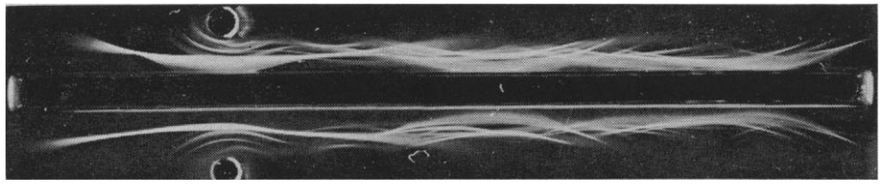

Fig. 2. Photograph of immunoelectrophoresis in 4.25-percent Cyanogum gel (140 × 90 × 1 mm), polymerized by photocatalysis, with the use of borate buffer of *p*H 7.5. Electrophoretic separation of the antigen: 320 volts for 60 minutes (40 ma), anode in the right. Antigens: 6 μl of normal human serum concentrated 2 times by means of lyophilization (upper reservoir); 6 μl of normal human serum in normal concentration (lower reservoir). Immune serum: 0.2 ml of duck specific antihuman serum.

rate of diffusion of proteins is not only decreased generally, but that of certain protein categories is completely arrested.

The catalyst system (photocatalysis by riboflavin), for 100 ml of 4.25-percent Cyanogum, consisted of a freshly prepared mixture of the following: stock solution of 30-percent Cyanogum, 13.98 ml; 0.64-percent solution of $Na_2S_2O_8$, 0.74 ml; 0.005-percent solution of riboflavin (*7*), 11.68 ml; water or buffer, 73.6 ml.

Since polymerization is initiated by light, the gel is exposed to a 6-minute period of lighting with two 500-watt photoflood lamps, well centered and placed at 0.40 m above the Cyanogum gel. The photocatalysis of thin layers of Cyanogum is correctly achieved only in the presence of a CO_2 atmosphere. However, the role of CO_2 is not only to suppress the air-oxygen inhibitory action, since its replacement by an N_2 atmosphere does not result in identical photocatalysis. The specific effect of CO_2 is probably due to the transitory decrease in *p*H, which returns to the initial level a few moments after the gel is reexposed to atmospheric air.

Slight qualitative and quantitative differences in immune precipitates are frequently observed between identical gel plates, depending upon whether they have been exposed to daylight or kept in a dark chamber (*8*). Darkness also prevents the nonspecific precipitates around unstable products, a frequent observation after many days of immune reaction. The photocatalytic action of riboflavin does not seem to alter the immune precipitates once they are formed.

After elimination of the excess proteins by a 48-hour immersion of the gel strips in a water bath, enhancement of the precipitated bands is obtained either by overprecipitation with dilute ethanol or ebullition, or by use of the standard staining procedures of ordinary agar gel. Washed and dyed gel strips can be kept in the hydrated form for a long time or they can be plasticized according to the following method. The gel strips are immersed for 2 hours in an aqueous solution of 2-percent glycerin. Then they are covered with a sheet of cellophane previously dipped in the glycerin solution, avoiding the trapping of any air bubbles. Finally, they are dried at 40°C for a few hours. The resulting films are smooth and translucid, and can be kept indefinitely.

Immunological study of small quantities of normal human serum by means of a specific horse antiserum (*9*), when the reservoirs are separated by a distance of 4 mm, results in the formation of 11 different precipitates. The immunoelectrophoretic study of 6 μl of normal human serum by means of a specific duck antiserum shows 25 different bands of precipitation (Fig. 2). Excellent immunological precipitates are observed with 1-mm-thick strips of Cyanogum gels, with or without preliminary electrophoresis at 4.25 to 4.5 percent concentration, and with the use of borate buffer (*p*H 7.5; 0.14*M*). When the reservoirs of the antigens and the immune sera are separated by a distance of 4 mm, the first immune precipitates appear after 3 hours. They correspond to the most rapidly diffusible and the most concentrated antigens. After 72 hours, all the immune reactions are completed and definitive. This prolonged period calls for the maintenance of the gels in a humid atmosphere.

Because of their particular physical and chemical properties, Cyanogum gels may have numerous practical advantages for the study of immunological precipitations, with or without preliminary electrophoretic fractionation. These include: the possible utilization of any buffer solutions of any *p*H; the lack of affinity toward dyes or reagents; the high capacity for resolution of even microscopic immune precipitates. For these reasons, Cyanogum gels seem especially well adapted for the immunological study of unstable biological materials with complex antigenicity, such as tissue homogenates (*10*).

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1 October 1962

Visual Observations of Nightglow from Manned Spacecraft

Abstract. The luminous band around the horizon noted by J. Glenn in the first U.S. manned orbital flight is attributable to airglow. Dip-of-the-horizon measurements on the star γ Ursae Majoris showed that the band is centered at an elevation of 91 kilometers or somewhat higher. The edge-on brightness of the airglow layer was 6×10^{-7} candles per square centimeter.

The luminosity of the night sky in places remote from artificial illumination was discovered a long time ago and has been studied by many researchers in many countries. The sources of the light are radiations and scattered sunlight from interplanetary space, radiation and starlight from intersteller space, and atomic and molecular radiation from the earth's atmosphere, generally from above the ozonosphere. These atmospheric sources of light are either the aurorae or the night airglow. The physics of these phenomena has recently been comprehensively reviewed by J. W. Chamberlain (*1*). One

of the most intense emissions of the nightglow is the strong oxygen green line at a wavelength of 5577 angstroms (Å). The height of this light has been measured, by various indirect means, from the ground. But the most reliable data, to date, come from direct measurements made in the 1950's from rockets passing through the nightglow layer at heights in the vicinity of 90 kilometers.

Glenn (*2*) reported from the first U.S. manned orbital flight (MA-6), observing with the naked eye a tan to buff luminous band some 6° to 8° above the horizon (*2*). It seemed to him that the starlight which passed through this layer might be reduced. This phenomenon was incorrectly attributed by O'Keefe to a reflection in the capsule window (*3*).

On the second U.S. orbital flight (MA-7) on 24 May 1962, this band was again observed. By means of an interference filter with a passband of 11 Å (half peak transmittance), centered at 5578 Å where transmittance was 44 percent, the luminous band was identified as attributable to the airglow, to which the emission at 5577 Å is a major contributor. At the moment of this observation, $17^h02^m39^s$ Universal Time, the sun was just rising on the third orbit and the horizon was illuminated. It was noted that, through the filter, the luminous band appeared bright, while the illuminated horizon was not seen.

Somewhat earlier in the flight, at $16^h48^m49^s$, it had been noted that the airglow band was approximately as bright as the horizon band, which was then illuminated by the moon, near last quarter. On the assumption that the atmosphere acts like a perfectly diffusing reflector and that the illuminance produced by the moon at last quarter is about 2×10^{-6} lumen/cm^2, it is found that the luminance (surface brightness) of the airglow layer is about 6×10^{-7} lumen/cm^2 per steradian (or 6×10^{-7} candle/cm^2). This is equivalent to about 30,000 Rayleighs if the radiation is assumed to be isotropic, a value consistent with previously reported visual measurements taken from the ground.

At about the same time it was noted that Phecda (γ Ursae Majoris) was about to pass through the airglow band, as seen from the capsule. From a replaying of the capsule tape, approximate times for apparent positions of Phecda have been determined as follows: Time just prior to entry into the airglow band, $16^h48^m49^s$; time of reaching approximate center of band, $16^h50^m8^s$; time of reaching apparent lower boundary of band, $16^h50^m41^s$.

Table 1. Angular zenith distances of Phecda and corresponding times, capsule heights, and heights of features of the airglow band.

Feature	Time (16^h +)	Angular zenith distances of Phecda (deg)	Capsule height (km)		Height of feature (km)
			Above feature	Above ground	
Outside layer	48^m49^s	100.86	118	237	119
Middle of layer	50^m08^s	101.88	141	232	91
Lower boundary of layer	50^m41^s	102.22	149	230	81

From the tabulated latitude and longitude of the spacecraft it was possible to determine the angular zenith distance of Phecda for each minute of the relevant time interval. The orbital data were obtained from the Goddard Space Flight Center. The angular zenith distances shown in Table 1 were then interpolated, and capsule heights above the given feature were found from the standard formulas for the dip of the horizon.

The apparent angular elevation of the horizon, after allowance had been made for refraction, was approximtely 106°; it was thus only about 4° below the middle of the nightglow layer. This figure is supported by an observation as follows.

At $17^h03^m37^s$ the capsule happened to be rolled at such an angle that a reticle cross, scribed on the window, was seen against the background of the luminous band. The crossarm then spanned the angular distance from the luminous band to the horizon thus furnishing a measure of the angular distance. Subsequent measures show that the crossarm is 1.54 cm long and is at a distance of 26.2 cm from the observer's eye. At an angle of 45° it subtends a vertical arc of approximately 2.4°. This is reconcilable with the calculated arc of 4°, if it is assumed that the apparent horizon is produced by scattering and extinction of light in the atmosphere and is considerably above the true horizon.

The discrepancy between the measured height of 4° or less and the estimated heights of 6° to 10° above the horizon, estimated visually on both MA-6 and MA-7, may be attributable to the well-known illusion which makes the apparent diameters of the sun and moon seem largest near the horizon. If so, it is clear that this illusion can in no way be connected with the direction of gravity, as sensed by the inner ear.

These heights are somewhat lower than those previously reported from rockets (*4*). J. W. Chamberlain has kindly pointed out that observations made in the manner of the astronaut observations tend to give too low an estimate of the average height of the layer, for the following reason. A ray which passes through a thick uniform layer near the top lies within the layer only for a short distance, corresponding to the chord of a short arc. For rays which penetrate the layer at greater depths the chord is longer, until we reach the ray which is tangent to the bottom of the layer. Beyond this point a piece will be cut out of the middle of the chord. The approximate formula for the distance which must be traversed is the real part of the equation:

$$s = (2R)^{\frac{1}{2}} [h^{\frac{1}{2}} - (h\text{-}h_o)^{\frac{1}{2}}]$$

where R is the radius of the earth, h is the distance below the tip of the layer and h_o is the thickness of the layer. The second term is to be taken equal to zero until $h > h_o$. Then the distance s will be a maximum where $h = h_o$—that is, for a ray tangent to the bottom of the layer. Hence, this discussion implies that if the layer is 10 kilometers thick, the astronaut will find maximum luminance at a zenith distance which corresponds to a level 5 kilometers below the middle of the layer. In this way it is possible to explain the difference between the height reported here and that derived by rocket measurements.

The absorption in this layer which was suggested by the observations of Glenn in the MA-6 flight was not found in the MA-7 flight. In the latter, the passage of Venus through the layer was observed; there was no noticeable dimming. Fainter objects became more difficult to see, but it is our feeling

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.

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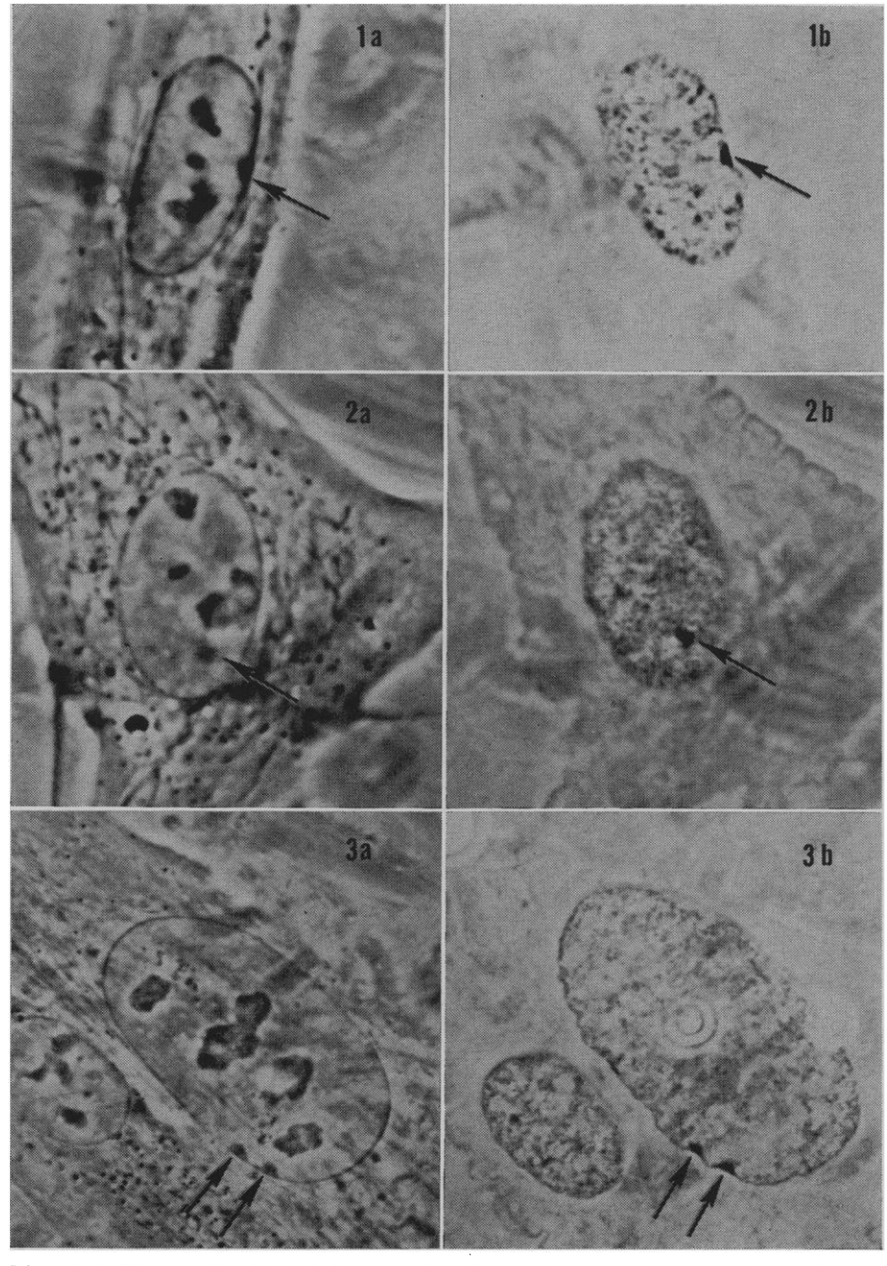
15 November 1962

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×, 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 1*a*, 2*a*, and 3*a* are photographs of living cells. Figures 1*b*, 2*b*, and 3*b* are photographs of the same three cells after Feulgen staining (see text).