THE DIAGNOSIS OF THE SEX CHROMOSOMES  
IN HUMAN TISSUES  

by  
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In the human species, as in most other mammals, men normally have the sex chromosome constitution XY (references in Sachs 1954a), and women the sex chromosome constitution XX. But there are also human intersexes and other types of sexual abnormalities, and some of these may have different combinations of X and Y chromosomes. 

A diagnosis of the sex chromosomes of such people could, until relatively recently, only be made by a study of the chromosomes in dividing nuclei, and this requires a detailed knowledge of the chromosomes, and biopsy material from special organs. There is, however, another method based on the differential behaviour of the sex chromosomes in non-dividing nuclei, that requires less specialized training, and that can be applied to cells found in easily accessible parts of the body. This method has been used for the diagnosis of the sex chromosomes originally in some insects (Geitler 1937, 1939, Smith 1945, Jucci 1949), in a number of mammals (Graham and Barr 1952, Moore and Barr 1953), and lately also in humans including people with abnormalities (Moore, Graham and Barr 1953, Moore and Barr 1954, Barr 1954, Barr and Hobbs 1954, Emery and McMillan 1954, Hunter and Lennox 1954, Polani, Hunter and Lennox 1954, Cruickshank 1955, and Tavares 1955). 

This latter method has so far been based in mammals, on the assumption that in an XX the two X chromosomes together form, in
most of the nuclei, a chromocenter (which has been called "sex chromatin"), whereas in an XY the X and Y together do not form such a chromocenter in most nuclei. This assumption, which would also make it difficult to diagnose possible cases of XXY, XYY, etc., seems however, to be at variance with evidence found in other studies on the differential behaviour of the sex chromosomes, and we therefore decided to re-investigate the basis for the diagnosis of the sex chromosomes in the non-dividing nuclei of mammals.

Our analysis on human material, like that of most other investigators, has been mainly made on the skin, since biopsies of this tissue can be readily obtained. We have also included in the present study an analysis of the polymorphonuclear neutrophil leucocytes in the human blood, since this is another type of cell that is easily available, and that has also been reported as showing a sex difference (DAVISON and ROBERTSON SMITH 1954).

MATERIAL AND TECHNIQUES

Pieces of skin were obtained by biopsy by courtesy of the Surgery Department of the Kaplan Hospital, Rehovoth, and fixed immediately in Smith's modification of Kahle's fixative (DARLINGTON and LACOUR 1947), which is composed of 100 parts absolute ethanol, 7 parts glacial acetic acid, and 40 parts 40% formaldehyde. This fixative can give a very good preservation of the nucleus, and for the present study we chose six males and six females whose skin showed the best degree of preservation.

The pieces of skin were embedded in paraffin, and sectioned at 10μ for observation and at 6μ for photography. The sections were stained by a Feulgen and fast green method, in which after the ordinary Feulgen staining, the sections were treated for 20 minutes with 80% alcohol saturated with Na₂CO₃; rinsed in alcohol; stained for 20 minutes in a saturated fast green solution diluted 1/4 with 80% alcohol; and then differentiated in 95% alcohol. Alternatively, staining with a 0.5% solution of toluidine blue at a pH of 3.5 also gave satisfactory results. After staining, the slides were dehydrated in the usual way and mounted in De Pe X. Material taken from other mammals was treated in the same way as the human skin, except for some