DETERMINATION OF GENETIC SEX
BY EXAMINATION OF EPITHELIAL CELLS IN URINE

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In 1949 Barr & Bertram reported morphological sex differences in the nerve cell nuclei of cats. In further studies Barr et al. (1950), Graham & Barr (1952) and Moore & Barr (1954) found sexual dimorphism of the nuclei also present in many tissues of human subjects.

Later (Moore, Graham & Barr, 1953), it was reported that skin biopsies were well-suited for studying chromosomal sex. This method rapidly gained ground in clinical practice as a diagnostic aid in various anomalies of sexual development, especially gonadal dysgenesis and hermaphroditism.

This method requires, however, a rather special technique, including well-differentiated stainings, and a wide experience. The sex chromatin is best observed in the cells in the deeper epithelial layers of the skin. The oral mucosa (Eskelund) appears to be somewhat better suited, but local anaesthesia is necessary for the removal of biopsy specimens, the preparation of which takes at least 3 days, and the evaluation of the results is not always easy.

It is considerably easier, as reported by Moore & Barr (1955) to examine scrapings of the oral mucosa. This is obtained by rubbing the edge of a wooden tongue depressor firmly against the buccal mucosa. The material obtained is smeared gently on microscopic slides coated with a thin film of Meyer's egg-albumin. The slides are immediately immersed (without drying) in Papanicolau's fixative (equal parts of 95 % alcohol and ether). The fixed smears are passed through decreasing concentrations of alcohol to water, stained in a 1 % solution of cresyl echt violet (Coleman & Bell) for five minutes, differentiated first in 95 % alcohol for five minutes and then in absolute alcohol under the microscope, cleared in xylene, and mounted in neutral balsam. The preparations are reported to be «easier than skin biopsies to interpret and they require less experience in cytology . . . The result obtained from a study of a smear from
the oral mucosa will no doubt have the same significance in differential diagnosis as does the interpretation of skin biopsy» (Moore & Barr, 1955).

By a slightly modified technique Marberger, Boccabella & Nelson (1955) arrived at the same result. The vaginal epithelium also appears to be suitable for the study of «sex chromatin» (Carpentier, Stolte & Visschers, 1956). In smears of the vaginal secretion stained by the Papanicolaou technique the sex chromatin body was easily demonstrable. Comparison with scrapings from the oral mucosa showed that in many cases the vaginal secretion was preferable. These authors state, however, that they have no experience of the staining procedure described by Moore & Barr.

PRESENT INVESTIGATIONS

The cytological test to be described below is based on desquamated epithelial cells from the bladder epithelium. The procedure is as follows: Morning urine is voided into or immediately mixed with 15–20 ml. of Davidson’s fluid and shaken. Allow the specimen to stand for about one hour, pour off the urine and centrifuge the sediment in conical centrifuge tubes. Again pour off supernatant, stir sediment with a wooden stick, and spread on a slide coated with a very thin film of serum (Priman, 1954). Fix for another 20–30 min. in methyl alcohol. Thereafter, the preparation may be allowed to dry without any risk and, if necessary, be dispatched by mail.

Stain in basic fuchsin in acid solution to which formol¹ has been added. The staining fluid must be at least two weeks old, but it will then keep for years. Stain the slides for about 10 min. in a staining dish. thoroughly differentiate in absolute alcohol, clear in xylene, and mount in dammar. By this procedure the sex chromatin stains distinctly, while other chromatin structures are less conspicuous. The structure is particularly distinct when using a green filter. The location of the sex chromatin varies somewhat, but in the majority of cases chromatin positive nuclei are of typical marginal location (Figs. 1 and 2). In chromatin negative nuclei, moreover, the structural pattern is often coarser (Fig. 3). In smears of the sediment there are, in addition to cells from the bladder epithelium, cells from the urethral and in females from the vaginal epithelium. These cells are also of diagnostic value. Furthermore, there will be bacteria and often large quantities of leukocytes and inorganic sediment. Occasionally there are spermatozoa too. Cells with blurred or shrunken nuclei are excluded as in the study of smears from the oral mucosa or vaginal secretion. The examination of more than 100 urines from subjects whose sex was unknown to the examiner gave the correct result in all. In the male specimens the sediment was sometimes very scanty, but sufficient.

¹ Carbol fuchsin 15 ml., glacial acetic acid 2 ml., 40% formaldehyde 2 ml.
Fig. 1.
Sex chromatin positive cell nuclei (× 1900) with a delicate chromatin network and sex chromatin in one nucleus in the typical marginal location, whereas in the other one it is more centrally located.

Fig. 2.
Sex chromatin positive cell nucleus (× 1900) showing the sex chromatin adjacent to the nuclear membrane.
**Fig. 3.**
Sex chromatin negative cell nucleus (× 1900) with a coarse chromatin network.

**DISCUSSION**

By the present staining method it is fairly easy to demonstrate the sex chromatin. As mentioned above, however, shrunken, folded, or partially autolysed nuclei must be excluded. The sex chromatin is extremely sensitive to drying, which is avoided by the procedure used. In sex chromatin positive nuclei the entire structure, apart from the sex chromatin, is extremely delicate and often almost invisible. The sex chromatin is usually of the typical marginal location, but a more central location does not exclude its demonstration. The coarser structure and more distinct staining of the sex chromatin negative nuclei affords another good clue. On the whole, the cytological evaluation is considerably easier and quicker than the histological assessment in which current staining methods frequently stain not only the sex chromatin, but also other chromatin structures so deeply as to give rise to misinterpretation. In routine use, cytological determination is without doubt preferable to the histological method. When using urine, the test may be carried out and repeated without the patient knowing anything about it. If the patients are infants or have not yet developed toilet training, the urine may be obtained by catheterization, or it may be collected in bags.
SUMMARY

A method for determining the genetic sex by examination of epithelial cells in the urine after staining with formol-fuchsin is described.

REFERENCES