INFLUENCE OF GENE DUPLICATION AND X-INACTIVATION ON MOUSE MITOCHONDRIAL MALIC ENZYME ACTIVITY AND ELECTROPHORETIC PATTERNS

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ABSTRACT

We have investigated, with and without the influence of X-inactivation, the relationship between autosomal gene-dosage and gene-product in a mammalian system, the mouse. The gene was mitochondrial malic enzyme (Mod-2), shown to lie on Chromosome 7 between the albino (c) and shaker-1 (sh-1) loci, and the enzyme was its product, mitochondrial malic enzyme (MOD-2). Gene duplication, with and without the influence of X-inactivation, was achieved using a translocation that involves the insertion of a portion of Chr 7, including Mod-2, into the X, T(X;7)iCt. A 1:1 relationship for Mod-2 dosage and MOD-2 activity was found in heart mitochondria. Evidence of X-inactivation of Mod-2 was noted in heart and kidney preparations from females carrying a Mod-2 duplication (one copy of Mod-2 in the X and two copies of Mod-2 on Chr 7). We conclude that the expression of an autosomal locus attached to X-chromatin depends upon whether the translocation is in a balanced or unbalanced state.

IN mammals there appears to be a one-to-one relationship between gene-dosage and gene-product (protein) when one or two functional copies of an autosomal structural locus are present (reviewed by KIRKMAN 1972). It is unknown whether this relationship still persists when three or more copies of a locus are present as a result of duplication or chromosomal trisomy.

It has been hypothesized that, although a direct relationship between gene-dosage and gene-product may exist as long as the involved autosomal loci remain surrounded by autosomal chromatin, this relationship may change when the autosomal loci are attached instead to inactive X chromatin (see review by EICHER 1970). To date, no evidence has accumulated at the protein level to confirm the hypothesis that autosomal loci attached to inactive X-chromosomal material are, in fact, inactivated.

Recently, a mammalian system was found that allowed questions to be answered concerning X-inactivation of autosomal loci and the relationship

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between gene-dosage and gene-product in a duplicated state, both with and without the influence of X-chromosomal inactivation. The mammalian organism is the mouse carrying the T'(X;7)1Ct (TCt) translocation, the enzyme is mitochondrial malic enzyme (MOD-2), and the locus is mitochondrial malic enzyme (Mod-2). We present here evidence that (1) the Mod-2 locus is located between the Chromosome 7 loci c (albino) and sh-1 (shaker-1) and is, therefore, translocated to the X-chromosome in the TCt translocation; (2) the specific activity of mitochondrial malic enzyme is increased in heart mitochondria in TCt animals carrying a duplication for the Mod-2 locus; and (3) inactivation of the Mod-2 locus occurs in unbalanced (those carrying the duplication), but not balanced female TCt mice.

MATERIALS AND METHODS

Linkage of Mod-2

SM/J is the only inbred strain known to contain an inherited electrophoretic variant at the Mod-2 locus, the Mod-2a allele. It also carries the Hbb+ (single) allele at the Hbb locus (β-hemoglobin chain). The FS/Ei strain is homozygous for the Chr7 alleles p (pink-eyed dilution), cch (chinchilla), Mod-2a, sh-I, Hbb+ (diffuse), and fr (frizzy).

In order to determine the exact location of Mod-2 in Chr 7, mice of the two inbred strains SM/J and FS/Ei were crossed to each other. The p cch Hbb+ fr/+ + Mod-2a + Hbb+ F1 offspring were crossed back to FS/Ei mice. Each offspring was classified for its phenotype at all six loci.

Production of MOD-2A stock

Several backcross mice from the previous cross that were p +/p cch, thus crossovers between p and cch, were mated together and their p/p offspring kept. Each p/p offspring was mated to an SM/J animal and 7 to 10 of their offspring were tested to determine whether they were homozygous Mod-2a and Hbb+. The presence of only homozygous Mod-2a and Hbb+ offspring indicated that their p/p parent was also homozygous for the Mod-2a and Hbb+ alleles. A proven homozygous p Mod-2a Hbb+ female was crossed to an identical male to produce the homozygous p Mod-2a Hbb+ stock, hereafter designated MOD-2A, used in these studies.

The TCt translocation

The nonreciprocal X-autosomal translocation TCt involves the insertion of a central region from Chr 7 into the distal medial region of the X chromosome (reviewed by EICHER 1970, and Figure 1). The X chromosome containing the inserted region of Chr 7 is commonly called the Xct or flecked X chromosome (Xfd). In relation to the autosomal genes of interest in this report, the Xct chromosome contains the wild-type allele at the p locus (+) and the Mod-2b allele at the Mod-2 locus. We have represented this X as Xct (p+ Mod-2a) to indicate the presence of the p+ and Mod-2a. It should be noted in Figure 1 that the Hbb locus was not translocated to the X chromosome.

TCt animals are of two common chromosomal types, balanced and unbalanced (Figure 2). The balanced type contains an Xct chromosome, a normal X (or Y), a normal Chr 7, and a Chr 7 that has a deletion for the region contained in the Xct chromosome, hereafter called Chr 7Df. In relation to specific genes carried on Chr 7 and Chr 7Df, if we symbolize an individual as p cch Mod-2a Hbb+/Df Hbbd, it indicates that this individual carries the p, cch, Mod-2a, and Hbb+ alleles on Chr 7, and Hbbd allele on Chr 7Df, and a deficiency for the p, c, and Mod-2 genes on Chr 7Df. The unbalanced type contains an Xct chromosome, a normal X (or Y), and two normal Chr 7. Thus, unbalanced TCt individuals have a duplication for the Chr 7 region contained in the Xct chromosome.
Figure 1.—Diagram of T(X;7)1Ct translocation. Two breaks occurred in Chr 7, as indicated by arrows. This chromosomal region was inserted into the X chromosome in region of X designated by the arrow. Actually, it is unknown whether the piece was inserted distal (as shown), or proximal to $jp$. It should be noted that the piece of Chr 7 is inverted in the X chromosome relative to the centromere. The loci shown on the X and Chr 7 are spf (sparse fur), Ta (tabby), Mo (mottled), $jp$ (jimpy), Hyp (hypophosphatemia), Es-8 (esterase-8), $pu$ (pudgy), $p$ (pink-eyed dilution), c (albino), Mod-2 (malic enzyme, mitochondrial), sh-I (shaker-1), $Hbb$ (hemoglobin $\beta$ chain), and fr (frizzy).

In order to produce the TCt mice and their normal sibs for analysis, females that were genetically $X^{c^t}$ ($p^+ Mod-2^b)/X p Mod-2^a Hbb^b/Df Hbb^d$ were crossed back to MOD-2A males. The six classes of expected offspring are listed in Table 1. The animals designated as Class 1 can be distinguished from those in the other two classes because of their nonpigmented eyes. The animals designated as Class 3 can be distinguished from those in Class 2 because of their HBB-S phenotype (see below).

In order to increase the number of Class 3 males for enzyme analysis, a few females of the genotype $X^{c^t}$ ($p^+ Mod-2^b)/X p Mod-2^a Hbb^b/Hbb^d$ (Class 3) were crossed to males of the MOD-2A stock. This cross produces offspring that are of the Classes 1 and 3.

**Hemoglobin determinations**

The $Hbb^b/Hbb^a$ or $Hbb^b/Hbb^d$ genotype (HBB-S and HBB-SD phenotypes, respectively) of most of the mice was determined using the method of Hutton (1969) for the preparation of mouse hemoglobin for starch gel electrophoresis, as modified in our laboratory for cellulose acetate electrophoresis. The modified method consists of collecting approximately 0.2 ml of whole blood in 2 ml 0.8% NaCl containing 1 drop of 5000 U/ml Heparin solution. The cell suspension is centrifuged and the supernatant removed. Lysis is accomplished using 1 part distilled H$_2$O and 1 part toluene per 1 part of packed RBC's. The lysate is centrifuged at 15,000 rpm for 30 minutes. Three parts of the supernatant are mixed with 2 parts of a 15.38 mg/ml solution of iodoacetate in 0.2 M phosphate buffer, pH 7.0, and left at room temperature for 2 hrs. Thirty minutes before electrophoresis, 1 drop of a 250 mg percent solution of potassium
Electrophoresis of MOD-2

Electrophoresis of heart or kidney extracts was performed on starch gels or cellulose acetate gels. Most starch gels were run according to a modified method of Shows, Chapman and Ruddle (1970). Single hearts or both kidneys were homogenized in 0.4 or 0.3 ml cold distilled H₂O, respectively. Two drops of a 10% Triton-X 100 solution was added to each tube and the contents mixed. Supernatants were collected after centrifugation in the cold at 15,000 rpm and used directly for electrophoresis or after a maximum of one week, if frozen. Electrophoresis was performed at 150 volts, cathode to anode, at 4°C for 18 to 20 hrs. The bridge buffer consisted of a one-to-one dilution of Shows’ buffer (54 g Tris base; 36.14 g citric acid, monohydrate; per liter, pH 6.2). Gels consisted of 58 g Electrostarch, 9 ml Shows’ buffer, and 491 ml distilled H₂O. The staining method was identical to that used by Shows, Chapman and Ruddle.
During the course of these studies, we were able to improve the above buffer system so as
 to avoid the heating problems encountered by the high ionic strength of Snows' buffer. The
 bridge buffer (TC buffer) consisted of a 0.1 M Tris citrate solution, pH 7.6, (12.1 g Tris base
 titrated to pH 7.6 with citric acid and then brought to one liter.) The gel buffer consists of 1:9
dilution of TC buffer. Electrophoresis was conducted at 180 volts, cathode to anode, 4° C, 18–22
 hours. Identical MOD-2 patterns were observed with TC buffer as with Snows' buffer.

Electrophoresis of MOD-2 was also performed on cellulose acetate gels (Titan III strips,
 Helena Laboratories) using a 1:9 dilution of the TC buffer. We obtained as detailed an MOD-2
 pattern with the cellulose acetate system as with the starch gel system.

We determined the MOD-2 electrophoretic pattern from lysates of heart, kidney, or both,
of more than 50 females each from Class 2 and 3, and 20 males each from Classes 2 and 3.

**Enzyme determinations**

Mice were killed by cervical dislocation and the hearts removed. Each heart was washed
in isotonic saline, minced, and homogenized in 1 ml 0.44 M sucrose. After 10 min centrifuga-
tion at 800 x g, the supernatants were removed and centrifuged at 10,000 x g in a Spinco
ultracentrifuge for 10 min. The supernatant was discarded. The resulting mitochondrial pellet
was washed in 1 ml 0.44 M sucrose and then centrifuged as before. Again the supernatant was
discarded. The mitochondrial pellet was suspended in 0.30 ml of mixture of 5 parts of 0.25 M
sucrose to 1 part 10% Triton-X 100. The pellet material and suspension solution were thoroughly
mixed on a vortex mixer, and then set aside for 10 min at room temperature. The mixture was
then centrifuged at 10,000 x g for 10 min to separate the soluble fraction from the membrane
fraction. The soluble fraction was removed and 0.1 ml taken for a protein determination by the
biuret method and the rest used to determine malic enzyme activity (MOD-2: EC 1.1.1.40
L-malate : NADP oxidoreductase, decarboxylating). Malic enzyme was determined essentially as
described by Hsu and Lardy (1969) except that 0.1 ml of a 0.03 M malic acid solution, rather than
0.05 ml, was used in the reaction mixture. All assays were conducted at room temperature using a
Beckman DB spectrophotometer. The conduct of these assays at ambient temperature in different
seasons of the year may have contributed to the different values obtained for male and female
mice studied at different times.

Like-sexed groups of 6 to 8 individuals of comparable ages were always analyzed together.
The investigator (DLC) conducting the enzyme determinations did not know how many or
which individuals were Class 2 or Class 3 within any individual group. Each individual group
contained at least one mouse of each of the three classes. All determinations were conducted
on freshly prepared soluble material obtained from mice killed the same day. The analysis of
MOD-2 levels of heart mitochondria isolated from individual females was conducted during the
first part of 1974 and that from individual males conducted during the second part of 1974.
A separate group of Class 1, 2, and 3 females and males were used for analysis of heart and
kidney MOD-2 electrophoretic patterns.

**RESULTS AND DISCUSSION**

**Location of Mod-2 in Chr 7 and the TCt translocation**

It became clear in the early part of these studies that Mod-2 was located either
between c<sup>ch</sup> and sh-1 or between sh-1 and Hbb. Therefore, we can focus our attention
only on the c<sup>ch</sup> to Hbb interval of Chr 7. Of the 478 mice produced in the
cross p c<sup>ch</sup> Mod-2<sup>b</sup> sh-1 Hbb<sup>d</sup> fr/p c<sup>ch</sup> Mod-2<sup>b</sup> sh-1 Hbb<sup>d</sup> fr × + + Mod-2<sup>a</sup> - +
Hbb<sup>b</sup> +/p c<sup>ch</sup> Mod-2<sup>b</sup> sh-1 Hbb<sup>d</sup> fr, 25 were recombinants between the c and
sh-1 loci, indicating that the percent recombination between c and sh-1 was
5.23 ± 1.02. Nineteen of these mice were successfully classified for MOD-2.
Fifteen were recombinants between the Mod-2 and sh-1 loci but not recombinants between the c and Mod-2 loci, and four were recombinants between both the c and Mod-2 loci and the Mod-2 and sh-1 loci. Thus, Mod-2 is located closer to the c locus than to the sh-1 locus. The relative distance from c to Mod-2 and from Mod-2 to sh-1 can be determined by dividing the recombination fraction from c to sh-1 (5.2%) into 4/19 and 15/19, respectively. The order of loci in Chr 7 with percentage recombination is c–1.1–Mod-2–4.1–sh-1.

Since one of the TCT breakpoints in Chr 7 is between Hbb and sh-1, the Mod-2 locus is carried on the X<sup>ct</sup> chromosome. As expected from the linkage study, Class 3 males had an MOD-2 isozyme pattern different from that of Class 1 and Class 2 males (see Table 1).

### TABLE 2

**Enzymatic activity of MOD-2 in heart mitochondria**

<table>
<thead>
<tr>
<th>Class</th>
<th>Dosage of Mod-2</th>
<th>Enzymatic activity</th>
<th>N</th>
<th>M.2&lt;sup&gt;*&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heart</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 Copies-Normal</td>
<td>12</td>
<td>15.92 ± 0.47**</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 Copies-Balanced TCT</td>
<td>19</td>
<td>15.13 ± 0.28**</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 Copies-Unbalanced TCT</td>
<td>20</td>
<td>22.84 ± 0.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per cent increase, Class 3</td>
<td></td>
<td>47.9</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2 Copies-Normal</td>
<td>13</td>
<td>21.68 ± 1.13††</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2 Copies-Balanced TCT</td>
<td>12</td>
<td>20.21 ± 0.96††</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 Copies-Unbalanced TCT</td>
<td>15</td>
<td>29.13 ± 1.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Per cent increase, Class 3</td>
<td></td>
<td>38.9</td>
<td></td>
</tr>
</tbody>
</table>

* Nanomoles NADP/mg protein/min room temperature ± S.E.
** Values not significantly different from each other according to the Student-Newman-Keuls multiple range test.
†† Values not significantly different from each other according to the Student-Newman-Keuls multiple range test.
Analysis of enzyme levels

The results of the enzyme studies are given in Table 2. It is appropriate to consider first the enzyme levels in males because of complication of inactivation of the Mod-2 locus by the X chromosome in Class 2 and 3 females.

Class 1 and 2 males differ in two ways in relation to the Mod-2 locus (see Figure 2 and Table 1): (1) Class 1 males are homozygous Mod-2*, whereas Class 2 males are heterozygous, and (2) Class 1 males have both their alleles on Chr 7, whereas Class 2 males have one of their alleles on the X chromosome and one on Chr 7. We had determined early in our investigation that mice having the MOD-2A and MOD-2AB forms of the enzyme did not show significant differences in activity. Therefore, if differences were noted between Class 1 and 2 males, the cause must be due to the influence of the X chromosome on the Mod-2 locus in Class 2 males (see difference number 2 above). Because Class 1 and 2 males contain the same MOD-2 activity in their heart mitochondria, we conclude that the presence of the Mod-2 autosomal locus in the X chromosome does not in itself influence its expression. In addition, we conclude that any increase in the level of MOD-2 enzyme in Class 3 males will be a direct reflection of their duplicated Mod-2 state.

As seen in Table 2, Class 3 males contain approximately a 50 per cent increase in MOD-2 activity in heart mitochondria, as compared to Class 1 and 2 males. This is strong evidence that in the duplicated state, the Mod-2 locus maintains a 1:1 relationship with its product in heart tissue.

It is pertinent to note that similar results on the effect of dosage of Mod-2 on MOD-2 levels were also noted by DIAMOND and ERICKSON (1974). They measured the MOD-2 levels in whole heart extracts of mice heterozygous for one of the radiation-induced albino mutations (c*sh) that has been shown to be a deletion covering the Mod-2 locus (ERICKSON, EICHER and GLUECKSOHN-WAEICHE 1974). Controls consisted of homozygous normal littermates. In the heart they found a 1:1 relationship between the number of Mod-2 genes (one versus two) and the MOD-2 enzyme level. That is, individuals heterozygous for the deletion had half the MOD-2 level compared to normal individuals. We conclude from their studies and our studies that the level of MOD-2 in heart mitochondria in relation to a Mod-2 gene dosage of 1:2:3 is 0.5:1:1.5, respectively.

Analysis of MOD-2 levels in the females is more complicated than in the males because of the influence of X-inactivation on the attached Mod-2 gene. Class 3 females showed a significant increase in heart mitochondrial MOD-2 levels compared to Class 1 and 2 females. This increase indicates that the extra Mod-2 copy carried on their X chromosome is increasing the level of MOD-2. If the Mod-2b allele carried on the X chromosome was always inactivated when the X chromosome was the inactive X and the X chromosome was inactive in 50 per cent of the heart cells, we would expect that Class 3 females would have half of the Class 3 male MOD-2 increase. The observed increase was 39 percent. This increase is greater than the 25 percent increase expected (half of that observed
in Class 3 males) and may indicate that in heart cells these females have either (1) the normal X preferentially inactivated, or (2) the $Mod-2^b$ allele in the $X^{fa}$ chromosome is active when $X^{fa}$ is the inactive X (in some cells $Mod-2^b$ is never inactivated or it can be reactivated). Further research is needed to clarify this point.

Contrary to what we expected, Class 2 females showed no decrease in heart mitochondrial MOD-2 levels, indicating they did not inactivate the $Mod-2^b$ locus in the $X^{ci}$ chromosome (see results from electrophoretic gels below.) There is evidence that autosomal loci attached to inactive X chromatin are not always inactivated. For example, evidence exists that the further removed an autosomal locus is from inactive X chromatin, the greater is the probability that it will escape inactivation (reviewed by Eicher 1970). This possibility is highly unlikely for the $Mod-2$ locus in the $X^{ci}$ chromosome. The order of Chr 7 loci in the $X^{ci}$ chromosome is centromere—X chromosome—sh-1$^+$—$Mod-2^b$—c$^+$—p$^+$—X chromosome such that $Mod-2^b$ is located between the sh-1$^+$ and c$^+$ loci, both of which are inactivated in both Class 2 and 3 females (Eicher and Washburn unpublished data; Eicher 1967; Deol and Green 1969; Cattanach 1961; Eicher 1970). Further, we have presented evidence here that the $Mod-2^b$ locus in Class 3 females can be inactivated (see results from electrophoretic gels below). Thus, in most heart cells of Class 2 females, either (1) the Chr 7 region in the $X^{ci}$ chromosome is not inactivated when the $X^{ci}$ is the inactive X, or (2) the normal X is always the inactive X, or (3) the $Mod-2^b$ allele is reactivated in the inactive $X^{ci}$ chromosome. Unfortunately, there are presently no X-linked isozyme variants in the mouse that can be used for an efficient test of possibilities 1 and 2.

Regarding possibility 3, Cattanach (1974) has presented evidence that there is reactivation of the inactive c$^+$ locus during the lifetime of Class 3 females. However, no such finding has been reported for Class 2 females. Until we have information on the MOD-2 levels of embryonic and very young Class 2 females to indicate whether they have an inactive $Mod-2^b$ allele in some of their cells during development that is reactivated by adulthood (or those cells are selected against), possibility 3 cannot be eliminated from consideration.

It is appropriate to note here that we obtained different levels of MOD-2 in heart mitochondria of Class 1 males and females (Table 2). As pointed out previously (see Materials and Methods), the heart determinations for males and females were done at room temperature at separate times in different seasons. At the end of these experiments (end of 1974), we analyzed the heart mitochondria MOD-2 levels for two groups of Class 1 individuals, each group consisting of four females and four males. No differences were noted between males and females. Therefore, the previous difference between sexes in some way reflects either changes in our efficiency of measuring MOD-2, or unknown environmental changes that affected the MOD-2 level, or both. Thus, although the absolute values of MOD-2 present in heart mitochondria are in question, the differences noted between Class 1, 2 and 3 females or Class 1, 2 and 3 males are not in question.
Figure 3.—Electrophoresis of heart MOD-2 cellulose acetate. The slot number, sex of animal, and phenotype are given. "A" represents the phenotype of a Mod-2n/MdF mouse, "B" a Mod-2n/Mod-2b mouse, and "AB" an Mod-2n/Mod-2b mouse. When the symbol is surrounded by parentheses, it indicates that the allele responsible for that part of the phenotype is in the X chromosome (X0). For example, "(B)AA" is the phenotype observed in the Xc' (Mod-2n)/X Mod-P/Md-2b female (or male, if indicated). Slot 3 is a mixture of slots 1 and 2; slot 4 of 1 and 6; slot 5 of 2 and 6. Note similarity of slots 4 and 7.

Starch gel and cellulose acetate electrophoretic patterns

We obtained the same gel patterns for MOD-2 as those reported by Shows, Chapman and Ruddle (1970): The MOD-2A form migrated faster than the MOD-2B form, with the MOD-2AB form migrating in an intermediate position (Figure 3). The same patterns have been observed for mitochondrial malic
enzyme in human brain and *Macaca nemestrina* brain (Cohen and Omenn 1972a,b). Mitochondrial malic enzyme is probably a tetramer (Povey et al. 1975).

The MOD-2 starch gel and cellulose acetate electrophoretic patterns observed from kidney and heart preparations of Class 1, 2, and 3 individuals were consistent with our findings of MOD-2 enzyme levels. As can be seen in Figures 3 and 4, the Class 2 males and females showed the MOD-2AB pattern typical of the *Mod-2a/Mod-2b* mouse indicating no influence of X-chromosomal material on the *Mod-2b* allele in either sex. Furthermore, as seen in Figure 3, the Class 3 males had a MOD-2 pattern similar to that obtained if supernatants from *Mod-2a/Mod-2a* and *Mod-2a/Mod-2b* individuals were mixed before electrophoresis.

It was very striking that each Class 3 female displayed a unique MOD-2 pattern, indicating that each contained different proportions of cells with the *Mod-2b* allele active (or inactive). As can be seen in Figure 5, some females had a pattern similar to Class 3 males (*Mod-2b* allele was active in all cells), some had a pattern showing increased activity in the MOD-2A region of the gel (*Mod-2b* allele was active in some cells and inactive in others), and some displayed a pattern similar to Class 1 females (*Mod-2b* allele was inactive in most cells).

A number of hypotheses related to the mechanism of X-inactivation have been postulated that rely on observations in females heterozygous for X-A translocations (Russell and Montgomery 1970; Russell 1963, 1964; Cattanach 1961, 1974; Lyon 1971, 1972, 1974; Gruenberg 1967a,b; Eicher 1970; Brown and Chandra 1973; Chandra and Brown 1975; Cooper 1971). Several of these

![Figure 5](image-url)

**Figure 5.**—Electrophoresis of heart MOD-2 on cellulose acetate. Note variation in phenotype of *Xct (Mod-2b)/X Mod-2a/Mod-2a* females. For example, phenotype of female in slot 2 is like that of male in slot 4 (Interpretation: *Mod-2b* in *Xct* chromosome is active in most cells), whereas that of female in slot 6 is like that of individual in slot 1, Figure 3 (Interpretation: *Mod-2b* in *Xct* chromosome is inactive in most cells).
hypotheses were based on the fact that lack of inactivation for X-linked or variegation-position effect for autosomal loci occurred in females heterozygous for one of several translocations. As previously pointed out, the lack of variegation in one tissue does not rule out the presence of variegation in other tissues (EICHER 1970). A case in point is the Class 2 TCt/+ females; they have variegation for c and p in melanocytes but not for Mod-2 in kidney or heart cells.

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