Sex Chromatin and Gene Action in the Mammalian X-Chromosome

MARY F. LYON

M.R.C. Radiobiological Research Unit, Harwell, Didcot, Berkshire, England

THIS PAPER describes in greater detail a hypothesis, which has already been put forward briefly, concerning gene action in the X chromosome of the mouse (*Mus musculus* L.) (Lyon, 1961-a), and at the same time extends it to cover the X chromosomes of mammals generally. The hypothesis was formed by the welding together of facts recently described in the two fields of mouse genetics and mouse cytology.

FACTS AND HYPOTHESIS

The cytologic evidence was provided by Ohno and Hauschka (1960), who showed that in cells of various tissues of female mice one chromosome was heteropyknotic. They interpreted this chromosome as an X chromosome and suggested that the so-called sex-chromatin was composed of one heteropyknotic X chromosome.

The hypothesis formulated on the basis of this and the genetic facts was that (1) the heteropyknotic X chromosome was genetically inactivated, (2) that it could be either paternal or maternal in origin in different cells of the same animal, and (3) that the inactivation occurred early in embryonic development.

The genetic facts used in formulating the hypothesis were: first, that mice of the chromosomal type XO are normal, fertile females (Welshons and Russell, 1959), showing that only one active X chromosome is necessary for normal development of the female mouse, and second, that female mice heterozygous for sex-linked genes affecting coat color have a mosiac phenotype. Several mutant genes of this type have been described under the names mottled, brindled, tortoiseshell, dappled, and 26K (Fraser, Sobey and Spicer, 1953; Dickie, 1954: Welshons and Russell, 1959; Lyon, 1960; Phillips, 1961). Some or all of them may be allelic with each other. In each case the coat of the heterozygous female has patches of white, normal color and an intermediate color. Most of these mutants are lethal when hemizygous, but brindled males live long enough to show that their coat is white. Thus the coat of heterozygous females may be considered to consist of patches of mutant color and of wild-type color. A similar phenotype, described as variegated or flecked, is seen in females heterozygous for autosomal coat color genes whose normal alleles have been translocated onto the X chromosome. Four such sex-linked translocations are so far known: one in which part of linkage group VIII including the

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normal allele of the gene brown (*b*) is translocated to the X chromosome, and three in which parts of linkage group I, including the loci of the genes chinchilla, c^{ch}, and pink-eye, *p*, are so translocated (Russell and Bangham, 1959, 1961; Cattanach, 1961). The color of the patches in each case corresponds to that of the mutant concerned. The distribution of the patches in these sex-linked conditions differs from that seen in mutants causing white spotting in the mouse. All the spotting mutants, including s, *bt*, *W*, *Sp*, *Sl*, Mi^{wh} and *Va*, have a certain basic similarity of pattern, in that certain regions of the body are more susceptible to spotting and tend to be affected first. These regions include the tail, feet, belly, forehead, nose and the dorsal lumbar region (Grüneberg, 1952). In heterozygotes for sex-linked color genes, however, the white regions seem to be distributed more or less evenly over the body. Characteristic features are that both the mutant and the fully pigmented patches rarely cross the mid-dorsal or mid-ventral line, and that the edges of the patches tend to be ill defined. In these features the pattern resembles that resulting from somatic mutation. Russell and Major (1957), studying radiation-induced somatic mutation, remarked on the ill defined edges of the mutant patches and the mingling of mutant and normal-colored hairs. They gave a diagram of the distribution of the mutant patches which seems to indicate that these rarely cross the midline. Cases of spontaneous somatic mutation include that of Dunn (1934) at the *c*-locus, in which the animal had several small areas of the mutant color on its back and head, with the gonads also partly affected, and a similar animal found recently by Lyon (1959) which carried a spontaneous gonosomic mutation from c^{ch} to *c*. On a background of color *aa* c^{ch} c^{ch}, patches of the mutant color, *aa* $c^{ch}c$, were scattered over the body, with ill defined edges and mingling of hairs of the two types. The animal bred as though the gonads were entirely of the type $c^{ch}c$.

This resemblance of the pattern of mottled, dappled, etc., females to that of somatically mosiac animals suggested that some event happened early in development which determined whether the normal or mutant gene should act in any particular cell (Lyon, 1960). This event is now thought to be the inactivation of one X chromosome. It is postulated that the pigment cells of the normal-colored patches are descended from cells in which the chromosome carrying the mutant gene was inactivated, and those of the mutant-colored patches from cells with the normal gene inactivated. Patches of intermediate color would arise by cell mingling in development, and the shape of the patches would depend on cell movement during growth. The proportions of active normal or mutant chromosomes would be expected to vary from one individual to another by chance.

TESTS OF THE HYPOTHESIS

Various expectations can be formulated from the hypothesis and used to test it.

The first is that all sex-linked color genes of the mouse, and all autosomal color genes translocated to the X chromosome should show the mottling effect in heterozygous females. This is obeyed so far.

A second expectation is that in female mice of chromosomal type XO the single X chromosome would not be inactivated, and therefore neither sex-chromatin nor mottling should occur in XO females. Both of these expectations are fulfilled. Ohno, Kaplan, Kinosita, and Welshons (1960) found no heteropyknotic X chromosome in XO females, and Cattanach (1961) and Russell and Bangham (1960, 1961) have reported that females carrying a sex-linked translocation and lacking one X do not show variegation. Similarly, males of the chromosomal type XXY would be expected to have one X chromosome inactivated and therefore to show variegation, and this also is obeyed in males with a translocation (Cattanach, 1961). In this respect variegation in mice differs from the V-type position effect in Drosophila. Sex-linked translocations in Drosophila may cause color variegation, but this still occurs in flies with a single X chromosome (Lewis, 1950), whereas two X chromosomes are essential for its expression in mice.

Another expectation, which it has not been possible to test so far, is that females heterozygous for two different color genes, one on each X chromosome, should show no normal-colored patches. They should also show no doubly mutant patches, but this might be more difficult to score. It is hoped to test this point using sex-linked translocations.

Rather more diffuse expectations can be formulated concerning the expression of sex-linked genes other than color genes in heterozygous females. Any gene with localized action would be expected to show a similar mosaic effect. This would include genes affecting hair and coat structure, of which there are two, tabby *Ta,* and scurfy, *sf* (table 1). Tabby heterozygotes do show a mosiac

TABLE 1. SEX-LINKED GENES IN THE MOUSE

Color	Skin	Other
Mottled	Tabby	Bent-tail
Brindled	Scurfy	Jimpy
Tortoiseshell		Gyro
Dappled		
Dappled-2		
26K		
4 translocations involving:		
Brown		
Albino alleles		
Pink-eye		

effect. Males hemizygous and females homozygous for this gene have an abnormal coat structure, while heterozygous females have a striped appearance which gives the mutant its name. Falconer (1953) showed that the normal-colored regions of the coat had a normal hair structure, while the black regions had a structure resembling that of the tabby homozygotes and hemizygotes. The gene scurfy, on the other hand, does not express itself in heterozygous females (Russell, Russell, and Gower, 1959).

Where the phenotype is not the result of localized gene action, heterozygous females might show various types of effect. Except in rare cases, the cells in which the gene action takes place will include both the type with the normal gene active and that with the mutant gene active, and the proportions of the

two types will vary by chance. Thus, it may be that the phenotype of the heterozygotes will be intermediate between those of the two homozygous types, or the presence of any normal cells may be enough to insure a normal phenotype (so that the gene behaves as a recessive), or the phenotype of heterozygotes may vary as the proportion of cells with active normal or mutant genes varies, leading to incomplete penetrance and variable expression. The gene bent-tail (Garber, 1952), is probably an example of this last type. The hemizygous males have bent and shortened tails; in the heterozygotes the expression of the defect varies from nearly normal to nearly equal to the hemizygotes, and the penetrance is only 80 to 85 per cent (Falconer, 1954; Grüneberg, 1955). The gene jimpy, *jp,* behaves as a recessive, but as mentioned in the earlier publication, there has been one exceptional jimpy female, heterozygous for tabby (*Ta*), which may represent an example of the rare case in which, by chance, all the cells responsible for the jimpy phenotype have the normal gene inactivated. Gyro, *Gy,* (Lyon, 1961-b) gets its name from the circling behavior of the affected animals. The males in addition are small, sterile, and have abnormal bone development. The female heterozygotes show a low penetrance of the circling behavior, but no other abnormalities have been detected.

In general the hypothesis predicts that females heterozygous for sex-linked genes with nonlocalized gene action will show incomplete penetrance, and variable gene expression, as the proportion of active normal and mutant chromosomes varies. This prediction is borne out. There is, of course, no need to invoke a similar explanation for incomplete penetrance of autosomal genes, since genetic theory has always allowed that variable gene manifestation might have many different causes in different instances.

Thus, so far, the hypothesis stands up to test in the mouse.

CAN THE HYPOTHESIS BE EXTENDED TO OTHER MAMMALS?

One obvious question is whether this phenomenon is peculiar to the mouse or is general in mammals.

Sex-chromatin is found generally in female mammals, and Ohno and colleagues have reported that it is formed from a single heteropyknotic X chromosome not only in the mouse, but also in the rat, opossum, and man (Ohno, Kaplan, and Kinosita 1959, 1960; Ohno and Makino, 1961). Thus the cytologic evidence suggests that inactivation of one X is the typical method of dosage compensation in female mammals. Does the genetic evidence point in the same direction?

The only other mammalian species in which an appreciable number of sex-linked genes are known is man. In mammals other than man there are only yellow, in the cat, and hemophilia, in the dog. As mentioned in the earlier publication the mosaic phenotype of the tortoiseshell cat is in accord with the hypothesis; hemophilia in the dog strongly resembles that in man except that no heterozygous effect has yet been recorded (Brinkhous and Graham, 1950).

To test the hypothesis for man, the two aspects of human genetics which have to be considered are the phenotype of XO individuals, and that of females heterozygous for color genes. An important fact in relating the hypothesis to

the mouse was that mice of type XO are normal fertile females. In man the XO type is female, but not normal; women of this chromosome type have Turner's syndrome, which includes small stature and gonadal dysgenesis (Ford *et al.*, 1959). Thus, in man one cannot say that only one active X chromosome is necessary for normal female development. However, it is still possible that inactivation of one X does take place in man, but that it differs in some way from the process in the mouse, e.g. it might occur later in embryogeny. Therefore, it is worthwhile to consider the second part of the evidence, the phenotype of heterozygous females. Only one sex-linked gene affecting color is at present known in man: sex-linked ocular albinism. Males carrying this gene lack retinal epithelial pigment, while the stromal pigmentation of the iris and choroid are normal. Heterozygous females have irregular retinal pigmentation, with patches of pigment and patches lacking pigment, so that the fundus has a stippled appearance (Falls, 1951; Francois and Deweer, 1953; Waardenburg and van der Bosch, 1956). Thus, the only color gene available for study shows the expected mosaic effect in heterozygotes. The further predictions of the hypothesis should now be considered.

Any other genes with localized action would also be expected to give the mosaic effect, while heterozygotes for genes with nonlocalized action would be expected to show incomplete penetrance and variable expression. The effects observed in heterozygotes for genes for which there is good evidence of sex-linkage (based on the progeny of affected males or linkages with a known sex-linked gene), and which are known by more than one pedigree, are shown in table 2. This table has been compiled from a study of the literature on human genetics including Gates (1946), Sorsby (1951-a), Stern (1960), and recent papers. In six conditions the heterozygotes typically show an intermediate effect, and in two of these, choroideremia and chronic hypochromic anemia, the effect is visibly mosiac. In choroideremia the hemizygous males have a pale grayish white eye fundus, while the heterozygotes have patches of paleness with overlying pigment, attributed by McCulloch and McCulloch (1948) to disruption of patches of the pigment epithelium. In chronic hypochromic anemia the males have abnormally shaped hypochromic red cells and an enlarged spleen. The heterozygous females have a small proportion (5 to 20 per cent) of typically abnormal red cells, the remainder appearing normal. This leaves nine conditions for which no typical heterozygous effect is recorded, but of these eight have reports of partial, occasional, or rare heterozygous expression, leaving only one condition, ichthyosis vulgaris, out of the fifteen mentioned which, according to the literature, is completely recessive.

The frequency and degree of heterozygous expression varies greatly from one disease to another, as would be expected. Diseases with relatively common heterozygous expression include nystagmus in which the percentage manifestation varies from zero to 78 per cent in different families; keratosis follicularis, in which many partly affected females are recorded in one of the two known pedigrees showing sex-linkage; and vitamin D-resistant rickets with hypophosphatemia, in which twelve out of forty-eight females showed the bony deformities which are characteristic in the male. In diseases in which heterozygous expression is rare, including both hemophilia and color blindness, some

TABLE 2. EFFECTS IN HETEROZYGOTES FOR VARIOUS HUMAN SEX-LINKED GENES

Condition	Heterozygous effect		References
	Frequency	Type of effect	
Color blindness	Typical	Schmidt's sign (decreased sensitivity to red light)	Schmidt (1934); Pickford (1947); both referred to by Walls & Mathews (1952)
Protanopia series	Occasional	Partial manifestation	Walls & Mathews (1952)
	Rare	Complete manifestation	Jaeger (1951)
Deuteranopia series	Rare	Complete manifestation	Jaeger (1951, 1952)
Retinitis pigmentosa	Typical	Tapetal reflex	Falls & Cotterman (1948); Sorsby (1951-b)
	Occasional	Complete manifestation	Sorsby (1951-b)
Choroideremia	Typical	Fundus has pale patches with overlying pigment	Goedbloed (1942)
			McCulloch & McCulloch (1948)
	Occasional	Partial night blindness and reduced visual field	Goedbloed (1942)
Nystagmus	Occasional	Penetrance in 0-73% heterozygotes of different families	Cuendet & Streiff (1957)
		Penetrance 5.6-78% in differe. t families	Franceschetti (1961)
Night blindness with myopia	Occasional	Manifestation	Gates (1946)
Megalocornea	Occasional	Manifestation	Gates (1946)
Ichthyosis vulgaris		None	Levit (1936)
			Gates (1946)
Keratosis follicularis spinulosa	Frequent	Partial expression	Levit (1936)
Anhidrotic ectodermal dysplasia	Occasional	Partial expression: missing teeth, dry skin.	Levit (1936); Seagle (1954); Bowen (1957)
		One report of mosaic patches on skin.	Roberts (1929)
	Rare	Complete manifestation reported but not genetically proven	Seagle (1954)
Hemophilia A	Occasional	Mild bleeding in a proportion of carriers	Merskey & Macfarlane (1951); Fantl & Margolis (1955); Taylor & Biggs (1957); Biggs & Macfarlane (1958)
		Abnormal laboratory findings in some carriers	Merskey & Macfarlane (1951); Graham *et al.* (1953); Biggs & Macfarlane (1958)
	Rare	Complete manifestation reported but not genetically proven	Wilkinson *et al.* (1957) Mellman *et al.* (1961)
Christmas disease	Occasional	Abnormal laboratory findings in some carriers	Ramot *et al.* (1955) Pitney & Dacie (1955)
	Rare	Complete manifestation reported but not genetically proven	Hardisty (1957)
Chronic hypochromic anemia	Typical	Small proportion of abnormal red cells	Rundles & Falls (1946)

TABLE 2 (cont'd)

Condition	Heterozygous effect		References
	Frequency	Type of effect	
Glucose-6-phosphate-dehydrogenase deficiency	Typical	Reduced enzyme activity. Much individual variation.	Childs *et al.* (1958); Beutler (1959)
Vitamin D-resistant rickets with hypophosphatemia	Typical	Hypophosphatemia	Graham, McFalls & Winters (1959)
	Occasional	Bony deformities	
Diabetes insipidus	Occasional	Sometimes slight signs or symptoms	Forssman (1955)
Duchenne type muscular dystrophy	Rare	One partly affected mother of affected sons.	Kryschowa & Abowjan (1934)
		Out of 107 cases, 2 were females not genetically tested, and 1 was an XO female.	Walton (1957)

authors have in the past expressed doubt that it occurs at all. These doubts are well justified since there are several other possible explanations for the manifestation of a sex-linked disease in a female. These explanations include:

1. The female is homozygous for the gene concerned, either as a result of mutation or of consanguineous mating.
2. The disease has been mistaken for a closely similar but autosomally inherited one. This possibility is especially important in hemophilia, which with modern methods of diagnosis can be separated into several different "hemophilioid states" (Brinkhous and Graham, 1954).
3. The female does not have the normal XX chromosome complement. As would be expected, women with the chromosome constitution XO manifest any sex-linked mutant genes that they carry, to the same degree as the corresponding XY male. Similarly, women with the condition of "testicular feminization" and the chromosome constitution XY manifest sex-linked genes as does a male. The literature on color blindness in such individuals has recently been reviewed by Polani (1961), while hemophilia in an XY girl has been reported by Nilsson *et al.* (1959). Turner's syndrome in an XO individual may be diagnosed fairly easily while the child is still young. Testicular feminization, and other types of chromosome anomaly, however, are often not suspected until adult life, when primary amenorrhea results (Jacobs *et al.*, 1961). Moreover, some women with this type of chromosome abnormality have been color-blind (Lindsten, 1961; Stewart, 1961), suggesting that perhaps the locus of color blindness had been deleted from the abnormal chromosome.

In view of all these possible alternative explanations for manifestation of a sex-linked gene in a female, the necessary criteria that must be fulfilled before she can be interpreted with reasonable certainty as a manifesting heterozygote are quite formidable. These criteria are: (1) The condition must have been diagnosed recently with modern methods for distinguishing closely related diseases. (2) The woman must be fertile and have produced both normal and affected sons, showing that she is neither homozygous nor hemizygous at the locus concerned.

With these criteria there are no instances known to the present writer of severe hemophilia in a manifesting heterozygote. Snyder (1932) found hemophilic women with normal sons, but it cannot now be known from what type of hemophilia they suffered. Wilkinson *et al.* (1957) knew the type of hemophilia, hemophilia A, of the two affected female cousins that they studied, but unfortunately since these children were aged only 4 and 2 years, it will be some time before their genotypes can be ascertained from their sons. Hardisty (1957) reported typical Christmas disease in a young woman whom he considered heterozygous. She had normal menstruation but had not yet married. On the other hand, there appears to be ample evidence of mild hemophilia in heterozygotes. Merskey and Macfarlane (1951) examined twenty-one mothers of hemophilic sons and compared them with normal controls. They found no test which would reliably distinguish the heterozygotes from the normals, but they stated that 47 per cent of the known carriers had excessive bleeding after tooth extractions as compared with 5 per cent of normals, and there were "three positive results out of 21 known carriers examined by the prothrombin consumption test." All these three bled excessively after tooth extraction. A large pedigree with fourteen males showing a very mild form of hemophilia, with bleeding only after injuries such as tooth extraction or tonsillectomy, was studied by Graham *et al.* (1953). Several females were genetically ascertained from their sons to be heterozygous, and they fell into two groups: some with plasma antihemophilic factor values in the normal range and others with values intermediate between normal and those of the affected males of this pedigree. None of them showed any abnormal bleeding. Similar instances of abnormal laboratory findings in otherwise unaffected heterozygous women have been reported for Christmas disease. One woman who was proved heterozygous by her normal and affected sons had a definitely abnormal thromboplastin generation test and "recalled having bled profusely after hysterectomy and also after a tooth extraction" (Ramot *et al.*, 1955). To sum up, the general picture among heterozygotes for hemophilia seems to be one of considerable variation in expression, which is in accord with the present hypothesis.

In color blindness it does seem possible to find instances of genetically proven manifesting heterozygotes. Jaeger (1951) gave the pedigree of two sisters who from their ancestry would have been expected to be doubly heterozygous for genes of the protanopia and deuteranopia series, on opposite chromosomes. (Such double heterozygotes usually have normal color vision.) One of these sisters manifested deuteranomaly and had both a protanopic and a deuteranomalous son; the other manifested protanopia and had a deuteranomalous son. In another pedigree Jaeger (1952) found a deuteranomalous woman who was the mother of one normal and one deuteranomalous son. She also had a bilateral macular degeneration which he thought might have precipitated the manifestation of color blindness. Walls (1959) considered that when one of a pair of monozygotic female twins is color-blind the manifesting twin may safely be considered heterozygous, and gave examples, including two of the Dionne quintuplets. This seems open to the objection, however, that X chromosome loss during cleavage might well occur in one only of a pair of twins, and they might still be regarded as monozygotic. As with hemophilia, although fully manifesting heterozygotes for color blindness seem very rare, partially

affected ones are quite frequently found, especially for genes of the protanopia series. Walls and Mathews (1952) mentioned the sister of a protanopic male who considered herself color blind but was only partly affected by their standards, and they reviewed the work of Pickford and Schmidt who have both shown that slight deviations from normality are common in the relatives of color-blind males.

Thus, the general picture concerning heterozygotes for sex-linked genes in man is one of variable expression, which accords with the predicted result of random inactivation of one or the other X chromosome. Moreover, in a few conditions the effect in heterozygotes is visibly mosaic.

Another prediction of the hypothesis is that if a female carries two non-allelic mutant genes affecting the same cells, one on each X chromosome, she should have no normal cells of this type. This fact would only be demonstrable for genes with localized effects. Since most of the conditions mentioned in table 2 are very rare the hope of finding two suitable ones together in the same pedigree is slight. Color blindness and glucose-6-phosphate-dehydrogenase deficiency, however, are both sufficiently common to occur in pedigrees together with other sex-linked diseases. Franceschetti (1961) has reviewed such pedigrees involving color blindness. A very interesting one is that described by Falls and Cotterman (1948). In a family with sex-linked retinitis pigmentosa they were able to detect the heterozygotes by a glistening property of the tapetum, the tapetal reflex. A heterozygous woman had three daughters by a color-blind man. Two daughters showed themselves heterozygous for retinitis pigmentosa by the tapetal reflex and these two both manifested the color blindness for which they were heterozygous; the third daughter did not show the tapetal reflex and did not manifest color blindness though she was presumed heterozygous like her sisters. The explanation of this, according to the present hypothesis, would be that in the sisters heterozygous for retinitis pigmentosa all the retinal cells were affected either by retinitis pigmentosa or by color blindness, so that the two sisters had no normal cells and were necessarily color-blind. However, until more pedigrees can be found showing two suitable sex-linked diseases, this particular example can only be considered an interesting one. Franceschetti mentions also one pedigree involving nystagmus and color blindness and one with night blindness and color blindness. In both instances the two mutant genes were carried on the same X chromosome, and so the point is not tested. In the night blindness pedigree one woman is shown as being a repulsion heterozygote, but from her ancestry this seems unlikely. The usually normal color vision of double heterozygotes for genes of the protanopia and deuteranopia series seems at first contrary to expectation, but it can be explained by the complementary nature of the two defects; the protanopic cones are normal for green light, and the deuteranopic ones normal for red.

RELATION OF THE HYPOTHESIS TO RECENT FINDINGS CONCERNING SEX CHROMOSOMES AND SEX CHROMATIN IN MAN

Recent progress in the understanding of sex chromosome abnormalities in man has been very rapid (Miller, 1960; Polani, 1961), and it is obviously important to consider how the new hypothesis fits in with these findings.

It is postulated that in a normal female with two X chromosomes one of the two is inactivated and forms a sex chromatin body, and that when there is only one X, as in a normal male or an XO female, it is not inactivated. This agrees with the finding that XO women are sex chromatin negative, and also with the fact that XXY males, with Klinefelter's syndrome, are sex chromatin positive. Recently, individuals have been found with three and four X chromosomes (Jacobs *et al.*, 1959; Ferguson-Smith *et al.*, 1960; Fraccaro and Lindsten, 1960; Miller *et al.*, 1961) and in each instance the maximum number of sex chromatin bodies present has been one less than the number of X chromosomes. This means that the hypothesis may be extended to postulate that all X chromosomes in excess of one normal one are inactivated. This would explain some hitherto rather puzzling features of cases such as the males with XXXXY chromosomes: that the Y chromosome has been able to produce a male phenotype in opposition to so many X's, and that the abnormalities of an individual with three extra chromosomes are not much greater than those of an XXY male with only one extra chromosome. These observations are readily understandable if the three additional X's are inactive. If the findings of small sex chromatin bodies in women with a partly deleted X, and large ones in a woman with presumptive isochromosome X, are substantiated (Jacobs *et al.*, 1961) this would indicate that if one of two X's is deficient, then the normal one remains active in all cells, with the abnormal one forming the sex chromatin body. This would provide an alternative explanation for the manifestation of color blindness in women with this type of chromosome abnormality, if they carry the gene for color blindness on the normal, active X. Stewart (1961) has suggested the explanation of deletion of the locus of color blindness.

Facts that remain unexplained are that an XO female and an XXY male show any abnormality, and that an XO female in man differs in phenotype from that in the mouse. There seem at least two possible explanations for these facts. The first is that the abnormal phenotype of XO etc., individuals in man is due to abnormal action of the sex-chromosomes before the time in development at which inactivation of one X occurs. Park (1957) found that sex-chromatin in human embryos was first detectable at sixteen days' gestation, and became more easily detectable up to eighteen days. Thus, one must presume that before this stage all X chromosomes are active. No similar observations are available for the mouse, but in the rat, which has an embryology similar to that of the mouse, sex chromatin is first detectable at seven days gestation (Zybina, 1960). This is an early egg cylinder stage, and probably corresponds to a rather earlier stage than that of the sixteen-day human embryo, which is just forming the primitive streak. Thus it seems quite possible that inactivation of X chromosomes takes place at a developmentally earlier stage in the mouse than in man.

The other possible explanation is that the X chromosome of man has a short pairing segment, that this is not normally inactivated, and that it is duplication or deficiency of this region which gives rise to the abnormal phenotypes observed. Controversy still remains concerning the presence or absence of a pairing segment in the sex chromosomes of particular mammalian species (Hamerton, 1958).

No other general hypothesis concerning the behavior of sex-linked genes in

mammals has been put forward. However, simultaneously with the original publication of the present hypothesis, Russell (1961) put forward a very similar but more limited one concerning the variegation due to sex-linked translocations in the mouse. She considered that this variegation was "presumably a heterochromatic effect," and from the fact that two X chromosomes were essential for its expression, together with cytologic evidence, postulated that "in mammals, genic balance requires the action of *one* X in a manner which precludes realization of its heterochromatic potentialities, so that only *additional* X's present assume the properties characteristic of heterochromatin."

CONCLUSION

Although it would be desirable to have far more evidence, that which has been collected already seems sufficient to warrant extending the hypothesis to cover gene action in the X chromosome of man and mammals in general. Efforts to obtain more evidence about mosaicism in heterozygotes, phenotypes of double heterozygotes, the appearance of sex chromatin in embryology, etc., will be pressed forward.

SUMMARY

The hypothesis is put forward that the normal method of dosage compensation in mammals, including man, is inactivation of one of the two X chromosomes of females. It is postulated that the inactive X forms the sex chromatin body, that either one of the two X's may be inactivated in different cells of the same animal, and that the inactivation occurs early in development. In adult life this leads to patches of cells some with one and some with the other X chromosome inactivated and hence to the mottled appearance characteristic of female mammals heterozygous for sex-linked color genes. The hypothesis stands up so far to test against the predictions which can be made from it, but more evidence is needed. Recent discoveries concerning sex chromosome abnormalities in man suggest that in such cases all X chromosomes in excess of one normal one are inactivated. The hypothesis thus explains some hitherto puzzling facts, such as the viability of XXXXY individuals with three extra chromosomes.

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