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MEDICAL BIOLOGY PRACTICALS. CYTOGENETIC.

Practical 2. Barr body observation in squamous epithelium cells from human throat lining.

Compiled by Boris M. Sharga, Diana B. Pylypiv, Volodymir P. Feketa

Theoretical background. Barr & Bertram (1949) reported that feline nerve cells consistently showed a small darkly staining body close to the nuclear envelope, while cell nuclei from cat males showed no such structure [2]. It was named as *Barr body (Bb)* after M. Barr. It is an inactivated X chromosome seen in female somatic cells usually near to the nuclear membrane. The chromosome is rendered inactive early in the embriogenesis of female mammals in a process called *lyonization*, in which it becomes highly condensed and visible as a densily staining spot.

Condensed state of densely stained chromatin signifies that in such cases DNA replication occurred at the later stage of S phase of cell cycle. *Bb* generally appear as basophilic structures with varying morphology (spherical, rectangular, planoconvex, biconvex, or triangular), 0.8 × 1.1 μm in size. Often they resembles various alphabetical letters in EM as V, W, S, or X. Special stains for nucleus such as Papanicolaou stain, Fëulgen stain, orcein, hematoxylin and eosin, cresyl violet, carbol fuschin, toluidine blue, and fluorescent staining are used to visualize *Bb* [1]. *Bb* can be observed in buccal smear, tooth pulp tissue, vaginal smears, hair follicle, blood neutrophils, etc.

By Lyon hypothesis, cells with several X chromosomes have all but one inactivated during embryogenesis of mammals [13]. This is random process [3] which can involve any of the X chromosomes in the cells. As a result, the female adult is *mosaic* body, having 2 genotypes, corresponding to the inactivation choice (Fig. 1). This may results in manifestation of some X-linked traits in heterozygous females, like in cases of calico (Fig. 2) and tortoiseshell (Fig. 3) furs in cat or anhydrotic (hypohydrotic) ectodermal dysplasia in human (*HED*), characterized by the absence of sweat glands (Fig. 4), heat intolerance, finely wrinkled skin, sunken nose, malformed and missing teeth, and sparse fragile hair [9].

A test using iodine to detect the presence or absence of sweat glands especially in those heterozygotic females who have only 1 defective gene for X-linked *HED* (2 defective genes are necessary for all the symptoms). The starch-iodine test consists of the application of an iodine-in-alcohol solution over the entire back. It is then followed by the application of a starch/castor oil suspension. The sweat glands thus become highlighted and appear as black dots. Characteristic streaks appear on the back specifying those areas that are devoid of sweat glands (usually, along the lines of Blaschko) [10].

The *mosaicism* is the presence of two or more cells populations with different genotypes in one individual, developed from a single zygote.

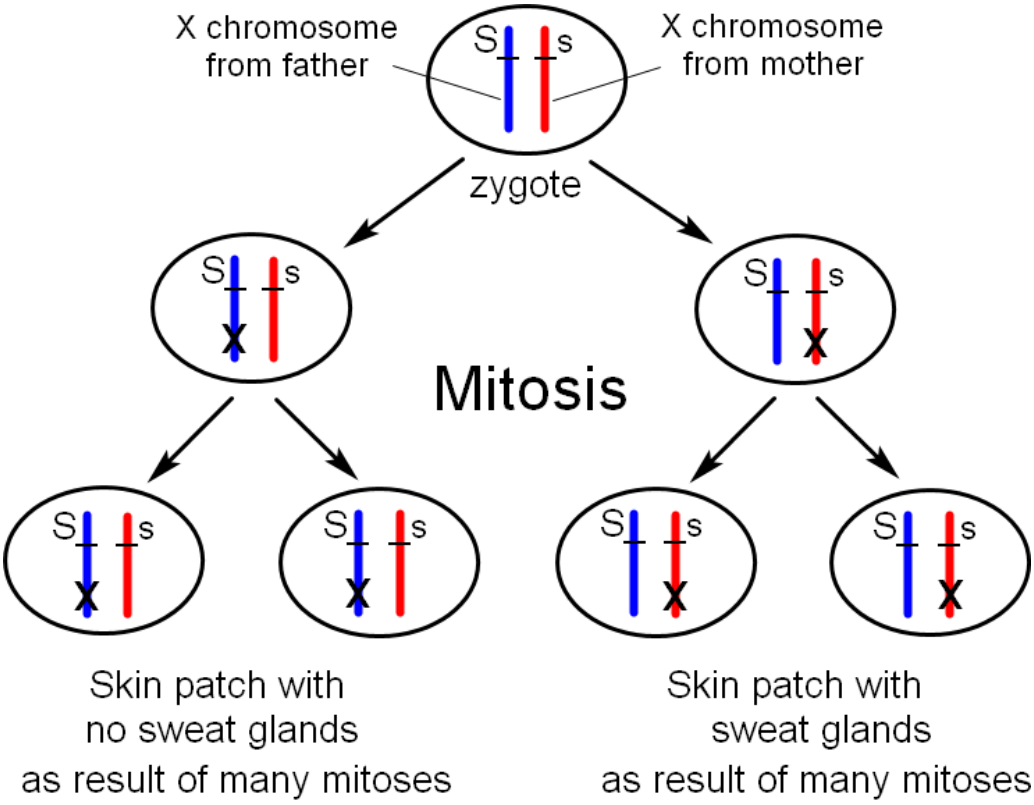

Fig. 1. Human X chromosome inactivation at 2-cell stage zygote, resulting in mosaic body. Female zygote is heterozygous (*Ss*) for theX-linked genes *S* (sweat glands presence) and *s* (anhidrotic ectodermal dysplasia sweat glands absence) becomes a mosaic women composed of 2 cell lines expressing one or the other of the alleles due to one or the other X chromosome inactivation. The latter can take place at other low cell numbers too. *By Diana B. Pylypiv*.

Mosaicism is present in up to 70% of cleavage stage embryos and in as high as 90% of blastocyst-stage embryos derived from *in vitro* fertilization. Genetic mosaicism can result from several mechanisms other, than X chromosomes inactivation also, particularly, by *non-disjunction*, *anaphase lagging* and *endoreplication*. Anaphase lagging is the most common mechanism by which mosaicism arises in the preimplantation embryo [5].

Mosaicism can also result from one cell mutation during development in which the mutation is passed on to only its daughter cells. Thus, the mutation is only going to be present in a fraction of the adult tissues [14].

Genetic mosaics may often be confused with *chimerism*, in which two or more genotypes from the fusion of two or more fertilized zygotes at the early stages of development, rather than from a mutation or alternative X chromosomes inactivation. Test for *Bb* presence in neutrophils was introduced into gender estimation of athletes by the International Olympic Committee in 1968. Regarding the possible XX/XY chimerism, Turner's Syndrome (45, XO), Klinefelter's Syndrome (47,XXY), SRY-gene (maleness gene) presence in some of XX persons or its absence in some of XY

athletes, it was regarded as controversial [16, 17]. However, this test remained the standard method of gender verification by IOC until 1992, when it was replaced by Y chromosome tests. However, neither of these tests is 100% accurate, because of above mentioned possibilities.

Fig. 2. Calico cat
By Felis_silvestris_catus.010_-_La_Coruña.JPG: Drow male [7]

Fig. 3. Short-haired tortoiseshell cat
By Luca Shawranke [7]

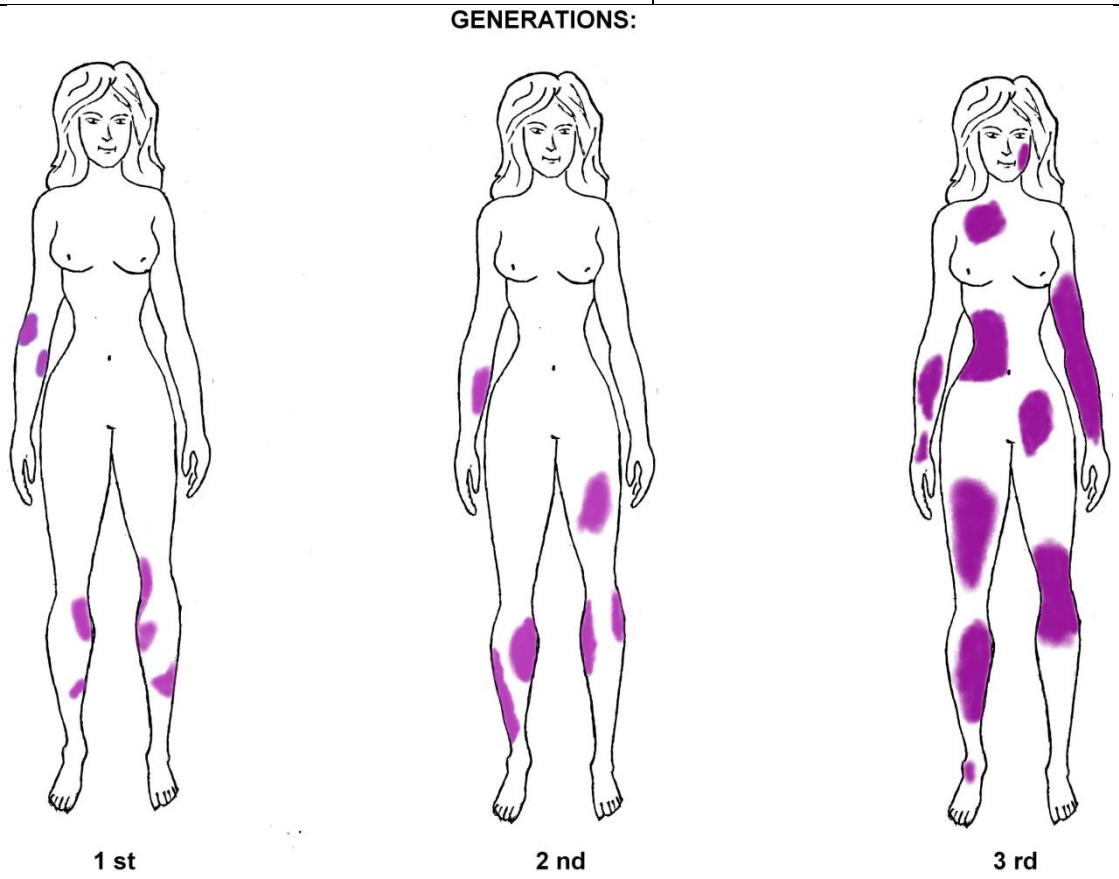

Fig. 4. Somatic mosaicism in 3 generations of females heterozygous for sex-linked anhydrotic ectodermal dysplasia, the absence of sweat glands. Areas without sweat glands are shown in violet. The extent and location of the patches is determined by chance. *By Diana Pylypiv*.

The number of *Bb* visible at interphase is always equal total number of X chromosomes minus one (Table 1).

Table 1. Number of Barr bodies at different genotypes

Condition	Sex Chromosomes	Number of Barr Bodies
Normal men	XY	None
Kleinfelter's syndrome men	XXY	1
Normal women	XX	1
Turner's syndrome women	X0	None
Trisomy X women	XXX	2

The X inactivation center, *Xic*, usually found near the centromere, regulates of X chromosome inactivation. It contains 12 genes, 7 of which code for proteins and 5 for untranslated RNAs, 2 of which, *Xist* and *Tsix*, play an active role in the process. Supply of an extra artificial *Xic* results in inactivation of the single X in male cells in early embryogenesis [15, 18]. The *Xist* and *Tsix* act as antagonists. The loss of *Tsix* expression on the future inactive X chromosome results in an increase in levels of *Xist* around the *Xic* (and *vice versa*)[12]. The *Xist* begin to coat the future inactive chromosome, spreading out from the *Xic* [13].

In non-random inactivation this choice is fixed and the maternally inherited gene may be imprinted [3]

This constitutes the mechanism of choice, and allows downstream processes to establish the compact state of the *Bb*. These include histone modifications, such as histone H3 methylation (i.e. H3K27me3 by PRC2 which is recruited by Xist) [6] and histone H2Aubiquitination and methylation of CpG sites in DNA [4]. These changes suppress the gene expression on X-chromosome and causes its compaction to form the *Bb*.

The marsupials (like kangaroos, wallabies, koalas, possums etc.) and some extra-embryonic tissues of some mammals, deactivate the father's X chromosome always [10].

Reactivation of a *Bb* has been seen in breast cancer patients. The frequency of Barr bodies in breast carcinoma were significantly lower than in healthy controls, indicating reactivation X chromosomes [15].

Experiment 1. Squamous epithelium cells staining by methylene blue

Materials and equipment:

0.3% methylene blue, pipette for 10-100 μL and sterile nose tips for it, cotton swabs, glass slides and cover slips, alcohol burner, matches, microscope.

Method:

1. Throats should be rinsed with sterile water to remove the food remnants and to decrease the bacteria presence in preparations. Gently scrap the inside both of girl’s (female’s) cheeks with a sterile cotton swab in one direction. Do the same with cheeks of boys (males).

2. Wrap and spread the collections onto a diameter of ~ 2 cm on glass slide. Let it air dry of or fix it gently over flame of alcohol burner.
3. Add 10 μL drop of methylen blue onto a glass slide and cover it with cover slip.
4. Let stain for 3-5 min.
5. Observe under a microscope using objectives 10 ×, 40 ×. When interesting cells are found turn to oil immersion objective 100× to increase magnification and resolution.
6. Compare the observations in smears from males and females. Why in normal males you can't find the *Bb*? Why *Bb* observed not seen in each cell from females?

Experiment 2. Squamous epithelium cells staining by thionin

Materials and equipment:

Stock solutions

1. *0.5% Thionin solution:* 0.5 g thionin in 100 ml distilled H_2O. Do not use a metal things to stir the thionin! Filter the thionin solution.
2. *1.0 M Sodium acetate solution:* 8.2g of anhydrous sodium acetate dissolved in 100 ml of distilled H_2O.
3. *1.0 M Glacial acetic acid solution:* add 6.1 ml glacial acetic acid to 100 ml distilled H_2O.

Thionin stain solution

Add 0.9 ml of Sodium acetate solution to the 18 ml of distilled water. Mix it and add 2.1 ml of Acetic acid solution. This buffer solution should have pH 4.3. Adjust, if necessary, by adding drops of either sodium acetate or acetic acid solution. Then add 1.8 ml of 0.5% thionin solution.

The pH strips or pH meter are useful for this experiment. Light microscope with oil immersion objective, the pipette for 10-100 μL and sterile nose tips for it, the cotton swabs, the glass slides and cover slips, alcohol burner, matches are necessary also.

Method:

1. Fix-dry the buccal cells smear over the flame as above.
2. Cool it and cover (**with great care!)** for 8 sec by drops of 6N HCl, while holding the slide over the tap sink. **(Caution!** HCl at this concentration may burn your skin or eyes. Do use protective eyeware and gloves).
3. Gently rinse off the slide with sterile distilled H_2O .
4. Applyfew drops of thionin stain solution. Place the slide into Petri dish to prevent evaporation for 15 min. (**Caution!** The solution stains fingers or laboratory coat).
5. Wash the slide in a little stream of distilled H_2O for 30 sec.
6. Soak the slide in 70% ethanol for 2 min to bleach the stain a little.
7. Rinse in distilled water. Remove excess water and place cover slip over the stained area.
8. Examine the preparations with oil immersion objective. Detect dark blue or almost black stained *Bb*, usually, close to the nuclear envelope.

9. Compare your slides with someone of the opposite sex. To make right conclusion, remember: about 2% of male buccal cells show dense chromatin, resembling *Bb*.

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