is moved until visible light passing through it is brought into sharp focus on a mirror placed in the object focal plane. The primary aperture is then moved so that its microimage is centered on the aiming cross hairs. Aiming the ultraviolet microbeam is then accomplished merely by focusing the microscope on the target and centering the target image on the cross hairs.

Suitable baffles minimize stray radiation. By interchanging primary apertures, we have used microbeam focal spots of various shapes and dimensions. Diffraction patterns of the Airy type set a practical lower limit of about 1 or 2 μ for the shortest dimension (*7*).

For the present experiments, we used a circular primary aperture 400 μ in diameter, which, with an optical path length of 180 mm, yielded a microspot about 7 μ in diameter. A Hanovia high-pressure mercury arc was the source. Selective absorption in the mica cover slip, especially of the shorter wavelengths, considerably altered the energy spectrum of the ultraviolet microbeam from that of the source. Selection of targets was restricted to cell parts only a few microns below the culture cover slip, thus minimizing scattering and absorption as well as chromatic aberration.

The cultures of newt (*Triturus viridescens*) heart and the cinematographic methods of recording the results obtained with each cell were the same as those described previously (*1*). For each experiment, a mitosis was selected that had a "centrophilic" chromosome with a clearly visible kinetochore. After preirradiation photography at 15 frames/min, the culture was transferred to the bombardment apparatus (Fig. 2) and a linear segment of the chromosome was exposed to the ultraviolet microspot. The culture was then returned to the observation microscope, and pho-

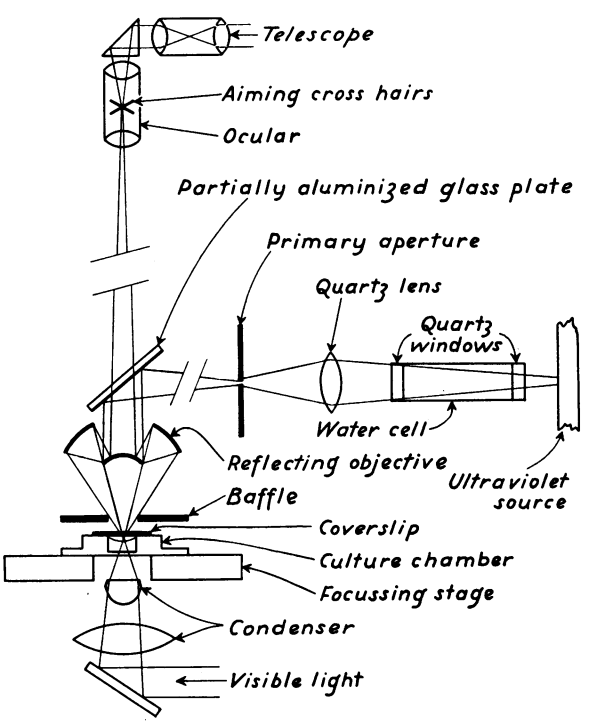

Fig. 2. Arrangement for irradiation with ultraviolet microbeam.

tography was resumed. Since the total length of the chromosome was 25 to 30 μ, the 7-μ microspot covered between one-third and one-fourth of its length.

Immediately after irradiation, the bombarded chromosome segment appeared "pale"; that is, its index of refraction was changed so that it no longer appeared blackish by medium-dark phase-contrast microscopy. This "paling" intensified for 20 or 30 min after irradiation and usually came to extend beyond the irradiated segment, so that frequently a considerable fraction of the irradiated chromosome appeared only as a colorless "ghost." Aside from its intrinsic interest, this phenomenon served as a valuable check on the accuracy of the aiming.

In each of 20 chromosomes (group A), the irradiated segment included the kinetochore; the exposure was 3 min. In 15 other chromosomes (group B), the kinetochore was excluded from the irradiated segment; 10 were exposed for 3 min, and the rest for 6 min. Without exception, those of group A failed to join the metaphase plate and drifted until anaphase (Fig. 1). After anaphase, each of these drifters was squeezed into one of the daughter cells by the constriction of the cytoplasm and formed either an accessory nucleus or a lobe on one of the main daughter nuclei. By contrast, all chromosomes of group B joined the metaphase plate before anaphase, even though some of them were exposed twice as long as group A. Moreover, this migration to the plate, in contrast with the drifting after kinetochore irradiation, was normally directed; that is the kinetochore proceeded foremost, with the two "legs" of the chromosome trailing behind.

This experiment shows that when parts of chromosomes are exposed to ultraviolet light, the normal directed movement of the chromosome from centrosome region to metaphase plate is inhibited only if the kinetochore is included in the irradiated part.

Work is in progress to improve visual observation during bombardment by equipping the apparatus with phase-contrast and dark-field illumination. This will permit the use of smaller microspots to determine how well the ultraviolet-sensitive entity corresponds to the morphological kinetochore. Work is also in progress to utilize monochromatic radiation and to measure the energy in the microbeam.

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* National Science Foundation Fellow.

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6. In case the amount of refracting material is not negligible, it can be compensated by suitably adjusting the position of the primary aperture.
7. Details of the apparatus will be described and discussed elsewhere.

Rudolf Höber: His Life and Scientific Work

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PHYSIOLOGY lost another of its great leaders when, on 5 September 1953, in Philadelphia, Rudolf Höber passed away. It was my privilege to work with him years ago in the Kiel laboratory and to see him frequently after he came to America. More than any other man, he has been to me my master in science. He was a fine and true spirit of intelligence and human sympathy, who stimulated the scientific work of a great group of students and colleagues. Through his books and publications, he has had a truly international influence.

Höber was born in Stettin, Germany, on 27 December 1873. From early childhood he was devoted to natural science, spending much time in collecting plants and minerals and in microscopic studies. He early read Darwin and other biological classics. He decided to study medicine, not to practice, but to prepare himself for a life of teaching and research. He attended the Universities of Freiburg, Berlin, and Erlangen. In 1898 he graduated in medicine at Erlangen.

Soon after taking his medical degree, Höber joined the staff of the Physiological Institute of the University of Zürich, directed by J. Gaule. His first scientific paper, an experimental study of wound shock, appeared in 1898. During the next 10 years there followed more than 30 papers from his own pen. At the same time, 22 graduate students wrote their doctoral theses under his direction. Höber's interest ranged broadly over his field. His work included studies of intestinal resorption, the mechanism of catalysis, the permeability of cells, the hydroxyl ion concentration of the blood, the mechanism of narcosis and its influence upon permeability, vital staining, the secretion of the urine, the physiological significance of the colloids, and the effects of ions upon the resting potential of nerve and muscle.

During this period, Höber's basic scientific interest