INTRODUCTION

Among the Amphibia there are numerous examples of the suppression to a varying extent of a larval stage in the life-history. In such instances the animal is freed by various means from the necessity of passing its early phases of development in open water. This evolutionary trend has nowhere proceeded further than in the Anuran genus *Eleutherodactylus*, which is distributed through the Caribbean and the adjacent mainlands. In *Eleutherodactylus*, development is direct and wholly embryonic, and many larval features have been suppressed.

In 1871 was published the first description of a West Indian frog which laid eggs in air, and from which young frogs with fully formed limbs were hatched (Bello y Espinosa, 1871). Since that time some twenty papers have been published on the embryology of different species of the genus, mainly in recent years by Dr. W. Gardner Lynn and his collaborators. The main account of the general development of *Eleutherodactylus* relates to the montane species *E. nubicola* (Lynn, 1942). Later papers have been concerned with the role of the thyroid gland in this Amphibian which develops without any metamorphosis (Lynn, 1948; Lynn & Peardon, 1955; Millott & Lynn, 1954).

In *Eleutherodactylus* the eggs are large and yolky and are enveloped in layers of tough jelly. The segmentation is complete, though for much of embryonic life the bulk of the embryo consists of a mass of large yolky cells surmounted by a cap formed by the smaller-celled dorsal region of the embryo. Waddington (1952) has remarked on the tendency towards the development of a blastoderm among the more yolky eggs of Amphibia. In this direction *Eleutherodactylus* approaches the limit consistent with holoblastic cleavage.

In the embryo of *Eleutherodactylus* the gill clefts are never open, and the only

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complete aortic arches ever to be formed are the carotid and systemic. There are no lateral-line organs or nerves; no tadpole-like mouth parts or coiled gut. In some species there are traces of external gills.

The present study is mainly concerned with some aspects of neural development in *Eleutherodactylus*. The nervous system of the developing Amphibian shows a number of primitive fish-like features which have persisted as larval adaptations. It is consequently of interest to examine how far they remain in an Amphibian with an embryonic type of development. One instance of the atrophy in *Eleutherodactylus* of such a feature has already been observed by Dr. Lynn. Not only have the lateral-line organs been lost, but also the two giant Mauthner neurones of the medulla are absent (Lynn, 1957). In fishes and larval Amphibia, these cells form the middle link in a three-neurone chain between the vestibular system and the trunk musculature. In most Anura the Mauthner cells disappear at metamorphosis, though they persist in the aquatic genus *Xenopus* (Stefanelli, 1949).

In the present paper are also described observations on the development of the dorsal root ganglia and ventral motor horns of *Eleutherodactylus*, the interest of which are neuro-embryological rather than phylogenetic. It is intended, however, in a second paper to return to the theme of larval and embryonic development by a comparison of the motor innervation of trunk and limbs in *Xenopus* and *Eleutherodactylus*.

**MATERIAL**

Eggs of two species of *Eleutherodactylus* were collected in the neighbourhood of the University College of the West Indies, Mona, St. Andrew, Jamaica. Two clutches each of about a score of eggs were found by Mrs. Valerie Croston, in her garden at College Common; they had been laid in coco-nut husks in which orchids were growing. With the second batch was a female of *E. ricordii*. These eggs were placed on moist filter paper in Petri dishes and were observed in the laboratory from day to day. Generally, three eggs from each clutch were fixed on each day. The first batch was obtained 5 days before hatching, and the second 11 days. The development of both hatches during the last 5 days of intra-oval life appeared to be identical. They have both been assigned to *E. ricordii*. Goin (1947) states that in Florida the average period of development of this species is 15·6 days.

In addition, one clutch of eggs was collected from the situation where Dr. Lynn has found that those of *E. martinicensis* are laid (Lynn, 1957), namely, in the axils of the leaves of the wild ‘pinguin’, a Bromeliad, ‘vigoroso and formidable’, as Gosse (1851) describes it, ‘the recurved spines with which the edges of the long leaves are set being exceedingly sharp, and inflicting terrible scratches’. This third batch of eggs was collected at a stage which corresponded in most respects with that of *E. ricordii* 5 to 6 days before hatching. These eggs of *E. martinicensis* were in process of hatching after 6 days in the laboratory. They were clearly
different from those of *E. ricordii* in that the embryos were more heavily pigmented and somewhat larger in size though the diameter of the outside of the egg was the same in both species, namely, 3.5 mm. According to Lynn & Peadon (1955) the normal developmental period of *E. martinicensis* is 13–14 days. As in *E. ricordii*, embryonic life ends with the greater part of the yolk yet to be absorbed.

**METHODS**

Each embryo was dissected free of its jelly-layers and fixed for 24 hours in a 1:1 mixture of Bouin’s fluid and cellosolve, as recommended by Lynn (1948). The material was then gradually dehydrated in ascending mixtures of cellosolve and water. From pure cellosolve the embryos were transferred to a 1:1 mixture of xylene and cellosolve, and then cleared in pure xylene. They were then embedded in paraffin wax of m.p. 60°C. Sections were cut at 9 μ. Some series were stained in Ehrlich’s haematoxylin and eosin while others were impregnated with silver by Bodian’s method, for which the silver proteinate of Établissements Roques of Paris was used.

Serial sections were also prepared from the central nervous system of the adult *E. ricordii*. The animal was killed in Bouin’s fluid and, after death, the dorsal surface was skinned and an axial strip of the body was cut out, some 5–6 mm. wide and less than 20 mm. in length. This contains the whole of the central nervous system. Fixation was continued for a further 24 hours, after which the specimen was decalcified in 1 per cent. nitric acid in 70 per cent. alcohol, dehydrated in ascending alcohols, and then cleared in xylene and embedded in paraffin wax. Sections were again cut at 9 μ and stained by the same methods.

**GENERAL DESCRIPTION OF DEVELOPMENT IN *E. RICORDII***

The second batch of eggs were collected on the 15th of January 1958, and those which remained unfixed, hatched on the 27th of the month. Following the usage of Lynn (1942), the stages of development of the material will be designated by the number of days which remained before hatching.

- **11 days.** The embryos were at a stage with small limb-buds. Most of them were rotating within the egg-envelopes in the way described by Gitlin (1944) for *E. portoricensis*. Of 14 embryos in movement, 2 were rotating clockwise and 12 anti-clockwise.

In section series deep clefts are seen in the surface of the embryo between the limb-buds and the upper part of the body-wall. The tail-bud is present. Eye-vesicles have appeared, and the hind-brain is thin-roofed. In the auditory sacs the endolymphatic duct is yet undilated. The fore-gut is a flattened diverticulum of the archenteron, with which it joins by a wide anterior intestinal portal. The heart is a simple tube with endo- and myocardium. The three pronephric tubules
TEXT-FIG. 1. Drawings from section series through embryo of *E. ricordii* at −10 days, to show transition from trunk region (top of page) to tail, flattened on one side against egg-membrane. *a*, archenteron; *d.a.*, dorsal aorta; *h.g.*, hind-gut; *h.l.b.*, hind limb-bud; *n.*, notochord; *p.c.v.*, posterior cardinal vein; *sp. c.*, spinal cord; *sp. g. IX, X*, spinal ganglion IX, X respectively; *W.d.*, Wolffian duct; *y*, yolky endoderm.
have open funnels into the coelom. The Wolffian duct extends backwards to the level of the hind limb-buds. The myotomes consist of elongated myoblasts, which as yet show no cross-striations.

-10 days. The limb-buds were larger. The embryos were no longer rotating, but muscular movement had begun; in sections cross-striations were evident in the trunk myotomes. Both spontaneously and after slight mechanical shock, the embryos would wriggle in a way corresponding to the 'early flexure' stage of activity in *Ambystoma* (Coghill, 1929) (in later stages this motion was never seen to progress any farther towards swimming movements). By -10 days the nasal placodes have appeared, and the eye-cup is two-layered; it contains a hollow lens vesicle. A large infundibular recess has appeared on the floor of the mid-brain. On the surface of the third pharyngeal arch at this stage is a blunt vascular papilla which may represent an external gill, though considerably smaller than Gitlin (1944) has described in *E. portoricensis*.

The stomodaeum is present, though it does not yet make contact with the pharynx. The anterior intestinal portal is much narrower; on its anterior surface are seen the first epithelial cords of the liver. Posteriorly, the archenteron leads into the hind-gut. The latter is continued by an open proctodeal tube. The tail, still closely applied to the surface of the egg, is twisted through a right angle, so that its neural tube and notochord lie side by side (Text-fig. 1). A deep cleft leads from the medial surface of one hind limb-bud beneath the lateral surface of the apposed tail.

-9 days. By this stage new features were apparent in the living embryos. The heart was beating and blood was in circulation. The whole egg could easily have been mistaken for that of an Amniote, with a network of blood-vessels over the yolk, and the vascular tail, now closely applied to the inner surface of the egg envelope, strikingly resembling an allantois. The eyes were pigmented. The olfactory placodes have become cup-shaped. The inner layer of the lens vesicle is thickened. The saccus endolymphaticus, now separated from the original auditory vesicle, is beginning to expand over the hind-brain as in other Amphibia (Whiteside, 1922).

-8 days. Over the whole body surface melanophores have developed pigment. The limbs now protrude stiffly from the body and are several times as long as broad. The alimentary tract consists of two narrow tubes, suspended by mesenteries, which lead respectively from pharynx and cloaca to separate junctions with the central mass of cellular yolk—as yet little reduced in volume. The lumen of the fore-gut tube in the oesophageal region is occluded. Oesophagus and trachea have become separate, and the latter ends in the dilated lung-buds.

-7 days. The hind limb is now rather longer than the forelimb, and ends in a spatulate tarsal region. The internal nares now open into the pharynx. In the eye the lens fibres are heavily cornified. The inner ear has acquired two semicircular canals, and the future position of the third is indicated. Within the heart the truncus arteriosus is divided by a septum, and the trabeculae of the
ventricular wall are developing. The auricle is yet undivided. The first mesonephric tubules have appeared.

—6 days. The first signs of layering within the retina are apparent. The auricular septum is present, and in the ventricle the trabeculae are much deeper. Within the thyroid, vesicles have developed.

—5 days. The egg-tooth at the tip of the upper jaw has appeared. The digits are visible in both pairs of limbs. A second fibre-layer can be traced within the retina. Chondrification has begun at several points within the skeleton; the hind limbs and pelvic girdle are more advanced in this respect than are the anterior members. Cartilage matrix has appeared within the first few vertebrae. No degenerative changes within the pronephros are yet seen.

—3 days. In both humerus and femur the cartilage of the shaft is hypertrophic, and invasion by osteocytes and blood-vessels has just begun. Two membrane bones are present in the head; the squamosal lateral to the auditory capsule, and the dentary along the inner surface of Meckel's cartilage. The mesonephros is much larger than at —5 days, and within the pronephric tubules vacuolation is apparent. The mucous glands within the skin now have acini.

—1 day. The first signs of ossification are visible in the distal limb segments. Within much of the chondrocranium cells have become hypertrophic. To external view the tail is yet undiminished, yet the first degenerative change is apparent in sections, in that the ectodermal cells of the tail have put out irregular amoeboid projections. The pronephros is now a mass of vacuoles.

Comparison of the rate of development of *E. ricordii* with *E. nubicola* (Lynn, 1942) with respect to the features described above indicates that —11 days in *ricordii* corresponds to —20 days in *nubicola*, and that the former hatches at a stage which in many respects resembles that of the *nubicola* embryo with 7 days of the intra-oval period yet remaining.

Free life in *E. ricordii* begins with the greater part of the abdominal cavity still occupied with yolky cells unchanged since the period of cleavage, and with the later stages of yolk absorption, such as Sampson (1904) and Lynn (1942) describe, still to be accomplished. In both *E. ricordii* and *E. martinicensis* the rate of development must be among the most rapid of all vertebrate ontogenies. Three days after the lens vesicle has invaginated its inner wall has formed heavily cornified lens fibres; the limb-skeleton begins to ossify 2 days after the first appearance of cartilage matrix. The same rapidity of development is seen within the central nervous system.

**THE DEVELOPMENT OF THE SPINAL CORD IN E. RICORDII**

At —11 days the spinal cord, as yet confined to the trunk region, consists of a thick wall on each side, joined by thin roof and floor plates (Plate, fig. A). The wall is about 80 μ in thickness at most. The nuclei are about 10 μ long, and occur in every position throughout the depth of the epithelium. Mitotic figures are
common near the ependymal surface; yolk granules are still scattered throughout the neural tube. This trunk cord of *Eleutherodactylus* differs in several ways from that of other developing Amphibia, but resembles the cord of other vertebrates with embryonic development, such as the dogfish or the chick. In an early Amphibian larva the inner cellular part of the cord consists only of a single inner ependymal layer, surrounded by a zone of functional neurones which soon are clearly differentiated from other cells; mitotic figures soon become infrequent among the ependymal cells. The embryonic type of cord, on the other hand, laterally consists of a thick neuroepithelium through which the nuclei migrate to divide at the ependymal surface, from which they afterwards retreat (Sauer, 1934). In *Eleutherodactylus* mitosis within the trunk cord does not cease until 5 days before hatching.

The tail cord of the *Eleutherodactylus* embryo is similar to that of other young Amphibia in calibre. It is first recognizable in *E. ricordii* at —10 days. The cord then sharply tapers at the junction between the two zones, where it twists through a right angle to run alongside the notochord of the tail.

At the surface of the trunk cord at —10 days are first seen a thin layer of longitudinal fibres which are part of a descending tract which originates in the medulla oblongata. Within the myotomes fine single nerve fibres are seen in the plane of the myocommata. At these sites in other Amphibia both the first sensory and motor fibres are found (Hughes, unpublished), though to which category in *Eleutherodactylus* these first myocommatal fibres belong, I am unable to decide.

The dorsal root ganglia, which on the previous day were part of a continuous tract of neural crest cells, are now, at —10 days, separately discernible. Within the anterior trunk ganglia some of the constituent neuroblasts have produced a short, outwardly directed primary nerve fibre.

At —9 days another set of sensory cells has appeared; these are the Rohon-Beard neurones, which are found in all lower vertebrates. They remain within the cord and form a primary and transitory sensory system. *Eleutherodactylus* is so far unique in that their appearance follows that of the dorsal root ganglia. At —9 days in *E. ricordii* they are found in dorso-lateral positions within the cord, in both trunk and tail (Plate, fig. D). They are pear-shaped, with a large nucleus, which differs from that of the surrounding neuroblasts in that there is a clear nuclear sap containing a large nucleolus. The apex of the cell is continued by a single nerve process.

A further feature at this stage is the outgrowth of the definitive spinal motor roots. At trunk levels bundles of extremely fine fibrils emerge from the ventral surface of the cord, opposite the dorsal root ganglia, beneath which, and accompanied by numerous future Schwann cells, they make their way to the myotomes. Each bundle of motor fibres then turns caudally to run along the inner surface of a myotome as far as the adjacent inter-myotomic boundary into which a few motor fibres can now be clearly traced. A primary myocommatal innervation of the somatic musculature has already been demonstrated in the larva of *Xenopus*
A. Hughes—The Embryology of Eleutherodactylus

(Lewis & Hughes, 1957) where a histochemical reaction for cholinesterase is first detectable at these sites (Lewis, 1958).

By — 9 days in E. ricordii a bundle of fine nerve fibres has appeared in each limb-bud (Plate, fig. J). Some of these fibres can be traced back through motor roots to ventral horn cells at limb levels in the cord. The ventral horns at this stage form an expanded lower zone of the mantle layer of the cord in which many cells have already developed into bipolar neuroblasts with a slender nerve process at each end (Plate, fig. E). Their axons point in a ventro-medial direction. These ventral horn cells are further distinguished from neuroblasts elsewhere in the mantle layer by their larger, clearer nuclei; each has a single large nucleolus. Elsewhere in the trunk cord motor neuroblasts are not conspicuous, though in the tail pairs of primary motor neurones are recognizable. Within the dorsal root ganglia differentiation of neuroblasts is proceeding, and occasional cell-bodies are conspicuous by the size of their nuclei. At — 8 days fibres from the dorsal root ganglia first penetrate the cord to enter the dorsal funiculus, within which dorsal root fibres bifurcate and give off branches which run longitudinally in both directions. At this stage the general form of the trunk cord in Eleutherodactylus is still further unlike that of corresponding stages in a larval Amphibian, where the white matter of the cord forms a broad even margin to the inner cellular layers. In Eleutherodactylus, however, the fibre tracts are much sparser, and dorsally do not reach beyond the dorso-lateral sensory funiculus. This feature is associated with the early development of the dorsal root ganglia relative to that of the Rohon–Beard system of neurones. At — 7 days in Eleutherodactylus the trunk cord is strikingly reminiscent of that of a 6-day chick embryo (Plate, fig. C).

Not until — 8 days in E. ricordii can fibres which belong to the Rohon–Beard cells be traced. From each Rohon–Beard cell two processes are given off; one continues as an afferent fibre which leaves the dorsal surface of the cord; from the second, a longitudinal fibre joins the dorsal funiculus (Plate, fig. I). Both processes may arise from a single short axon.

From — 7 days onwards Rohon–Beard cells migrate towards each other to meet in the mid-dorsal plane. This movement is part of a general inward folding of the dorsal part of the spinal cord which thereby greatly increases in thickness. For Xenopus this movement has already been described (Hughes, 1957). In Eleutherodactylus ricordii the shift in position of the Rohon–Beard cells is achieved in the tail at — 7 days, but is not completed in the thicker cord of the trunk region until 2 days later (Plate, figs. G, H). Towards the end of development Rohon–Beard cells become fewer in number, but in E. ricordii are still numerous at the root of the tail on the day before hatching.

Meanwhile, differentiation continues within the ventral horns of the cord. At — 9 days these centres are first recognizable as ventro-lateral expansions of the mantle layer (Plate, fig. B). As development proceeds they become increasingly distinct from the adjacent masses of neuroblasts. By — 6 days the main part of the ventral horn consists of a compact ovoidal mass of bipolar cells whose
axons converge towards the ventral surface of the cord. Below them is a smaller subsidiary group of neuroblasts. The ventral horn is now similar to that of late larval stages in *Xenopus*, whereas in its first appearance there was a resemblance in general form to that of a 3-day chick embryo. At −1 day the ventral horn cells have reached a condition of considerable maturity. Blocks of Nissl material are visible in the cytoplasm towards either pole of the cell. The linear dimensions of the nucleus are about twice that in neighbouring cells.

**CELL POPULATIONS IN VENTRAL HORNS AND DORSAL ROOT GANGLIA**

From −7 days onwards in *E. ricordii* pycnotic nuclei are found among the ventral horn cells. Here cell degeneration is at a maximum from −5 to −3 days, and becomes infrequent on the day before hatching. The loss of some maturing ventral horn cells in developing Amphibia has also been observed in *Xenopus* (Hughes & Tschumi, 1958). In *Eleutherodactylus*, however, pycnotic nuclei are especially frequent at these sites.

**Text-Fig. 2.** Stages of cell degeneration in differentiated neurones, from the second dorsal root ganglion of an embryo of *E. ricordii* at −5 days. Taken from contiguous sections. (a), nuclear membrane still present, and cytoplasm as yet unchanged. Chromatin within nucleus aggregated into lumps; (b) shrunken remains of cell enclosed by original capsule. Satellite cell still present.

In the dorsal root ganglia also degenerating cells are common during the same period of development. At −7 days pycnotic nuclei and mitotic figures occur together among neuroblasts which as yet show little differentiation. At later
TABLE 1
Counts of cells in dorsal root ganglia of E. ricordii. Two individuals were counted at −5 days, and also in the adult stage.

<table>
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<th>Days before hatching</th>
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<th>9</th>
<th>10</th>
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<td>151</td>
<td>175</td>
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<td>970</td>
<td>108</td>
</tr>
<tr>
<td>3</td>
<td>268</td>
<td>369</td>
<td>352</td>
<td>335</td>
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<td>4</td>
<td>202</td>
<td>254</td>
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<td>212</td>
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<td>5</td>
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<td>206</td>
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<td>369</td>
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<td>448</td>
<td>1,005</td>
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<tr>
<td>10</td>
<td>268</td>
<td>138</td>
<td>328</td>
<td>388</td>
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| Total                | 2,829 | 2,716 | 4,467 | 4,657 |

Adult individuals

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<tr>
<td>9</td>
<td>223</td>
</tr>
<tr>
<td>10</td>
<td>185</td>
</tr>
</tbody>
</table>

| Total                | 5,519 | 5,657 | 648 | 213 |

* Some sections missing.
stages of embryonic life the various phases of cell degeneration (Glücksmann, 1930) are well shown among the larger neurones of the dorsal root ganglia (Text-fig. 2). The frequency of pycnotic nuclei in the spinal ganglia and ventral horns of *Eleutherodactylus* suggested an inquiry into the effect of this loss of cells on their total numbers within these sites throughout development. These counts have been continued into the adult stage of the life-history.

Compared with other Anura the adult of *Eleutherodactylus* is very small. The central nervous system has been adapted to these reduced dimensions apparently in two ways—either by decrease in cell size or by reduction in total numbers as with the motor neurones within the cord and the cells of the dorsal root ganglia. Thus whereas in *Xenopus* at metamorphosis the larger spinal ganglia may contain about 10,000 cells, the corresponding number in an adult *Eleutherodactylus* is about a twentieth of this figure. The largest mature neurones of the dorsal root

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**Text-fig. 3.** Average totals of cells in spinal ganglia in *E. ricordii* at the stages indicated, together with cell numbers in ventral motor horns.
ganglia are about 40\(\mu\) in diameter and hundreds of times the volume of the majority of cells within the cord.

The results obtained in these counts of cell populations within the central nervous system of *Eleutherodactylus* are shown in Tables 1 and 2. As in a previous study on cell populations in the dorsal root ganglia of *Xenopus* (Hughes & Tschumi, 1958) only nucleoli were counted in each section, with the object of minimizing error due to the spread of cells through adjacent sections. In

**Table 2**

Counts of cells in brachial and lumbar ventral horns of *E. ricordii*.  
Two individuals were counted at –5 days, and also at the adult stage

<table>
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<th>Lumbar ventral horn</th>
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<td>783 704</td>
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<td>693 666</td>
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<tr>
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<td>899 854</td>
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<tr>
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<td>528 493</td>
<td>598 687</td>
</tr>
<tr>
<td>1</td>
<td>553 506</td>
<td>656 654</td>
</tr>
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</table>

*Eleutherodactylus* there are ten pairs of dorsal root ganglia belonging to the trunk, including the morphological first pair, the ventral root of which gives off the hypoglossal nerve before joining the brachial plexus.

Table 1 and Text-fig. 3 show that the numbers of cells in the dorsal root ganglia of *Eleutherodactylus* from –9 days onwards do not greatly change during the remainder of the embryonic period. They reach a maximum between 5 and 7 days before hatching. At first cell production by mitosis more than compensates for the loss of cells by degeneration; after –5 days mitotic figures disappear from the ganglia while pycnotic nuclei continue to be found until the end of embryonic life. Table 2 and Text-fig. 3 indicate that in the ventral horns also there is a maximum cell population at –5 days. Here, however, cell degeneration is at first opposed not by mitosis, but by differentiation of fresh ventral horn neuroblasts from the adjacent mantle layer of the cord. Pycnotic nuclei are most frequently seen at the inner border of the ventral horn (Plate, fig. F).

**DISCUSSION**

The significance of the results which have just been described is not confined to questions of cell numbers. In *E. ricordii* the dorsal root ganglia are first separately recognizable at –10 days. Throughout the series the numbers of constituent cells are from the first closely similar to those of the adult animal (Text-fig. 4). The main limb ganglia are already larger than are the other members of the series. Yet this stage of development precedes the entry of nerve fibres
into the limbs by one day. Consequently, the general pattern of the spinal ganglia in *Eleutherodactylus* must be attained in the first place independently of any trophic influences derived from the limbs, and with respect to cell numbers the development of the ganglia is a process of self-differentiation.

![TEXT-FIG. 4. Average cell numbers in each spinal ganglion in *E. ricordii* at the three stages indicated.](image)

These results may be compared with those obtained for the dorsal root ganglia of the chick in a series of studies by Levi-Montalcini & Levi (1942, 1943) and Hamburger & Levi-Montalcini (1949). In the first two of these papers the 25th was chosen as an example of the lumbar ganglia of the chick. Here, the full number of cells, over 10,000 in all, is attained by about 8 days of incubation, though in one embryo 11,000 were counted on the 5th day (Levi-Montalcini & Levi, 1942). The proportion of differentiated neurones among these steadily rises to nearly 90 per cent. at the end of the incubation period. If, however, the hind limb is removed at the end of the 3rd day, cells degenerate within the lumbar ganglia, so that the total number within the 25th ganglion is then decreased by about two-thirds. A second consequence of the operation is that the number of differentiated cells remains at a low level such as is seen in a normal 6-day embryo. These results have led to the conclusion that in some way the developing limb exerts a trophic influence which not only maintains the cells within the
ganglion, but stimulates their differentiation into mature neurones. This conclusion is supported by comparison within the normal embryo of ganglia at limb and non-limb levels (Hamburger & Levi-Montalcini, 1949). In the latter, from 6 to 9 days of incubation, not only is the mitotic rate at a level lower than in limb ganglia, but also degenerating cells are conspicuous. Thus, as Hamburger (1952, p. 125) says, 'It is of considerable interest to note that the regressive changes produced in limb ganglia as a reaction to limb extirpation are identical with changes which occur in cervical and thoracic ganglia as part of the normal pattern.' However, as Ernst (1926) has observed in a series of normal Amniote embryos, cell degeneration is also to be found within the dorsal root ganglia at limb levels.

The reduction in size of limb ganglia which are prevented by various means from innervating a limb has been observed in a series of studies on various vertebrates (Piatt, 1948; Hughes & Tschumi, 1958), though the effect has nowhere been studied in greater detail than by Levi-Montalcini and her colleagues.

In *Xenopus*, however, before the trophic influence of the limbs is exerted upon the limb ganglia, the effect of intrinsic factors is already recognizable (Hughes & Tschumi, 1958). As is often true, self-differentiation precedes dependent differentiation. The self-differentiating factors in the limb-ganglia of *Xenopus* are in operation before any nerve fibres have entered the limbs; already these ganglia contain a larger number of cells than do the adjacent members of the series, and in them the maximum cell size is restricted to a level below that seen elsewhere among the dorsal root ganglia.

In *Eleutherodactylus* the relative differences in cell number among the dorsal root ganglia at limb and non-limb levels are already evident as soon as the ganglia are separately recognizable and a day before any nerve fibres enter the limb-buds. Here neither differential rates of mitosis nor selective cell-degeneration play any part in shaping the final relative sizes of the spinal ganglia. Within them the mitotic rate never rises above a relatively low level, and cell degeneration is found to an equal extent throughout the series of ganglia. Cells degenerate both in the limb ganglia and in the ventral horns of *Eleutherodactylus* notwithstanding any trophic influences which emanate from the limbs. Glücksmann (1951), in a valuable and comprehensive review, has shown that cell death is associated with many aspects of vertebrate development in a range of tissues and at various stages of differentiation. Cell degeneration must constitute an element in several distinct aspects of the development of the nervous system, and these must vary in importance from one example to another.

*Eleutherodactylus* is admittedly something of a special case. Its ontogeny is adapted not only to an embryonic mode of development, but also to the minute size of the future adult in such respects as the small number of cells in dorsal root ganglia and ventral horns. Furthermore, development in *E. ricordii* is particularly rapid, as has already been noted. In other vertebrates, as far as is known, the number of cells in the dorsal root ganglia is mainly controlled by peripheral
factors, such as the formative stimuli which emanate from the limb-buds. Such dependent differentiation must need a minimum time-scale on which to operate. To determine how far the rapid development of E. ricordii restricts the scope of these influences, however, demands the crucial experiment of limb ablation. The relative importance of each of Roux' complex components in various patterns of ontogeny would be an important topic in a comparative neuro-embryology.

**SUMMARY**

1. In the West Indian Anuran genus *Eleutherodactylus* the larval stage is wholly suppressed. As in other systems of organs there are features in the spinal cord which are correlated with this embryonic type of development.

2. The cord in the trunk region resembles more that of a chick embryo than that of a larval Amphibian. Within the thick neuroepithelium mitoses in the ependymal zone persist much longer than in larval forms. There is a relatively thin layer of white matter.

3. The Rohon–Beard system of sensory neurones persists, but does not precede the development of the dorsal root ganglia. In the trunk there are no conspicuous primary motor-cells as in larval Amphibia.

4. The numbers of cells within the dorsal root ganglia and ventral motor horns have been counted both in the embryo and in the adult. From the first, the numbers of cells present in both are similar to those in the adult. During development, increase by mitosis is opposed by cell degeneration. Pycnotic nuclei are conspicuous in both spinal ganglia and ventral horns in the embryo.

5. As soon as the spinal ganglia are recognizable, their relative sizes are similar to those of the adult. The ganglia at limb levels are already larger than those elsewhere a day before nerve fibres first enter the limb-buds.

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**REFERENCES**


A. HUGHES—THE EMBRYOLOGY OF ELEUTHERODACTYLUS


EXPLANATION OF PLATE

All figures are of sections through embryos of E. ricordii.

Figs. A, B, and C. Transverse sections of spinal cord in trunk region. A. —11 days. Haematoxylin and eosin. ×140. B. —9 days, showing early stage of lumbar ventral horn (cf. Fig. E). Bodian. ×150. C. —7 days, through lumbar ventral horn at second stage. Haematoxylin and eosin. ×140.

Fig. D. Transverse section through half of spinal cord at —9 days, to show Rohon–Beard cell (extreme top of figure) with axon root. Central canal at left margin. Bodian. ×640.

Fig. E. Transverse section through part of spinal cord at —9 days. Same section as in Fig. B showing lower half of right-hand ventral horn, below which are the first fibres of the longitudinal motor column. Many neuroblasts in ventral horn already bipolar. ×640.

Fig. F. Transverse section through part of spinal cord at —5 days to show lumbar ventral horn
and adjacent mantle layer to left. End stages of cell degeneration between them (arrow). Haematoxylin and eosin. ×640.

**Fig. G.** Transverse section through dorso-lateral part of spinal cord at −7 days. The section is taken through the trunk region, near the junction with the tail, and shows a Rohon-Beard cell still in the original position. Haematoxylin and eosin. ×600.

**Fig. H.** Transverse section through spinal cord in tail region of same embryo as in Fig. G to show Rohon-Beard cell in dorsal position. Haematoxylin and eosin. ×640.

**Fig. I.** Part of horizontal section through dorsal region of spinal cord at −6 days. The axis of the embryo lies across the picture. At upper and lower margins are shown the dorsal funiculi on each side; inwards from each are a row of Rohon-Beard cells. One in upper part of picture (arrow) receives an afferent fibre from the skin. Rohon-Beard cell at lower margin gives off a longitudinal fibre. Bodian. ×650.

**Fig. J.** Part of section through hind limb-bud at −9 days, showing the first nerve fibres which have just entered the base of the limb. Bodian. ×620.

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