The average of 1142 basophil counts at different times of day was 516.4 ± 10.8/μl blood (st. dec. ± 366). The mean of 426 basophil counts of females was 545.6 ± 19.3 (st. dev. ± 399), which is insignificantly higher than the mean of 716 basophil counts of male rabbits, showing 499 ± 12.9 (st. dev. ± 344).

During pregnancy, 6 females demonstrated a clear tendency towards a fall in basophil count, most pronounced on the day of delivery and the third day after delivery (Fig. 3).

Variations in the morning basophil count in rabbit blood (counts per mm³ blood, at 9 a.m.) on successive days.

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of counts</th>
<th>Males: Mean ± standard error</th>
<th>Standard deviation</th>
<th>Females: No. of counts</th>
<th>Mean ± standard error</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>128</td>
<td>475 ± 23.6</td>
<td>267</td>
<td>43</td>
<td>552 ± 72.0</td>
<td>472</td>
</tr>
<tr>
<td>2</td>
<td>128</td>
<td>528 ± 20.5</td>
<td>232</td>
<td>43</td>
<td>453 ± 49.9</td>
<td>327</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>422 ± 34.0</td>
<td>284</td>
<td>43</td>
<td>524 ± 46.1</td>
<td>302</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>428 ± 35.8</td>
<td>272</td>
<td>43</td>
<td>509 ± 32.9</td>
<td>374</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>422 ± 45.0</td>
<td>318</td>
<td>43</td>
<td>509 ± 32.9</td>
<td>374</td>
</tr>
<tr>
<td>Total</td>
<td>434</td>
<td>469 ± 12.9</td>
<td>269</td>
<td>129</td>
<td>509 ± 32.9</td>
<td>374</td>
</tr>
</tbody>
</table>

count of 0-4%. Although a wide individual variation in the counts was observed, they differed but slightly from day to day under standard conditions. The higher level of basophils in the female group and the afternoon rise in both counts are in accordance with reported findings in normal humans. Even the decrease observed during pregnancy agrees with the decreasing tendency reported in pregnant women. An influence of adrenocortical as well as female sex hormones on the number of circulating basophils has been suggested previously, and may also be responsible for these variations.

Discussion. By indirect counting methods, the number of basophils in rabbit blood ranged from 0–950 cells/μl blood, with differential counts of 0–12%. Direct counts of the present study, however, revealed a minimum count of 45 basophils/μl blood, corresponding to a differential count of 0-4%. Although a wide individual variation in the counts was observed, they differed but slightly from day to day under standard conditions. The higher level of basophils in the female group and the afternoon rise in both counts are in accordance with reported findings in normal humans. Even the decrease observed during pregnancy agrees with the decreasing tendency reported in pregnant women. An influence of adrenocortical as well as female sex hormones on the number of circulating basophils has been suggested previously, and may also be responsible for these variations.

A.-W. A. BOSEILA

Connective Tissue Research Laboratory, University Institute of Medical Anatomy, Copenhagen (Denmark), January 19, 1959.

Zusammenfassung


The Embryonic Origin of the Intrinsic Limb Musculature in Amphibia, Salientia

The mesodermal cells that develop into the intrinsic limb muscles, in tetrapods, are generally considered to be exclusively somatopleural in origin. MILAIRE has recently suggested that the limitation of the primitive intrinsic muscle is to the region of the hinge of the leg. The present study shows that the intrinsic muscle of the leg is developed from the same region as the extrinsic muscle of the leg. The extrinsic muscle of the leg is developed from the same region as the extrinsic muscle of the leg.

1 E. F. BYRNES, J. Morph. 14, 105 (1898).
8 On leave from the Histology Department, Faculty of Medicine, Cairo University, Egypt.
cently contested this interpretation for reptiles. In view of Milaire's results and the earlier report of Field* that myotomes contribute to the limb musculature in frogs, it seemed pertinent to re-examine the problem in the latter group.

**Normal series.** Normal limb bud development was followed in Bufo calamita, Megophrys pelodytides, and Rana ridibunda. A closely spaced series extending from limb bud stages I–IX (Taylor and Kollross*) was employed for each genus. The primary limb protuberance forms by the accumulation of mesenchyme cells which leave the coelomic epithelium and migrate outwards to establish contact with the adjacent thickened epidermis. Subsequent growth of the fore-limb (except for nerves and blood vessels) is entirely by the in situ proliferation of these parietopleural cells. In the hind limb region, however (at about stage IV), a bud separates from the medioventral edge of the 8th trunk myotome. This extends towards the hind limb 'anlage', and then itself constricts a much smaller secondary bud, which grows into and supplements the mesenchyme of the limb (Fig. 1). The residuum of the primary bud makes no contribution to the future limb, but bends ventrally to contribute to the abdominal musculature.

**Transplantation experiments.** Early fore and hind limb primordia (stage III–IV) were implanted in the abdominal wall of the same individual. At the end of metamorphosis the explanted and normal limbs were compared topographically and histologically. Fore limb explants differed from the controls only in their slightly slower growth rate and in a variable degree of distortion of their skeletal elements. Hind limb grafts, however, consistently showed a marked paucity of thigh musculature (Fig. 2), although the distal musculature of the same limb and its skeleton as a whole were quite normal.

**Discussion.** Harrison* showed, in Anura, that the nerve elements which normally innervate the muscles of hind limbs play no part in the morphogenesis of those muscles. The transplantation results, therefore, cannot be attributed to inadequate innervation. In any event, such an explanation could not account for the normal muscle condition in the distal segments of the same limb. The results are interpreted, therefore, as indicating that the dual origin of the hind limb mesoderm, observed in the normal series, has a real developmental significance, and that a primordium deprived of its myotomic component cannot adequately rectify this deficiency by compensatory proliferation of the somatopleural factor. This accords with recent experimental work** which indicates that the development of the anuran limb is of the mosaic type.

Failure to trace carbon particles from somites to limbs forms one of the main arguments for a purely somatopleural interpretation of limb muscles. But marking the ground tissue of a localized area does not preclude the possibility of cells migrating past the labelled zone, particularly if they arise by secondary constriction from within the main mass. In such cases it is suggested that the carbon method should be employed only in a confirmatory role, and significance attached only to positive results. Interpreted in this way, the few positive results gained during this investigation provide at least some support for the conclusions based on normal development.

Fig. 1.—Transverse section through the hind limb bud of Rana ridibunda. A = myotome; B = residuum of primary muscle bud; C = secondary muscle bud; D = limb bud

**Normal series.** Normal limb bud development was followed in Bufo calamita, Megophrys pelodytides, and Rana ridibunda. A closely spaced series extending from limb bud stages I–IX (Taylor and Kollross*) was employed for each genus. The primary limb protuberance forms by the accumulation of mesenchyme cells which leave the coelomic epithelium and migrate outwards to establish contact with the adjacent thickened epidermis. Subsequent growth of the fore-limb (except for nerves and blood vessels) is entirely by the in situ proliferation of these parietopleural cells. In the hind limb region, however (at about stage IV), a bud separates from the medioventral edge of the 8th trunk myotome. This extends towards the hind limb 'anlage', and then itself constricts a much smaller secondary bud, which grows into and supplements the mesenchyme of the limb (Fig. 1). The residuum of the primary bud makes no contribution to the future limb, but bends ventrally to contribute to the abdominal musculature.

These results were tested by two experimental techniques. For each experiment 25 specimens of Bufo calamita and of Rana ridibunda were employed.

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**Marking experiments.** Stages just prior to the formation of the limb bud protuberance were employed. Fine carbon particles were pressed into the ventral and ventro-medial borders of trunk myotomes 7–9. The larvae were killed at the late paddle stage, fixed in 70% ethanol and cleared in N/10 potash solution. Particle recoveries were made from the tissues of the abdominal wall only, in 39 individuals, and from the proximal segments of the hind limb as well as the abdominal wall in 4 others. In the remaining 7 specimens, no particles at all were located.

Regarding the purely somatopleural nature of the fore limb mesenchyme as compared with the situation in the hind limb of frogs, it is noteworthy that Harrison* described a comparable condition in the salmon. He showed that the pectoral fin originates exclusively from the somatopleure whilst most of the other