CC/NUMBER 7
FEBRUARY 14,1983

This Week's Citation Classic

Moorhead P S, Nowell P C, Mellman W J, Battips DMA Hungerford D A.
Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* **20**:613-16, 1960. [Wistar Inst. Anat. and Biol.; Dept. Pathol., Sch. Med., Univ. Pennsylvania; Dept. Pediat., Hosp. Univ. Pennsylvania and Sch. Med., Univ. Pennsylvania; and Inst. for Cancer Res., Philadelphia, PA]

A combination of cytological and leukocyte culture techniques is described for a convenient, reliable approach to chromosome studies of humans. Advantages are: ease of obtaining a small volume of blood, adequate mitotic yield, and high proportion of quality metaphases for critical analysis of chromosome morphology. [The *SCI*® indicates that this paper has been cited in over 3,445 publications since 1961.]

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January 6, 1983

"This publication described the basic 'method' for cytological preparations of mitotic chromosomes from cultures of blood cells and contributed much to the explosion of published findings in human and animal cytogenetics in the 1960s. In essence, the method involved the combination of two prior contributions and a simple improvement of one aspect of each. Peter Nowell's discovery of phytohemagglutinin (PHA) as a mitogen[1] was combined with the technique of Rothfels and Siminovitch[2] for air drying to spread metaphase cells.

"The finding regarding PHA emerged from its original use in the separation of buffy coat from red blood cells. Osgood and Krippaehne,[3] pioneers in the cultivation of cellular elements of blood, had found mitoses in cells which attached to a slanted glass surface in their cultures. Mitotic activity seemed restricted to a certain level on the slide and this was attributed to an oxygen gradient in the culture. David Hungerford of the Institute for Cancer Research and Peter Nowell of the University of Pennsylvania were using such 'gradient cultures' for the study of human chromosomes. Bill Mellman, a pediatrician at the University of Pennsylvania, approached me regarding a similar collaboration and introduced me to Nowell and to gradient cultures. Disappointed with the small numbers of cells that attached to the glass slide, I examined the unattached cells on the floor of the culture vessel. Among them were high numbers of mitoses which had escaped attention, perhaps due to the wide acceptance of the importance of oxygen tension.

"Once it was established that the cells of interest did not attach to glass, a cytological method for suspended cells was sought. I had been impressed with the quality of chromosomes prepared by a cell suspension method published earlier by Rothfels and Siminovitch.[2] This method eliminated difficulties of the 'squash' technique and provided superior preparations, all chromosomes being in a single focal plane. However, air drying had repeatedly failed in my hands. Serendipity was involved in that cells of the mouse lymphoma, L-5178-Y, were available from Lionel Manson's laboratory near my own at the Wistar Institute. The ease of cultivation and high growth rate of these nonattaching cells permitted the testing of several variables each day. Surprisingly, minor departures from the usual air-drying procedure greatly affected cytological quality and it became apparent that rapidity of drying was a major factor. After weeks of trial and error involving hair dryers, dry ice, and other variations with little rationale, wetting the slide in cold distilled water prior to rapid evaporation of fixative dropped on the slide produced excellent results.

"These improvements were simple, but taken altogether they comprised a reliable and rapid method for karyological sampling of human subjects. The many citations to this paper were due to its practical value to a growing army of researchers, primarily pediatricians, who were drawn to cytogenetics by classic papers such as the determination of the true chromosome number in humans[4] and the demonstration that trisomy 21 was the cause of Down's syndrome.[5] A review in the field of cytogenetics has been published by H.J. Evans."[6]

1. **Nowell P C**. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. *Cancer Res* **20:**462-6, 1960. [Citation Classic. *Current Contents* (42):13, 17 October 1977.]
2. **Rothfels K H & Siminovitch L.** An air-drying technique for flattening chromosomes in mammalian cells grown in *vitro. Stain Technol* **33**:73-7, 1958.
3. **Osgood E E & Krippaehne M L**. The gradient tissue culture method. *Exp. Cell Res.* **9:**116-27, 1955.
4. **Tjio J H & Levan A.** The chromosome number of man. *Hereditas* **42:**1-6. 1956.
5. **Lejeune J, Gautler M & Turpin R.** Etudes des chromosomes somatiques de neuf enfants mongoliens. *C.R Acad Sci.* **248**:1721-2. 1959.
6. **Evans H J.** Some facts and fancies relating to chromosome structure in man. *Advan Hum Genet* **8**:347-438. 1977.