The average of 1142 basophil counts at different times of day was 516·4 ± 10·8/µl blood (st. dev. ± 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).

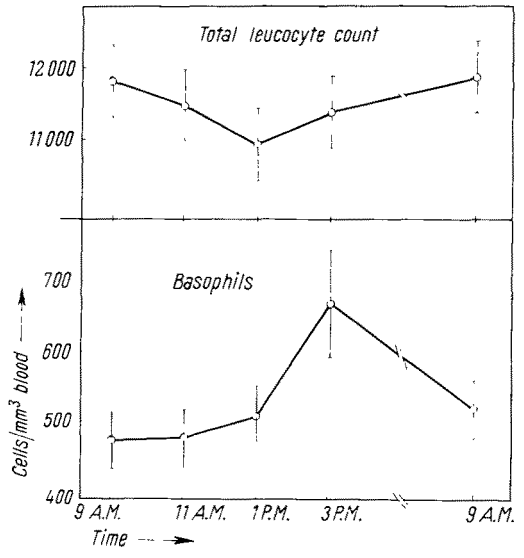

Fig. 2. – Diurnal variation of blood basophil and total leucocyte counts for 58 male adult rabbits ($\frac{\delta}{1}$: mean ± standard error).

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).

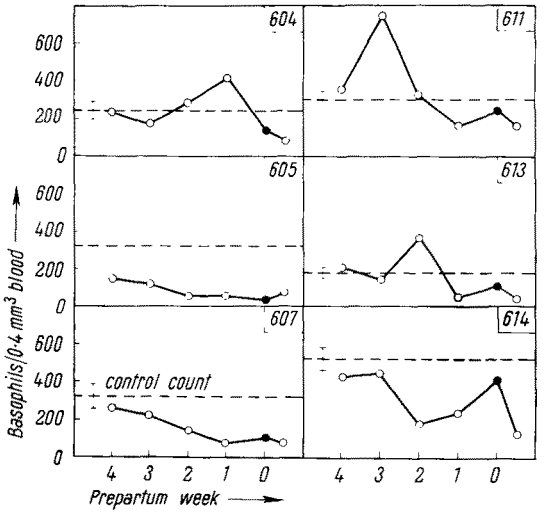

Fig. 3. – Blood basophil count variations for 6 rabbits during pregnancy (o: counts before or after delivery, •: count on day of delivery; – – –: mean of 4 control counts taken on 4 successive days before copulation with standard error indicated by vertical line).

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%[5]. 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[6]. 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[7], and may also be responsible for these variations.

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

Day	Males			Females		
	No. of counts	Mean ± standard error	Standard deviation ±	No. of counts	Mean ± standard error	Standard deviation ±
1	128	475 ± 23·6	267	43	552 ± 72·0	472
2	128	528 ± 20·5	232	43	453 ± 49·9	327
3	70	422 ± 34·0	284	43	524 ± 46·1	302
4	58	428 ± 35·8	272			
5	50	422 ± 45·0	318			
Total	434	469 ± 12·9	269	129	509 ± 32·9	374

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Zusammenfassung

Direkte Zählung von basophilen Leukozyten 195 männlicher und weiblicher Kaninchen in einer Blutkammer ergab eine Durchschnittszahl von 516 pro mm³ Blut. Eine signifikante Zunahme von Basophilen findet nachmittags statt. Während der Schwangerschaft wurde eine Abnahme der Basophilen beobachtet.

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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[1–6]. MILAIRE[7] has re-

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cently contested this interpretation for reptiles. In view of MILAIRE's results and the earlier report of FIELD[8] (later denied by BYRNES[1]) that myotomes contribute to the limb musculature in frogs, it seemed pertinent to re-examine the problem in the latter group.

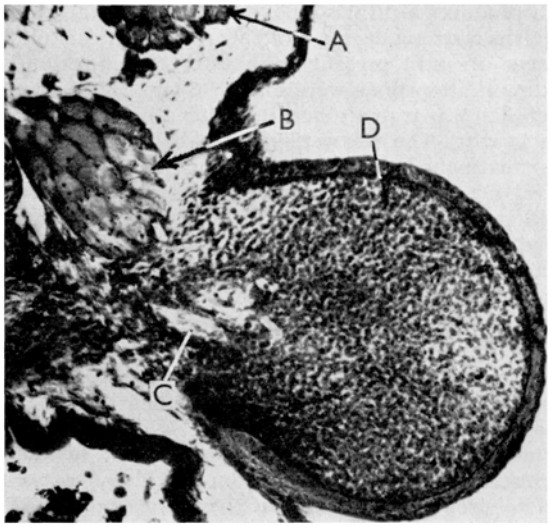

Fig. 1.—Transverse section through the hind limb bud of *Rana ridibunda*. *A* = myotome; *B* = residuum of primary muscle bud; *C* = secondary muscle bud; *D* = limb bud

Normal series. Normal limb bud development was followed in *Bufo calamita*, *Megophrys pelodytoides*, and *Rana ridibunda*. A closely spaced series extending from limb bud stages I–IX (TAYLOR and KOLLROSS[9]) 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.

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.

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.

Discussion. HARRISON[10] 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[11,12] 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[6,13,14]. 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.

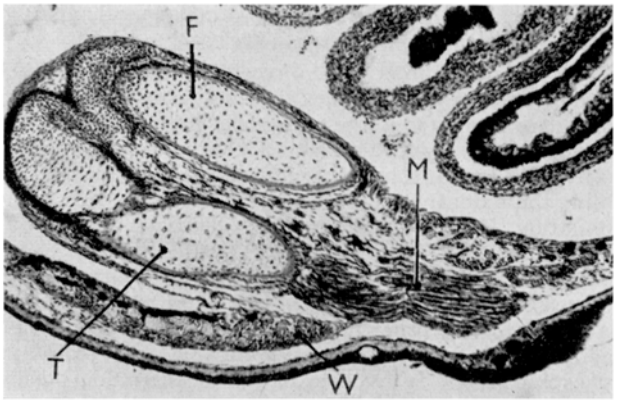

Fig. 2.—Transverse section through the abdominal wall and implanted limb bud of *Rana ridibunda*. *F* = femur; *M* = tibio-fibular musculature; *T* = tibio-fibula; *W* = abdominal wall musculature

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[15] described a comparable condition in the salmon. He showed that the pectoral fin originates exclusively from the somatopleure whilst most of the other

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fins receive direct myotomic contributions. He demonstrated, also, that the posterior half of the dorsal fin derives buds from the myotomes, but that the anterior half of the same fin receives no myotomic tissue whatsoever. The possibility of expressing the myotomic role, in limb and fin formation, in terms of a cephalocaudal gradient, therefore, should not be excluded.

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Zusammenfassung

Das Blastem, welches sich zur freien, hinteren Gliedmassenmuskulatur entwickelt, stammt bei Anuren von parietalem Mesoderm und von Ursegmenten ab. Das Vorderbein-Mesoderm dagegen ist ausschliesslich parietal. Diese Feststellungen werden durch Befunde an transplantierten Beinknospen und durch Markierungsversuche mit Kohlepartikeln gestützt.

Die Brauchbarkeit des Kohlemarkierungs-Verfahrens wurde speziell in ihrer Anwendung auf die Feststellung der Wanderrouten von Muskelzellen geprüft.

p-Aminohippuric Acid Accumulation in Kidney Slices in Cloudy Swelling

The clearance of *p*-aminohippuric acid (PAH) is generally considered as one of most important tests of kidney tubular function. Injected PAH is brought with the blood to the tubule cells, and then passes into the cells and is finally excreted in the urine. The peculiar mechanism of this phenomenon is, however, not clear.

CROSS and TAGGART[1] have recently shown that PAH is accumulated within kidney slices respiring *in vitro* when these are incubated in the presence of this substance. The same authors have also shown that 2,4-dinitrophenol (DNP), as well as some other related substances which inhibit the oxidative phosphorylation, prevents this accumulation. Other substances which decrease the respiration rate such as cyanide, azide, arsenite, fluoride, iodoacetate, fluoroacetate, and mercuric hydrochloride also decrease the accumulation of PAH within the slices. Acetate, on the other hand, has a powerful stimulating activity. It thus seems very probable that oxidative phosphorylation is involved in the accumulation phenomenon.

Oxidative phosphorylation is decreased in tissues showing cloudy swelling [2,3]. It therefore seemed interesting to study the influence on the accumulation of PAH within kidney slices *in vitro* of treatments capable of producing cloudy swelling in this organ. The results of such an investigation are described in this paper.

Several types of damaging treatments were used: (1) Intraperitoneal injection in the rat of the toxin of *Salmonella typhi murium* (0·5 ml of a 24 h culture in broth, killed by heating at 70°C for 1 h). The animals were killed 24 h after the injection. This type of treatment has been found able to produce a typical cloudy swelling in rat kidney[4,5]. ATP concentration of the organs of treated animals is decreased[6]. (2) Intraperitoneal injection of the α-toxin of *Staphylococcus pyogenes aureus* (10 hemolytic combination dosis; the toxin was kindly supplied by the Istituto Sieroterapico Vaccinogeno Toscano, Siena). This toxin produces a diffused cloudy swelling in rat kidneys, as well as a strong decrease of P/O ratio[7]. The histological changes are still present 24 h after the injection, but maximum alterations were found 96 and 120 h after the injection. (3) Intraperitoneal injection of DNP (3 mg/100 g body weight). The rats were killed 24 h after the injection. This treatment was found to produce cloudy swelling in several rat organs[8,9], as well as decrease of P/O ratio[8]. (4) Intraperitoneal injection of thyroxine (Hoffmann-La Roche, 1 mg/100 g body weight, for 2 days). The rats were killed 24 h after the last injection. Thyroxine produces decrease of P/O ratio in liver mitochondria[10–12] as well as cloudy swelling[13].

Albino rats weighing 250–300 g were used. They were killed by bleeding. Their right kidney was immediately taken out and weighed. A small fragment was used for nitrogen determination (method of Kjeldhal). Another fragment was used for the preparation of histological specimens. The remaining portion of kidney cortex was used to prepare hand cut slices for the study of PAH accumulation. This was made by the method described by CROSS and TAGGART[1], using Warburg manometers at 25°C, with 90–100 mg of slices (wet weight) and 0·001 *M* sodium *p*-aminohippurate (S.I.M.E.S., Milano). Oxygen was the gaseous phase. When added, sodium acetate had the final concentration 0·01 *M*. Oxygen uptake was recorded for 1 h. After this time, the flasks were removed and immediately transferred to the cold room at 2°C. The slices were collected on a metal nest filter and then homogenized with a gum pestle. PAH concentration in both slices and suspension fluid was determined according to the procedure of CROSS and TAGGART[1]. Readings of the optical density were made at 450 mμ in a Beckman Mod. DU spectrophotometer. The following data were calculated in each experiment: (1) QO_2: this was obtained by dividing the microliters of O_2 consumed in 1 h by the mg (wet weight) of the used amount of tissue. (2) the S/M ratio between the micromoles of PAH found within the slices (S) per g and those remaining in the suspension medium (M) per ml. (3) the quotient between this value and QO_2 (S/M: QO_2). This is indicated in the Table as Q. The standard deviation was calculated for each average. The significance of the differences between two averages was estimated by calculating the '*t*' value of Fisher. The Table reports the results. It is clear from the Table that QO_2 is significantly increased after all types of treatments. The increase was, however, particularly high after the injection of thyroxine. The significance of the results is less when the values are referred to the nitrogen content, but remain particularly high for the rats treated with thyroxine or with DNP. In fact, in last cases, nitro-

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