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Sex Chromatin Demonstration by A Modified Hair Root Sheath Technique

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BARR and Bertram (1949) were first to observe a fairly distinct chromatin mass in neurones of female cats while studying the basic aspect of fatigue (nerve) at high altitude. This nuclear mass was often found to be juxtranucleolar; therefore they named it a nucleolar satellite. Later they found it to be often lying near the nuclear membrane as a rounded body, about 1μ in diameter, and it was labelled as Sex Chromatin. Now it is well established that the sex chromatin can be seen in cells of females only since it represents one of the paired X-chromosomes which differs from its homologue and is heterochromatic. The sex chromatin is not seen in males where the single X-chromosome resembles non-heterochromatic X-chromosome of the females.

Sex chromatin can be studied either in sections or in smears (from oral mucosa, blood etc). Barr and Moore (1953) used skin biopsy for demonstrating sex chromatin. Later in 1955 they introduced buccal smear technique. Since then a large number of workers have used different body cells for demonstrating sex-chromatin.

But in clinical practice, the buccal smear technique remains as the widely used method for demonstration of sex-chromatin. The difficulty to get a proper specimen in uncooperative patients is a big draw back of this method. To overcome this the hair root sheath method was introduced by Schmid Katz (1970) and Culbertson (1969) further improvised this method. The need for finding an easier and predictable technique has been enhanced by a much wider application of this investigation in clinical practice (Sinha et al. 1971).

Material & Methods :

The present study was conducted on a group of patients admitted in the Department of Plastic Surgery, King George's Medical College, Lucknow. The criterion for selection was that these should not be suffering from any disorder of sexual differentiation. It constituted of 75 cases of either sex & of varying age groups. The following technique was employed to demonstrate the sex-chromatin in hair root sheath.

Technique Employed :

To obtain hair root sheath few hair were plucked with epilation forceps from the scalp. Savlon hair bath was given to patients prior to epilation in order to obviate the interference by dirt and dandruff. Hair root sheath with the attached hair (cut short to

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about 2 cms.) was put on a thin and clean slide. Two drops of 60% glacial acetic acid were poured over the hair. A cover slip with the edge projecting beyond the slide was put on it. After two minutes the preparation was squashed between the cover slip and the slide. The cover slip was then briskly lifted off the slide and the preparation was fanned to dryness.

The dried specimen was subjected to the routine haematoxylin and eosin staining and finally the hair root sheath was mounted in canada balsam. Fig. 1 & 2 show the photomicrograph of cells of hair root sheath

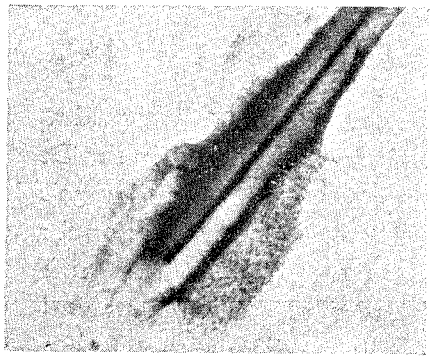

Fig. 1—Photomicrograph of hair root sheath under low power magnification : showing the relation of hair and its root sheath.

under low and oil immersion magnifications respectively. Under oil immersion magnification 100 nuclei were subjected to examination for the demonstration of sex chromatin.

Observations :

The observations made are shown in the following tables.

Table I. Showing the average incidence of sex chromatin positive cells in females.

% +ve cells	below 65	66-75	76-85	above 85
No. of subjects	Nil	17	6	2

Table II. Showing the average incidence of sex chromatin positive cells in males.

% +ve cells	Nil	below 2	2-4	above 4
No. of subjects	8	12	5	Nil

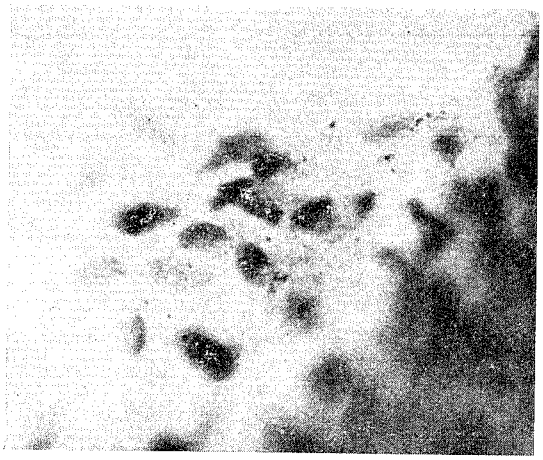

Fig. 2—Photomicrograph of hair root sheath under oil immersion magnification : showing the sex chromatin body.

Table III. the percentage of sexchromatin positive cells in cases with labelled sex.

Sex	No. of cases	% +ve Cells Range	Average
Female	25	66-87	73.8
Male	25	0.4	1.2

Table IV. Showing the results of blind trial in 25 cases.

Case Nos.	% +ve cells	Sex Interpretation	Confirmation
1,3,4,6,9,10, 11,13,15,16, 22,24,25	72-82	Female	100%
2,5,7,8,12,14, 17,18,19,20, 21,23	0-2	Male	100%

Discussion :

It was Schmid (1967) who for the first time advocated the use of hair root sheath for the demonstration of sex-chromatin in the nuclei of the cells. He used dissecting microscope for mincing and separating the sheath & stained the preparation with Aceto-orcein. Katz (1970) used bard-parker knife & Culbertson (1969), the dissecting microscope for separating the sheath from the hair root. Both these workers used Lacto-orcein for staining the preparation. Tedious and lengthy process with release of pigment granules were the disadvantages common to all these techniques and this prevented the common usage of hair root sheath for histological demonstration of sex chromatin.

The property of swelling of hair root sheath with glacial acetic acid for separating the sheath from the hair root was utilised in the present work; proper squeezing by cover slip enabled us to get a flat specimen. The glacial acetic acid also provided for the necessary acidification of the cell and its nucleus, a pre-requisite for proper nuclear staining.

As haematoxyline gives quite satisfactory nuclear differentiation we did not use any other specific nuclear stain.

Total time taken in the preparation of slide and staining varied from 20-25 minutes and at a time if many slides are made it further saved the time. The advantages of using this techniques were— a) easily obtainable clinical material, b) no interference from bacteria, c) many nuclei are available for examination in one field and d) sharp definition of chromatin body in the nuclei.

Now we are employing this technique in all cases of disorders of sex differentiation and male infertility*.

Summary

A modified technique for demonstration of sex chromatin in nuclei of hair root sheath has been described and its advantages are discussed.

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