cytes are often found in amniotic fluid obtained from hysterotomy samples between the 16th and 20th weeks of pregnancy (*17*). If sufficient numbers of fetal erythrocytes are also present in routine amniotic fluid samples obtained by transabdominal amniocentesis during this period, these techniques should be applicable to the antenatal detection and management of hemoglobinopathies such as sickle cell anemia.

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14. Tryptic peptides are numbered according to the distances of their lysyl or arginyl residue from the NH_2 terminus of the unhydrolyzed chain.
15. The term chymotryptic peptide is used because the trypsin preparation has chymotryptic-like activity in cleaving the β chain at asparagine residue 140.
16. After digestion of A and F globins, the radioactive peptides solubilized by trypsin were analyzed. The expected radioactivity in the peptides isolated was calculated from the ratio of the number of leucine and valine residues in those specific peptides to the total number of those residues solubilized by trypsin. By this method, the recovery of radioactivity in peptides of hemoglobins A and F was very similar: 20 to 25 percent of the calculated value.
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Polymorphism of Human Constitutive Heterochromatin

Abstract. *Genetic polymorphism has been demonstrated in man for many characteristics including blood groups, serum proteins, tissue enzymes, and hemoglobins. A class of chromosomal polymorphism involving constitutive heterochromatin has now been found. Through the use of a special technique that permits visualization of heterochromatin, seven heterochromatin variants have been found among four individuals. These results suggest a very high frequency of variability of heterochromatin in the population.*

Arrighi and Hsu (*1*) have described a method by which constitutive heterochromatin can be identified in human chromosomes. The technique, first used with mouse chromosomes (*2*), involves an alkali treatment of the cells in situ and subsequent incubation in a salt solution. This procedure is believed to denature the DNA and then allow partial annealing of the DNA strands during incubation. When the slides are then treated with Giemsa stain, a darkly stained area is produced at the centromere of every chromosome. In addition, very prominent blocks of heterochromatin are stained in the secondary constriction regions of chromsomes No. 1, 9, and 16. The Y chromosome is unique in that it has a large heterochromatin block that covers the distal half of the long arm. We used the procedure of Arrighi and Hsu for the original purpose of differentiating between morphologically similar chromosomes. However, a high frequency of variation was found among individuals, results which suggested a new class of polymorphism in humans.

Slides were made from cultures of human lymphocytes prepared according to the method of Moorhead *et al.* (*3*). Basic protein (histones) was removed by treating cytological preparations with 0.2*N* HCl for 15 minutes. The slides were treated with 0.07*N* NaOH for 1 minute and then incubated in a saline-citrate solution (*4*) at 65°C for 24 hours. The slides were finally stained in buffered Giemsa (*5*) for 15 minutes.

Analyses of 25 cells from each of five individuals revealed some differences among chromosome pairs in groups D, E, and G; however, these differences were often so poorly defined that it was difficult to consistently classify specific pairs. No clear-cut differentiation could be made among the pairs of groups B, C, and F (*6*).

An unexpected result was the discovery that a high frequency of heterochromatin polymorphism occurs within homologs. Only one of five individuals studied had a "normal" heterochromatin pattern in all 46 chromosomes. In the remaining four subjects, we observed seven variant patterns involving chromosomes No. 1, 9, and 16 as well as groups D, F, and G. These seven variants are shown in Fig. 1.

One individual had a chromosome No. 9 (Fig. 1b) with a block of heterochromatin at least twice as long as that normally seen for pair No. 9. The heterochromatin region of its homolog, readily identifiable with this technique, was normal in size. A second individual had two different variants, one chromosome in group D with a heterochromatin block larger than usual (Fig. 1d) and a group G chromosome in which no satellites or stalks were visible in any of the cells examined (Fig. 1f, lower row). Though the short arms do not usually appear darkly stained with this procedure, the treatment tends to loosen the compaction of the stalks and satellites of the acrocentric chromosomes. Thus, greater detail of the short arm morphology is revealed, and a loss of chromatin from this region is more readily apparent.

Each of the remaining two individuals also had two different variants. One individual had a chromosome No. 1 with an unusually long block of heterochromatin (Fig. 1a) and a chromosome No. 16 in which the heterochromatic region was decreased to approximately one-third its normal size (Fig. 1c). The last person had two less obvious though equally consistent deviations, one F chromosome that had an exaggerated amount of centromeric heterochromatin extending into both arms (Fig. 1e) and a G chromosome in which very prominent satellites were unusually dark (Fig. 1f, upper row). Neither of these two patterns is normally seen in groups F and G.

Each of the seven variants was found only in the heterozygous state and was consistently seen in every cell examined; repeat cultures from one individual confirmed the original observations. In addition, ten cells of each of the five individuals were karyotyped from untreated, coded slides (acetoorcein stain). No morphological variations could be identified in any of these slides. Therefore, conventional cytology failed to demonstrate the heterochromatin variants detected with the denaturation-renaturation technique.

An increase in total length is concomitant with the heterochromatin variant of chromosomes No. 1 and 9 (Fig. 1, a and b). This could be interpreted as either differential uncoiling of the variant chromosome or an addition of heterochromatin material. We feel the latter explanation to be more likely, because there appears to be an increase in total amount of heterochromatin in all cells, instead of simple extension of the heterochromatic region.

The exact nature of heterochromatin has yet to be determined, but biochemical studies in other mammals have shown that constitutive heterochromatin (visible in both homologs of a chromosome pair) is composed primarily of highly repetitious DNA (*7*). The same is true for man, as is indicated by the in situ hybridization studies of Arrighi *et al.* (*8*), who showed that the highly repetitious DNA of human chromosomes is localized, at least in part, in the heterochromatin regions. There is little doubt, therefore, that the constitutive heterochromatin made recognizable with the denaturation-renaturation procedure has a specific molecular organization. This material consists of nucleotide sequences repeated many times, and represents DNA that is distinct from the unique or single copy DNA of euchromatin.

The repetitive structure of the heterochromatin suggests a mechanism which would explain the occurrence of polymorphism. The most likely origin of the heterochromatin variants is from unequal crossing over in the tandemly repetitive sequences. Since many sites would be available for the pairing of homologous regions within families of repetitious DNA, there would be an increased risk of unequal crossovers at these regions. For example, within a sequence *abcabc*, pairing could take place between position 1 of one strand and position 4 of the second strand. A subsequent exchange would then result in two unequal crossover products, *abcabcabc* and *abc*. Hence, the more repeating units within a sequence, the more variants to be expected; the longer the sequence, the greater the difference between the resultant crossovers.

The significance of constitutive heterochromatin is still unknown. It is possible that these areas do not include active or functional genes. Nevertheless, it is difficult to envision such ubiquitous DNA with no genetic activity. Several possible roles of repetitious DNA have been suggested (*7*). One possible function that has not been considered is that the centromeric heterochromatin codes for microtubule formation and assembly. This seems plausible, since microtubules are assembled at all centromeres from small specialized elements to form the chromosomal fibers of the spindle apparatus (*9*). In addition, constitutive heterochromatin is localized at the centromeres of all organisms thus far stud-

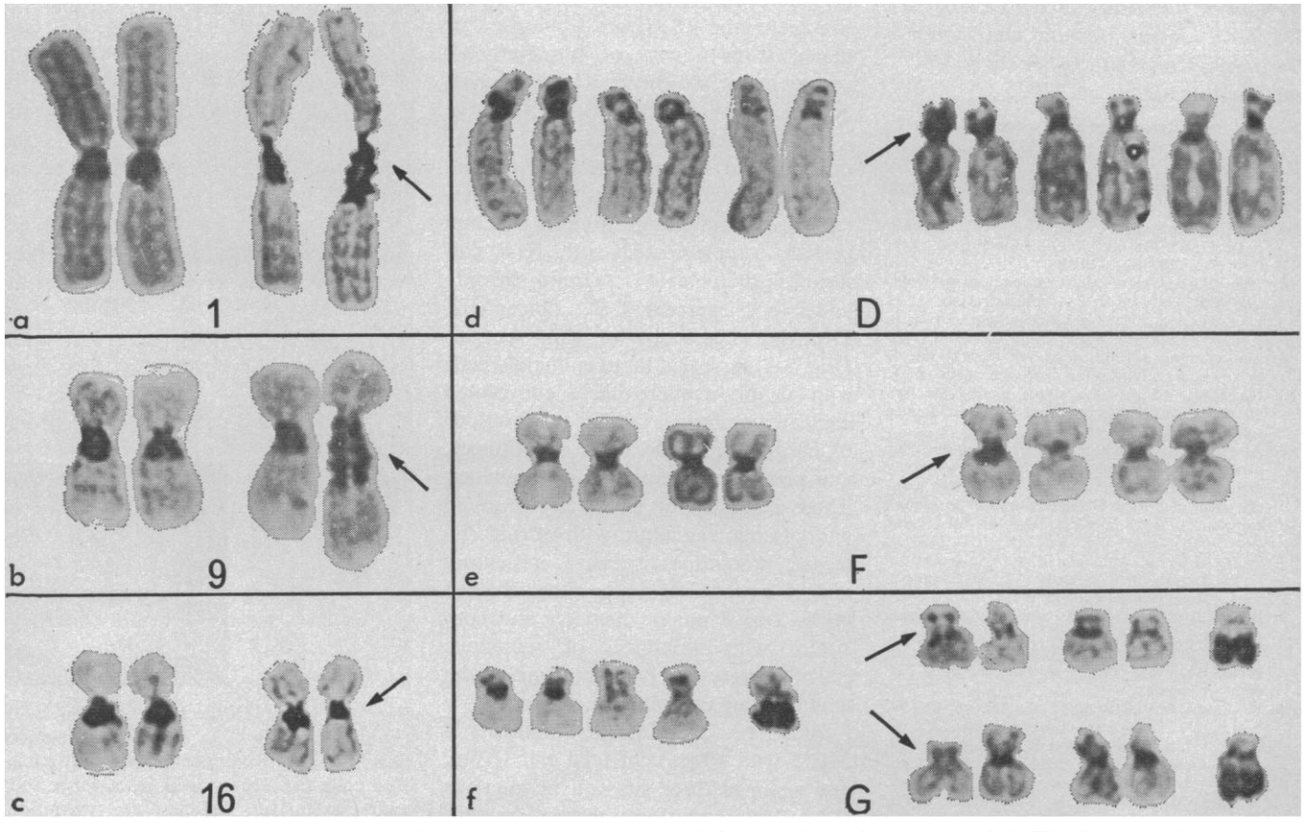

Fig. 1. Normal and variant heterochromatin patterns. Autosome pairs 1, 9, and 16 are shown on the left. The first two chromosomes depict the normal heterochromatin pattern in homologs; the second two show the variant compared to its normal homolog. Groups D, F, and G are similarly shown on the right side of the figure. Arrows pointing upward represent a variant with an increase in heterochromatin; arrows pointing downward show a decrease.

ied (*10*). This specialized function would not apply to the heterochromatin in the secondary constriction regions or in the long arm of the Y chromosome, and other functions will undoubtedly be assigned to these regions.

The type of variation which we have described has previously been reported only as a rare phenomenon. For example, asymmetry of chromosomes No. 1, 9, and 16 and variation in the length of the Y chromosome have been observed in standard leukocyte preparations and are usually inherited (*11*). It is probable that these variations, detectable by conventional cytological methods, simply represent one end of the spectrum of heterochromatin polymorphism. The sensitivity of the denaturation-renaturation technique in detecting heterochromatin variants has, therefore, led to the significant discovery that these variants occur with a much higher frequency than was heretofore suspected. Additional studies of heterochromatin variability, both in populations and within families, may determine how much tolerance the genome has for such variants.

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5. The staining solution consists of 1.5 ml of citric acid adjusted to *p*H 6.8 with 0.2*M* Na_2HPO_4, 1.5 ml of absolute methyl alcohol, 50 ml of distilled water, and 5 ml of Giemsa stock solution (Curtin).
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Herpesvirus Type 2 Isolated from Cervical Tumor Cells Grown in Tissue Culture

Abstract. *A herpesvirus has been isolated from spontaneously degenerating cultures of cervical tumor cells grown in vitro. The virus was identified as a type 2 herpesvirus on the basis of biologic properties, including plaque morphology and microtubule formation in infected HEp-2 cells, and of immunologic specificity as determined by neutralization. Herpesvirus antigens and virus particles were not seen in duplicate cultures of viable cervical tumor cells.*

Two types of herpesvirus, biologically and antigenically distinct (*1*, *2*), have been isolated from humans: herpesvirus type 1 (HSV-1) associated with facial lesions, and herpesvirus type 2 (HSV-2) isolated from smegma and cervical lesions (*3*) and shown to be venereally transmitted (*4*). Recent data indicate that patients with invasive (*5*, *6*) and preinvasive (*6*) cervical neoplasia have a significantly higher prevalence of antibody to HSV-2 than a matched control population, and that exfoliated dyskaryotic cells from these patients possess HSV-2 antigens as determined by immunofluorescence (*7*). In this report we describe the isolation of a herpesvirus with immunologic specificity of HSV-2, from cells of carcinoma of the cervix grown in tissue culture.

A line designated S332G was established by the Maximow double cover slip technique (*8*) from a biopsy obtained from a case of intraepithelial carcinoma of the cervix. The line, characterized by atypical cells with large lobulated nuclei and occasional multiple nucleoli and approximately 10 percent multinucleated cells (Fig. 1A), was established in minimum essential medium (MEM) supplemented with fetal calf serum (20 percent) (Grand Island) under a 5 percent CO_2 atmosphere. Electron microscopy of thin sections (Fig. 2A) revealed large epitheloid cells with elaborate microvilli, a convoluted nuclear membrane, small condensations of chromatin at the nuclear periphery, and prominent membranous organelles. These consisted of an extensive rough endoplasmic reticulum with dilated cisternae, smooth endoplasmic reticulum and a large Golgi apparatus. Packed fibrils 5 to 8 nm in diameter and consistent with tonofibrils of squamous cells (*9*) were observed surrounding the membranous cytoplasmic elements.

After ten transfers (6 months) in MEM with 10 percent fetal calf serum, and again at three subsequent intervals (12th, 15th, and 18th transfer), the cells in one out of replicate culture flasks began to round up, and within 48 hours cell degeneration consisting of swollen refractile cells (Fig. 1B) became prominent. The cells were scraped, collected by centrifugation at 400*g* for 10 minutes, and resuspended in 1 ml of MEM. Duplicate cultures established from the same seeding of cells and that had not degenerated were used as controls; they were designated "viable" and studied at the time of degeneration. Cultures of viable cells were also studied (3rd, 5th, and 8th transfer) before onset of degeneration. Two series of experiments were done to test for virus in degenerated cells. In the first series, one-half of the cell suspension was frozen and thawed, and the extract was used to in-

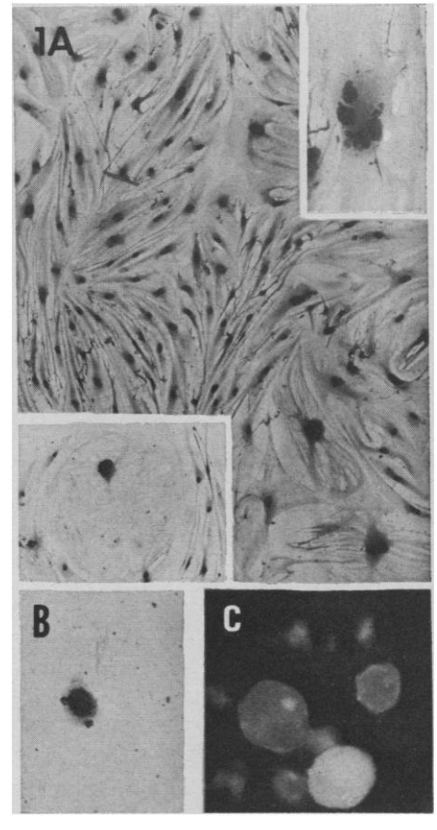

Fig. 1. (A) S332G cells stained with Giemsa display characteristic pleomorphism (× 110); upper inset, multinucleated cells (× 220); lower inset, large cells with extensive filamentous cytoplasm (× 110). (B) Degenerated S332G cell stained with Giemsa (× 220). (C) Degenerated S332G cells stained with Ra-2 serum (× 480).