The development of bovine syndactylism

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INTRODUCTION

Despite its cloven hooves, the normal cow, like other ruminants, shows a mild degree of syndactylism. In the adult, metacarpalia and metatarsalia III and IV are intimately fused with each other to form the cannon bone, but they arise as separate blastemata which undergo separate chondrification. Inherited syndactylism with a single hoof on the forelimbs and occasionally also on the hindlimbs has been described in cattle on several occasions, and it might be thought that this is simply an exaggeration of the normal process so as to include the phalanges. With as cumbersome an animal as the cow, such a suggestion might appear to be fairly safe from an observational test. This paper will show that this is by no means the case, and that with a rather modest number of embryos the course of events can be traced with some confidence. This was mainly due to two favourable circumstances. In the first instance, work with similar entities in the mouse which has been summarized elsewhere (Grüneberg, 1963) gave hints as to the developmental stages to be examined and features to be looked for. Secondly the work was greatly aided by a technique of preterminal Caesarian sections by means of which the same cow could yield a series of successive embryos (Huston et al., 1964; Noordsy, Huston, Smith & Oberst, 1964); this considerably reduced the time for the collection of the material, and the number of animals which had to be maintained along with the costs of the investigation. In general, the mode of action of genes affecting the appendicular skeleton is less well understood than that of genes affecting the axial skeleton. The bovine material was thus a welcome addition to the conditions available for study.

MATERIAL AND METHODS

The autosomal gene for bovine syndactylism (symbol $sy$) used in the present study has been described in Holstein–Friesian cattle in the United States of America (Eldridge, Smith & McLeod, 1951; Huston, Eldridge & Mudge, 1961;

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Huston, 1968). As classified by eye, the condition is recessive with variable manifestation. The forelimbs are much more often affected than the hind limbs; in the embryonic material examined, syndactylism was confined to the forelimbs. There may be differences between the sides; and in the forelimbs, at any rate, the right tends to be rather more severely affected than the left. Some animals which must be presumed to be sy/sy show no syndactylism at all and are normal overlaps as judged by that criterion. However, according to Adrian (1961), externally normal limbs from syndactylous cattle may have minor internal abnormalities, and the same may be true for the limbs of normal overlaps. Apart from three +/+ embryos in Table 1, all but two embryos have been sired by the normal overlap bull 062B, who in turn produced more normal overlaps; the two embryos sired by another (manifesting) bull (014B) are the syndactylous one of 44 days and the normal overlap of 40 days. The embryos were obtained by Caesarean section as described by Huston et al. (1964) and Noordsy et al. (1964) and, with two exceptions, fixed in Bouin’s solution.

Table 1. Summary of embryonic material sectioned

(All limbs were sectioned in the palmar-plantar plane, except that embryos in italics had their left limbs sectioned transversely.)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype</th>
<th>Age (days)</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>+/+</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>? +/sy</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>? sy/sy</td>
<td>43</td>
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<tr>
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<td>42</td>
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<td></td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>sy/sy</td>
<td></td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Syndactylo(s)  sy/sy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* One of these known to be +/sy from ancestry.
† From one of these, only a fore limb is available.

(Two embryos were fixed in 10 % buffered formalin.) In the timing of embryos, account was taken of the fact that the cow ovulates about 12 h after oestrus and that fertilization occurs even later. The animals are usually bred in late oestrus, and the Kansas cow embryology group follows the practice of counting the day of breeding as −1; the day after breeding as day 0, and the second day after breeding as day 1. Prior to processing for histology, all embryos were photographed in toto, and separate photos were also taken of all the limbs after they had been severed from the body. The limbs were subsequently embedded by Peterfi’s method, sectioned at 12-5 μ and stained with haematoxylin and eosin.
In addition to the embryos listed in Table 1, there were a few specimens which were either too young or too old to give useful information.

RESULTS

In homozygous normal cattle (Text-fig. 1) the blastemata for metacarpalia and metatarsalia III and IV are laid down as separate structures which at first considerably diverge from each other. Soon, in preparation for their eventual fusion, they move closer together and at the same time turn so as to become approximately parallel to each other. While these movements are taking place, the blastemata grow out distally to form the material for the phalanges which remain widely separate from each other until the outgrowth is complete.

Text-fig. 1. Palmar sections through the right forelimbs of three +/+ embryos (a, b and c; aged 37, 39 and 41 days; C.R.L. 16-3, 20-8 and 21-5 respectively). The development of the hind limbs follows a similar course, but is retarded as compared with that of the forelimbs; thus, in a, chondrification has started in the metacarpals, but not yet in the metatarsals. Camera lucida drawings made at magnification ×50; final magnification ×20.

The situation is quite different in limbs destined to become syndactylous. From the very beginning (day 37), the blastemata of the metacarpals are laid down much closer together and virtually parallel to each other (Plate 1, fig. B); the developmental age of the embryo is actually somewhat less than that of the normal (Plate 1, fig. A) in which chondrification of the metacarpals has already started. In the abnormal limb, the blastemata of digits III and IV are beginning to coalesce distally, whereas in the normal limb they remain widely separate from each other.

In the normal 39-day embryo (Plate 1, fig. C) the blastemata of the phalanges...
remain far apart from each other. In the abnormal limb (Plate 1, fig. D) the blastemal material normally destined to form two sets of phalanges, having run together previously, now grows out form to a single massive syndactylous digit which only near the proximal end of the basal phalanx shows a slight suggestion of its composite nature.

Text-fig. 2. Transverse sections through the left limbs of a +/+(a and b) and of an sy/sy embryo (a' and b'), 39 days old (C.R.L. 20-8, and 17-0 mm respectively). a and a' are forelimbs, b and b' hind limbs. In each case, the 10th, 20th, ..., 70th section as counted from the free margin drawn by camera lucida at magnification ×50; final magnification ×20.
At the 41-day stage (Plate 1, figs. E, F) chondrification has spread distally to the level of the basal phalanges in both normal and syndactylos limb. In this case the coalescence of the blastemata for the basal phalanges is much less complete, but more distally the syndactylos digit formed shows no signs of its dual origin.

In transverse sections, syndactylos limbs (Text-fig. 2, $a'$) are much thicker

Text-fig. 3. Transverse sections through the left limbs of $+/+(a \text{ and } b)$ and of a normal overlap $sy/sy$ embryo ($a'$ and $b'$), 41 and 40 days old respectively (c.r.l. 21.5 and 19.3 mm respectively). Otherwise as in Text-fig. 2.
in dorso-palmar direction, but correspondingly narrower from side to side. The same situation was found in the other syndactylyous limb (39-day embryo) which was sectioned transversely. In common with the other sy/sy embryos of this series, there was no syndactylyism in the hind limbs. But the digital rays are closer together (Text-fig. 2, b') than in the corresponding normal limb (b), and the limb bud is thicker but narrower.

The same situation is encountered in normal overlaps (Text-fig. 3, a', b'); the limbs of that embryo should be compared with those of the homozygous normal in the same figure and with that in Text-fig. 2, which are respectively 1 day older and 1 day younger than the sy/sy normal overlap in question. It thus appears that the non-syndactylyous limbs of partly manifesting sy/sy embryos as well as those of normal overlaps can be distinguished from those of homozygous normal embryos.

The same type of mildly abnormal limb buds without manifest syndactylyism was found in a 'normal' embryo of 39 days which, from its ancestry, could have been either +/sy or sy/sy. It may thus have been a normal overlap like the embryo in Text-fig. 3. Alternatively, there is the possibility that sy is not fully recessive.

DISCUSSION

The embryological material, small though it is (and perhaps because it is so limited), gives a consistent picture of events leading up to bovine syndactylyism. Syndactylyism is the result of a more or less complete coalescence of the blastemata destined to form the phalanges. Syndactylyism is thus primary and hence quite different from the physiological syndactylyism of metacarpals and metatarsals, which is secondary if not tertiary, having been preceded by separate blastemata and separate cartilaginous models prior to the ultimate union of the respective skeletal elements.

The coalescence of skeletogenic material which gives rise to bovine syndactylyism is clearly a consequence of the proximity between the respective blastemata. The situation is indeed the same as in shaker with syndactylyism in the mouse (Grüneberg, 1962), where the corresponding blastemata are also laid
down too close to each other and where a similar coalescence near their distal ends leads to the formation of a single blastema which gives rise to composite phalanges. In the mouse mutant, such fusions may involve digits II and III, or III and IV, or all three of them. In both cases the coalescence of blastemata which are too close to each other may be explained in terms of competition (Tschumi, 1954). When blastemata are formed, they attract mesenchyme cells from the surrounding tissues. When two blastemata are close together they may mutually attract mesenchyme cells from each other, with the result that a cross-traffic to and fro leads to their coalescence.

Whereas syndactylism of the phalanges is clearly a consequence of the proximity of digital rays III and IV, it is not quite certain why the blastemata are formed in such close juxtaposition. If the abnormal shape of the limb buds—too thick but at the same time too narrow from side to side—is present from the beginning, it would result in the blastemata being laid down too close to each other. In two mutants in the mouse, syndactylism and oligosyndactylism (Grüneberg, 1960, 1961), it has indeed been shown that abnormalities of the shape of the limb buds are detectable before any blastemata have been formed: under such circumstances it is difficult to avoid the conclusion that the anomalous configuration of the limb buds is the cause for the skeletal anomalies which subsequently arise in it. In bovine syndactylism, it is not certain whether the bloated shape of the limb buds is present before the formation of the blastemata. We are inclined to believe that it is. If it were not, one would have to assume that the anomalous arrangement of the blastemata is primary and that it leads to the abnormal shape of the limb buds, and that the early blastemata have enough mechanical strength for that to happen. If, as seems more likely, the atypical shape of the limb buds is the basic anomaly, its origin remains obscure.

Bovine syndactylism is a threshold character which comes into being if, and only if, the digital blastemata coalesce with each other. The \textit{sy} gene, in all the four limbs of homozygous embryos, creates a situation in which this may happen. It occurs more often in the forelimbs than in the hind limbs, and not rarely it fails to happen altogether. If so, separate blastemata form separate digits with separate hooves, and the limb ultimately appears superficially quite normal.

**SUMMARY**

In homozygous condition, the gene for syndactylism (\textit{symbol sy}) in Holstein–Friesian cattle has a variable manifestation. Affected limbs typically have a single digit with a single hoof. This happens more often on the fore than on the hind limbs, and some \textit{sy/sy} animals show no syndactylism at all. Whereas normally the blastemata for digits III and IV at first diverge widely from each other, those of \textit{sy/sy} embryos arise close to and approximately parallel to each other and have a tendency to coalesce distally. If coalescence is complete, a single but rather massive digit is formed; incomplete fusion may give rise to limbs with
incomplete syndactylism, and if coalescence fails to happen the digits remain separate and have separate hooves. The limb buds of sy/sy embryos are thicker but correspondingly narrower from side to side, and it is probable (but not certain) that the atypical shape of the limb buds is the cause of the close juxtaposition in which the digital blastemata are laid down in the first instance.

ZUSAMMENFASSUNG
Die Entwicklung der Syndaktylie beim Rinde

Das Gen für Syndaktylie beim holstein-friesischen Rindvieh manifestiert sich in Homozygoten unregelmäßig. Betroffene Gliedmassen haben eine einzelne Zehe mit einem einzelnen Huf. Dies kommt öfter vorne als hinten vor, und manche sy/sy-Rinder zeigen überhaupt keine Syndaktylie. Während in der Normalentwicklung die Blasteme für die Strahlen III und IV zunächst erheblich von einander divergieren, entstehen sie bei sy/sy-Embryonen nahe bei einander und annähernd parallel, und sie haben eine Neigung, distal mit einander zu verschmelzen. Im Extremfalle entwickelt sich eine einzelne aber verästelte Zehe; sonst unvollständige Syndaktylie oder, falls Verschmelzung der Blasteme ganz unterbleibt, entstehen getrennte Zehen mit getrennten Hufen. Die Extremitätenknospen von sy/sy-Embryonen sind dorso-palmar dicker aber entsprechend schmaler von Seite zu Seite, und es ist wahrscheinlich (aber nicht sicher), dass die atypische Form der Extremitätenknospen die Ursache dafür ist, dass die Blasteme für die Strahlen III und IV abnorm nahe zusammen entstehen.

The embryos were collected as a part of the North Central States, U.S.A., Regional Dairy Cattle Breeding Research Project (NC-2). In the collection of the embryos by Caesarean section, we have been aided by Dr E. P. Call and Mr S. T. Smith. The microscopical preparations were made by Miss June Denny and by Miss Jean M. Gray, who has also taken the photomicrographs for the plate. The text-figures were made by Mr A. J. Lee. To all of them, we wish to express our sincere thanks.

REFERENCES
Bovine syndactylyism


(Manuscript received 3 October 1967)