Enzyme ontogeny in normal and hemimelic limbs of mice

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The appearance of electrophoretically separable isozyme patterns is specific both to type of tissue and to its stage of development (Markert & Möller, 1959). In abnormally developing embryonic systems there are alterations in the time of initial appearance and the persistence of isozymes from a variety of metabolic areas (Johnson & Spinuzzi, 1966, 1968). In malformed embryos from folic-acid-deficient rats the altered zymogram patterns of esterases from abnormal limbs were different from those of abnormal kidneys, yet differentiation of zymograms from the normal hearts of these embryos was unaltered. This implies a correlation between altered tissue-specific zymogram patterns and abnormally developing systems, though no causal relationship has as yet been shown (Johnson, 1968). Moreover, when tibial hemimelia in the rat was elicited by two different teratogens—a folic acid antagonist, 9-methylpteroyl-glutamic acid (PGA), and the alkylating agent N-nitroso-N-methylurea (NNMU)—there were similar changes in the non-specific esterases after electrophoretic separation of homogenates from the defective limbs (Johnson & Lambert, 1968).

Dagg (1960, 1967) was able to elicit tibial hemimelia in C57BL/10 mice with an injection of 500 μg 5-fluorouracil (5-FU) on the tenth day of gestation. This defect was structurally indistinguishable from that produced by the homozygous luxate gene (lx/lx) (Carter, 1951). A dose of 250 μg 5-FU produced polydactyly, the phenotype of lx/+ mice, and tibial hemimelia was produced when 250 μg 5-FU was given to females carrying heterozygous embryos.

The present study was designed to determine whether the enzymic ontogeny is similar for the homozygous mutant luxate and the 5-fluorouracil-induced defect, and whether or not any interaction of the effects of the genes and chemical agents could be detected by electrophoretic techniques.

The enzymes included alkaline phosphatases, which have been found characteristically in the apical ectoderm ridge of the limb bud (Milaire, 1962), adenosine monophosphatases, which are active in interdigital areas (Milaire, 1965), non-

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specific esterases, which are in mesenchyme (Johnson, 1968), and the dehydro-
genases of glucose-6-phosphate, malate and lactate, because these occur in a
variety of metabolic pathways and have shown alterations of isozyme patterns
in other teratogenic situations (Johnson & Spinuzzi, 1966, 1968).

MATERIALS AND METHODS

Mice of the C57BL/10J strain were obtained from the Jackson Laboratory,
Bar Harbor, Maine, and a colony of homozygous luxate mice was derived from
several C57BL/10Dg-lx/+ mice, a strain in which the lx gene had been back-
crossed to the C57BL/10 inbred strain for thirty-three generations by C. P. Dagg.
The diet consisted of Rockland or Purina Mouse Breeder Chow supplemented
weekly with horsemeat and lettuce. The animals were kept at a relatively constant
temperature (25° C) in a room with 12 h light-and-dark cycle which became
dark at 9.30 p.m. Proesterous, nulliparous females were selected between 7.30
and 9.00 p.m. and caged overnight with males. To produce litters entirely of
one genotype the following matings were used ♀+/+ × ♂+/+, lx/lx × lx/lx, and
lx/lx × +/+ . The next morning was day zero of gestation for females with
copulation plugs, who were anesthetized with ether, ear-notched and assigned
to an experimental group.

On day 10 of gestation the females to be treated were anesthetized at 10.00 a.m.
and received an intraperitoneal injection of 0.5 ml. aqueous solution containing
either 420 µg uracil, 370 µg 5-FU or 500 µg 5-FU. There were seven experi-
mental groups:

1. +/+ females carrying +/+ offspring (untreated).
2. +/+ females carrying +/+ offspring and injected with 420 µg uracil
   (420 µg uracil).
3. +/+ females carrying +/+ offspring and injected with 370 µg 5-FU
   (370 µg 5-FU).
4. +/+ females carrying +/+ offspring and injected with 500 µg 5-FU
   (500 µg 5-FU).
5. lx/lx females carrying lx/+ offspring (lx/+).
6. lx/lx females carrying lx/lx offspring (lx/lx).
7. lx/lx females carrying lx/+ offspring, injected with 370 µg 5-FU (lx/+ and
   5-FU).

Each pregnant female was killed by cervical dislocation between 10.15 a.m.
and 11.00 a.m. on days 12–17 of gestation and the uterus removed. The embryos
were dissected free of the decidua, and placed in a small dry Petri dish. The yolk
sac and amnion were removed while the dish was kept on an ice bath in the
field of a dissecting microscope. At this time the hind limbs of each embryo were
examined. The limbs were removed at the knee and segregated into homogenizer
tubes according to their genotype and whether they were normal, polydactylyous
or hemimelic. If there were obvious maturational differences within a homo-
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The characteristics of the litters and frequency of limb defects in the experimental groups are listed in Table 1. Tibial hemimelia occurred in all \(\text{l}_x/\text{l}_x\) young, though occasionally it appeared unilaterally. Polydactyly occurred in 53% of \(\text{l}_x/+\) offspring.

The majority of malformed offspring exhibited bilateral limb defects. When the hind limbs of one embryo were affected differently the whole embryo was classified according to the more severe defect, but each limb was placed in the homogenizer tube assigned to its special phenotype and genotype. Unilateral polydactyly was found more often on the right side (28 out of 44 cases) in \(\text{l}_x/+\)
offspring but on the left (12 out of 17 cases) in the 370 µg 5-FU group. There was no apparent preference as to side affected among hemimelic offspring from either the lx/lx or 500 µg 5-FU groups. When the effects of the gene and fluorouracil were combined, the directional influence of the mutant gene appeared to predominate and the more severe defect—hemimelia—was found on the right side in eight out of ten cases (not significant) and the less severely affected limb was polydactylous on the left.

Table 1. Incidence of polydactylous and hemimelic fetuses

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Litters (no.)</th>
<th>Total conceptions (no.)</th>
<th>Live fetuses (no.)</th>
<th>Resorbing fetuses (%)</th>
<th>Live fetuses with malformed limbs</th>
<th>Polydactylous fetuses* (%)</th>
<th>Hemimelic fetuses* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>28</td>
<td>146</td>
<td>117</td>
<td>20</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>420 µg uracil</td>
<td>12</td>
<td>64</td>
<td>48</td>
<td>25</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>370 µg 5-FU</td>
<td>20</td>
<td>110</td>
<td>101</td>
<td>14</td>
<td>45 45</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>500 µg 5-FU</td>
<td>36</td>
<td>216</td>
<td>134</td>
<td>38</td>
<td>108 81</td>
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<tr>
<td>lx/+</td>
<td>20</td>
<td>126</td>
<td>107</td>
<td>15</td>
<td>57 53</td>
<td>53</td>
<td>0</td>
</tr>
<tr>
<td>lx/lx</td>
<td>29</td>
<td>187</td>
<td>160</td>
<td>14</td>
<td>160 100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>lx/+ &amp; 5-FU</td>
<td>17</td>
<td>103</td>
<td>81</td>
<td>13</td>
<td>71 88</td>
<td>29</td>
<td>59</td>
</tr>
</tbody>
</table>

* The embryos were classified according to the more severely affected limb.

Limbs appearing normal from heterozygous embryos or embryos in experimental groups (420 µg uracil, 370 µg 5-FU or lx/+ & 5-FU) had normal enzyme patterns. Similarly, zymograms from polydactylous limbs (from lx/+ or lx/lx young and from the 370 µg 5-FU, 500 µg 5-FU or lx/+ & 5-FU groups) were indistinguishable from those of normal limbs from the untreated normal controls. Maturation differences among limbs of one group were reflected in the isozyme patterns for the more mature and less mature limbs, but these do not show in the zymogram composites in the figures.

As described above, hemimelic limbs were derived by three means: (1) +/+ embryos treated with 5-FU, (2) homozygous luxate young, and (3) lx/+ embryos treated with 5-FU. The sequence of isozyme changes in these three groups is contrasted with the normal patterns of enzyme differentiation during days 12–17 of gestation in Figs. 1–6.

Phosphatases

A maximum of eight electrophoretic bands which would hydrolyze alphannaphthyl acetate in an alkaline medium were demonstrated in homogenates of hind limbs from mouse embryos between days 12 and 17 of gestation (Fig. 1). On day 12, five were present and by day 15 the maximum complement was present. As gestation progressed, the four slow-moving enzyme forms became
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increasingly intense in staining reactivity and the bands became so wide that they appeared to coalesce by day 17.

The zymograms of alkaline phosphatases from hemimelic limbs did not differ noticeably from this pattern of normal differentiation on any day of development.

Fig. 1. Alkaline phosphatases. Composite zymograms from normally developing hind limbs of the mouse during the 12th–17th days of gestation.

Normal

Fig. 2. Adenosine monophosphatases from normally developing hind limbs of the mouse.

Adenosine monophosphatases

Of these phosphohydrolases which utilize alpha-naphthyl acid phosphate, the adenosine monophosphatases can be identified by substituting adenosine-5-phosphate for the usual substrate. Using this approach, Milaire (1965) examined sections of hemimelic limbs from the mutant Dominant hemimelia and noted a striking absence of AMPase activity in the affected portions of the foot. Because of this observation, adenosine monophosphatase isozymes were included in this survey. In normal limbs the differentiation of the four enzyme bands which reacted with adenosine monophosphate (Fig. 2) corresponded to the differentiation of the four fastest-migrating forms of the alkaline phosphatases depicted in Fig. 1. These enzymes in the hemimelic limbs did not differ from normal in their sequential development of general activity.

Non-specific esterases

During the stages of limb development examined in the present study there were eleven multiple molecular forms of esterases (Fig. 3). In serum from 16- to
18-day fetuses, six fetal esterases as well as most adult esterases were observed (Pantelouris & Arnason, 1966). When the esterases of mouse serum and fetal limb homogenates were compared, esterases 1 and 5 of mouse limbs appeared to correspond to fetal serum esterases associated with fetal proteins. These zones of enzymic activity disappeared from serum during the third and fourth weeks after birth. In the limbs, band 1 had disappeared by the 16th day of gestation and the fifth band began to diminish in intensity on the 16th and 17th days of gestation. These coincidences supported the likelihood that bands 1 and 5 correspond to the zones of fetal serum proteins.

There were alterations in esterase ontogenesis associated with hemimelia. The third enzyme band was precociously intense in \( Ix/lx \) and \( lx/+ \) 5-FU embryos on day 14, lost this intensity on the next day, but regained normal intensity on day 16 in contrast to normals. The fifth esterase band in 5-FU hemimelicls retained its intense activity through day 16. However, in the \( lx/+ \) & 5-FU animals this band was delayed a day in developing its intensity. Among the slower-migrating esterase forms, the eighth band showed considerable variability among all hemimelic groups and bands 9 and 10 appeared more intense than normal in the 5-FU treated hemimelics on day 14, and in \( lx/+ \) & 5-FU combination hemimelics on day 16.

There also were changes in multiple esterase forms in abnormally developing limbs of rat fetuses (Johnson, 1968; Johnson & Lambert, 1968). Tibial hemimelia could be elicited in rats by two different teratogens, an acute folic acid deficiency on days 10–12 of gestation or \( N\)-nitroso-\( N\)-methylurea (NNMU) on day 13 of pregnancy. The enzymic forms which were most noticeably absent or delayed in appearance in malformed limbs in the rat may correspond to bands 6, 7 and 8 of mouse limbs. It is interesting that bands 6 and 7 were unaltered by the mutant or by 5-fluorouracil treatment in the mouse, whereas in the rat with the same structural malformation there were pronounced changes at this region.

Glucose-6-phosphate dehydrogenases

Five isozymes of glucose-6-phosphate dehydrogenase were evident (Fig. 4). The most obvious difference in these isozymes between hemimelic and normal embryos was in the differentiation of the first band. In \( lx/+ \) & 5-FU fetuses this band was only slightly reactive until the 15th day, and by the next day it became disproportionately intense. In the \( lx/lx \) limbs the first band appeared to develop more or less as in the normal controls during all the days examined, except that on day 15 it was missing altogether. In 5-FU hemimelics this fast zone began to disappear precociously on day 15 and was very faint on day 16. The fourth isozyme, which normally appeared on the 13th day, did not appear in \( lx/+ \) & 5-FU fetuses. The variability in intensity of the second band in the hemimelic fetuses is not considered significant, because of the inconsistency in detecting this isozyme.

In normal mouse limbs the differentiation of glucose-6-phosphate dehydro-
Fig. 3. Non-specific esterases from developing hind limbs of mice. Normal: differentiation in normal limbs of control and experimental groups and in polydactyous limbs of experimental groups. 5-FU hemimelics: the maternal mice received 500 μg 5-fluorouracil on day 10 of gestation. Ix/lx hemimelics: the fetal mice were homozygous for the gene luxate. lx/+ & 5-FU hemimelics: the maternal mice were pregnant with lx/+ offspring and received 370 μg 5-fluorouracil on day 10 of gestation. VAR: a zone of enzymic activity which varied from sample to sample as regards its presence and/or staining intensity. X: a zone of enzymic activity present from normal limbs but absent from hemimelic limbs. Blanks: no experimental tissue was available. Origin-staining: at base of zymograms, anode at top of zymograms.
genase isozymes was remarkably similar to that in the normal rat yolk sac (Johnson & Spinuzzi, 1968). A striking similarity also existed between these isozyme patterns in the rat and mouse from abnormally developing tissue. In

<table>
<thead>
<tr>
<th>Days of gestation</th>
<th>Normal</th>
<th>5-FU hemimelics</th>
<th>1/1 hemimelics</th>
<th>1/3 &amp; 5-FU hemimelics</th>
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<tr>
<td>12</td>
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Fig. 4. Glucose-6-phosphate dehydrogenases from four classes of fetal mouse limbs.
both cases the first and second isozymes appear inconsistently and with variable intensities.

In addition there were three or four faster-migrating and very light-staining

Normal

5-FU hemimelics

Ix/Ix hemimelics

Ix/+ & 5-FU hemimelics

Days of gestation

Fig. 5. Malate dehydrogenases from four classes of fetal mouse limbs.
zones which occurred in both the glucose-6-phosphate and malate dehydro-
genases. These were not described, because of their inconsistent and faint-
staining characteristics.

Fig. 6. Lactate dehydrogenases from four classes of fetal mouse limbs.
Malate dehydrogenases

The eight malate dehydrogenase isozymes (Fig. 5) from mouse limbs which are described here were slowly migrating forms. In the hemimelic limbs the seventh isozyme did not appear on days 13 and 14 in the 5-fluorouracil-treated and \( lx/lx \) fetuses and had very weak expression in the \( lx/+ \) & 5-FU offspring. However, in the \( lx/lx \) fetuses it was present on day 16 although absent in the normal tissue at this time. The first isozyme band did not appear in \( lx/lx \) hemimelics on days 12–15 and in the 5-FU hemimelics on day 14. The sixth isozyme was variable in 5-fluorouracil-induced hemimelics on days 14–16 and \( lx/+ \) & 5-FU hemimelics on days 14 and 15.

The isozymes altered in the hemimelic mouse limbs corresponded to the isozymes that showed susceptibility to alteration in the rat yolk sac (Johnson & Spinuzzi, 1966). In rats subjected to PGA deficiency the isozyme corresponding in mobility to the mouse isozyme 6 disappeared early and the isozyme 7 appeared earlier than normal. Even though these two isozymes in hemimelic mouse limbs did not show the same alterations, it is apparent that these two enzyme forms were generally less stable than other MDH isozymes when the fetuses were subjected to these teratogenic procedures.

Lactate dehydrogenases

All five isozymes of lactate dehydrogenase were present at the stages of limb development examined (Fig. 6). In the mouse limb the fastest isozyme was the narrowest and each of the succeeding slower isozyme bands was wider than the last. There was enzyme staining-reaction at the origin of the spacer gel. The sixth band is probably a sieving artifact at the spacer/hard-gel interface.

Changes in intensity were the major changes in isozyme patterns from hemimelic limbs. In the 5-fluorouracil-induced hemimelics, the first isozyme zone became markedly less intense on the 14th through 16th days of gestation. In the \( lx/lx \) hemimelics the first isozyme was darker than normal on the 16th day, the third isozyme varied in intensity on the 14th day and was lighter than normal on the 15th day, and the fifth isozyme was lighter than normal on the 14th day. Among the \( lx/+ \) & 5-FU hemimelics the two fastest isozymes did not decrease in staining intensity until the 15th day, and at this stage the fifth isozyme was lighter than normal. On the 17th day the second isozyme stained more intensely in both the \( lx/lx \) and \( lx/+ \) & 5-FU hemimelics.

In hemimelic limbs and tails of folic-acid-deficient rat fetuses there was no alteration in LDH patterns (E. M. Johnson, unpublished data). However, in abnormally developing limbs of rats subjected to the teratogen NNMU the first isozyme never appeared and the intensity of the second isozyme was greatly reduced.

To determine if all the isozymes were present in homogenates of the hemimelic mouse limbs the gels were often allowed to overstain. In all cases all five isozymes
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were evident. Yet the first isozyme was consistently less intense in the 5-FU hemimelics and the third isozyme was variable in the Ix/Ix. These differences suggest that the B monomer was affected. This would coincide with the effect of NNMU on the B monomer in vivo and in vitro, as shown by Johnson & Lambert (1968) in hemimelic limbs of rat fetuses.

**DISCUSSION**

The multiple electrophoretic forms of six enzymes changed sequentially in normally developing hind limbs of mice. In severely abnormal limbs, displaying tibial hemimelia, the ontogeny of multiple enzyme patterns was often altered. However, for limbs displaying a minor defect, polydactyly of the hallux, there were no changes in zymogram pattern. Perhaps this is because the affected hallux represents only a small proportion of the distal limb, or perhaps polydactyly is simply excess normal tissue, and whatever abnormal biochemical changes were involved occurred prior to the time of examination.

The hemimelic hind limbs of the homozygous mutant luxate mice were structurally similar to hemimelic limbs of embryos treated with the teratogen 5-fluorouracil. A third group of similar hemimelic limbs were derived from Ix/+ embryos exposed to 5-fluorouracil. In spite of the similar structural malformation, the altered enzyme patterns were not similar. There was no case in which a single enzyme was altered in the same manner for the same period of development for all three groups—5-FU, Ix/Ix, and Ix/+ & 5-FU hemimelics. There was one instance, however, where the same alteration occurred in all three groups (esterase bands 9 and 10 were unusually intense on day 15), but the duration of this change was different for each class of hemimelics. Sometimes one enzyme moiety would be altered in a different way for each type of hemimelia (i.e. the first band of glucose-6-phosphate dehydrogenase underwent three different sequences of intensity changes). In other cases an alteration might be the same for two types of hemimelia but unlike the third group. For instance, 5-FU and Ix/Ix limbs had similar alterations of glucose-6-phosphate dehydrogenase band 4 on days 13 and 14, of MDH band 7 on days 13 and 14 and of band 1 on day 14, all in contrast to the state of the enzyme forms in Ix/+ & 5-FU fetuses. The Ix/Ix and Ix/+ & 5-FU fetuses showed similar alterations in contrast to 5-FU hemimelics (i.e. esterase band 3 on days 14–16). And for the last possible combination the 5-FU and Ix/+ & 5-FU hemimelics showed a similarly variable sixth band in MDH on days 14 and 15.

The diversity of these patterns is perplexing. It would be tempting to speculate that the three types of hemimelia develop by different biochemical routes, but the data do not establish this. It has been shown that altered isozyme patterns may precede the appearance of structural malformations (Johnson, 1965) but a causal relationship has not been established. In this study the enzyme alterations were observed after the structural malformations appeared. The skeletal defects
were indistinguishable from each other and any enzyme alterations which occurred secondarily might be expected to be similar, not different as was found in these classes of hemimelia. The possibility remains that certain multiple molecular forms of enzymes might be cell-specific and that these might vary with each type of hemimelia.

Dagg (1967) has suggested that the presence of the heterozygous luxate gene or a low dose of fluorouracil might disturb the system leading to differentiation of the tibial-fibular region, but not enough to reach the threshold necessary to produce hemimelia. When the amount of luxate effect or fluorouracil was increased or they acted together, then the threshold would be crossed and hemimelia would appear. If this were true and if the isozyme changes were causally related to these skeletal defects, then these patterns might indicate that the subthreshold phenomena are different in each case.

Landauer (1959, 1965) has noted that the effect of a teratogen may mimic the expression of a mutant gene though the final effect may develop by entirely different mechanisms. When, however, there is an interaction between a heterozygous gene or polygenes of low penetrance and the teratogen to yield an additive effect, or when modifying genes within a strain have similar action on the mutant and the teratogen, then the teratogenic product is considered to be a phenocopy, since the metabolic pathways of the mutant and phenocopy are ‘sufficiently related to permit interaction during development’.

The luxate-fluorouracil system fits Landauer's criteria for a phenocopy because fluorouracil increases the penetrance of $l x / +$, the actions of fluorouracil and $l x / +$ interact to produce the homozygous phenotype (Dagg, 1965a) and modifier genes within the BALB/c strain suppress both the penetrance of luxate and the effect of fluorouracil compared to the C57BL/10 strain (Dagg, 1963, 1965b).

Landauer's suggestions imply that the metabolic pathways involved might be similar for the luxate and fluourouracil-induced hemimelias, and that the combination of gene and teratogen should demonstrate interactions between them.

These data do not completely support this tenet. It is true that specific isozymes among the repertoire were altered in these classes of hemimelia, but the types of alterations were not similar for the mutant and ‘phenocopy’, and the enzyme alterations for the $l x / +$ & 5-FU enzymes did not indicate a general pattern of interaction between the other groups. This may indicate differences at the biochemical level of phenocopy development, or that the changes in isozyme pattern observed are coincidental to cell types which may not be related directly to the skeletal defect.

**SUMMARY**

1. Differentiation of isozyme patterns was examined in hind limbs of mice with tibial hemimelia. This defect occurred in 100% of homozygous luxate mice C57BL/10 Dg-$l x / l x$, in 38% of the offspring of normal C57BL/10J mice injected
intraperitoneally with 500 μg 5-fluorouracil (5-FU) on the 10th day of gestation, and
59% of the offspring of mice carrying lx/+ young which were injected with
370 μg 5-FU on the 10th day of gestation.

2. Preaxial polydactyly occurred in hind limbs of 53% of lx/+ young and this defect was also produced in normal mice by 370 μg 5-FU, a dose which did not elicit hemimelia.

3. Hind limbs from fetuses were removed on days 12 through 17 of gestation, classified as normal, polydactylous or hemimelic, homogenized and electrophoresed in acrylamide gels. The following multiple molecular forms of enzymes were examined: alkaline phosphatases, adenosine monophosphatases, non-specific esterases, glucose-6-phosphate dehydrogenases, malate dehydrogenases and lactate dehydrogenases.

4. Polydactylous limbs and normal limbs from lx/+ embryos or 5-FU treated mice showed no changes in isozyme patterns from those of normal control limbs.

5. Hemimelic limbs showed no changes from normal in the differentiation of alkaline phosphatases and adenosine monophosphatases.

6. The three classes of hemimelic limbs showed alterations in the ontogeny of zymograms for non-specific esterases and the dehydrogenases of glucose-6-phosphate, malate and lactate. The alterations were specific for each class of hemimelia and for each enzyme family studied.

7. A comparison of the altered enzyme patterns from hemimelic mouse and hemimelic rat limbs showed that lactate and glucose-6-phosphate dehydrogenases were altered in a similar manner. Differences in isozyme patterns for alkaline phosphatases, esterases and malate dehydrogenases were not comparable for hemimelic rat and mouse limbs.

RÉSUMÉ

L'ontogenèse des enzymes chez les membres normaux et hémiméliques de Souris

1. La différentiation des zymogrammes a été étudiée dans les membres postérieurs des souris affectées par une hémimélée tibiale. Ce défaut apparaît chez 100% des souris 'luxates' homozygotes C57BL/10Dg-lx/lx, et chez 38% de la progéniture des souris normales C57BL/10J injectées avec 500 μg de 5-fluorouracile (5-FU) pendant la dixième journée de gestation. Ce défaut apparaît aussi chez 59% de la progéniture des souris portant des embryos de génotype lx/+ injectées avec 370 μg de 5-FU au dixième jour de gestation.

2. La polydactylie préaxiale du membre postérieur apparaît chez 53% des jeunes lx/+ . Cette anomalie a aussi été produite par 370 μg de 5-FU chez les souris normales +/+ , une dose qui ne produit pas l'hémimélée.

3. Les membres postérieurs des fœtus ont été prélevés du douzième jour au dix-septième jour de la gestation. Les membres ont été classifiés comme normaux
polydactyles ou hémiméliques, ils ont été ensuite homogénisés et l'électrophorèse 
de l'homogénot a été faite sur du gel acrylamide. Les multiples formes molé-
culaires des enzymes suivant ont été étudiés : phosphatases alcalines, adénosines monophosphatases, estéras non spécifiques, déshydrogénases glucose-6-
phosphate, déshydrogénases maliques et déshydrogénases lactiques.

4. Les membres polydactyles et normaux des embryons  $lx^+/+$ ou des souris 
traitées avec le 5-FU ne montrent aucun changement dans des zymogrammes 
relativement aux contrôles effectuées avec les membres normaux.

5. Pour les membres hémiméliques, il n'y a pas de changement dans la diffé-
rentiation des phosphatases alcalines et des adénosines monophosphatases.

6. Les trois classes de membres hémiméliques montrent des changements dans 
l'ontogénie des zymogrammes des estéras non spécifiques et des déhydro-
génases: glucose-6-phosphate, malique et lactique. Les changements étaient 
spécifiques pour chaque classe de membres hémiméliques et pour chaque famille 
d'enzyme étudiée.

7. Une comparaison des zymogrammes modifiés chez les souris hémiméliques 
et les rats hémiméliques a démontré que la déshydrogénase lactique et la déshydro-
génase glucose-6-phosphate étaient modifiées de façon similaire. Les différences 
dans les zymogrammes pour la phosphatase alcaline, les estéras et la 
déshydrogénase malique n'étaient pas comparables pour les rats et les souris 
hémiméliques.

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