that this is a contrast effect and not a true absorption.

Although time did not permit careful scrutiny of the band around the horizon, it was nevertheless possible to examine sections about 30° in length as they crossed the window. No structure, either vertical or horizontal, was noticed.

MALCOLM SCOTT CARPENTER Manned Spacecraft Center,

Houston, Texas

JOHN ALOYSIUS O'KEEFE III LAWRENCE DUNKELMAN Goddard Space Flight Center, Greenbelt, Maryland

## References

1. J. W. Chamberlain, Physics of the Aurora and Airglow (Acadmic Press, New York, 1961)

1961).
J. H. Glenn, Science 136, 1093 (1962).
J. A. O'Keefe, *ibid.* 136, 1095 (1962).
M. Koomen, R. Scolnik, R. Tousey, J. Geophys. Res. 61, 304 (1956); R. Tousey, Ann. Geophys. 14, 186 (1958); J. P. Heppner and L. H. Meredith, *ibid.* 63, 51 (1958).

15 November 1962

## Sex Chromatin Mass in Living, **Cultivated Human Cells**

Abstract. Cells taken from human skin were observed with a phase contrast microscope before and after Feulgen staining. Living cells with the sex chromosome karyotypes XX and XXY (but not XO or XY) manifested, in late interphase, an object that corresponded in number, location, and shape to the sex chromatin mass found in the same cells after staining.

Information about the "sex chromatin mass" of mammals has recently been thoroughly reviewed (1). It suffices here to briefly summarize the properties commonly attributed to it. The sex chromatin mass is found in interphase nuclei of somatic cells of mammals having more than one X chromosome per normal or near-normal diploid set of autosomes. It usually looks like a compact mass and has the specific staining properties of DNA. Its position in the nucleus varies within and among cell types, but its most common position in cells cultured from human skin is on the circumference of the rather flattened nuclei. Each sex chromatin mass is formed by a single X chromosome, and the maximum number found is one less than the total number of X chromosomes in basically diploid cells and two less than the total number of X's in basically tetraploid cells. Evidence for and

qualifications of these statements can be found in the symposium cited above.

Miles has briefly mentioned (2) that the sex chromatin mass could be seen in vivo with a phase contrast microscope. The present report contains evidence showing that the object visible in living cells has the attributes listed above and is indeed the sex chromatin mass. The cells used were of the fusiform variety commonly cultivated from skin biopsies; they were propagated in Puck's medium F4 (3)

supplemented with 15 percent fetal bovine serum. Observations were made at 37°C with cells growing on cover slips forming the walls of Sykes-Moore chambers (4). A phase contrast microscope with a  $40\times$ , oil immersion, apochromat objective and a condenser with a front lens of long working distance was used. Photographs were made with high-contrast film. Living cells were photographed, and the stage coordinates defining their positions were recorded. The culture me-



Figs. 1-3. Three cells derived from an XX human female. Arrows indicate the sex chromatin masses. All photographs were taken with a phase contrast microscope. Figures 1a, 2a, and 3a are photographs of living cells. Figures 1b, 2b, and 3b are photographs of the same three cells after Feulgen staining (see text).

dium was then quickly removed from the chamber; the cells were washed with 0.85 percent NaCl and fixed with 95 percent ethanol. Hydrochloric acid  $(1N, 60^{\circ}C)$  was injected into the chambers, and the entire assemblies were immersed in a water bath at 60°C for 8 minutes. Fuelgen staining (5) was then executed in the chambers, and the cells were relocated by the original stage coordinates. The photographs of the stained preparations shown in Figs. 1-3 were also made with phase contrast optics and show more than one would see if ordinary optics were used. In particular, cell outlines could be readily discerned with phase contrast microscopy, giving added assurance that the cell observed after Feulgen staining was identical with the cell observed in the living state. The same preparations observed in bright field showed the usual Feulgen specific effect unambiguously.

Three pairs of photographs of cells from a human female are shown. Figures 1a, 2a, and 3a show living cells and Figs. 1b, 2b, and 3b show the same cells, respectively, after staining. The arrows in Figs. 1a, 2a, and 3a indicate objects observed in the living cells; these objects occupy the positions corresponding to those of the conspicuous sex chromatin masses found in the same cells after staining. Twenty-seven observations of this sort have been made with the same results, and the cells shown were selected as being representative in showing the sex chromatin mass in its most common form and position (Fig. 1), in a position away from the nuclear circumference (Fig. 2), and in a polyploid (probably tetraploid) nucleus (Fig. 3). Parallel observations of living human cells with the sex chromosome karyotypes XO, XX, XY, and XXY revealed sex chromatin masses in cells that were of XX and XXY origin but not in the others. There is little doubt that the object observed in the living cells is the sex chromatin mass.

Several hundred observations of the sort described in this report have been made of female cells of known age relative to the division in which they originated. They indicate that the sex chromatin mass observed in living cells and in stained preparations occurs only at a stage of interphase corresponding to the last few hours prior to the next division. Cells at earlier stages of interphase lack an object that can clearly be identified as a sex chromatin mass. Such cells often have one or more Feulgen positive condensations that might be identified as a sex chromatin mass but these condensations are invariably smaller than the unambiguous sex chromatin mass found at late interphase (6).

**ROBERT DEMARS** 

Department of Medical Genetics, University of Wisconsin School of Medicine, Madison 6

## **References** and Notes

- 1. "Symposium on sex chromatin," Acta Cytol.
- symposium on sex chromatin," Acta Cytol. 6, 1-142 (1962). C. P. Miles, Exptl. Cell. Res. 20, 324 (1960). R. Ham and T. T. Puck, in Methods in En-zymology, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1961), vol. 5.
- vol. 5. Bellco Glass, Inc., Vineland, N.J. C. D. Darlington and L. F. La Cour, *The Handling of Chromosomes* (Allen and Un-win, London, ed. 3, 1960), p. 156. This research was supported by grants Nos. RG-6983 (C-2) and RG-8217 from the U.S. Public Health Service. This report is paper No. 893 from the Genetics Division; Univer-sity of Wisconsin School of Medicine. 6.

27 September 1962

## **High-Speed Microcinematographic** Studies of Blood Flow in vitro

Abstract. The macroscopically steady flow of human blood at varying hematocrit values and flow rates through small glass capillaries has been studied by following in detail the motion of individual erythrocytes. At the microscopic level the flow was found to be nonuniform and unsteady. The shape of the average velocity profile of the erythrocytes was found to be in general more blunt than the Newtonian profile, and to be affected by concentration and flow rate, particularly in the capillaries of smaller diameter.

Determination of the detailed flow characteristics of blood flowing in vitro. and the relation of these to the gross characteristics, such as the relationship of pressure and flow, is a problem of fundamental significance in blood rheology, one which has been the subject of several studies. Comprehensive critical reviews have been presented by Bayliss (1).

One of the most important aspects of the problem is that of obtaining information about the velocity distribution across the cross section. Investigations such as those of Thoma (2), of Coulter and Pappenheimer (3), and, more recently, of Haynes and Burton (4) have led to the formulation of velocity distributions on the basis of the gross characteristics of the flow or of other

indirect evidence. Direct measurements of the velocity profiles have been, however, very scarce.

A systematic study of the detailed flow characteristics of blood in vitro has been undertaken, with particular regard to the measurement of the velocity of individual erythrocytes at various stations across the diameter of glass capillaries. The results described in this report are still exploratory in nature but afford some further insight into the nature of the flow and permit some comparison with the results of a very recent study by Bloch (5) on the detailed characteristics of blood flow in vivo.

Human blood of various hematocrits, treated with anticoagulant acid citrate dextrose solution (ACD), was caused to flow steadily at room temperature through horizontal glass capillaries 35 to 84  $\mu$  in diameter. The driving force was provided by air issuing under pressure from a reservoir devised for the purpose.

A phase-contrast microscope with oil-immersion objective was focused on the vertical diametral plane of the tubes. This arrangement permitted observation of sedimentation effects which would go undetected if the flow were studied in the horizontal diametral plane. The microscope was connected to a Beckman-Whitley Magnifax camera capable of operation at speeds up to 3000 frames per second. The total magnification of the process was approximately 970.

The light source was a 1400-watt alternating-current carbon arc. Although heating of the test section by the light beam could not be completely eliminated, it was reduced by interposing, between the arc and the microscope condenser, a water cell and a screen synchronized with the camera (so as to limit the exposure time to that strictly required for exposing the film). Comparison of measurements of pressure and flow made with and without heating of the objective showed an increase in the average viscosity of the flow, due to heating, of approximately 10 to 15 percent in the smaller tubes.

The films made were projected on a screen and analyzed frame by frame. In general, observation became more difficult as concentration, tube diameter, and flow velocity increased. Thus the maximum hematocrit value for which observation of the entire diametral plane was possible over a range of ve-