J. Zool., Lond. (1967) **153**, 139–152

Embryonic behaviour in the lizard, *Lacerta vivipara*

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(*Accepted 11 April 1967*)

(With 6 figures in the text)

The behaviour of embryos of the lizard *Lacerta vivipara* has been studied in cultured eggs, removed from the mother. Spontaneous movements begin at a time when the embryo is still unresponsive to touch, and about two days before the appearance of reflex responses. The earliest movements consist of lateral flexion which gradually gives place to dorsiventral flexion. Embryonic movements are not necessarily related to contractions of the amnion. The activity of the embryo, as measured by the number of times movements were initiated and the amount of time spent in activity during a half hour period, rises to a plateau and then decreases sharply as the time of hatching approaches. Activity of the tail is prominent, particularly at times when other movements occur in rapid succession. Serpentine movements were not observed in embryos younger than stage 39; some prematurely born animals showed such activity in response to contact with the substratum. During embryonic life any part of the animal may move individually or in combination with any other part, such combined movements being unco-ordinated. In these respects the spontaneous motility of the lizard embryo closely resembles that of the chick. Statistical analysis of the embryonic movements provides evidence of rhythmic activity within the developing central nervous system.

Contents

Introduction

Observations on embryonic behaviour have now been made on members of most classes of vertebrates and are of interest for the light which they throw on the developing nervous system. A fresh approach to the problem has been made by the recent studies of

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Hamburger and his collaborators on the spontaneous activity of the chick embryo (Hamburger, 1963, 1964; Hamburger & Balaban, 1963; Hamburger, Balaban, Oppenheim & Wenger, 1965; Hamburger, Wenger & Oppenheim, 1966). These workers have shown that the embryo spends much of its time in making movements of the head, trunk and limbs which are totally unco-ordinated. They have found that this spontaneous motility forms a distinct pattern of behaviour from that elicited in response to external stimuli; it begins at an earlier period of development than the first reflex responses. A comparable duality of embryonic behaviour has been described in embryos of the anuran *Eleutherodactylus martinicensis;* in this species, however, the responses to stimuli gradually develop into bilateral swimming movements, while spontaneous activity builds up into a pattern of walking movements (Hughes, 1965, 1966).

Opportunities for observing the behaviour of undisturbed amniote embryos have hitherto been very limited, since the latter are concealed not only by their embryonic membranes but also by a shell, or by the wall of the maternal reproductive tract. Previous knowledge of such behaviour in reptiles has been confined to the highly specialized Chelonia in which early trunk movements are soon inhibited by the formation of the shell (Tuge, 1931; Smith & Daniel, 1946). Methods are now available, however, for maintaining the embryos of certain viviparous lizards in culture after removal from the mother (Panigel, 1956; Raynaud, 1959: Holder & Bellairs, 1962). Embryos of the Common lizard (*Lacerta vivipara* treated in this way will often survive throughout the greater part of ante-natal life and ultimately hatch from their membranous eggs. They provide most favourable material for the study of the development of behaviour in a comparatively generalized type of amniote vertebrate.

Material and methods

Studies on the development of *Lacerta vivipara* have been facilitated since the description by Dufaure & Hubert (1961) of a normal series of embryonic stages. Reference to these numbered stages has been made throughout this paper but in some cases it has been necessary to distinguish stages intermediate between those described; designations such as 32/33 for stages between 32 and 33 in the series have been adopted.

Our observations have been made on a total of 14 embryos removed from pregnant lizards collected in the south of England during June and July 1966. After removal from the oviducts the eggs were placed in sterile Petri dishes on gauze moistened with 0·8% saline to which a little chick albumen had been added to reduce the risk of infection (see Raynaud, 1959). The embryos were examined under a Bausch & Lomb Stereozoom dissecting microscope at magnifications of 10 to 20 diameters. The time and nature of every movement was noted over a series of half-hour periods on successive days; in some cases it was found convenient to use a tape recorder. Observations were made at room temperature, mainly within the limits of 22 to 24°C; between observations the embryos were kept either at room temperature or at 28°C, the latter accelerating the rate of development. The earliest embryos studied were at stage 31 (roughly equivalent to a chick embryo of 4½ days) and the oldest at stage 40, the last stage in the Dufaure-Hubert series before hatching (see Fig. 2).

Within its egg the embryo is partly visible through the thin shell and embryonic membranes, but it cannot be seen sufficiently clearly to enable one to distinguish where each individual movement is occurring. Liquid paraffin dropped on the shell rendered it immediately transparent so that the head, trunk and tail, and the limbs on the uppermost side of the body could clearly be seen. Development continued for some days in eggs treated in this way but movement of the embryos ceased after 3 to 7 days; the embryo usually died after a further 1 to 4 days. These eggs failed to increase in size, unlike the untreated ones; probably this was because they were unable to absorb fluid from the medium. It was found that the application of saline to the egg surface rendered it nearly as transparent as the paraffin, though it was necessary to repeat it at intervals of about 10 min. The saline produced no fatalities and several eggs on which it was used hatched after being studied over periods of 4 to 11 days. Comparison between specimens treated with paraffin and saline showed that since the deleterious effects of the former did not become apparent for several days, many of the observations made with the help of this technique could be regarded as valid.

Reflex movements in response to touch and other stimuli were studied either after exposure of the embryo through a hole in the shell and membranes, or after the embryo had been dissected from the egg.

Observations

Movements of the amnion

As in birds and certain other reptiles the amnion is motile (Bautzmann, 1956). Owing to lack of early embryonic material we were unable to ascertain the stage at which movements of the amnion began. They were clearly visible, however, at stage 32 and continued until near-hatching stages (39 to 40). These contractions of the amnion appeared to shift the whole embryo, causing it to pivot round the umbilical region. Each contraction, as indicated by displacement of the embryo, lasted for 30 to 60 seconds; the frequency of the contractions was highly variable, ranging from one to 14 in half an hour. Slight nodding and turning movements of the head were also observed at stage 32; these were perhaps caused by disturbance of the amniotic fluid.

In the later stages (37 to 39) contraction of the amnion was sometimes seen to follow a phase of prolonged embryonic movement, as if the latter had been responsible for setting off the former. Lightly touching or pinching the amnion through a hole in the shell and chorio-allantois also usually initiated amniotic contractions.

In most cases movements of the amnion could be clearly distinguished from those of the embryo and were separately recorded in our data. As in the chick (Hamburger, 1963) the two types of movement are not always causally related; there is no evidence that movement of the embryo is initiated by amniotic contraction, although as previously suggested, the converse may be true. The occurrence of amniotic and prolonged embryonic movement, which sometimes coincided and sometimes occurred separately, is shown in Fig. 1.

Spontaneous movements of the embryo

Undoubted spontaneous movements of the embryo itself were first seen at stage 32/33 but were not observed in slightly younger embryos. These movements consisted of lateral flexions of the trunk which were short in duration (about two seconds) and infrequent, occurring about 15 times in half an hour. They could be directly perceived from the side, but when viewed from above they were evident mainly as changes in the focal level of the embryo during observation. At about stage 33 or 34 these lateral flexions were gradually superseded by dorsiventral movements in which the head jerked upwards, the trunk straightened and the tail partly uncoiled. The first signs of limb motility appeared in embryos of stages 34 and 35. These were at first confined to movements of the whole limb at its base in a plane either transverse or longitudinal to the body. During the succeeding days activity of the limb gradually extended distally and by stage 36 involved movements of the digits. A plucking action of the foot was often seen, with closure of the toes over the plantar surface combined with plantar flexion at the ankle. In some cases only a single part of the embryo moved at once, but in others movements of the head, trunk, limbs and tail occurred together or in various combinations. Activity of the tail was most prominent when movement in general was at its height.

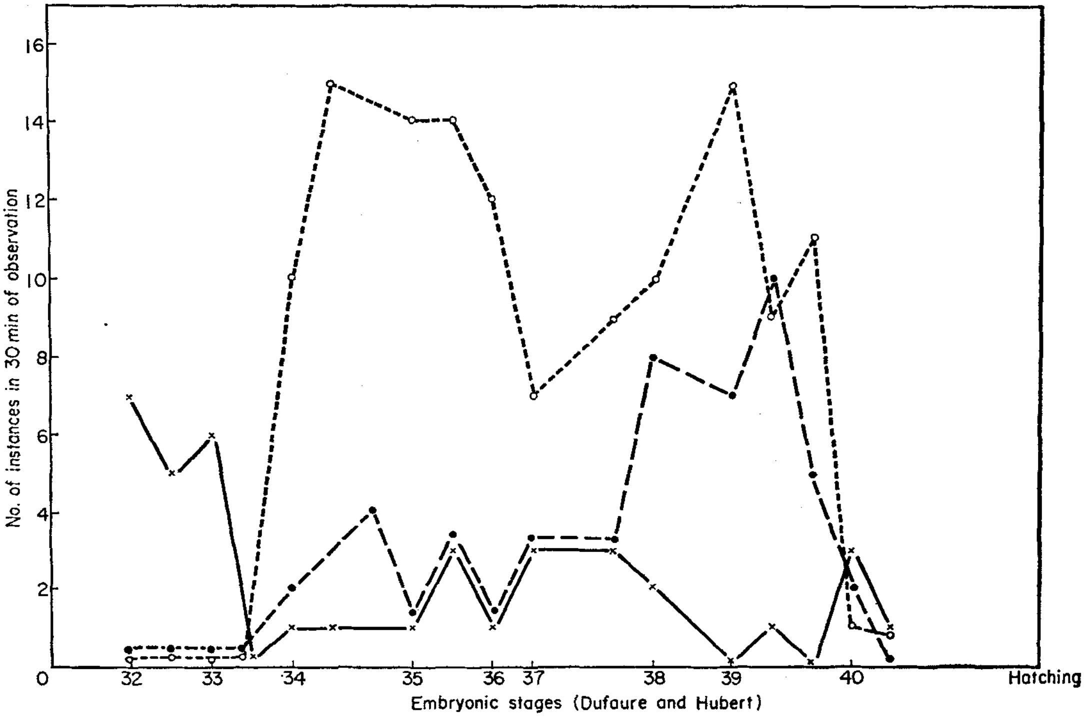

FIG. 1. Graph showing numbers of recorded instances of (a), [○ ---- ○], prolonged movements of embryo, (b), [×———×], contractions of amnion only, and (c), [●— — —●] prolonged movements accompanied by amniotic contraction, at various embryonic stages. Between stages 37 and 39 movements of type (b) are few and of (c) are numerous, suggesting that the amnion is being stimulated by the sustained activity of the embryo.

Many of the movements described, both those involving single parts of the embryo and those which involved two or more parts at the same time were of short duration. It was not possible to time them accurately but they generally lasted for one or two seconds. These may be referred to as brief movements and were most evident at stage 37. Embryos of stage 34 and older, however, also showed phases of prolonged movement which occurred most often at stages 35 and 39. They lasted for periods of five to 40 seconds, during which some or all parts of the embryo motile at the relevant stage were active.

Neither the brief nor the prolonged movements conformed to any constant recognizable pattern or showed evidence of coordination. They were apparently unrelated to locomotion or to any other organized behaviour observed in the newly hatched young.

These types of spontaneous activity, both brief and prolonged, were maintained until nearly the end of embryonic life. They disappeared, however, after the beginning of stage 40, when the embryo entered a phase of quiescence in which almost no movement was observed. This continued until the onset of a final short burst of intense activity which led almost immediately to hatching. The act of hatching in Panigel culture was observed in one specimen. The shell and embryonic membranes were ruptured by powerful thrusts of the snout and it was impossible to say whether the egg-tooth was used. The young lizard remained with its head projecting for 16 minutes, and then crawled out.

Amount of spontaneous activity

An attempt was made to express quantitatively the total amount of spontaneous activity exhibited by embryos at different stages. In order to do this, the activity was assessed in two different ways. First, a count was made of the number of times during any half hour period that a movement, either brief or prolonged, began. The results of this assessment for all the embryos studied at various stages is shown in Fig. 2. Secondly, the time during each half hour period which the embryos spent in activity was calculated. The duration of each of the brief movements, which was too short for accurate measurement by the techniques available, was estimated for the purpose of this calculation to be two seconds long. Accurate measurements of the duration of the prolonged movements had been made at the time of observation. The results of this method of assessment are illustrated in Fig. 3.

It can be seen from Figs 2 and 3 that spontaneous activity increases at about stage 34/35 and declines at stage 40. The number of times which movements are initiated has begun to decrease by stage 39.

Distribution of spontaneous movements

Hamburger (1963) has shown that spontaneous movements of the chick embryo do not occur at random but conform to a rhythmic pattern. This consists of cycles of activity and inactivity which can be directly observed and recorded. In the lizard, however, no similar pattern was directly discernible, and a statistical analysis was made in an attempt to determine whether or not the movements had a random distribution.

Each half hour of observation time was arbitrarily divided into 90 periods of 20 seconds each, and histograms were constructed showing the numbers of these periods in which zero, one, two or more movements, either brief or prolonged, were initiated (see Fig. 4).

Any of the prolonged movements could occupy the whole of a 20-second period; if no compensation was made for this fact, such a period would appear on analysis as one in which no new movement had occurred. Indeed it would seem equivalent to one in which the embryo had not moved at all. It was therefore decided to compensate for the duration of each prolonged movement by extending the 20-second period in which it occurred by the number of seconds which it had lasted. Consequently, the number of periods shown in the histogram (Fig. 4) in which zero movements took place truly represents the number during which the embryo was motionless.

The numbers of periods in which zero, one, two or more movements took place were then compared with those which might have been expected if each movement had occurred

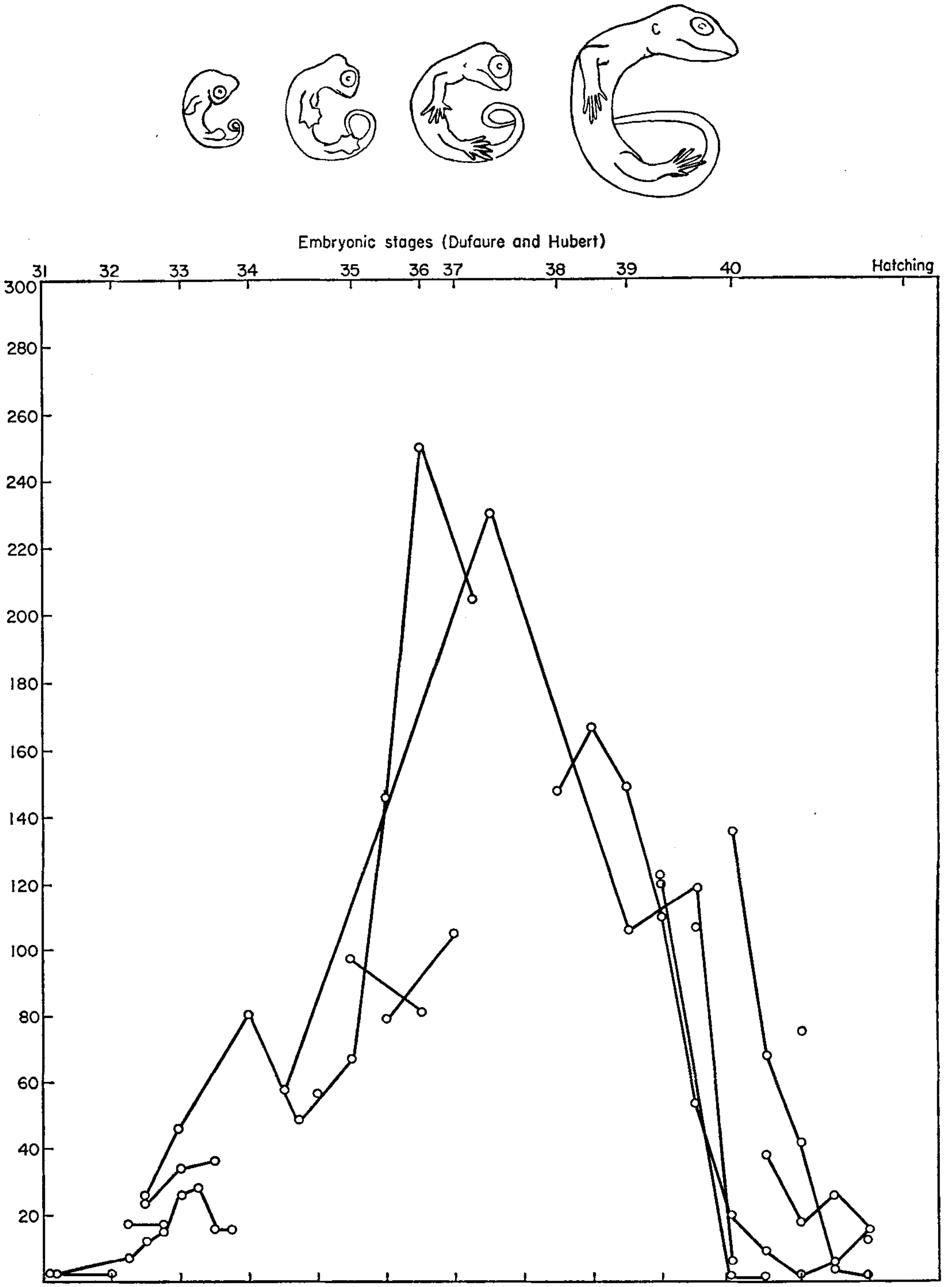

FIG. 2.—*See caption opposite.*

at random, without relation to previous events. Such expected probabilities form a Poisson series (Moroney, 1962), and can be calculated given an overall expectation based on the average number of movements in each period. This analysis was applied to all the embryos studied, with essentially similar results. A graph showing the total number of movements,

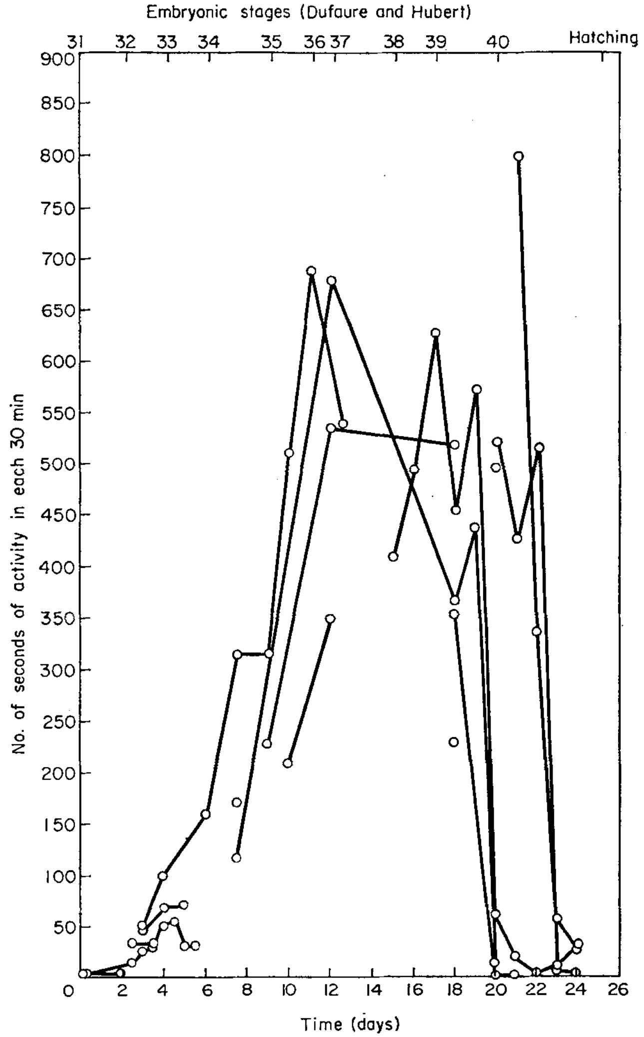

FIG. 3. Graph showing the amount of time spent in spontaneous activity of all embyros studied at all stages between 32 and hatching. Points connected by lines represent successive observations on the same embryo. Isolated points represent single observations on single embryos.

FIG. 2. Graph showing the number of times spontaneous movements were initiated in all embryos studied, at all stages between 32 and hatching. Points connected by lines represent successive observations on the same embryo. Isolated points represent single observations on single embryos. Drawings of embryos at stages 32, 34, 36 and 39 are shown above the graph.

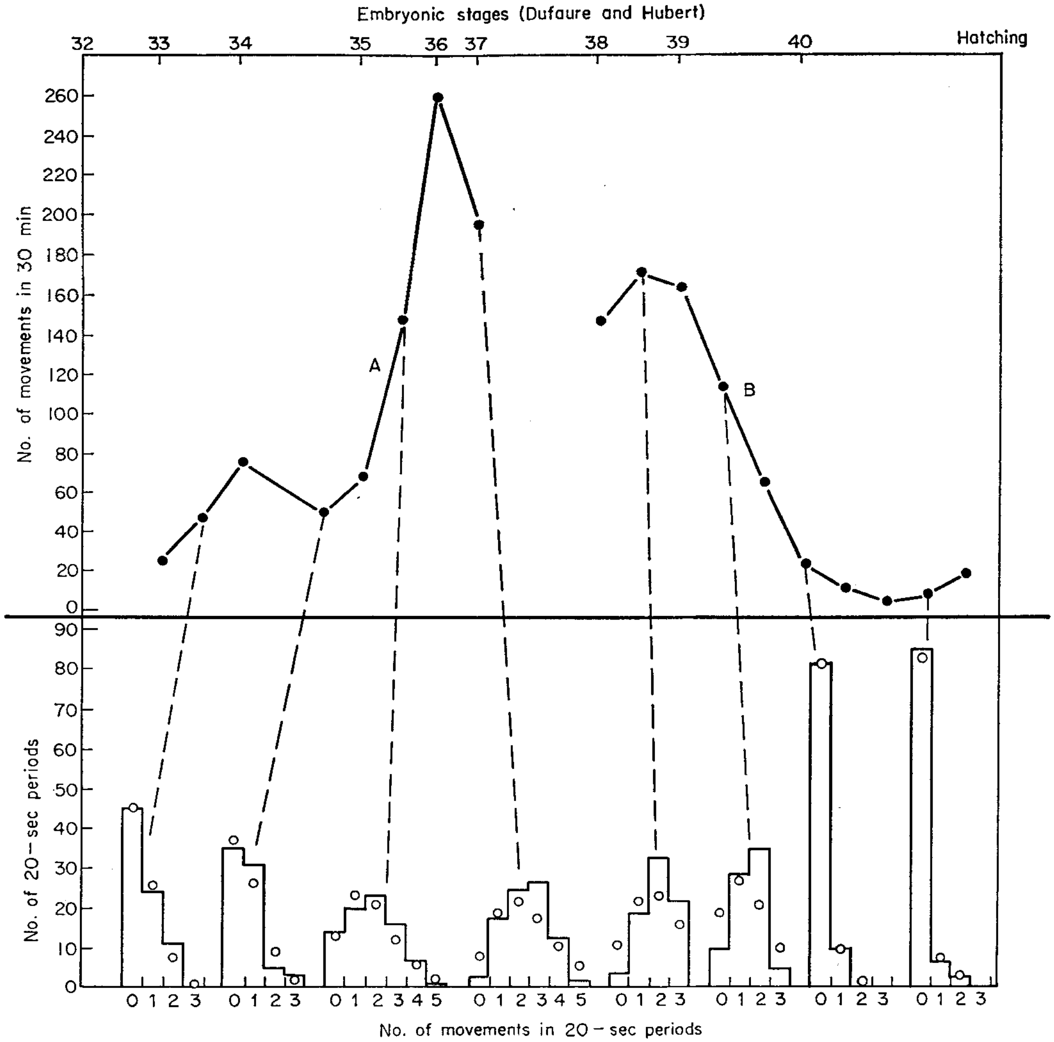

FIG. 4. Diagram to show frequencies of movements. The top graph above the thick line shows the number of recorded movements of two embryos (A & B) at different stages of development. Each point on the graph represents the total number of movements seen in each 30 min of observation.

Below is a series of histograms connected by broken lines with some of the points on the graph; the histograms derived from the other points are not shown for the sake of pictorial clarity. The histograms show the pattern of the movements within the 30 min of observation. Each 30 min has been divided into periods of 20 sec, and the numbers of movements in each of these is recorded. Thus, in the 30 min represented by the histogram on the extreme left, there were 45 periods of 20 sec during which 0 movements took place, 24 in which 1 movement occurred, and 11 in which there were 2 movements.

The frequencies of occurrence of 0, 1, 2, 3 or more movements per 20-sec period which would be expected according to the Poisson distribution are shown as small circles. Where these frequencies correspond with those observed (i.e. as shown by the histograms), a random distribution of movement is indicated. Between stages 35 and 39 they tend to differ more than at other stages from the observed frequencies, indicating that movement here was not random.

both brief and prolonged, seen during each half hour of observation, together with histograms indicating their observed and expected distribution for two embryos is illustrated in Fig. 4. These were the two embryos whose combined life-spans virtually extended from stage 32 to hatching.

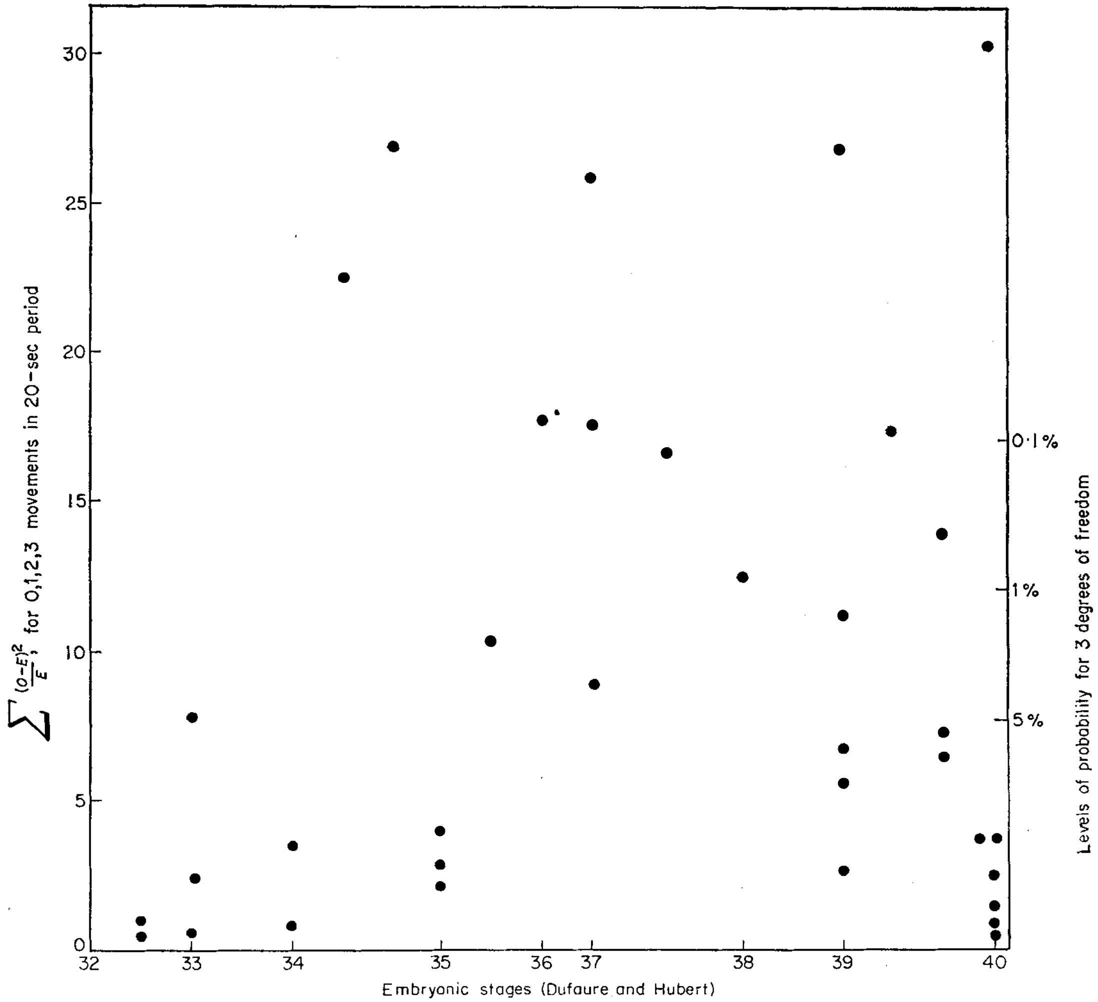

FIG. 5. Chi-square test. Diagram showing significance of the differences between observed and expected values of numbers of 20-sec periods in which 0, 1, 2 and 3 movements were seen, at various embryonic stages. Each point represents the sum of the squares of the differences between observed (O) and expected (E) values of numbers of periods, divided by the expected values. Points between stages 34/35 and 39 are significant.

In Fig. 5 the differences in distribution from that predicted by the Poisson series for all embryos studied is shown by an application of the Chi-square test. Up to stage 34 and after stage 39 most values of Chi-square are consistent with a Poisson (i.e. random) distribution, but between these stages they diverge widely from it.

A further analysis was attempted in order to determine the nature of the non-random behaviour. The mean values of the divergences from the expected, expressed as $O-E/\sqrt{E}$, are shown in Fig. 6, for each number of movements per 20-second period, at three groups of embryonic stages. Each mean value is accompanied by its standard error. It can be seen that from stage 34/35 onwards, there is a less than expected number of periods in which zero movements occurred, and a greater than expected number in which one and two movements occurred.

As the result of our method of compensation for the duration of the prolonged movements, the number of periods in which no new movements were initiated are less than the number which would have been obtained if the 20-second periods occupied by prolonged movements were counted as ones in which no movements occurred. Similarly, the number of periods in which one movement occurred would be greater. The possibility that the tendency towards a non-random distribution of movements during a large part of embryonic life might be a misleading result of this method of compensation could not be ignored. Hence, for two embryos which between them covered almost the whole period of embryonic life, the distribution of movements was recalculated, making no compensation for the duration of prolonged movements. As might be expected, the number of periods in which zero movements occurred was greater in most cases, but once more the differences in distribution of the movements from that predicted by the Poisson series were found to be significant between stages 34 and 39.

It may therefore be concluded that the movements themselves are non-random and have a rhythmic or periodic character during a substantial part of embryonic life.

Reflex movements

Reflex activity in response to external stimuli was found to begin about two days after the onset of spontaneous movement, at stages 34 or 35. Light touch on the trunk would then cause lateral flexion of the body, while stimulation of the tail was followed by lashing movements of this appendage. The limbs at these stages were not responsive. In embryos of stage 36 stimulation of the limbs evoked a withdrawal reaction, or, if it was more severe, flexion of the trunk as well. At stage 37 the embryo would bend dorsiventrally if its back was touched. Jaw movements could also be provoked by touching the head. These responses became stronger at stage 39 but remained essentially of the same character.

At stage 40 the embryos closely resemble the newborn young in appearance. If left undisturbed such embryos would remain for up to a week in culture before hatching. The blink reflex of the eyelids was present, and autotomy of the tail was readily elicited by lightly pinching the organ. Autotomy involves active contraction of the tail muscles and can usually be distinguished from passive rupture which sometimes occurs after manipulation at the earlier stages. As in post-natal lizards, the autotomized portion of tail wriggles for several minutes.

Any severe interference with embryos at stage 40 was likely to be followed by violent thrusts of the head similar to those which normally occur at hatching. In some cases hatching actually took place. Such premature young could often perform walking movements immediately, but others made only a few steps and then tried to wriggle along by serpentine undulations. These latter individuals usually died quite soon, but the more mature ones often began feeding on *Drosophila* larvae within 48 hours, and were probably as viable as normal newborn.

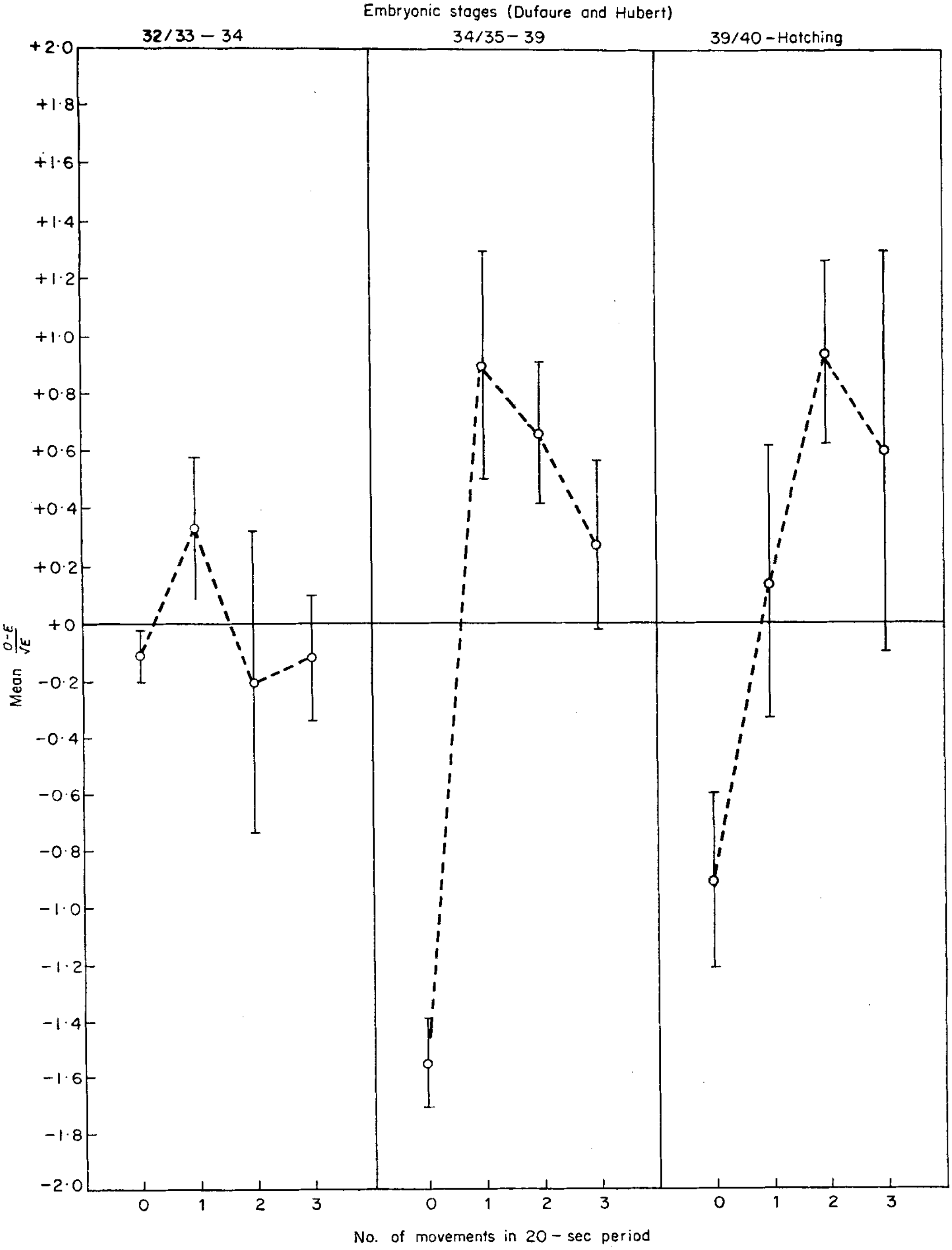

FIG. 6. Diagram showing the direction of the divergences, at three stages of embryonic development, of the observed (O) from the expected (E) number of periods in which there were 0, 1, 2 and 3 movements, expressed as mean $O-E/\sqrt{E}$. Each mean value is accompanied by its standard error. Values above the line at nought are positive divergences, and those below negative ones. It can be seen that after stage 34/35, large divergences occur, and the number of periods in which no movements take place is less than expected, while that in which 1 and 2 movements occur is greater.

Discussion

Our observations show that in embryos of the common lizard spontaneous movements precede reflex responses, as in the embryos of all other forms where distinction between two types of activity has been drawn (*Terrapene*, Tuge 1931: chick, Hamburger, 1963: *Eleutherodactylus*, Hughes, 1966). In all these the earliest spontaneous movements consist of lateral trunk flexion of a type originally described by Coghill (1929) in the larva of *Amblystoma*. In this animal such 'C flexion' is followed by the appearance of a more elaborate swimming movement known as 'S flexion' in which the body is thrown into lateral undulations which progress in a cranio-caudal direction. In the chick, too, there is a phase of 'S flexion', between four and a half and six and a half days of incubation, but this is transient and is succeeded by another phase when movements become uncoordinated (Hamburger, 1964).

Lateral or serpentine undulations form an important component of the locomotor pattern, both aquatic and terrestrial, of many of the more primitive tetrapods such as urodeles and long-bodied reptiles. This pattern was presumably taken over from piscine ancestors, and one might expect it to appear both consistently and early in ontogeny. The fact that we failed to observe it, either as a spontaneous or as a reflex movement in *Lacerta* embryos much younger than stage 39 is surprising. Possibly such movements occur very transiently, or are inhibited by the cramped surroundings of the egg; they were apparently absent, however, even in specimens which had been dissected free of their membranes. Confirmation of this point and comparison with the embryonic behaviour of a limbless lizard or a snake would be of interest.

Smith (1964) has described the hatching of *Lacerta vivipara* under normal circumstances, when the young may escape from their eggs immediately after these are laid, or after an interval of hours or even days. His account strongly suggests that hatching movements are co-ordinated and purposeful in the sense of being directed towards rupture of the membranes. Co-ordinated movements were certainly possible in the premature young which we obtained, but in our single specimen which was seen to hatch from an undisturbed egg, the perforating thrusts of the head could have been interpreted as random movements. Presumably the initiation of hatching activity after a phase of quiescence depends upon some inherent physiological mechanism, as it must in typically oviparous reptiles and in birds. It is also possible, however, that it is sometimes naturally provoked by mechanical disturbance when the eggs are expelled from the cloaca; as we have found, interference may lead to hatching in culture.

Apart from the apparent absence of 'S flexion' in *Lacarta*, its embryonic behaviour resembles that of the chick in most respects. There is the same lack of co-ordination between movements of each part of the body; unlike the amphibians studied by Coghill (1929) and by Hughes (1965, 1966), these embryos do not develop integrated patterns of behaviour, at least until very late in embryonic life. Again, activity rises to a peak or plateau in the older stages and then declines as the time of hatching approaches. Finally, for a considerable period, the movements of the embryo have a non-random or rhythmic frequency. These similarities are evident from comparison of our results with those of Hamburger and his collaborators. It is true that overall frequency of movement is lower in the lizard than in the chick, but this may be due merely to the fact that the former has a lower metabolic rate, at least at the temperatures at which our observations were made.

These resemblances suggest that the underlying neural mechanisms are much the same in the lizard as in the chick. Hamburger *et al.* (1965, 1966) have shown that neither transection of the spinal cord nor removal of the sensory input substantially affect the pattern of movement of the chick, and have concluded that the alternating periods of activity and inactivity originate from rhythmic excitations of the spinal cord. Alconero (1965) has also shown that in grafts of chick spinal cord grown on chorio-allantoic membrane with the associated musculature a similar alternation of movement and rest is apparent.

Such rhythmic activity is not confined to forms with prolonged embryonic development. The earliest swimming movements of the larvae of *Xenopus* and *Rana* are seen during bursts of activity which are separated by quiescent periods. Similar patterns have been observed both in larvae where the medulla and cord have been isolated from the rest of the brain, and in explants of the larval cord and related somites (Corner, 1964; Corner & Crain, 1965). It seems likely that these observations are all indicative of a basic pattern of activity in the developing spinal cord of vertebrates, upon which more complex relationships are imposed during ontogeny. One of these is presumably the advent of overall inhibition leading to the phase of quiescence before hatching.

We are most grateful to Dr H. G. Britton, Professor F. Golby, Mr M. Prestige and Dr G. A. Rose for advice. This work was aided by a grant from the Medical Research Council.

REFERENCES

Alconero, B. B. (1965). The nature of the earliest spontaneous activity of the chick embryo. *J. Embryol. exp. Morph.* **13**: 255–266.

Bautzmann, H. (1956). Fruchthüllenmotorik und Embryokinese. Ihre Natur und ihre Bedeutung für eine physiologische Embryonalentwicklung bei Tier und Mensch. *Arch. Gynaek.* **187**: 519–545.

Coghill, G. E. (1929). *Anatomy and the problem of behaviour.* Cambridge: University Press.

Corner, M. (1964). Rhythmicity in the early swimming of anuran larvae. *J. Embryol. exp. Morph.* **12**: 665–671.

Corner, M. & Crain, S. M. (1965). Spontaneous contractions and bioelectric activity after differentiation in culture of presumptive neuromuscular tissues of the early frog embryo. *Experientia* **21**: 422–424.

Dufaure, J. P. & Hubert, J. (1961). Table de développement du lézard vivipare: *Lacerta (Zootoca) vivipara* Jacquin. *Archs Anat. microsc. Morph. exp.* **50**: 309–327.

Hamburger, V. (1963). Some aspects of the embryology of behaviour. *Q. Rev. Biol.* **38**: 342–365.

Hamburger, V. (1964). Ontogeny of behaviour and its structural basis. In *Comparative neurochemistry:* 21–34. Richter, D. (Ed.) London: Pergamon Press.

Hamburger, V. & Balaban, M. (1963). Observations and experiments on spontaneous rhythmical behaviour in the chick embryo. *Devl Biol.* **7**: 533–545.

Hamburger, V., Balaban, M., Oppenheim, R. & Wenger, E. (1965). Periodic motility of normal and spinal chick embryos between 8 and 17 days of incubation. *J. exp. Zool.* **159**: 1–14.

Hamburger, V., Wenger, E. & Oppenheim, R. (1966). Motility in the chick embryo in the absence of sensory input. *J. exp. Zool.* **162**: 133–160.

Holder, L. A. & Bellairs, A. d'A. (1962). The use of reptiles in experimental embryology. *Br. J. Herpet.* **3** (3): 54–61.

Hughes, A. (1965). The development of behaviour in *Eleutherodactylus martinicensis* (Amphibia, Anura). *Proc. zool. Soc. Lond.* **144**: 153–161.

Hughes, A. (1966). Spontaneous movement in the embryo of *Eleutherodactylus martinicensis. Nature, Lond.* **211**: 51–53.

Moroney, M. J. (1962). *Facts from figures.* Harmondsworth: Penguin Books Ltd.

Panigel, M. (1956). Contribution à l'étude de l'ovoviviparité chez les reptiles: gestation et parturition chez le lézard vivipare *Zootoca vivipara. Annls Sci. nat.* (Zool.) (11) **18**: 569–668.

Raynaud, A. (1959). Une technique permettant d'obtenir le développement des oeufs d'orvet (*Anguis fragilis* L.) hors de l'organisme maternel. *C. r. hebd. Séanc. Acad. Sci., Paris* **249**: 1715–1717.

Smith, K. U. & Daniel, R. S. (1946). Observations of behavioural development in the loggerhead turtle (*Caretta caretta*). *Science, N.Y.* **104**: 154–156.

Smith, M. (1964). *The British amphibians and reptiles.* (3rd ed.). London: Collins.

Tuge, H. (1931). Early behaviour of embryos of the turtle, *Terrapene carolina* (L.). *Proc. Soc. exp. Biol. Med.* **29**: 52–53.