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DETERMINATION OF SEX CHROMATIN

A New Concept

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SUMMARY

Instead of the random activation and/or inactivation of the X-chromosome in sex determination, as suggested by the Lyon's hypothesis, a proposal is made here that crossingover between the sister- and/or nonsister-strands at the sticky or nonsticky loci, at heterochromatinizing regions and at the inactivating centers of the centromere, be responsible for the heterochromatinization and/or heteropyknotization of the X-chromosome. (This proposal will be called the Mustafa hypothesis.)

Such would be the basis for the activation and/or inactivation of the X-chromatid(s), which would then replicate into a normal or a heterochromatic X-chromosome respectively. The heterochromatic X-chromosome may be transformed into a heteropyknotized mass of sex chromatin (Barr body). Translocation of the Y-chromosome and of some of the autosomes could also result in the same effect. Hence, the number of heterochromatinized X-chromosomes, and/or of heteropyknotized masses (Barr bodies), in each daughter-cell is directly proportional to half the number of chromatids involved in crossingover and/or translocation in the mother-cell.

INTRODUCTION

Frequent heterochromatinization of sex chromosomes and their occasional heteropyknotization into a condensed state to form a single nucleolus, similar to the "karyosphere" in the resting nuclei, was originally demonstrated by Wilson (1911). The sex chromosomes were further characterized by the formation of a permanent highly condensed state (heteropyknosis) and relative genetic inactivity by Heitz (1928, 1929). Sexual dimorphism of nuclei in the interphase stage, known to biologists since Wilson's time, was later confirmed by Geitler (1937). The sexual dimorphism exists in favor of either sex. Where the male is heterogametic it is expressed by an XY set-up of sex chromosomes, where female by a ZW system. However, only the female nuclei are known to have a significant mass of nuclear satellite (Beckert 1962). Incidently, the details of the formation of a nuclear satellite in a ZW system have not been thoroughly worked out. However, in the majority of the interspecific crosses of birds the viable hybrids have been found to be chromosomal males (Mott et al. 1968) unlike the male mule and hinny, which are heterogametic and sterile, obtained from the reciprocal crosses of horse and donkey. Probably the females of hybrid birds and the males of mule and hinny are sterile because of the lack of synapsis between their sex chromosomes. The female mule and hinny, on the other hand, are occasionally fertile (Mukherjee and Sinha 1964, Isaacs 1970). Since the hybrid males in birds and females in mammals are homogametic and fertile, the importance of synapsis

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between all the chromosomes of their maternal and paternal genomes is emphasized as an essential factor for fertility. Moreover, the allotetraploidy in *Raphanobrassica* is a very good example of fertility based on the synapsis between the genomes of *Raphanus* and *Brassica* origin (Karpechenko 1928).

Since the discovery of the formation of nuclear satellite in the neurons of a female cat (Barr and Bertram 1949), there has been intensive research to find its parallels in other species having an XY set-up of sex chromosomes. Barr and Bertram suggested that the presence of " nuclear satellites " was dependent on the presence of two X-chromosomes. Graham and Barr (1952) used the term Sex Chromatin for the first time to designate the nuclear satellite.

Melandrium dioicum, Drosophila melanogaster, Platypoecilus xiphidium, and human beings are the best subjects in an XY set-up to use in exploring the variations of the apparent sex and sex chromatin formation. While XY is a normal male in *Melandrium, Drosophila, Platypoecilus*, and humans, XXY is an apparent male (occasionally hermaphrodite) in *Melandrium* (Warmke 1946) and humans (Turpin et al. 1962) and a functioning female in *Drosophila*. Hermaphroditism has however been associated with the progressive reduction of the Y-chromosome in some frogs and snakes (Ohno et al. 1964). The hermaphroditism in some species of XY set-up is therefore understandable for the individuals of XXY karyotype having a low Y-chromosomal content, unlike the Y-chromosomal content in a normal XY male. Consequently, the sex chromosomes alone in an XY set-up are not by themselves sufficient enough to impart the same apparent sex to all similar karyotypes. While the cause of the variations is not well established in humans, it is very well documented for *Melandrium, Drosophila*, and *Platypoecilus*, where the apparent sex is decisively based on the autosomal/sex-chromosomal ratio (Warmke 1946). Since sex chromosomal alterations in humans, based on spontaneous polysomy and polyploidy, fit more closely the experimental observations on *Melandrium*, the principles of XY-autosomal relationship in each species (*Melandrium* and humans) could be considered to be similar to each other (Russel 1961). However, nothing is identical in nature. Identicalness may apply to the two strands of a DNA molecule, or to MZ twins (Bauke 1970). Although the formation of sex chromatin in *Drosophila, Melandrium, Platypoecilus*, and humans, has been associated with the number of X-chromosomes, irrespective of their apparent sexes, Witschi (1956: pp. 133-138) believes that it is not necessarily true for other species besides mammals. However, Moore et al. (1953) and Moore and Barr (1954) confirm that both X-chromosomes in a normal human female are responsible for it. Whatever the facts may be, it is well established that the sex-chromosome complex of XY males fails to form a chromatin mass of comparable size. Until 1959 Ohno et al. (1959a) thought that X_p (patroclinous X) was always the inactive one in the formation of the Barr body in a normal female. However, Ohno and Hauschka (1960) provided the cytological evidence that one of the two X's in various tissues of the normal female mouse was heterochromatic and suggested that the sex chromatin was composed of such a heteropyknotic X-chromosome. Its usage in the identification and verification of a normally potentiated, or an abnormally hidden, or an apparent sex, was recognized by

Russel and Bangham (1959, 1960, 1961) and was extended by Lyon (1961, 1962a, 1962b, 1963). The modifications and highlights of the Lyon-Russel Hypothesis are as follows:

1. Only one X-chromosome functions and remains active in the somatic cells of mammalian tissues, regardless of the number of X's present;

2. The choice of activation/inactivation of the patroclinous/matroclinous X-chromosome is random;

3. Differentiation occurs at embryonic level, and once it has occurred it remains fixed in the following generations of that particular cell with respect to that specific X-chromosome.

The author of this review does not agree with the random X-chromosome activation/inactivation concept of Lyon and Russel, but would like to bring to light the works of Barr and Bertram (1949), Graham and Barr (1952), Moore et al. (1953), and Moore and Barr (1954), where both the X-chromosomes in a normal female have been proposed to form a mass of sex chromatin.

DISCUSSION

The two most important questions in connection with the formation of apparent sexes, based on the additional number of X- and/or Y-chromosomes in humans, and the Lyon-Russel hypothesis concerning the forces in a cell responsible for the activation of a certain X-chromosome leaving the other X's inert, nonfunctional, inactivated, or converted to sex chromatin, are unsolved mysteries. With our present knowledge of cyto- and histochemistry, no answer can be given to these questions. In this manuscript, however, the author would like to discuss the possible explanation of the second question, i.e., activation and/or inactivation of X-chromosomes in a normal mammalian female and its transformation into a mass of sex chromatin.

The study of a living cell at the time of its division discloses morphological changes of a nucleus into a number of different chromosomal configurations leading to crossingover, translocation, inversion, deletion, duplication, isochromosome formation, etc. Out of these chromosomal configurations, crossingover at the centromere and/or at regions other than the centromere is the most frequently seen phenomenon and is more natural. In the opinion of Swanson (1964: p. 269), crossingover is considered to be more widespread than it is usually believed, and is detectable only under careful observation. On the basis of these and other findings, the author proposes *association* between the sister- and nonsister-strands and/or *crossingover* at the chromatinic portions of the sister and nonsister strands, and also most probably at the centromeric positions, after approximately the first two weeks of embryonic growth, as the basis for heterochromatinization of the X-chromosome or its heteropyknotization into the

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mass of chromatin known as sex chromatin. The proposal will be called the Mustafa Hypothesis.[1]

It is already known that crossingover in somatic or germ cells is a process of profound biochemical activity (Pontecorvo 1952). An interesting case showing the cytological details of crossingover at the centromere has been mentioned by Cuevas-Sosa (1967) where half-incorporation of tritiated thymidine is demonstrated by grain deposition on the opposite arms of each chromatid of a chromosome. Similarly, if studied using the X-chromosome in mammals, such an experiment may show the incorporation of tritiated thymidine on opposite arms of the half-incorporated X-chromosome. Complete incorporation of tritiated thymidine in both the strands of the X-isochromosome long arm has however been reported by Muldal et al. (1963).

The zygote is the first somatic cell. Crossingover, once having taken place in the zygote, at the centromere or any other part of the X-chromosome, affects future cell lines.

The possibility of crossingover in the zygote, at the centromere of the X-chromosome, has not been reported but can be postulated on the basis of the above finding. Crossingover at another part of the X-chromosome can however be explained using the example of the cis and trans position of the deutan and protan genes in two MZ twin sisters. One of them is normal while the other is colorblind for red-green vision (Hirschhorn and Firschein 1964). Details will follow in the section on colorblindness in this review, but this case is mentioned here as an indication that crossingover between the two X-chromosomes can take place at the zygote level.

It has already been suggested by Giannelli (1970) that the inactivation center should be located in the centromere of the X-chromosome. The heterochromatinizing genes have also been reported to be present adjacent to the centromere (Reitalu 1957, Serre et al. 1958) and on the long arm of the X-chromosome in man (Grumbach and Morishima 1962). Therefore, such genes have a greater influence on the centromere of the X-chromosome. Reitalu (1957) has also mentioned that there are certain loci on the X-chromosome which are " sticky " in nature. It appears that the heterochromatinizing and sticky genes are not sufficiently mature to function during early embryogenesis. It may be postulated that such genes begin to function by the six-sixteenth day of embryogenesis in the human embryo (Park 1957). The inertness and inactivation of the X-chromosome will be referred to as *heterochromatinization* and its conversion into a mass of sex chromatin as *heteropyknotization* in the following discussion. Both of these states of the X-chromosome are attributed to be due to one or all of the following phenomena.

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[1] This hypothesis has been dedicated to the memory of my father, Mustafa Hasan, who died in October 1953 and who explained me " Nature " as the scientific name of God. For the solution of the natural problems he would emphasize the understanding of the principles of Nature.

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1. ASSOCIATION

A. Association Between the Sister-Chromatids of the X-Chromosome

Despite lateral attachment of the sister-strands of a chromosome at the centromere, their inactivation is very rare. This may be due to the fact that, since the two strands of a DNA molecule are complementary in nature, their assembly — the microfibrillar units and ultimately the chromatids — have to be complementary too. Hence, their occasional inactivation may be proposed to be based on their association due to the seldom occurrence of transition and transversion in the DNA molecules at one or many loci in one or both strands. This may be enough to make them late in replication. Half chromatids or the subchromatids have not been reported in human beings (Schwarzacher 1970).

B. Association Between the Nonsister-Chromatids of the X-Chromosome

While sister-chromatids of any chromosome are complementary in nature, the nonsister-strands cannot be practically so at each locus. Hence, the close association between the nonsister-strands may lead to the struggle of synapsis at almost each locus; more so at the sticky loci. The chromatids involved in this phenomenon may have more to do at the molecular level than the chromatids not involved. Those involved may be attributed to replicate late.

2. CROSSINGOVER

A. Crossingover Between the Sister-Chromatids at Centromere and Regions Other than Centromere

Crossingover between the sister chromatids at any regions is an advanced stage of their association. Its occurrence at the centromere and regions other than centromere might bring changes in the sequence of bases of the DNA molecules resulting in a new combination of changed and unchanged microfibrillar units in the sister-chromatids. Such alterations might also accelerate the frequency of transition and transversion in the DNA molecule. Hence the abnormality in the biochemical activity of the genes affected is expected. It can therefore be concluded that there are more chances of late replication of the chromatids involved in the process as compared to their inactivation due to association.

B. Crossingover Between the Nonsister-Chromatids at Centromere and Regions Other than Centromere

Crossingover between the nonsister-strands is a further stage of " association between the nonsister-strands" to facilitate their synaptic behavior. Thus, they may become entangled in a successful attempt to cross-over at the sticky or nonsticky loci. Those involved in an unsuccessful crossingover at the sticky loci or successful crossingover at the nonsticky loci are proposed to form the heterochromatinized state of the

X-chromosome. Those involved in successful crossingover at the sticky loci further the process through heterochromatinization to the formation of heteropyknotized mass of sex chromatin — the Barr body. Hartman and Suskind (1969) have very clearly explained such expectations. Therefore, evidence for inter- and intraspecific crosses should be thoroughly investigated in the literature pertaining to this subject. The work of Benirschke et al. (1962), Mukherjee and Sinha (1964), Isaacs (1970), and others, and discussion of Moore (1960: p. 31) should be reviewed from this point and more so at the level of the sex-linked genes in the species concerned.

Moreover, association and/or attempted crossingover at centromere or portions other than centromere between the sister- or nonsister-strands of chromatids at the sticky loci is expected to create strain in the chromatids in an effort to separate at the time of their mitotic pull toward the opposite poles in the cell. Such stretching of the chromatids may also affect the physical properties of the microfibrillar units to cause change in their physiological effects. Therefore, the inactivation of certain loci may also be a result of the force which separates the chromatids toward the opposite poles. Chromatids involved in this situation may be the ones which replicate late and become heterochromatic or heteropyknotized. The chromatids not involved in this complex situation are considered to replicate at the normal time and develop into a normal X-chromosome. So, in a normally dividing cell, one of the chromatids of each X-chromosome will be late replicating while the other one will replicate normally. This may also be the basis for the heterochromatinization of one of the two X-chromosomes in a normal female cell (Fig. 1).

As explained earlier, the involvement of association and/or crossingover at the centromere or sticky loci of the chromatids at about the first two weeks of embryogenesis is expected to form a heteropyknotized mass of sex chromatin. Thus the Barr body is expected to be present in those embryonic cell-lines which have undergone association, crossingover between the sister- or nonsister-strands of the X-chromosomes, and/or translocation between the X- and Y-chromosomes and autosomes at sticky loci during embryogenesis.

There is a greater chance of crossingover between any two homologous chromosomes in a normal mammalian female because of her XX make-up. Unlike a normal mammalian male which is either XY or XYY (rat-kangaroo: Hsu and Benirschke 1967: p. 1), the possibilities of crossingover between the sister-strands have always been found to be very rare as compared to the nonsister-strands. Therefore, crossingover between the nonsister-strands of the X-chromosome, and consequently the probability of the heterochromatinization and heteropyknotisation of the X-chromosomal material to form a heterochromatic X-chromosome or heteropyknotic mass of X-chromosome (Barr body), would be more prevalent in the cells of a normal mammalian female than a normal male. However, in all mammals the occasional presence of a Barr body in a normal male, as stated by Ferguson-Smith et al. (1964) and others, may be interpreted as being due to rare occurrences of sister-strand crossingover of the X-chromosome at the centromere, or regions other than centromere, at the time of
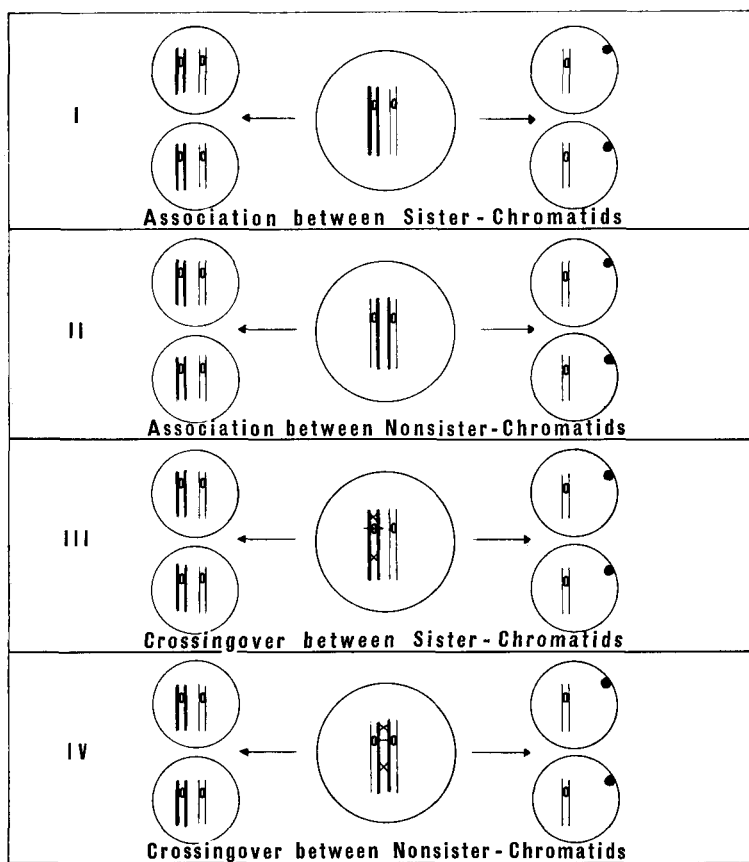
FIG. 1. *Diagrammatic representation of a normal female cell is shown in the centre. Columns I and II show association, III and IV crossingover. On the right and left of the mother-cell in each column are shown two daughter-cells as the result of mitotic division. On its right, each daughter-cell is shown to have one normal and the other heterochromatic X-chromosome with heavy lines. On the left, each daughter-cell has one normal X-chromosome and a heteropyknotized mass of sex chromatin of the other X-chromosome. Heterochromatic X-chromosome and/or sex chromatin are the result of the chromatids involved in association and/or crossingover.*

embryogenesis. The formation of a Barr body in patients having Turner's syndrome of the XO karyotype, as stated by Grumbach et al. (1960), Grumbach and Morishima (1962), Pawliger et al. (1970), and others, may be based on the same principles of crossingover.

Translocation between the X- and Y-chromosomes may also produce the same result. End-to-end association between the short arm of the X-chromosome and the Y-chromosome in man (Ferguson-Smith et al. 1964; McKusick 1964: p. 40, Fig. 27; Sebaoun et al. 1969) and mouse (Ohno et al. 1959b), and partial lateral synapsis of the short arm of the X-chromosome and the Y-chromosome in man (François 1968:

Fig. 4; Rao 1970) have been reported. Ohno and Weiler (1962) explain that in mammals, where the Y- is much smaller in relation to the X-chromosome, the pairing is end-to-end; whereas with relatively large Y-chromosome, the pairing is side-by-side. As mentioned earlier, the sex chromosomes in man have been observed in both positions. Rao (1970) has sketched the homologous regions on either side of the centromere of the X- and Y-chromosomes in man. Hence, the usual absence of Barr body in a normal mammalian male should not be explained on the basis of the activation of the X-chromosome (Lyon-Russel hypothesis), but rather as the result of the lack of chances of exchange between the greatly nonhomologous X- and Y-chromosomes at the time of embryonic growth. A unique member of mammalian species is the creeping vole (*Microtus oregoni*) where both the sexes are gonosomic mosaics (Ohno 1963). Males are known to develop from XY zygote and maintain OY/XY constitution in their somatic tissue, while females are formed from XO zygote and constitute XX/XO karyotype. Since two chromocenters are reported in the males as compared to one in the females of this species, the Y-chromosome has been shown to be completely heteropyknotized (Moore 1966: p. 31), unlike the usual pattern in other species. While OY individuals or such a somatic tissue are not traceable in any other animal species, due to the absence of genes controlling blood clotting factors on the Y-chromosome (Clarke 1970: p. 51, Fig. 8-3a), this tissue had been reported in the germ cells of the creeping vole. In the light of this observation it could be suggested that, in addition to the chromatinizing genes on the X- and Y-chromosomes of creeping vole, there may be some other vital homologous loci on the Y-chromosome of this species which enhance the possibilities of synapsis to facilitate translocation. This may lead to reciprocal heterochromatinization/heteropyknotization of the sex chromosomes, unlike other species where exchange of segments between the X- and Y-chromosomes is rare. Heteropyknotization of the Y-chromosome in man has however been reported within normal limits (Ford and Evans 1964). Translocation of the X-chromosome with an autosome and its heteropyknotization has been reported by Ohno and Weiler (1962) and Russel (1963). In the cell lines following these phenomena one could anticipate the formation of a Barr body.

Moore et al. reported, as early as 1953, that sex chromatin was found in 69% females and 5% males, with a range of 52-85% and 1-14% respectively. According to the Lyon hypothesis the number of sex-chromatin bodies is one less than the total number of X-chromosomes present (number of X's — 1 = number of sex-chromatin bodies). This solution was not found workable in other than polysomic cases. Harnden (1961), when confronted with more complex situations, amended Lyon's principle to " number of X's — P/2 = number of sex-chromatin bodies ", where P stands for ploidy number, to have his theoretical expectations coincide with the practical observations of polysomy as well as polyploidy. Both of these principles explain the arithmetical limit of the sex chromatin formation in the somatic cells, but neither of them confirms the cause of their variation in size and number in various tissues. Small or increased size has been interpreted as due to the X-chromosome's deletion and short-arm isochromosome formation, or duplication and long-arm isochromosome forma-

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tion, respectively. Variation in number of sex-chromatin bodies reported by Joannides and Tsenki (1969) is usually attributed to mitotic nondisjunction. These arguments could further be supplemented in that variation in size and number of sex-chromatin bodies probably also depends on the number of chromatids involved in crossingover at the centromere or the sticky loci and translocation with any of the chromosomes in the cells at the embryonic level.

Although the sister-strand crossingover is rare, it could be more frequent in the abnormal X-chromosome due to the fact that the sister-strands are abnormal. Therefore, the present author has the following explanation for the different kinds of abnormalities pertaining to the heterochromatinization and/or heteropyknotization of the abnormal X-chromosomes.

1. Deletion in the Long Arm of the X-Chromosome

Deletion in the long arm changes the morphology of the X-chromosome from submetacentric to metacentric. It may possibly expose the sticky loci providing an increased potential for the sister-strands to associate and fuse to one another. Such fusion could either render the X-chromosome inert and heterochromatic or convert it to heteropyknotized mass, forming a Barr body. The chances of crossingover at centromere may also be altered because of the change in the morphology of the X-chromosome.

2. Deletion in the Short Arm of the X-Chromosome

Short-arm deletion decreases the length of the short arm. It may, therefore, increase the potential of the inactivating centres in the centromere and/or the heterochromatinizing genes adjacent to the centromere and/or the sticky genes on the large arm, due to the loss of the segment of the short arm. This could enhance the probability of fusion of the sister-strand to become inert and heterochromatinized or be heteropyknotized to form a Barr body.

3. Duplication in the Long Arm of the X-Chromosome

This type of X-chromosome will have additional sticky and chromatinizing loci. Therefore, there are more chances for the sister-strands to associate and cross-over to heterochromatinize or heteropyknotize the chromosome. The crossingover possibilities will also be altered at the centromere.

4. Duplication in the Short Arm of the X-Chromosome

The length of the short arm is increased, changing the morphology of the X-chromosome from submetacentric to metacentric, rendering increased possibilities of association and crossingover between the sister-strands in both arms unlike a normal X-chromosome.

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5. Isochromosome Formation of X-Chromosome

Isochromosome formation of any of the arms of the X-chromosome may make the chromosome metacentric or telocentric in nature. No normal or abnormal telocentric chromosome has however been reported in human being as yet. Thus, in man, the only shape that an X-isochromosome could adapt is metacentric. Some genes are completely omitted while others are present in double doses depending on the arm.

(a) *Isochromosome formation of the long arm:* The explanation is essentially that given for point 3. The increased number of sticky loci, as well as the metacentric structure, increases crossingover possibility between the sister-strands at and on either side of the centromere.

(b) *Isochromosome formation of the short arm:* The chances of sister-strand crossingover could be increased due to metacentric nature.

6. Ring Formation of the X-Chromosome

This structure has no chance for synapsis with the normal X-chromosome due to its structural abnormality. Consequently, the sister-strands of the ring chromosome have a unique chance to coalesce into a sex chromatin body.

As mentioned earlier, the zygote is the first somatic cell. The evidence of crossingover at regions other than centromere at the zygote level, with respect to the X-chromosome, may be observed in the following example. The phenotypic difference between the two MZ twin sisters (Hirschhorn and Firschein 1964) for redgreen colorblindness could be possibly based on the cis and trans position of deutan and protan genes like the garnet eye-color in *Drosophila* (Crow 1968: p. 72) (Fig. 2). The cis and trans position of these genes in these twins could be obtained by the nonsister-strand crossingover between deutan and protan genes in the zygote. Similarly, the unilateral red-green colorblindness, frequently described as a result of mosaicism, could actually be the result of somatic crossingover at the time of embryogenesis for the development of both the eyes from a common or relatively similar cell for this trait.

As well as the deutan and protan genes, the quantitative expression of varied percentage in the population of red cells with respect to G6PD enzyme has been reported in cases of heterozygous females, as well as in hemizygous males, carrying the wild-type allele (Papayannopoulou and Stamatoyannopoulos 1964). Terms like multiple allelism, pseudoallelism, mosaicism, pseudomosaicism and low- and high-capacity alleles have been used to explain this situation. The author explains such variation on the basis of (1) normal association and crossingover at centromere and regions other than centromere between the sister- and nonsister-strands of the X-chromosome(s), or (2) unequal crossingover at the molecular level of G6PD gene on the X-chromosome. Similar variations based on reciprocal crossingover at centromere and equal and unequal crossingover at regions other than the centromere have been reported by Hartman and Suskind (1969).
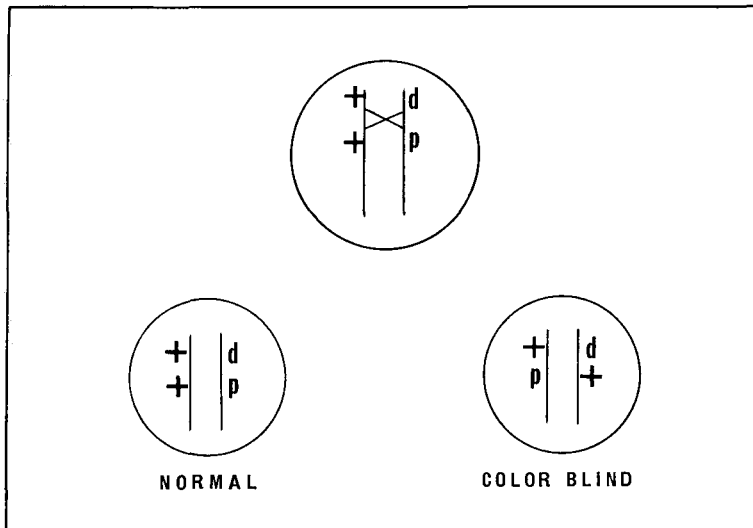
FIG. 2. *Nonsister-strand crossingover resulting in cis and trans position for deutan and protan genes. In this example the cis position is shown as normal, the trans position as colorblind.*

1. Crossingover at Centromere or Equal Crossingover at Various Loci of the X-Chromosome

As mitotic division occurs in the cells of an ambryo, under normal conditions a somatic heterozygous daughter-cell may have 1 chance in 28 of obtaining a particular pair of alleles for a particular gene (Fig. 3). Thus, if G stands for the normal condition of G6PD and g for a recessive allele, about 21/28 of the total population of red cells would have the gene for dominance while about 7/28 would have the gene for recessiveness. This expectation is based on their recombination possibilities. Among the recombinants which have G, 8/28 of them will be the result of crossingover at centromere and 16/28 at regions other than centromere; 4/28 of them will have the unchanged combination of the parental chromosomes. It could therefore be assumed that the X-chromosome, if attaining crossingover at the nonsticky loci or associating at the sticky loci in an unsuccessful attempt to cross-over at those points or at the centromere, may have become inert or heterochromatic. On the other hand, if the crossingover at the centromere or one of such sticky loci is completed, the chromatids involved become heteropyknotic and transform themselves into a mass of sex chromatin. Thus, genotypically, there will be six different kinds of red blood cells with respect to this enzyme in the somatic tissue:

1. Chromosome carrying G as a result of no crossingover (unchanged combination of parental chromosomes);

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FIGS. 3 and 4. *In a woman heterozygous for any of the genes mentioned in this synopsis, the arrangement of chromatids following mitotic division in her somatic cells could possibly take place in 14 different ways (I-XIV), resulting in 28 different combinations in daughter-cells. Diagrammatic representation for the formation of a normal, heterochromatic and heteropyknotic X-chromosome with respect to G6PD on the basis of association and/or crossingover is shown. In some columns of these figures crossingover is shown to take place at the centromere and regions other than the centromere between sister- and nonsister-chromatids. The recombinants in the daughter-cells based on such a change have to be genotypically different from the chromatids where the sequence of bases in the DNA molecules remains parental. Since each recombinant is unique, pheno-*

I	G g	GG	GG NORMAL	**VIII**	G g	$^{+}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (cross-over recombinant with parental "g")
		$\mathscr{E}\mathscr{E}$	$\mathscr{E}\mathscr{E}$ DEFICIENT			$^{+}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (same as above)
II	G g	G$\mathscr{E}$	G$\mathscr{E}$ NORMAL	**IX**	G g	$^{C}_{}$GG	NORMAL (cross-over recombinant with parental "G")
		G$\mathscr{E}$	G$\mathscr{E}$ NORMAL			$^{C}_{}\mathscr{E}\mathscr{E}$	$\mathscr{E}\mathscr{E}$ DEFICIENT
III	G g	$^{C}_{}$GG	DIFFERENT OR INTERMEDIATE (cross-over recombinants)	**X**	G g	G$\mathscr{E}$	NORMAL (parental recombinants)
		$\mathscr{E}\mathscr{E}$	$\mathscr{E}\mathscr{E}$ DEFICIENT			$^{C}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (cross-over recombinants)
IV	G g	$^{c}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (cross-over recombinant with Parental "g")	**XI**	G g	$^{x}_{}$GG	NORMAL (cross-over recombinant with parental "G")
		$^{c}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (same as above)			$^{x}_{}\mathscr{E}\mathscr{E}$	$\mathscr{E}\mathscr{E}$ DEFICIENT
V	G g	$^{+}_{}$GG	DIFFERENT OR INTERMEDIATE (cross-over recombinants)	**XII**	G g	G$\mathscr{E}$	NORMAL (parental recombinants)
		$\mathscr{E}\mathscr{E}$	$\mathscr{E}\mathscr{E}$ DEFICIENT			$^{x}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (cross-over recombinants)
VI	G g	$^{+}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (cross-over recombinant with parental "g")	**XIII**	G g	$^{x}_{}$GG	NORMAL (cross-over recombinant with parental "G")
		$^{+}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (same as above)			$^{x}_{}\mathscr{E}\mathscr{E}$	$\mathscr{E}\mathscr{E}$ DEFICIENT
VII	G g	$^{+}_{}$GG	DIFFERENT OR INTERMEDIATE (cross-over recombinants)	**XIV**	G g	G$\mathscr{E}$	NORMAL (parental recombinants)
		$\mathscr{E}\mathscr{E}$	$\mathscr{E}\mathscr{E}$ DEFICIENT			$^{x}_{}$G$\mathscr{E}$	DIFFERENT OR INTERMEDIATE (cross-over recombinants)

typical variation is natural. The respective signs on the letters G or g indicate the nature of crossingover on that chromatid; their position above and in between the letters expresses the involvement of both the chromatids. The columns without such signs are the result of simple recombination.

c = Crossingover at the centromere between sister-chromatids
C = Crossingover at the centromere between nonsister-chromatids
+ = Crossingover between sister-chromatids at regions other than the centromere
× = Crossingover between nonsister-chromatids at regions other than the centromere

2. Chromosome carrying G where the chromatids have crossed at the nonsticky loci or associated at one of the sticky loci;

3. Chromosome carrying G where the chromatids have crossed-over at the centromere or at one of the sticky loci;

4. Chromosome carrying g as a result of no crossingover (unchanged combination of parental chromosomes);

5. Chromosome carrying g where the chromatids have crossed at the nonsticky loci or associated at one of the sticky loci;

6. Chromosome carrying g where the chromatids have crossed-over at the centromere or at one of the sticky loci.

Phenotypically, there may be either two or three different kinds of red blood cells with respect to this enzyme. When there are only two phenotypical classes (Fig. 3) the explanation is as follows:

1. The chromosome carrying G as a result of no crossingover whatsoever in its parental form, will be undisputedly normal;

2. The chromosome carrying G as a result of association or attempted crossing-over at centromere or nonsticky loci, if theoretically considered functional and activated, would have a phenotype exhibition of G, but if nonfunctional it will exhibit a g phenotype and the X-chromosome will be heterochromatic in nature. The heteropyknotic stage can be achieved only if the crossingover has taken place at the centromere or at one of the sticky loci and will also phenotypically resemble g.

Either way, only two phenotypical classes would be thus observed unless one considers the ratio difference between the two.

There could also be three phenotypical classes (Fig. 4), the explanation being as follows:

1. G located on unchanged combination of parental chromosome producing a normal enzyme;

2. G located on a chromosome which is the result of association and/or crossingover at sticky or nonsticky loci, producing a different class of G6PD;

3. g having complete deficiency of the enzyme.

Therefore, the 80% value of the enzyme calculated by Stamatoyannopoulos et al. (1967) applying the technique of Kleihauer and Betke (1963) should not be interpreted as surprising, but as similar to the arithmetical expectations mentioned earlier.

2. Unequal Crossingover and Translocation at Regions other than Centromere of the X-chromosome

Another explanation for the mosaicism and chimerism pertaining to the aforementioned genes could be based on their cumulative effect due to unequal crossingover in the sister- or nonsister-strands of the X-chromosome (Fig. 5), or to the translocation between the homologous parts of the X- and Y-chromosomes (François 1969: Fig. 4). The linear accumulation of genes due to unequal crossingover, resembling the stacking of uniform coins, could be arranged in any order to show a different phenotypic effect in each case. So, instead of expecting a chemical gene mutation of a certain G6PD mutant to form the low- or high-capacity alleles for G6PD (Stamatoyannopou-
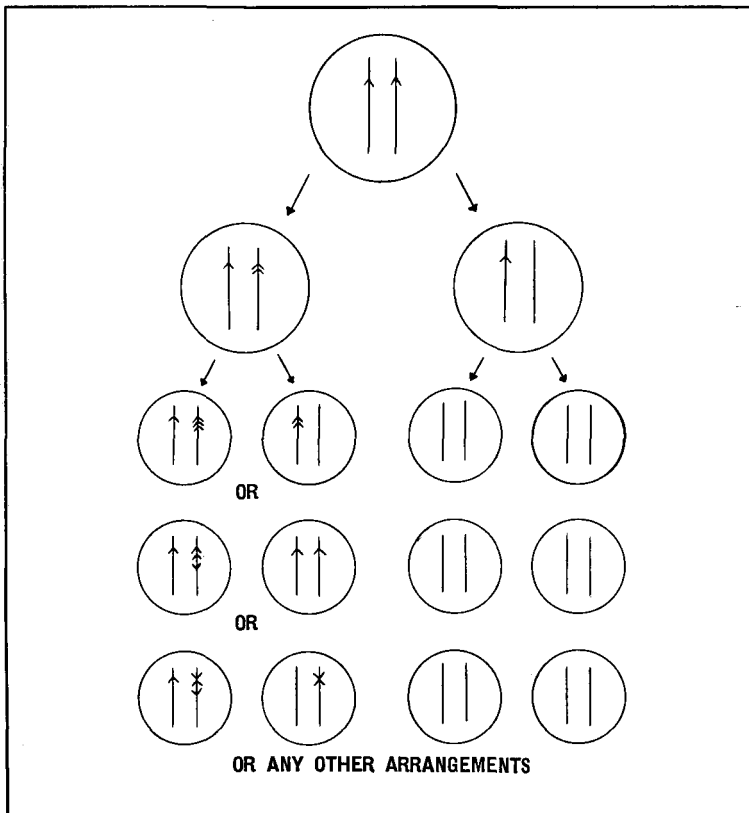

FIG. 5. *Diagrammatic representation of inter- and intracumulative effect of different arrangements of G6PD genes with respect to their positions showing possible phenotypical differences from their respective genotypes due to unequal crossingover.*
∧ = *normal position of the G6PD gene*
∨ = *inverted position of the G6PD gene*

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los et al. 1964), there could be a linear cumulative effect due to unequal crossingover causing a segment mutation like the *V* (variegated) effect in *Drosophila* (Stern 1936). Hence, if sister- or nonsister-strand unequal crossingover takes place in the X-chromosome, persons of XO and XX karyotype could have a number of different physiologic cell lines with respect to any of the genes located on it. The same could be true in a male if unequal translocation is taking place between the expected homologous segments of the X- and Y-chromosomes. The cumulative effect of some of the genes, such as G6PD, could be variably functional with different phenotypic expression for each cell line unlike the interpretation of Motulsky (1967). The pseudomosaicism for G6PD in some males and its mild deficiency in Greek males as reported by Papayannopoulou and Stamatoyannopoulos (1964), is proposed to be due to unequal crossingover in the sister-strands of the X-chromosome or to its translocation with the homologous part of the Y-chromosome to heterochromatinize and/or heteropyknotize the chromatids. It is however assumed that these studies were performed on the normal XY males carrying wild-type allele for this enzyme.

The phenotypic differences of nystagmus conditions are further examples to follow the genetic activity in various tissues. The percentage values for nystagmus phenotype (Lyon 1962a) could be explained on the same principle as mentioned in connection with unequal crossingover of the gene for G6PD. This gene is also presumed to be located on the X-chromosome, possibly near the sticky heterochromatic regions; hence, there are more chances of heteropyknotization. The G6PD and nystagmus genes are the examples where all the possible cumulative effects are expected to be viable.

There is a possibility that for some genes such as hemophilia A and B some of the cumulative arrangements in a cell may be viable while others lethal. The chances of survival of hemizygous XY and XO individuals and of homozygous females having any such trait should therefore not be questioned. Also the survival of a female dog, homozygous recessive for hemophilia (Brinkhous and Graham 1950), could be expected on the basis of variation of cell lines. Variation such as $X_h X_h$ to XX, $X_h X$, and $X_{hh} X$, etc., due to unequal crossingover, could have different cumulative effects for each cell line. The inter- and intracumulative effects of some of the above-mentioned genes could also vary in viability and/or lethality.

So far, only the genes adjacent to the centromere of the X-chromosome in man have been mentioned. Genes such as ichthyosis and X_g antigen (Wells et al. 1966) are believed to be located relatively far away from the centromere, almost on the extreme end of one of the arms of the X-chromosome (Sanger 1971). However, it is not yet clear whether the gene for hemophilia B (factor IX deficiency) is adjacent to the centromere or near the X_g locus (Whittaker et al. 1962, Davies et al. 1963). Due to the presence of heterochromatinizing genes at and adjacent to the centromere of the X-chromosome, the genes located at a distance from the centromere have a lower chance of being effected. Consequently, the possibility of heterochromatinization and heteropyknotization of the genes near the tip of either arm may be considered almost nil.

The lack of mosaicism in the carriers of galactosemia, needed for the performance

of the normal activity of uridine-diphosphate-galactose-transferase, and X_g antigen blood formation in a heterozygous female where their wild alleles are presumed to completely dominate their respective recessive alleles, could be based on one of the following principles:

1. Lack of chances of crossingover at the inactivating centres of the X-chromosome;

2. Incompleteness of certain loci (Reed et al. 1963) and their inability to function;

3. Completeness of certain loci and their probable ability to function even in the heterochromatic and heteropyknotic state of the X-chromosome. It may be postulated that the genes away from the centromere on either arms of the X-chromosome may not be deactivated as are those on or near it.

CONCLUSIONS

1. The sister- or nonsister-strands of the chromatids of X-chromosomes involved in close association at their sticky loci, or an attempt of crossingover at the centromere, or crossingover or translocation at nonsticky loci, become inactivated. Such chromatids develop into a heterochromatic X-chromosome.

2. Successful crossingover at the centromere or at sticky loci, or translocation at one of such loci makes the chromatids heteropyknotized. They are then transformed into a mass of sex chromatin.

3. The number of sex-chromatin bodies in a cell is equal to half the number of chromatids involved in crossingover or translocation in its mother-cell.

4. The genes adjacent to the centromere, heterochromatinizing zones, and sticky loci of the X-chromosome (such as G6PD, colorblindness of the deuteranopia and protanopia kinds, or hemophilia A and also probably hemophilia B) have more chances for heterochromatinization or heteropyknotization due to their presence in those regions. They show varied phenotypes with different cumulative combinations to mimic the phenomenon of mosaicism and chimerism. Some of them are viable in various cumulative forms, such as G6PD, while others may be probably lethal, like hemophilia A and B.

5. Genes located away from the centromere, heterochromatinizing zones, and sticky loci (such as X_g blood on almost the extreme end of one of the arms of the X-chromosome in man) have little or no possibility of heterochromatinization and heteropyknotization due to their separation from the inactivating regions.

APPENDIX

MUSTAFA HYPOTHESIS TEST

DESIGN OF THE EXPERIMENT

Healthy virgin female chinese hamsters should be selected. This species is utilized because the haploid number of chromosomes is small (n = 11).

They should be allowed to mate at their heat-period in order to insure pregnancy. Between the middle and the end of the second week the embryos should be tested for the inactivation and/or formation of the Barr bodies. Just at the transformation period when the inactivation and/or Barr-body formation is anticipated, the embryo should be subjected to the following experimentation in vitro and in vivo.

CONTROL

For the study of controlled embryos the cells should be cultured by any standard method (e.g., Moorehead et al. 1960). After the cells are cultured in a culture-medium:

1. Count the cells which lack crossingover at the centromere and at regions other than the centromere;

2. Count the cells where crossingover is shown taking place at the centromere and/or regions other than the centromere in the X-chromosome;

3. Count the cells with Barr bodies.

IN-VITRO TESTS

For the in-vitro study the cells following about 72 hours of culture should be incorporated with tritiated thymidine (specific activity 1.9 cc/mM) with a final concentration of 0.06 cc/ml of culture medium for about 8 hours. This should be followed by replacement of the radioactive medium with medium containing nonradioactive thymidine. The cells should be left in culture for an additional period of about 40 hours to give them the opportunity to divide again after the pulse, to obtain half-labeled X-chromosome. After 4 hours prior to harvesting, any arresting medium like colchicine, with an appropriate concentration, should be used. The cells should be removed from the bottle with 0.25% trypsin solution prepared for chromosome examination according to the method of Moorehead et al. (1960).

The growing cells should be looked for:

1. Cells which lack configurations of crossingover;

2. Cells where half-incorporation of H^3 is accomplished in the alternate X-chromosome confirming the crossingover at the centromere and regions other than the centromere;

3. Cells with Barr bodies with or without the deposition of tritiated thymidine.

IN-VIVO FINDINGS

Inject the tritiated thymidine in different doses and concentrations into the ten-days pregnant females and the embryos developing into females should be tested for the incorporation of tritiated thymidine and for the inactivation of the X-chromosome or the Barr-bodies formation in the following way:

1. Count the cells which lack configurations of crossingover;

2. Count the cells where half-incorporation of H^3 is accomplished in the alternate X-chromosome confirming the crossingover at the centromere and regions other than the centromere;

3. Count the cells with Barr bodies with or without the deposition of H^3.

Since crossingover or lack of crossingover are two different conditions we should not have the activation and/or inactivation (heterochromatinization and/or heteropyknotization) of the X-chromosome to form a Barr body for each condition. Therefore, count the cells showing crossingover at the centromere or the chromatinic portions of the X-chromosome in the control, in vitro and in vivo. Either the number of crossed-over cells or the number of cells not involved in crossingover should be statistically equal to the number of cells having heterochromatic X-chromosome. This will confirm whether the crossingover or lack of crossingover is responsible for heterochromatinization of the X-chromosome which leads to its heteropyknotization to form the sex chromatin. I expect that it is crossingover.

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RIASSUNTO

Viene avanzata l'ipotesi che, nella determinazione del sesso, invece della casuale attivazione o inattivazione del cromosoma X (ipotesi di Lyon), l'interscambio a livello dei loci agglutinanti, delle regioni eterocromatinizzanti e dei centri inattivatori del centromero, possa essere responsabile dell'eterocromatinizzazione o dell'eteropicnotizzazione del cromosoma X. Tale proposta viene chiamata ipotesi di Mustafa.

Sarebbe questa la base dell'attivazione o dell'inattivazione del cromatide X che si raddoppierebbe allora in un cromosoma X rispettivamente normale o eterocromatico. Il cromosoma X eterocromatico può essere trasformato in una massa eteropicnotizzata di cromatina sessuale (corpo di Barr). Lo stesso effetto si può avere

in seguito a traslocazione del cromosoma Y e di alcuni degli autosomi. Di conseguenza, il numero di cromosomi X eterocromatinizzati o di corpi di Barr in ciascuna cellula-figlia è direttamente proporzionale alla metà del numero di cromatidi implicati nell'interscambio o nella traslocazione nella cellula-madre.

RÉSUMÉ

L'hypothèse est avancée que, dans la détermination du sexe, au lieu de l'activation ou inactivation casuelle du chromosome X (hypothèse de Lyon), le crossingover au niveau des locus agglutinants, des régions hétérochromatinisantes et des centres inactivateurs du centromère, soit responsable de l'hétérochrcmatinisation ou de l'hétéropycnotisation du chromosome X (hypothèse de Mustafa).

Cette-ci serait la base de l'activation ou inactivation du chromatide X, qui se dupliquerait alors dans un chromosome X respectivement normal ou hétérochromatique. Le chromosome X hétérochromatique peut être transformé dans une masse hétéropycnotisée de chromatine sexuelle (corps de Barr). Le même effet peut être produit par translocation du chromosome Y et de certains autosomes. Par conséquent, le nombre de chromosomes X hétérochromatinisés ou de corps de Barr dans chaque cellule-fille est directement proportionnel à la moitié du nombre de chromatides impliqués dans le crossingover ou la translocation dans la cellule-mère.

ZUSAMMENFASSUNG

Es wird die Vermutung geäussert, dass für die Heterochromatinisierung oder Heteropiknotisierung des X-Chromosoms bei der Geschlechtsbestimmung nicht die zufällige Aktivierung oder Inaktivierung des X-Chromosoms (Lyons Hypothese), sondern vielmehr der Austausch der Agglutinations-Loci, der Heterochromatinierungszonen und der Inaktivierungszentren des Zentromeren verantwortlich seien. Dieser Vorschlag wird Mustafa Hypothese genannt.

Dies würde die Grundlage für die Aktivierung oder Inaktivierung des X-Chromatids darstellen, das sich dann in ein normales bzw. heterochromatisches X-Chromosom verdoppeln würde. Das heterochromatische X-Chromosom kann in eine heteropiknotisierte Masse aus Geschlechtschromatin (Barr'scher Körper) verwandelt werden. Die gleiche Wirkung lässt sich durch Translokation des Y-Chromosoms und einiger Autosome hervorrufen. Folglich entspricht die Zahl der heterochromatinisierten Zellen oder der Barr'schen Körper in jeder Tochterzelle direkt der Hälfte der Zahl der Chromatiden, die bei Austausch oder Translokation der Mutterzelle mitwirken.

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