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*DEMONSTRATION OF TWO POPULATIONS OF CELLS IN THE HUMAN FEMALE HETEROZYGOUS FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS**

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In 1932, Muller coined the term "dosage compensation"[1] to account for the equality of phenotypic expression in males and females for most genes located on the X chromosome. Over the years several explanatory theories for this phenomenon have been considered, and these have been recently reviewed by Stern[2] and by McKusick.[3]

In 1961, a unique hypothesis was developed by Lyon[4] and by Russell.[5] The hypothesis, often referred to as the "Lyon Hypothesis," proposes that in each somatic cell of the female, one of the two X chromosomes is genetically inactive. The inactivation must occur early in development, and it is a matter of chance whether the maternal or paternal X is inactivated. Once an X chromosome is inactivated in a developing cell, all progeny of that cell presumably maintain the same inactive X.

Examination of single cells in a female who is heterozygous for an X-linked gene(s) with a measurable effect would provide direct evidence bearing on the hypothesis. In such a case, the female would be expected to produce a mosaic of X chromosome activity. In some of her cells, one allele would be active; in the remainder, the other allele would function.

The enzyme, glucose-6-phosphate dehydrogenase (G-6-PD), provides both a quantitative and a qualitative tool for testing the hypothesis. It has been shown that in a Caucasian male whose erythrocytes have deficient G-6-PD activity cultured skin cells are also clearly deficient.[6] Our own studies have confirmed this and have shown the ranges of activities for skin cells of the various genotypes.[7] If the "Lyon Hypothesis" holds, the heterozygous Caucasian female should produce two quantitatively different cell populations, one with G-6-PD activity in the mutant range, and one with activity in the normal range.

Starch gel electrophoresis has demonstrated qualitative variants of erythrocyte and leucocyte G-6-PD in the American Negro population, but not among Caucasians.[8, 9] Negro males can have a fast G-6-PD band, A, or a slow one, B. Females can have A, B, or both A and B. These studies have proved that the electrophoretic variants are inherited, and that the responsible genetic locus is on the X chromosome. Thus, if the "Lyon Hypothesis" applies, the female who is heterozygous for the two electrophoretic variants should also be a mosaic—some of her cells producing A type G-6-PD, some the B type, but none producing both.

The quantitative and qualitative variants are genetically related, but the precise relationship is not clear. With rare exceptions, among Negroes, all males with erythrocyte G-6-PD deficiency have the A electrophoretic variant. However, not all males with A variant are enzyme-deficient. To date, all Caucasians have the B electrophoretic band.

This paper reports the results of quantitative and qualitative analyses of clones derived from single cells from females heterozygous for the G-6-PD variants.

Materials and Methods.—Individuals studied: Skin biopsies from 17 Caucasian males and females were obtained in Sardinia, and these were studied for G-6-PD deficiency.[7] The genotype of each individual was designated in a previous family analysis.[10]

The Negro females presumed to be heterozygous for the qualitative electrophoretic variants were selected on the basis of appearance of two electrophoretic G-6-PD bands in extracts of cultured cells. Four of the six women were also studied for erythrocyte G-6-PD deficiency[10] and are heterozygous for the enzyme deficiency as well. Two of the women with the AB phenotype were found among Negro female employees at Sinai hospital.

Experimental procedures: (1) *Skin biopsies:* Biopsies were obtained without anesthesia using a high-speed electric rotating biopsy punch.[11] A core drill of 3 mm in diameter was employed. (2) *Culture technique:* The cores of skin were minced into 20 or 30 tiny fragments and cultured in Eagle's "Minimum Essential Medium" with 20% human AB or B serum and 5% beef embryo extract ultrafiltrate.[12] Plastic culture dishes were used, and the tissue was incubated at 37°C in an atmosphere of 5% CO_2 in air. Clones were developed from single cell platings of cultured cells according to the techniques described by Puck *et al.*[13] (3) *Assay of G-6-PD activity:* Suspensions of cultured cells were disrupted by sonication, and enzyme activities were measured by a modification of the method of Kornberg and Horecker.[14] A Beckman DU spectrophotometer, equipped with a Gilford optical density converter, model 220, was used. G-6-PD activities are expressed as Δ O. D. per mg of protein per hr. (4) *Electrophoresis:* Sonicates of cultured cells were subjected to starch gel electrophoresis, using the Smithies technique.[15] The continuous buffer system of Kirkman[8] was used, substituting 0.02 *M* sodium barbital for this, and the gels were developed after 15–16 hr using the procedure described by Boyer *et al.*[9]

Results.—Quantitative study: In Table 1, G-6-PD activities are given for two proved heterozygous Caucasian women. Four clones were analyzed from each, and in each case one clone was clearly in the deficient range and three in the normal range. The established ranges of G-6-PD activity in skin cell culture for normal and deficient Caucasians are given below the table.[7]

TABLE 1

CLONE RESULTS—HETEROZYGOUS CAUCASIAN FEMALES (G-6-PD ACTIVITY/MG PROTEIN)

Individual	Original activity*	Clone number	Clone activity
Sardinian #3	6.3	1	12.9
		3	8.7
		5	9.0
		2	1.2
Sardinian #5	2.4	13	2.7
		2	9.7
		15	9.1
		16	10.3

Ranges of G-6-PD activity for cultured Caucasian skin cells

Normal (males and females): 5.2–16.2

Deficient ⟨Hemizygous males / Homozygous females⟩ : 0.7–3.4

* Refers to the activity of the cultured cell strain prior to cloning.

In Table 2, original and clonal G-6-PD activities are compared for various genotypes. It is quite evident that deficient individuals produce clones with deficient enzyme activities, and clones derived from normal individuals have normal enzyme activities.

TABLE 2

CLONE RESULTS—NORMAL AND DEFICIENT CAUCASIANS

Individual	Sex	Genotype	Original activity	Clone number	Clone activity
Sardinian #4	Male	Hemizygous mutant	1.8	1	1.3
				2	1.2
				5	2.1
Sardinian #13	Female	Homozygous mutant	2.3	4	1.6
Sardinian #12	Male	Hemizygous normal	10.4	1	13.7
Sardinian #14	Female	Homozygous normal	11.1	4	11.3

Qualitative (electrophoretic) study: Clones have been developed from single cell platings from six Negro women found to have the two G-6-PD electrophoretic bands; that is, they are all presumed heterozygotes for the electrophoretic variants. With no exceptions, the clones have yielded only single bands, some of which are A and some B. Table 3 shows the number of A and B clones for each heterozygote. Figure 1 depicts two heterozygous females (double bands), and three individuals with single bands, 2 A's and 1 B. Figure 2 shows an original double band with nine clones beside it, 3 A's and 6 B's.

Discussion.—Both the quantitative and qualitative clonal data show that, with respect to the G-6-PD locus, there are indeed two distinct populations of cells in the heterozygous female. This is direct evidence in favor of the "Lyon Hypothesis"—in each single cell of the female only one G-6-PD locus is operative. Thus,

TABLE 3

RESULTS OF STARCH GEL ELECTROPHORESIS OF CLONES FROM WOMEN WHO POSSESS THE AB ELECTROPHORETIC G-6-PD PHENOTYPE

	Mrs. Bi.	Mrs. Mi.	Mrs. Wi.	Mrs. Bo.	Mrs. De.	Mrs. Ha.
Type A	8	1	0	8	7	0
Type B	2	8	8	0	7	5
Type AB	0	0	0	0	0	0
Total number of clones	10	9	8	8	14	5

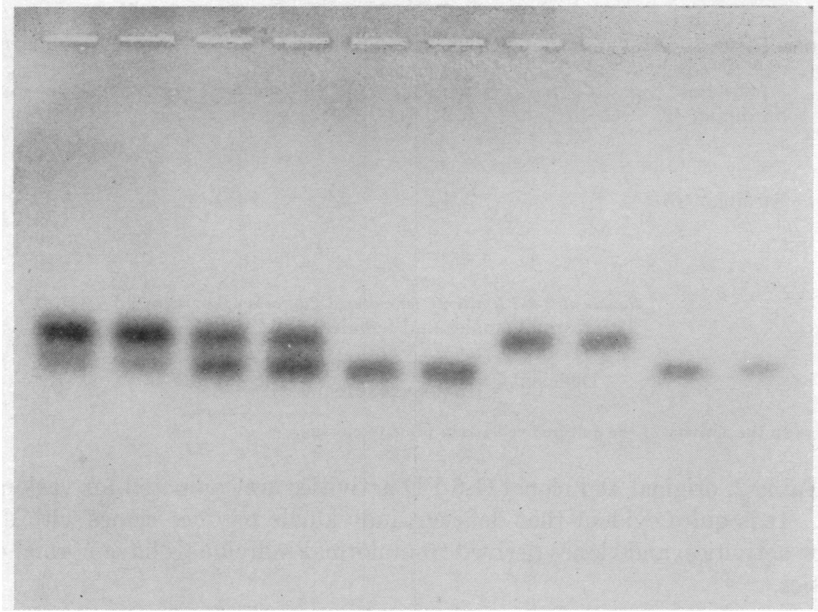

FIG. 1.—Starch gel electrophoresis of G-6-PD from sonicates of cultured skin cells. Samples were run in duplicate, starting from the origin which appears at the top of the figure. From left to right are shown the AB phenotype of 2 Negro females (double bands) and 3 individuals with single bands, 2 A's (fast band) and 1 B (slow band).

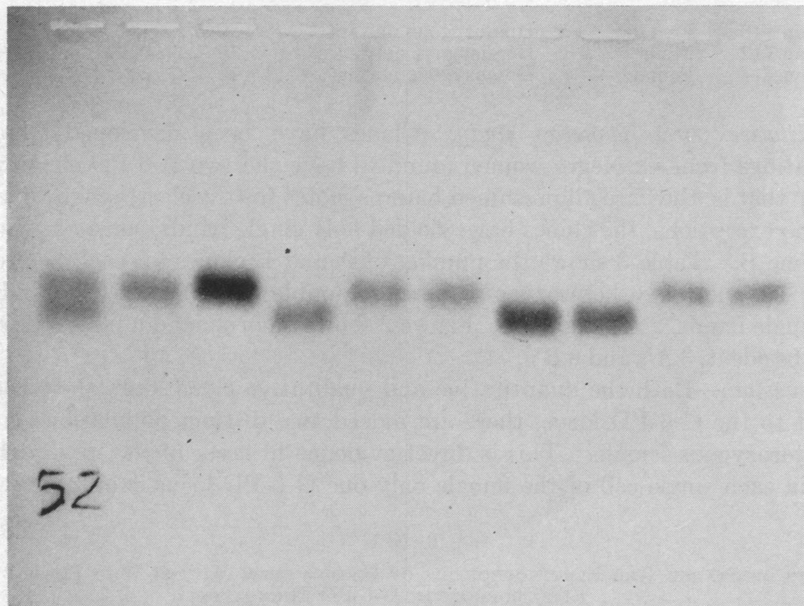

FIG. 2.—Electrophoretic pattern of G-6-PD from sonicates of cultured cells. Samples were run singly, starting from the origin at the top of the figure. From left to right are the AB phenotype of the cell culture from Mrs. De. prior to cloning, and the single bands of nine clones derived from the original cell lines. Variation in intensity of staining is due to inequality of enzyme concentration applied to the starch gel.

the inactivation of one, or at least part of one, X chromosome is most probably the mechanism of dosage compensation.

However, these findings do not imply that an entire X chromosome is inactivated in the female. Recent experiments involving the sex-linked Xg^a blood group have failed to show mosaicism.[16]

The probable mechanism of inactivation of an X chromosome is its transformation into heterochromatin. This belief is based on the observation that in the female one X chromosome is allocyclic,[17] replicates late,[18, 19] and probably forms the heterochromatic sex chromatin body.[20] However, inactivation of chromosomes by conversion to heterochromatin is not an all or none phenomenon either. In Drosophila, several genes are known to function even though located in the midst of heterochromatin,[21] and in a recent paper, Russell[22] has described experiments using translocations of marked autosomal fragments to the X chromosomes of female mice. It was shown that heterochromatin does not inactivate the entire attached fragment. Instead, there is a gradient of inactivation spreading only over limited distances.

Summary.—The appearance of two distinct populations of cells in the female heterozygous for both quantitative and qualitative G-6-PD variants is direct evidence in favor of the "Lyon Hypothesis." As far as the locus for G-6-PD is concerned, in each single cell only one X chromosome is functional. However, these data do not imply that one entire X chromosome is inactivated.

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