OBSERVATIONS ON THE SO-CALLED SEX CHROMATIN

By

J. JAMES

With 18 Figures in the Text

(Received December 22, 1959)

I. Introduction

After Barr’s initial discovery (Barr and Bertram 1949), the existence of a sexual dimorphism in various mammals and the human has become well established. There is some evidence that Barr’s “sex chromatin” in the female is of a chromosomal nature and probably represents fused heteropycnotic portions of both X-chromosomes (Lennox 1956, Danon and Sachs 1957, Ohno et al. 1958), although other hypotheses have been advanced as to the last point (Witschi 1957, Nelson 1958). There remain some conflicting facts, however, which seem to be at variance with the acceptance of such a simple cytogenetic explanation of the phenomenon in question; these can be summarized as follows:

1. Why should the “female sex chromatin” be found only in a smaller or larger portion of the nuclei but never in all? It is found in 60—80% of the nuclei in sections of human tissues and in 40—60% in smears of oral mucosa (Moore and Barr 1954, Marberger et al. 1955, Moore and Barr 1955). In a few instances (embryonic membranes) (Graham 1954, Klinger 1957), larger percentages have been reported, but this does not seem to be the rule. Slizinsky’s hypothesis (1957) that X-chromosomes may be lacking in some tens per cent of nuclei, cannot be maintained for normal tissues. Dykstra (1958) advanced the hypothesis that, in oral mucosal smears, the sex chromatin in cells of women may be invisible when forming a thin sheet lying parallel with the optical axis of the microscope.

In tissue cultures the situation is also obscure. In kidney cultures from female cats and dogs, Burlington (1959) describes a “sex chromatin” in 38—43% of the nuclei of a newly established culture; Fraccaro and Lindsten (1959) observed such a structure in 37—74% of the nuclei in a series of cover glass cultures of different organs from female human embryos.

2. What has one to think of such corpuscles as are found in the human in 3—13% of the nuclei in male individuals (Moore and Barr 1954) and rather vaguely described as having morphological features similar to those of the “female sex chromatin?”

If this corpuscle is an analogon, in the male sex, of the corpuscle found in nuclei of female cats and humans, then one would expect to find it in at least as large a percentage as the sex chromatin in the female sex. Otherwise there must be some doubt as to the counts made in female nuclei.

A probably related situation is encountered in some rodents (e.g. rats and mice), where Barr’s phenomenon cannot be traced in cell groups of the central
nervous system or liver cells (Moore and Barr 1953, Hinrichsen and Gothe 1958), the nuclei in both sexes containing a variable number of chromatin particles of the size of the sex chromatin. In other tissues in these rodents, however, the nuclei show a demonstrable sexual dimorphism similar to that seen in man, cats and apes, e.g. in the nuclei of ameloblasts in newborn rats (de Castro et al. 1956) or of the Purkinje cells in the cerebellum (Hinrichsen and Gothe 1958).

On the basis of the abovementioned facts some authors (Hienz 1957, Hertl 1957) independently reached the conclusion that, in fact, only a quantitative difference has been demonstrated between the cell nuclei of both sexes. They regard the sexual dimorphism described by Barr et al. as a result of a sex-linked difference in nuclear metabolism. This theory is also used to explain local variations in the percentage of "positive" nuclei in various tissues of female animals, and the conflicting evidence in rodents.

Although most authors do not go so far as the abovementioned investigators, this controversy is nevertheless a good starting-point for an attempt to establish somewhat more firmly a number of quantitative and qualitative facts concerning the "sex chromatin".

II. Material and techniques

Cats were used as test animals for the main part of the investigation (8 male, 10 female cats aged 4 months to 2 years). As a rule the animals were sacrificed under chloroform anaesthesia after a night's fasting. Material from 6 male and 6 female rabbits aged 6 to 12 months was fixed under the same conditions. Human material: oral mucosal smears from 12 normal male and female subjects.

The tissue fragments were fixed immediately after having been obtained. Carnoy's solution was chiefly used as a fixative, with half strength acetic acid (absolute ethanol, chloroform, glacial acetic acid 60:30:5 instead of 60:30:10). For small fragments with a diameter of a few millimetres, excellent fixation of nuclei is attained after 1½--2 hours. Overfixation with Carnoy's mixture must be avoided lest nucleoproteins be extracted (Pearse 1953); this risk is less marked when the original Carnoy solution is replaced by one with a lowered strength acetic acid. Also, we used the Smith mixture of 100 parts absolute ethanol, 7 parts glacial acetic acid and 40 parts 40% formaldehyde.

The handling of total preparations of fragments of mesentery from cats and rabbits posed some peculiar problems, as these delicate membranes (3--8 μ in their thinnest parts) tended to retract and tear when pinned on to cork rings during hydrolysis for the Feulgen reaction. They were therefore stretched in sets of two ebonite rings fitting into one another, in the manner of a drumskin; they were thus fixed and hydrolysed at 37° instead of 60° C.

Staining methods Feulgen reaction (after fixation in modified Carnoy solution, optimal hydrolysis at about 10 minutes in N HCl at 60° C., one hour at 37° C.); gallocyanin according to Einarsen (1951) and Giemsa's solution, applied in a buffered 3% solution with pH 6.8--7.1, applied once or twice, followed by differentiation in distilled water. Finally Weigert's haematoxylin was used, especially for material fixed in the Smith solution. All specimens were mounted in the synthetic medium "crystallite" (Gurr).

The tissue cultures were explanted, from joint capsules and subcutaneous tissue of young cats, into roller tubes in the conventional manner, using the following medium: 5 parts Tyrode solution and 5 parts of a mixture of 1 part chick embryo extract, 1 part Tyrode solution and 4 parts human umbilical cord serum. When growth was observed (generally after 5--7 days), subcultures were made on glass strips and fixed after 1--3 days. We also studied specimens of human fibroblast cultures from the collection of the Histological Laboratory (fixed in Maximow, stained with Grüber's Kernechtrot).

For phase-contrast observations we used a culture chamber of the conventional type (Leitz phase optics with a condensor of the Heine type).