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This Week's Citation Classic

Hungerford D A, Donnelly A J, Nowell P C & Beck S. The chromosome constitution of a human phenotypic intersex. *Amer. J. Hum. Genet.* **11:**215-36, 1959.
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An individual of outwardly male appearance, clinically diagnosed a 'true hermaphrodite,' was found to have an XX sex chromosome complement. The first full description appears here of our adaptation to cytogenetic use of a method for culturing human leukocytes. [The *SCI*® indicates that this paper has been cited over 370 times since 1961.]

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"The case was ascertained by Andy Donnelly, late pathologist to the American Oncologic Hospital, with whom I shared a lab at the Institute for Cancer Research. He suggested this study and contributed the clinical and histopathological findings. The subject was male in appearance, except for gynecomastia of recent origin. The initial diagnosis was supported by biopsy of an intrascrotal ovotestis and confirmed by examination of internal structures later removed surgically by Sidney Beck. Leukocytes from circulating blood were cultured by Peter Nowell of the University of Pennsylvania School of Medicine. Squash preparation and the metaphase chromosome studies were my responsibility. A normal chromosome number and XX sex chromosome constitution, without evidence of a Y, were demonstrated. Periodic examination of the subject continued thereafter, and skin biopsies were obtained from a number of sites. Cells cultured from these provided cytogenetic results which confirmed and augmented our original findings.[1] The subject's enlistment in the armed forces precluded further study.

"This report briefly interrupted a collaborative project that Pete and I had initiated late in 1957, soon after he had found mitosis in human leukemic cells cultured according to Osgood's 'gradient' method. This observation prompted him to inquire whether anyone in the Philadelphia area was then active in human chromosome studies. At the time, I was apparently the only one who answered that description. Our investigation of chromosomes in cultured leukemic leukocytes followed, and it was soon clear that normal leukocytes could also divide in this system. Within a short time Pete was to make the classic discovery that phytohemagglutinin (PHA), used to agglutinate erythrocytes and facilitate their removal from the inoculum, was also a mitogen, a finding that made this investigation possible. Within a short time, a major technical improvement was made by Paul Moorhead, who substituted the air-drying method for squashing.[2] Leukocyte culture quickly became the method of choice in human cytogenetics, remaining so to the present. My introduction of KCl as an agent for hypotonic treatment accomplished further significant improvement,[3] gradually becoming standard in preparing mitotic cells from other sources.

"I suspect that the methods described in this early paper have had more to do with its frequent citation than have our findings, even though they are the first published on an intersex of this kind. The proliferation of lymphocytes *in vitro* is accompanied by their retention of specific functional capacities, and for this reason the method has profoundly influenced many other fields.

"The paper is also particularly memorable to me as the basis on which I was to participate in the 1960 Denver conference, at which the initial proposals were formulated for a standard nomenclature of human mitotic chromosomes. These recommendations, since revised and greatly expanded, remain a foundation of international agreement."

1. **Hungerford D A, Donnelly A J & Nowell P C.** The chromosome constitution of a human phenotypic intersex: reconfirmation of a 46-chromosome, XX, apparently non-mosaic "true hermaphrodite." *Hereditas* **52:**379-86, 1965.
2. **Moorhead P S, Nowell P C, Mellman W J, Battips D M & Hungerford D A.** Chromosome preparations of leukocytes cultured from human peripheral blood. *Exp. Cell Res.* **20:**613-16, 1960.
3. **Hungerford D A.** Leukocytes cultured from small inocula of whole blood and the preparation of chromosomes by treatment with hypotonic KCl. *Stain Technol.* **40:**333-8, 1965.