The phenotypic interdependence of the musculoskeletal characters of the mandibular arch in mice*

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SUMMARY

Evidence from studies of craniofacial anomalies and the evolutionary transition from reptiles to mammals suggests that the temporomandibular joint (TMJ), bony zygomatic arch, middle ear ossicles and mandibular muscle pattern may form a correlated suite of characters. To test the degree of phenotypic interdependence among these features, mandibular arch defects were analysed in prenatal mice. Retinoic palmitate was administered to pregnant mice on day 8-7 to produce test foetuses with malformations of the mandibular arch. A rating scale was developed for each of the four characters so that numerical values could be assigned to each phenotype encountered. Control animals were used to establish normal phenotypes for each character which were assigned a value of 1. Data from each test age, 16, 18 and 19 days postconception, were pooled and Spearmann rank correlation coefficients between each of the traits were calculated.

Coefficients (R) range from a high of 0.87, between the TMJ and zygomatic arch, to a low of 0.67 between the zygomatic arch and the mandibular musculature showing highly significant correlations (P<0.0001) among all characters. Therefore, the data suggest that the musculoskeletal features of the mandibular arch are phenotypically interdependent during development.

INTRODUCTION

Changes in the skeleton and muscle of the mandibular arch are of interest to evolutionary biologists and clinicians alike. Clinicians who deal with craniofacial anomalies are often struck by common, even stereotyped, features that occur in many syndromes. For syndromes involving the mandibular arch, musculoskeletal anomalies often include middle ear defects, micrognathia, absent zygomas, anomalous musculature and aplastic mandibular condyles (Herring, Rowlatt & Pruzansky, 1979; Pauli, Graham & Barr, 1981; Goodman & Gorlin, 1983).

These same first arch musculoskeletal elements (i.e. temporomandibular joint, zygomatic arch, middle ear ossicles and mandibular musculature) create a problem for the evolutionary biologist who must explain the relatively rapid acquisition

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of a secondary jaw joint (between the dentary and the squamosal portion of the
temporal bone), the mammalian zygomatic arch, diversified adductor musculature
and middle ear ossicles for sound conduction, during the transition from reptiles to
mammals. Much has been written about the musculoskeletal homologues of these
mammalian features (Parrington & Westoll, 1940; Watson, 1953; Shute, 1956;
Crompton, 1963, 1972; Barghusen, 1968, 1972, 1973; Allin, 1975; Presley & Steele,
1978), but the demonstration of homologies does not explain how or why so
many phenotypic characters changed so suddenly. Even the popular theory of
punctuated equilibrium (Eldredge & Gould, 1972; Gould & Eldredge, 1977)
avoids mentioning what mechanism(s) might bring about such a spurt in the
evolutionary process.

If the musculoskeletal elements of the mandibular arch form a suite of characters
that change their phenotype in some interdependent manner, the explanation
of both the reptile–mammal transition and craniofacial anomalies would be
facilitated. It is hypothesized here that the muscular and skeletal elements of the
mandibular arch are indeed phenotypically linked. While this developmental
relationship is often assumed, it has never been demonstrated. The goal of this
study is to assess the association among phenotypes of the temporomandibular
joint, zygomatic arch, middle ear ossicles and mandibular musculature of mice.
Variation in these features was induced by foetal hypervitaminosis A, which
has been demonstrated to cause craniofacial abnormalities in mice (Kalter &
Warkany, 1961; Sherman, 1971; Keith, 1981). Indeed, the four characters under
investigation did exhibit a variety of dysmorphologies which have been reported
elsewhere (Kay, 1986). It is not claimed that hypervitaminosis A, or any teratogen,
simulates evolutionary change. Teratogen models merely allow measurement of
the developmental change which is feasible when morphogenesis is disturbed.
Although only one teratogen model was used to test the hypothesis other studies
support the assumption that the morphological variation is not unique to hyper-
vitaminosis A (Sessions & Stalling, 1972; Black, Myers & Rorke, 1973; Herring
et al. 1979; Burck, 1983; Juriloff & Harris, 1983). Therefore, although a teratogen
does not simulate evolutionary change, this model is appropriate to test a general
hypothesis of character linkage. Statistically significant correlations among the
characters would support the hypothesis of their linkage during the process of
ontogeny.

MATERIALS AND METHODS

(A) Animals

Sexually mature 129/Re mice (Mus musculus) from a pre-existing colony at the University of
Illinois at Chicago were housed under conditions of controlled temperature and humidity with a
12 h light/dark cycle. They were fed a diet of rat pellets and water ad libitum.

Pregnancy was assumed to have occurred if a vaginal plug was present at the end of a mating
period. The time of conception was arbitrarily recorded as 3 p.m. for afternoon matings and
12 o’clock midnight for overnight matings and was designated day 0 of gestation.

One injection of retinol palmitate was used to induce the effects of hypervitaminosis A on the
offspring (Kalter & Warkany, 1961; Sherman, 1971). A 0.2 ml solution of corn oil containing
Phenotypic linkage in mice

Table 1. Temporomandibular joint scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Morphological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal dentary–squamosal joint</td>
</tr>
<tr>
<td>2</td>
<td>Alteration of one trait</td>
</tr>
<tr>
<td>3</td>
<td>Alteration of two traits</td>
</tr>
<tr>
<td>4</td>
<td>Alteration of three traits</td>
</tr>
<tr>
<td>5</td>
<td>Absence of a recognizable TMJ</td>
</tr>
</tbody>
</table>

Traits

- Condylar cartilage shape (length/width ratio)
- Presence/absence of the condylar cartilage
- Presence/absence of the joint cavities
- Location at the root of the zygomatic arch
- Glenoid shape

10000 i.u. of retinol palmitate (Sigma Chemical Co.) was administered by gavage on day 8-7. Nineteen pregnant mice were controls and not given any injections as previous studies (Cohlan, 1953; Newall & Edwards, 1981; Tassin & Weill, 1981) have demonstrated that control mice receiving gavage injection of either water or oil do not differ from controls in craniofacial morphology.

Test and control foetuses were collected at three ages: 16, 18 and 19 days postconception. Foetuses were decapitated and the heads fixed by immersion in 10% formalin. Specimens were randomly selected from each litter for histological processing and scoring. In many cases all littermates were utilized because the total number of offspring was small. When litter size was large, only three offspring were selected, so that larger litters would not be overrepresented.

(B) Histological processing

All fixed heads selected for scoring were hemisected along the sagittal suture. Right halves were skinned and rinsed in distilled water before double staining with alcian blue for cartilage and alizarin red S for bone (Kay, 1985). Left hemi-heads were serially sectioned in the coronal or frontal plane. Odd-numbered slides from each specimen were stained using Harris' haematoxylin and eosin as given in Humason (1979). Even-numbered slides were stained with haematoxylin and then counterstained with picric indigocarmine (0.25% indigocarmine in water saturated with picric acid).

(C) Morphological scales

Morphological scales were developed so that all possible phenotypes of each anatomical feature (i.e. TMJ, ossicles, zygomatic arch and mandibular musculature) could be assigned scores or ranks for later correlation. A list of potential character states was made for each of the four features and a progression of severity was chosen (see below). Because no differences between sides were observed in this or other hypervitaminosis A studies (Keith, 1981), both head halves were used to establish one set of scores per specimen.

Scoring was performed using five-point scales, where a score of 1 represented the normal phenotype observed in control specimens and a score of 5 marked the most severe phenotype possible and represented severe anomalies such as the complete absence of a feature. Scores of 2 to 4 represented a continuum of severity in phenotypes. No attempt was made to standardize the scales of different features with each other except at the two extreme ends of the scale (i.e. 1 and 5). No standard increment of phenotypic change was present between each score on each scale. Each scale merely represented five observable phenotypes placed in order of increasing severity.

The TMJ scale (Table 1) measures the severity of character involvement by combining any of five unweighted trait alterations. During morphological assessment of the TMJ (Table 1) the shape of the articular cartilage was measured as a ratio of cartilage length to cartilage width. Any
change from the control ratio value and any change in both dimensions (which may not affect the ratio) were scored as a trait alteration. Absence of the condylar cartilage was an alternative criterion because absent cartilage was not deemed to have undergone a shape change, only a loss. Evaluation of other traits for scoring was straightforward, resulting in easily repeatable scores.

The zygomatic arch (Table 2), middle ear ossicles (Table 3), and mandibular musculature (Table 4) had fewer morphologic parameters to undergo change so their scales used weighted traits. Specific traits were judged to show increasing severity as denoted by their increasing scores. Ectopic cartilages are very common in vitamin A specimens so their inclusion in the zygomatic arch (Table 2) was judged to be the smallest morphological change possible. Ectopic cartilage was considered present if it occurred anywhere along the arch except at the margin of either root (maxillary or temporal) where its presence on the arch itself may have been questionable. Shape changes in the arch were considered more severe because they occur when ectopic cartilages enlarge the full length of the arch or when one of the dermal bone components was actually altered. Shape change included all grossly observable alterations in arch width, or in the orientation and shape of any bone component of the arch. Absence of any normal bone rated as the next most serious defect. Absence of bony portions was determined by the inspection of histological sections where early cellular condensation or ossification sites could be detected, thus verifying bone presence.

The smallest morphological change for the middle ear ossicles (Table 3) was judged to be any change in shape or orientation. The middle ear ossicles were scored for abnormal shape and/or orientation if any or all ossicles were affected. A change of this sort could be quite subtle and involve only a small area or structure. Abnormal fusion to a nonossicle structure(s) involved at least two structures (one ossicle and one nonossicle) and most likely more than one of the ossicles since they are continuous with each other. Abnormal fusion was scored when any or all of the ossicles were fused to any nonossicle structure(s). Absence of any ossicle was judged to be a more severe dysmorphology than any change in one or more ossicles.

The same reasoning was followed in the scale for mandibular musculature (Table 4). The degree of dysmorphology was judged to increase as the number of attachment alterations increased, but any loss of a whole muscle was considered more severe. Muscles were identified by their innervation and physical relationship to nerves. Abnormal attachments or omissions of muscles were then assessed based on skeletal identifications.

When more than one of the anomalous traits were present in a specimen, the highest applicable score was awarded. All morphological criteria for scoring were defined enough to allow the same scorer to rescore the data at a later date and achieve exactly the same results.

Since maturational stages can vary within, as well as between litters, neither immaturity nor precocity of development was considered abnormal. Each specimen was compared to a control of comparable maturity when scored. Therefore, scores were considered comparable for specimens of different ages.

(D) **Statistics**

Control specimens were excluded from statistical analysis because they were used to define the 'normal' phenotype, i.e. every score was 1 by definition. Therefore, the perfect correlations which would have existed between all control specimen features are a byproduct of the fact that they are controls, not of some biological process.

<table>
<thead>
<tr>
<th>Score</th>
<th>Morphological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal arch morphology</td>
</tr>
<tr>
<td>2</td>
<td>Presence of ectopic cartilage(s)</td>
</tr>
<tr>
<td>3</td>
<td>Any shape change in the arch</td>
</tr>
<tr>
<td>4</td>
<td>Absence of any of the arch’s three bony portions</td>
</tr>
<tr>
<td>5</td>
<td>Absence of the entire arch</td>
</tr>
</tbody>
</table>
Table 3. Middle ear ossicles scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Morphological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal ossicles</td>
</tr>
<tr>
<td>2</td>
<td>Abnormal shape or orientation</td>
</tr>
<tr>
<td>3</td>
<td>Abnormal fusion to nonossicle structure(s)</td>
</tr>
<tr>
<td>4</td>
<td>Absence of one or two ossicles</td>
</tr>
<tr>
<td>5</td>
<td>All ossicles absent</td>
</tr>
</tbody>
</table>

All test foetuses were scored on the five-point morphological scales. These data were analysed using the Statistical Analysis System (SAS) package (Barr, Goodnight, Sall & Helwig, 1976) to calculate the Spearman correlation coefficients \( R \) and their level of significance \( P \). The nonparametric Spearman statistic was selected, despite its reduced ability to reject the null hypothesis when it is false, because the data are defined only on an ordinal scale. Since the Spearman correlation coefficient is determined by the ranks of two variables, each feature (TMJ, zygomatic arch, middle ear ossicles and mandibular musculature) was correlated with every other feature.

RESULTS

Data from each specimen were plotted for each of the six possible pair combinations of features (Fig. 1). To aid in visualizing the data a reference line has been drawn on each graph to show where all points of perfect correlation would lie.

Spearman's rank correlation coefficients were calculated for each of the six possible feature couplings, and are given in Table 5. The correlation coefficients range from a high of 0.87, between the TMJ and zygomatic arch, to a low of 0.67 between the zygomatic arch and mandibular musculature. Despite this range of values, correlations for all six combinations of characters are highly significant \( (P < 0.0001) \) statistically.

Although all features are significantly correlated, the graphed data (Fig. 1) require further explanation. The first three graphs (Fig. 1A–C) plot skeletal features against each other and show that the data are distributed along the full length and on either side of the reference line. The relationship between the TMJ and the zygomatic arch (Fig. 1A) shows the least scatter while plots of TMJ versus ossicles or zygomatic arch versus ossicles (Fig. 1B,C) show more points to the right of the reference line. This latter pattern suggests a greater involvement of the ossicles than the other two skeletal features.

Table 4. Mandibular musculature* scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Morphological criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal musculature</td>
</tr>
<tr>
<td>2</td>
<td>One or two attachment variations</td>
</tr>
<tr>
<td>3</td>
<td>Three or more attachment variations</td>
</tr>
<tr>
<td>4</td>
<td>Absence of one or more, but not all, muscles</td>
</tr>
<tr>
<td>5</td>
<td>All muscles absent or unidentifiable</td>
</tr>
</tbody>
</table>

*The muscles used in scoring are the anterior digastric, mylohyoid, masseter, temporalis, medial pterygoid and lateral pterygoid.
Skeletal features are plotted versus muscle scores in Fig. 1D–F. In the cases of either TMJ (Fig. 1D) or ossicles (Fig. 1E) plotted versus musculature, all data points lie to the left of the reference line. In other words, both the TMJ and the

Fig. 1. Plots of six possible data pairs. a equals one observation, b equals two observations, etc. A reference line shows where all points of perfect correlation would lie.
ossicles are consistently disturbed as much as, or more than, the musculature of the same specimen. Only in the case of the zygomatic arch versus musculature (Fig. 1F) are there any examples of specimens that exhibit greater muscle than skeletal involvement and these are a small minority.

A general pattern of greater alterations in the phenotypes of skeletal features (TMJ, zygomatic arch and ossicles) than the musculature is apparent. Among the skeletal features, the ossicles appear to be more severely involved than either the TMJ or zygomatic arch. The median values were calculated for each feature, from their morphological scores, in order to quantify the pattern of involvement noted in the graphed data. The middle ear ossicles, the most affected character, have the highest median value, 4·0, while the least affected feature, muscle, has the lowest median score, 2·0. Both the TMJ and zygomatic arch show midrange effects and corresponding median values of 3·0. The significance of the differences among the medians was assessed using the nonparametric Mann-Whitney-U test. The results from the six possible pair comparisons are given in Table 6. Because this statistic is very conservative, any z score (or P value) less than or equal to 0·1 was considered to indicate that the two features used in the calculation had significantly different median values. The results of this test verify that the median values of all skeletal structures are indeed significantly greater than the muscle values, that the ossicles’ median is significantly different from the zygomatic arch median and that the median of the TMJ is not significantly different from that of the zygomatic arch.

Table 5. Spearman correlation coefficients (R)

<table>
<thead>
<tr>
<th>Character</th>
<th>Zygomatic</th>
<th>Ossicle</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMJ 0·868 18* 0·798 73 0·837 12</td>
<td>0·000 1 0·000 1 0·000 1</td>
<td>37 36 30</td>
<td></td>
</tr>
<tr>
<td>Zygomatic arch 0·798 60 0·672 11</td>
<td>0·000 1 0·000 1</td>
<td>37 31</td>
<td></td>
</tr>
<tr>
<td>Ossicles 0·746 15</td>
<td>0·000 1</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

* The first value is R, the correlation coefficient. The second number gives the significance of the correlation coefficient above it. The third number is n, the number of specimens.

Table 6. Mann-Whitney-U test results

<table>
<thead>
<tr>
<th>Character</th>
<th>Ossicle</th>
<th>TMJ</th>
<th>Zygomatic</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ossicle 0·36</td>
<td>—</td>
<td>0·06*</td>
<td>—</td>
<td>0·01*</td>
</tr>
<tr>
<td>TMJ —</td>
<td>—</td>
<td>0·16</td>
<td>—</td>
<td>0·03*</td>
</tr>
<tr>
<td>Zygomatic arch —</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0·10*</td>
</tr>
</tbody>
</table>

* Median values are significantly different.
† Zygomatic arch.
However, the median values for the middle ear ossicles and temporomandibular joints are not significantly different statistically. Therefore, the dysmorphogenetic pattern is one where all skeletal features are more involved than the muscles. Among the skeletal features, the ossicles are more involved than the zygomatic arch, but the TMJs are statistically indistinguishable from either the ossicles or zygomatic arches.

**DISCUSSION**

The fundamental hypothesis of this study is that the phenotypes of mandibular arch musculoskeletal characters are developmentally interdependent. If true, the hypothesis provides the basis for an explanation for the stereotyped changes observed in the craniofacial region. The existence of specific character linkages between numerically assigned phenotypes of the temporomandibular joint (TMJ), zygomatic arch, middle ear ossicles and mandibular musculature was proposed and tested.

Spearman rank correlation coefficients revealed the existence of highly significant correlations between the phenotypes of the temporomandibular joint (TMJ), zygomatic arch, middle ear ossicles and mandibular musculature; thus there is statistical support for a hypothesis of character linkage. Before concluding the existence of phenotypic interdependence some aspects of the study must be reviewed. First, it must be shown that the data are consistent with the results of existing hypervitaminosis A studies in order to demonstrate that the defects are not unique to the strain of mice used in this study. Second, data from each individual must be inspected for consistency with the hypothesis. Although the pooled data are supportive of the hypothesis individual data points could still offer evidence of hypothesis refutation. Consistency with the other hypervitaminosis A literature will be addressed first.

A pattern of phenotypic involvement was found to occur following hypervitaminosis A. This pattern of skeletal defects being greater than muscular defects could be the result of unequivalent morphological ranking scales. However, the pattern is consistent with observations made by other authors. The greater involvement of chondrogenic tissue than myogenic tissue in response to vitamin A was also noted by Kwasirogroch, Skalso & Church (1984), in fact, chondrogenesis has been proposed to be the primary target of developmental interruption in hypervitaminosis A (Kwasigroch & Kochhar, 1980; Kwasigroch & Neubert, 1980; DeSimone & Reddi, 1983).

The gross craniofacial defects noted in this study are also consistent with the anomalies reported by other investigators (Kalter, 1960; Kalter & Warkany, 1961; Kochhar, 1968; Keith, 1981; Tassin & Weill, 1981). Weidenbecher (1981) even shows a trend towards mandibular arch character interrelationships in rats receiving vitamin A. Malformations of the mandible, ossicles and middle ear muscles were reported in 16, 14 and 17% of the offspring, respectively. This is a low rate of occurrence compared to the present study. The difference is directly attributable
to the experimental protocol which included a wide range of dose times and levels many of which were incompatible with life or the creation of any craniofacial anomalies. No information about co-occurrence of recorded defects in the same individuals is given. However, the similarity in the percentage rates of the three dysmorphologies is consistent with the hypothesis of phenotypic interdependence of mandibular arch features.

The discoveries of a dysmorphogenetic pattern that is consistent with the known actions of vitamin A, craniofacial defects similar to those reported by previous studies and the finding of a similar rate of dysmorphogenesis across features by another author all validate that the present morphological data are representative of other hypervitaminosis A studies, justifying a closer evaluation.

The hypothesis states that all four musculoskeletal traits (TMJ, zygomatic arch, middle ear ossicles and mandibular musculature) selected from the mandibular arch are linked phenotypically during development. Therefore, if one trait changes they must all do so. This implies first, that a normal phenotype (score of 1) should not exist in a specimen where other phenotypes are abnormal (scores of 2–5), and second, that there should not be a great disparity in the rankings of different traits within the same specimen.

With regard to the first implication, a review of the purely skeletal data (Fig. 1A–C) reveals that there are 10 instances (out of 143 scores) where an abnormal morphological score (scores of 2–5) appears with a normal score (score of 1). The occurrences are uniformly distributed over the three graphs and represent data from five of the thirty-eight specimens. In each case the highest abnormal score involved is a 2, which represents only a minimal change in character morphology. Abnormal scores, in otherwise normal specimens, were received by the ossicles three times, the zygomatic arch two times, and the TMJ one time. One specimen had scores of 2 for both the ossicles (slightly misshapen manubrium of the malleus) and zygomatic arch (ectopic cartilage present).

While abnormal morphologies should not appear in an otherwise normal specimen, it is acknowledged that the anomalies encountered are minor although obviously outside the normal range of variation. It is concluded that in these five specimens the foetal level of retinoic palmitate created minimal morphological changes in individual characters, but was too low to trigger any dysmorphogenetic sequence that would dictate character linkage. Thus, if only the skeletal data are considered, the results are reasonably supportive of the hypothesis.

However, a review of the muscle versus skeletal data (Fig. 1D–F) reveals that normal musculature frequently appears in specimens with abnormal skeletal systems. In fact normal musculature can be coupled with even the highest scale score of 5 (Fig. 1E), which represents the most severe morphological change possible. However, the ossicles are the only skeletal character that can actually be lost and still leave the mandibular musculature unaffected. The zygomatic arch and TMJ could only reach a score of 4 without altering the attachments for the masseter and lateral pterygoid muscles respectively. These results refute the first implication of the hypothesis, at least with regard to the musculature. Musculature
appears to be only indirectly affected by retinoid activity. For instance, alterations in muscle location may have occurred in response to changes in the shape and location of the skeleton to which it attaches. In addition, the method of identifying muscles by their innervation, even when skeletal form is abnormal, creates a very conservative system for designating musculature as abnormal. Since the medial and lateral pterygoids share an innervation, they were identified as separate, based on their insertions and/or the presence of the lingual and inferior alveolar nerves between them. Probably because of these two factors, the indirect effects of vitamin A and a conservative scoring system for muscle, the musculature does not conform to predictions of the hypothesis of character linkage.

The second implication is that there should not be a great disparity in the rankings of different traits within the same specimen. There are, of course, a considerable number of score disparities of greater than 1 involving musculature, but these are not included here since it is already clear that the muscle data are in conflict with the hypothesis. An examination of the skeletal data alone (Fig. 1A–C) shows thirteen instances representing seven foetuses with a disparity of greater than 1 scoring level between two abnormal characters in the same specimen. Six of the seven specimens have two disparate data points each and will be discussed first. Two specimens had similar character scores with maximal anomalies of the TMJ (score 5) and moderate changes (score 3) in both the zygomatic arch and ossicles. The jaw joint changes differed in the two specimens; one lacked a true point of articulation between the two jaws (i.e. muscles lay between the mandible and the cranium) while the other showed jaw ankylosis. Defects of the zygomatic arch (broad ossification with ectopic cartilages present) and middle ear ossicles (malleus rotated and fused to the greater wing of the sphenoid) were shared by the two specimens. The third specimen had a maximal deformity of the middle ear ossicles (score 5) with moderate changes (score 3) in both the TMJ and zygomatic arch while the fourth specimen had similar scores for the ossicles (5) and zygomatic arch (3), but not for the TMJ (score of 2). The absence of all ossicles means that the caudal (posterior) end of Meckel's cartilage (malleus), the quadrate cartilage (incus), and the hyoid arch precursor to the stapes all failed to form. Involvement of the stapes, which is not a mandibular arch derivative, probably occurs because of the spatial and temporal proximity of its precartilagenous precursor to that of the other ossicles (Anson, Hanson & Richany, 1960; Hanson, Anson & Bast, 1959; Richany, Bast & Anson, 1956). The jaw joint is displaced with a greatly reduced condylar cartilage and the zygomatic arch is enlarged by ectopic cartilage. The fifth and sixth specimens had identical morphological scores with maximal changes (score 5) in both the TMJ and ossicles, but only moderate changes in the zygomatic arch (score 3). Both specimens were agnathic so Meckel's cartilages and mandibles are completely absent. The zygomatic arches were represented by large ectopic cartilages. One specimen had a single pair of features that scored more than one level apart. That specimen lacked a TMJ (score 5) secondary to changes in the zygomatic arch (score 4), but had only moderate defects in the ossicles (score 3).
A review of all seven specimens shows a common element – all of the disparities can be related to alterations in chondrogenesis. Morphological features like the TMJ (specifically the mandibular condyle) and middle ear ossicles, that rely on endochondral formation and/or some property of Meckel's cartilage, showed more severe dysmorphogenesis than the zygomatic arch, which is a dermal bone formation. However, even the zygomatic anomalies are related to chondrogenesis, because in hypervitaminosis A histologically normal, ectopic cartilages form and then ossify at the arch's normal location. This pattern in the disparities is consistent with the statistical findings shown in Table 6. Since discrepancies in the data were used to calculate the statistically significant correlations which support the hypothesis of character linkage and because the actual discrepancies of concern are consistent with the statistically established pattern of dysmorphogenesis that occurs in hypervitaminosis A, individual data points do not refute a hypothesis of phenotypic linkage.

A recent clinical study also supplies some evidence of phenotypic linkage. Although only the ossicles received more than a gross clinical examination, producing an underestimation of face and jaw defects, Kumakawa & Funasaka (1985) found significant statistical correlations between anomalies of the ossicles, face and jaw. Since it is unlikely that a majority of these subjects were exposed to retinoids, these data represent a second dysmorphogenic model. Therefore, a hypothesis of phenotypic interdependence during development of the temporo-mandibular joint, zygomatic arch, middle ear ossicles and mandibular musculature is generally supported.

Now that it has been demonstrated that phenotypes of the TMJ, ossicles, zygoma and to a lesser degree the trigeminal musculature, are interdependent, it is of interest to speculate about how they are linked developmentally. Their common origin from cranial neural crest tissue is often cited as the cause of multiple anomalies in the craniofacial region (Johnston, 1975; Hassell, Greenberg & Johnston, 1977) and can be proposed as a potential source of linkage. Chick studies have shown that the skeletal elements of the mandibular arch (i.e. branchial arch skeleton including Meckel’s cartilage, mandible and presumably the mammalian zygomatic arch, and middle ear ossicles), as well as the muscle tendons of the trigeminal musculature, arise from cranial neural crest cells (Hall, 1980; Noden, 1980, 1983a,b, 1984). However, any proposal involving neural crest cells can be readily criticized. For in order to selectively affect neural crest cells, a teratogen would have to be administered before the cells merge with the mesoderm to form facial process mesenchyme. Both Keith (1981) and Nichols (1981) have shown that this merger occurs on day 8:3 in the mouse, a time when the administration of hypervitaminosis A is incompatible with survival (Kalter & Warkany, 1961).

Another potential source of phenotypic linkage are the myriad of muscle-skeleton interactions (e.g. muscles affect on skeletal growth) which are known to occur during development. While these actions doubtless have some affect on the final phenotype, they can be excluded as causal because they occur too late
in development. These data demonstrate that a disruption in prechondrogenic differentiation, which precedes myogenesis, created the craniofacial anomalies observed here. It has been shown in the chick limb that myogenesis begins at stage 25 (Hilfer, Searls & Fonte, 1973) when skeletal primordium is already detectable and peripheral mesenchyme loses its chondrogenic potential (Solursh, Reiter, Ahrens & Vertel, 1981). The earlier onset of chondrogenesis than myogenesis, supports the secondary involvement of musculature noted here and refutes any proposal of tissue interactions as causal.

It is proposed here that the developmental linkage between the muscular and skeletal elements of the mandibular arch originate with the onset of chondrogenesis. The mode of action by which hypervitaminosis A creates craniofacial anomalies has been discussed elsewhere (Kay, 1986), but is consistent with a disruption of prechondrogenic differentiation. While it is not yet clear how vitamin A alters chondrogenesis, it is of interest to note that other teratogens like glucocorticoids (McDevitt, Gautieri & Mann, 1981; Mosier et al. 1981) and phenytoin (McDevitt et al. 1981) have also been reported to disrupt chondrogenesis. Future work should focus on if, and how, teratogens share a common pathway for disrupting chondrogenesis resulting in consistent alterations in craniofacial morphology and the apparent linkage of those characters.

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REFERENCES

BARGHUSEN, H. R. (1968). The lower jaw of cynodonts (Reptilia, Therapsida) and the evolutionary origin of mammal-like adductor jaw musculature. Postilla 116, 1–49.
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