tion medium, the resulting extracts exhibited little if any MPF activity (Table 1). Addition of Ca^{2+} to final extracts at a concentration of $10^{-5}M$ completely inactivated MPF. Therefore, low levels of endogenous Ca^{2+} released by the eggs during centrifugation may account for the rapid loss in MPF activity during storage on ice. This possibility is supported by the observation that when ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N'*,*N'*-tetraacetic acid (EGTA), a selective chelator of Ca^{2+}, was added to either the extraction medium or the final extract, MPF was still active for 24 hours.

In contrast to the effect of Ca^{2+}, the addition of Mg^{2+} to the extraction medium caused MPF activity to last significantly longer (Table 1). Extracts made with medium containing 10 mM $MgSO_4$ retained MPF activity for at least 24 hours, although most activity was lost after the extracts had been stored on ice for 48 hours. When ethylenediaminetetraacetic acid (EDTA), which chelates Mg^{2+} as well as Ca^{2+}, was added either to the extraction medium (Table 1) or to the final extract, MPF was immediately inactivated. Therefore, it seems that both the extractability and stability of MPF are increased in the presence of Mg^{2+} and the absence of Ca^{2+}.

Addition of Mg^{2+} to the extraction medium is indispensable when tris-maleate buffer is used instead of phosphate. Extracts made with tris-maleate buffer never exhibited MPF activity unless Mg^{2+} was added to the extraction medium at a concentration of 5 to 10 mM. Addition of Mg^{2+} to tris-maleate extracts made in the absence of Mg^{2+} failed to recover any MPF activity. Perhaps, maleate may chelate endogenous Mg^{2+} in the eggs as EDTA does to reduce the extractability of MPF.

The duration of this activity in both the maleate- and phosphate-buffered systems is dependent on the *p*H of the extraction medium. The optimum *p*H was between 6.4 and 6.8.

On the basis of these findings, in our later experiments we used an extraction medium that contained 0.2M NaCl, 0.25M sucrose, 0.01M $MgSO_4$, 0.002M EGTA, and 0.01M Na_2HPO /NaH_2PO_4, *p*H 6.5. Activity of MPF in extracts made with this improved medium has been detected for at least 48 hours and sometimes for 72 hours or more when the extracts have been kept on ice. However, activity was unstable at higher temperatures. There was no activity after 4 hours at 25°C or 15 minutes at 37°C.

MPF completely lost its ability to break down germinal vesicles when it was exposed to carboxymethyl cellulose-bound protease for 1 hour at 25°C, whereas there was no loss when it was exposed to polyacrylamide gel–bound ribonuclease (Table 2). This suggests that MPF activity is associated with protein but not with RNA.

The extracts were centrifuged on linear sucrose density gradients to determine the molecular size of MPF. After centrifugation, 18 fractions were collected, and each fraction was assayed for MPF activity. Three distinct peaks of activity (Fig. 2) were observed repeatedly after centrifugation at 150,000*g* for 10 hours or 85,000*g* for 17 hours at 2°C. The sedimentation coefficient (*S*) values for MPF in these fractions were estimated by comparing them to enzymes with known *S* values, which were centrifuged on separate gradients (*15*). The *S* values of the three MPF peaks were approximately 32*S*, 15*S*, and 4*S*. Therefore, under these in vitro conditions, MPF activity seems to be associated with heat-labile protein having three different molecular sizes. These may represent the association of MPF with other molecules or different degrees of aggregation of MPF molecules.

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High Resolution of Human Chromosomes

Abstract. *G-band prophase chromosomes from synchronized cells permit a high degree of resolution not previously attained in the study of chromosome structure and birth defects in man.*

The G-band patterns of human chromosomes visualized at various stages of mitosis demonstrate that the major bands of metaphase result from the progressive coalescence of numerous smaller bands uncovered in late prophase. This phenomenon occurs by a simple process of chromosomal contraction that clarifies our understanding of chromosome structure during cell division. In this study, the use of cell synchronization with amethopterin and a brief exposure to Colcemid as mitotic inhibitor allowed the achievement of consistently large numbers of excellent quality mitoses in prophase, prometaphase, and metaphase. In addition, in late prophase chromosomes, 1256 bands per haploid set were observed, which represents four times the number of bands previously found in metaphase. Since it is believed that man has approximately 30,000 genes per cell (*1*) and prophases would therefore average out to have 23 genes per band, the examination of late prophase chromosomes in the study of gene mapping and in individuals with birth defects may facilitate the localization of phenotypic characteristics to specific minute chromosome segments, extending our understanding of the organization and function of the human genome.

Peripheral blood (0.2 ml) was cultured for 72 hours at 37°C in 5 ml of RPMI 1603 medium (Grand Island Biological) supplemented with 20 percent fetal calf serum (Grand Island Biological) and 0.2 ml of phytohemagglutinin M (Difco). Amethopterin (methotrexate, Lederle), at a concentration of $10^{-7}M$, was then added to cultures to synchronize lymphocytes by a modification of the technique used by Rueckert and Mueller (*2*) for HeLa cells. The amethopterin block was released 17 hours later by the addition of $10^{-5}M$ thymidine-rich medium, after the cells were washed three times with culture medium and centrifuged at 60*g* for 8 minutes after each washing. The cells were then allowed to grow at 37°C for 5½ to 6½ hours and treated during this period for only 15 minutes with Colcemid (0.2 μg/ml) (Grand Island Biological) at 37°C to arrest cells in metaphase and inhibit the formation of spindle fibers of late prophase and

prometaphase chromosomes. Cells were harvested, and air-dried chromosome spreads were prepared and stained with Wright stain (*3*). Under these conditions, it is common to observe 12 to 15 percent of the cells in mitosis arrested in approximately equal numbers in prophase, prometaphase, and metaphase. The high number of unusually well spread mid- and early metaphases (with 320 to 554 bands), late and early prometaphases (with 555 to 842 bands), and late prophases (843 to 1256 bands) suggests the utilization of this technique as a possible method of choice for chromosome analysis.

The relatively high frequency of well-banded and spread mitoses demonstrates that the major dark and light bands of metaphase chromosomes result from the close apposition of smaller and multiple units found in the more elongated late prophase and prometaphase chromosomes. Such a phenomenon is illustrated in Fig. 1, where selected chromosomes are shown in mid-metaphase, early metaphase, early prometaphase, and late prophase. In addition, when the banding patterns of metaphase and late prophase chromosomes are represented schematically, in accordance with the recommended method (*4*), it becomes obvious that the great majority of the 320 bands of mid-metaphase chromosomes subdivide into several well-defined discrete subbands, totaling 1256 units (Fig. 2). This finding supersedes the previous observation by Yunis and Sanchez of 921 bands in nonsynchronized cultures (*3*). The higher resolution was possible because cell synchronization obtains

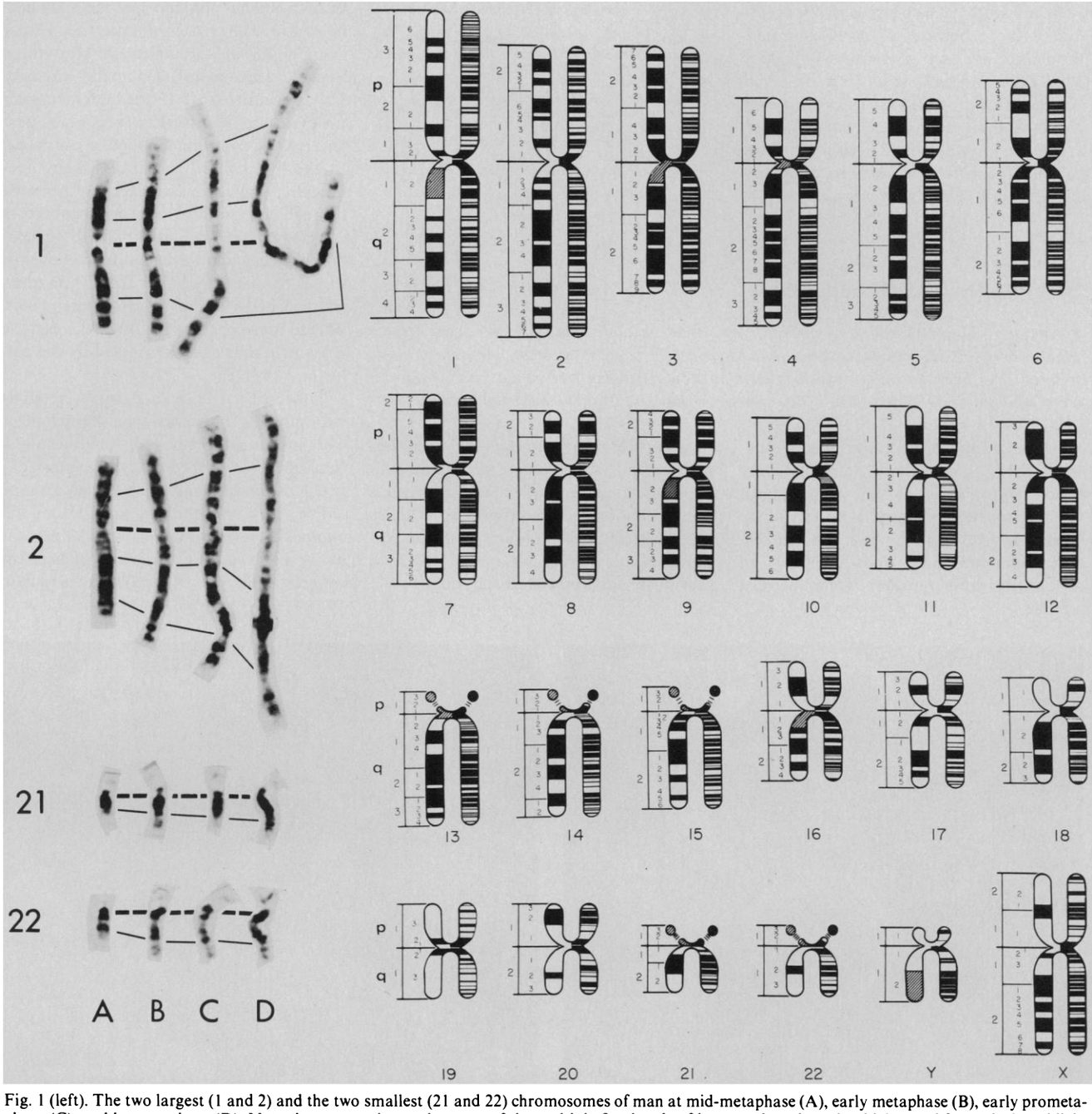

Fig. 1 (left). The two largest (1 and 2) and the two smallest (21 and 22) chromosomes of man at mid-metaphase (A), early metaphase (B), early prometaphase (C), and late prophase (D). Note the progressive coalescence of the multiple fine bands of late prophase into the thicker and fewer dark and light bands of metaphase. Fig. 2 (right). Comparative representation of human chromosomes according to the Paris Conference nomenclature (*4*). In each chromosome, the left chromatid represents the banding pattern observed in mid-metaphase and the right chromatid represents the G-banding pattern observed in late prophase.

late prophases of a frequency, spread, and quality not attained by the experimental technique previously described.

With the prophase-synchronization technique, we have been able to detect minute chromosome defects previously unidentified. This includes three patients with the cat-cry (5p-) syndrome and deletion of the small dark and light subbands of the distal end of the short arm of chromosome 5 (*5*), and one patient with moderate mental retardation and deletion of the minute distal light and dark subband of the short arm of chromosome 9 (Fig. 2).

In my study, it was also possible to obtain a large number of early and mid-prophases in which approximately 2000 to 3000 dark and light units were observed. At this stage of cell division, however, chromosomes remained highly extended, and their optimal spread is limited by the persistence of nuclear membrane and nucleoli. Efforts should now be placed toward the development of a suitable technique for their analysis in order to obtain a resolution comparable to that observed in the giant chromosomes *Drosophila* and thus begin to bridge the gap between genes and chromosomes in man.

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Stratospheric Aluminum Oxide

Abstract. *Balloons and U-2 aircraft were used to collect micrometer-sized stratospheric aerosols. It was discovered that for the past 6 years at least, aluminum oxide spheres have been the major stratospheric particulate in the size range 3 to 8 micrometers. The most probable source of the spheres is the exhaust from solid-fuel rockets.*

Although much is known about submicrometer stratospheric aerosols, very little is known about particles larger than a few micrometers. The spatial density of the larger particles is so low that most collection techniques have been incapable of adequately sampling them. Over the past 5 years we have flown very sensitive, high-volume air sampling collectors into the stratosphere to collect extraterrestrial dust. Although extraterrestrial particles were collected (*1*) the majority of the collected particles were spheres of Al_2O_3, a previously unknown stratospheric aerosol.

In 1970 and 1971 we flew two balloon-borne collections at 34 km, sampling 1.1×10^4 m^3 of ambient air (*2*). The flights were launched from Palestine, Texas, and the collections occurred over the southeastern United States. In March 1974 we began collections at 20 km, using U-2 aircraft of the National Aeronautics and Space Administration. To date, we have logged 100 hours of collection time with the U-2's, sampling 10^5 m^3 of ambient air. The U-2 collections crisscross the United States, and typical flight runs consist of consecutive exposures of ~ 4 hours each. In both the balloon and U-2 methods particles are collected by inertial deposition from a 200-m/sec airstream onto thick films of silicone oil (kinematic viscosity 5×10^5 centistokes). In the balloon collector (Vacuum Monster) air is pumped past an array of cylindrical collection surfaces. In the U-2 collector a single 18-cm^2 rectangular collection surface is rammed through the ambient air. Both collectors are clean, have negligible particle bounce-off problems, and have high impaction efficiency for particles larger than 3 μm (density = 3 g/cm^3). Two micrometers is considered the lower cutoff limit of the particle size range that can be sampled by the collector.

Collected particles (> 2 μm) were studied optically, and more than 600 particles were individually removed from the collection surfaces, washed in xylene, and analyzed in a scanning electron microscope (SEM) for morphology and elemental composition. In all collections, 90 percent of the particles < 6 μm were transparent spheres. Analysis of typical spheres both in

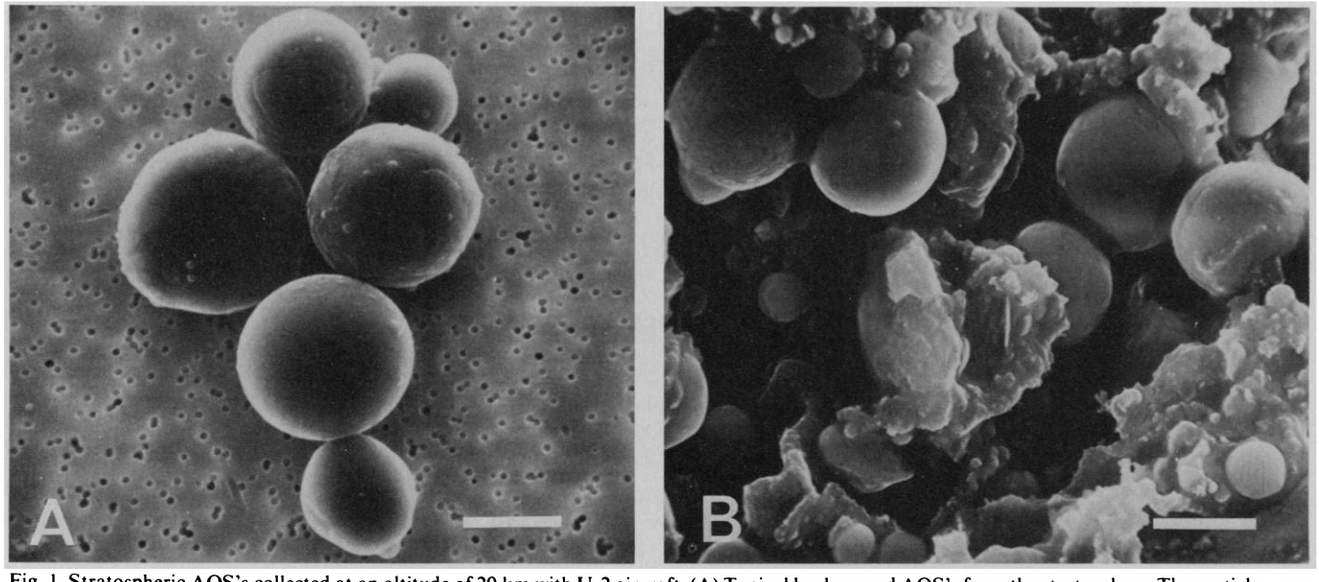

Fig. 1. Stratospheric AOS's collected at an altitude of 20 km with U-2 aircraft. (A) Typical background AOS's from the stratosphere. The particles were collected on silicone oil and were individually moved to the Nuclepore mounting medium for photography in the SEM. The small holes are part of the Nuclepore filter substrate material. (B) Aluminum oxide spheres collected from the smoke plume of a Titan III rocket. The particles were collected on cellulose filter paper, which was ashed at 450°C. The spheres are from the rocket and the other material is filter paper ash. Scale bars, 5 μm.