from the basal turn of guinea pig cochlea by means of vestibulo-tympanic leads, in the manner of Tasaki (*12*). When TEA was introduced into either the hair-cell region of the organ of Corti or the scala media, with a current of 8×10^{-6} amp for 10 minutes, there was a gradual irreversible decrease of the CM responses, which lost about 50 percent of their initial value within 30 minutes (Fig. 1). The decrease in CM responses, caused by TEA, was almost the same for these two regions, although remarkable differences are evident when acetylcholine is administered to the same two regions (*4*). The effects of TEA on the N_1 responses, not marked, resembled those following application of acetylcholine (*4*). The endocochlear d-c potential was decreased to 50 percent of its original value after the introduction of TEA into the scala media, but no change followed introduction into the hair-cell region.

Following iontophoretic introduction of tetrodotoxin into the hair-cell region, the CM showed almost no change, while the action potentials diminished gradually to less than 50 percent of their original amplitudes 30 minutes after the application commenced (Fig. 2). This depression of N_1 was irreversible.

From these results we can draw certain inferences concerning the receptor mechanism of the hair cells in the cochlea. Tetrodotoxin caused no change in the CM responses, which we regard as receptor potentials; this finding suggests that CM involves an ionic mechanism different from that of nerve action potential; it also agrees with results obtained with other receptor organs (*11*) in which the receptor-generator potentials were not affected by tetrodotoxin.

If TEA suppresses the increase in K-conductance, depression of the CM responses by TEA suggests that the CM represent changes in the membrane potential of hair cells, caused by changes in the rate of movement of potassium ions through this membrane, controlled by movements of the hairs on the cells.

When applied to the hair-cell region TEA did not decrease the endocochlear potential; in this instance the reduction in CM response was presumably caused only by the change in K-conductance of the hair cells. When TEA was introduced into scala media, however, the reduction in endocochlear potential probably contributed to the decrease in CM response. Presumably K-conductance is implicated in maintenance of the endocochlear potential by stria vascularis.

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1 February 1966

Electroretinogram of the Frog during Embryonic Development

Abstract. *Eyes removed from frog embryos at various stages of development gave a definite pattern of change in the electroretinogram. From the 7th to 9th days the electroretinogram consisted of slow, purely cornea-negative potentials. From the 9th to 10th days the responses were negative but included a prominent fast, negative component superimposed on the slow potentials. During the 11th to 17th days positive potentials appeared and developed. From the 20th day on, the typical electroretinogram of the adult obtained.*

During development of the vertebrate retina the electroretinogram (ERG) undergoes striking changes in form. In the house mouse, for example, the first appearance of the ERG at the 13th to 14th postnatal days of development revealed a response comprised of a prominent, initial, cornea-negative *a*-wave which was elicited even at low light-intensities (*1*). At similar intensities for the eye of the 21-day-old mouse the ERG consisted of large positive *b*- and *c*-waves without preliminary negative activity. The ERG of the 10-day-old albino rat was also significantly different from that of the adult, consisting of a small, duplex, negative response containing an initial fast wave followed by a slower wave (*2*). Predominantly negative activity in the early ERG was observed in the dog, rabbit, and chick (*3*). Contrary to these results are reports that the *b*-wave, rather than the *a*-wave, is the first to appear in the kitten and the albino rat (*4*).

We are here concerned especially with the results reported for the developing frog eye. The early ERG from *Rana temporaria* showed no negativity but only a small, monophasic, positive wave (*5*). For the same species of frog, Muntz (*6*) made the definite statement that "as soon as the ERG could be recorded at all, the components of the adult ERG were immediately apparent. . . ." Is the frog, then, an exception to the developmental sequence observed in the mouse, rat, dog, rabbit, and chicken? Our work was initiated to clarify this point. We have found that the earliest ERG of *Rana pipiens* does, in fact, consist only of negative components, that there is both a slow and fast wave in this ERG, and that a very orderly sequence of change occurs in the ERG of the developing eye.

We studied 141 isolated eyes obtained from embryos of accurately known age. Four different batches of eggs were employed; fertilization times were known to be on 23 April 1964, 13 February 1965, 10 March 1965, and 29 March 1965. Starting with the

6th day of development, the eyes were removed and the responses to flashes of white light were recorded. For recording, each eye was placed, cornea side up, on a bed of solid Ringer-agar in a petri dish. Glass electrodes, of tip size 100 microns, were filled with Ringer-agar and shielded from light by a coat of flat black paint on the outside. Silver wire coated with silver chloride was used to contact the Ringer-agar. The electrodes, one in gentle contact with the cornea, the other in contact with the agar at the base of the eye, were connected to an amplifier with a long time constant (10 seconds). Recording was done in the usual manner, with a cathode-ray oscillograph.

For purposes of description the responses have been classified as falling within four periods, according to their properties.

Period I: 7- to 8-day-old animals. Electrical responses appeared first in eyes from 7-day-old embryos. These responses, though somewhat variable with respect to magnitude of potential and detailed wave form, were invariably cornea-negative and consisted of slow waves (Fig. 1). Individual eyes responded to light flashes for periods varying from 15 to 60 minutes, after which the potentials declined and disappeared. No electrical activity was observed on illumination of the electrodes alone or on illumination of an eye that had been deliberately inactivated by pressure applied by means of the corneal electrode. Similar, slow, negative potentials were also recorded from eyes taken from 8-day-old animals (Fig. 1). In no eye from period I was any evidence obtained of positivity or of fast-wave activity.

Period II: 9- to 10-day-old animals. In some of the eyes from 9-day-old embryos and all the eyes from 10-day-old animals a new feature appeared in the electrical responses. This was a fast, negative wave occurring early during the rising phase of slow-wave negativity (Fig. 1). This fast component was of varying prominence in records from different eyes and was either distinctly separate from the slow wave or partially fused with it.

Period III: 11- to 17-day-old animals. On the 11th day of development a new feature, corneal positivity, made its appearance. At first inconspicuous (Fig. 1), this positivity increased during the next 6 days to become the dominant feature of the ERG (Fig. 1). The ERG during this period was similar to that of the fully developed larva, except for one property, and that was the striking tendency for the responses to transform from positive to negative, or, conversely, from negative to positive. With some eyes the potentials, at first positive, as shown in record 1 (Fig. 2), changed slowly and spontaneously to purely negative activity (record 2, Fig. 2). For other eyes, the reverse change—negative to positive—occurred. These changes occurred during periods of 20 to 50 minutes while the eyes were on

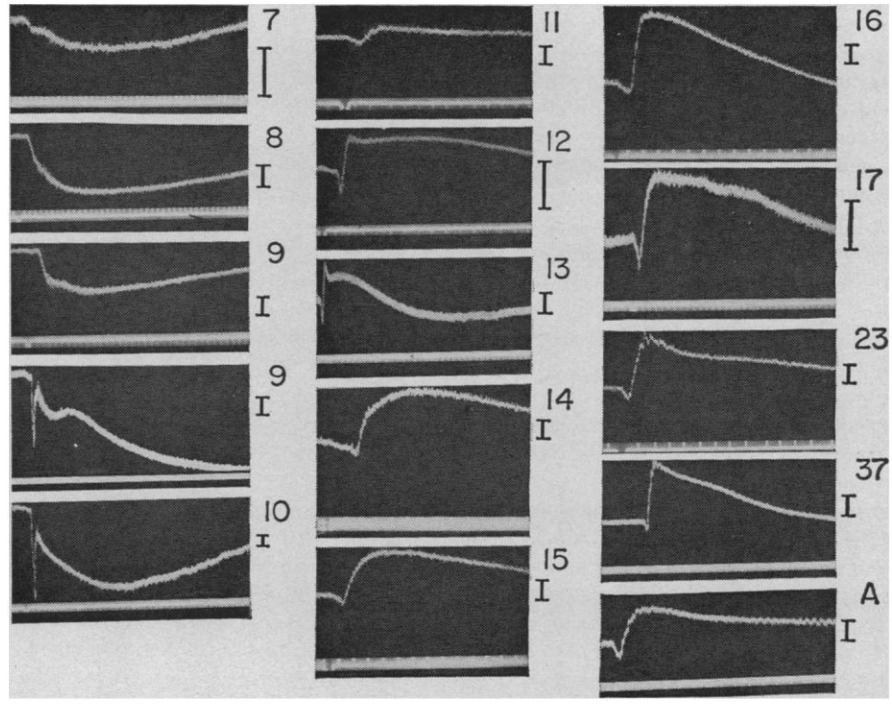

Fig. 1. Electrical responses, each to a 33-msec flash of white light, at various stages in development, these being indicated by numbers next to each record which give the days after fertilization of the eggs. Record A (bottom right) indicates the response from the eye of an adult. Development occurred in pond water in the laboratory, at a temperature of 20°±2°C. The lower line beneath some records indicates 100-msec intervals as well as the stimulus signal. Vertical lines drawn next to each record indicate 100-μv calibrations. The energy of the stimulus light, measured at the position of the eye, was 200 erg cm^{-2} sec^{-1}. This figure was obtained with a calibrated Kettering radiometer.

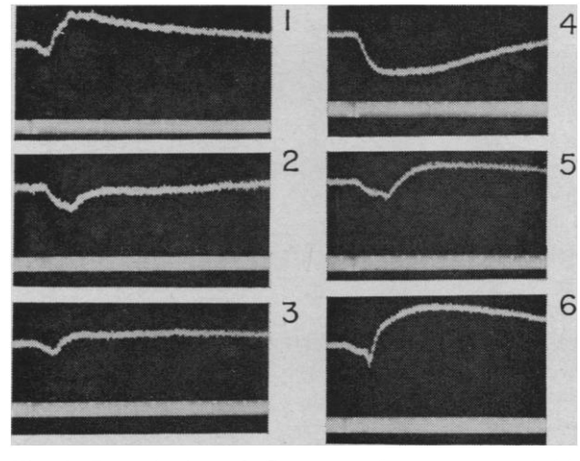

Fig. 2. Records 1 to 6. Responses from an eye at 14 days of development, showing the negative-positive lability characteristic of period III.

the agar in the dark. In addition, it was possible, by means of adaptation, to manipulate the responses so as to produce negative or positive potentials. An example of such a manipulation is pictured in Fig. 2. Starting with the response of record 2, the eye was illuminated with light at 600 nm, used because eyes at this stage are very sensitive to these longer wavelengths; during this steady illumination the potentials elicited by flashes of white light superimposed on the orange adapting field were observed. Over a period of 10 minutes of light adaptation, evidence of a growing positivity was obtained, the record secured at the 10th minute being shown as record 3 (Fig. 2). The adapting field was then turned off; 1 minute later the response to the white light flash had become quite large and purely negative (record 4). The eye was then allowed to remain in the dark, during which time it gave responses which changed again to a form (records 5 and 6) consisting of a positive wave following an initial negative deflection. We do not understand the basis for this complex lability at this stage. Light and dark adaptation appear to be involved, but other factors, as yet undetected, also may be concerned. All that we wish to point out at this time is that this period of lability is unique and that this behavior may be employed to differentiate the eyes prior to period III and following period III.

Period IV: after the 17th day. In larvae older than *about* 17 days (this time has not been critically examined) the ERG showed all the characteristics of the ERG of the adult larva. No lability like that associated with period III was noted, and the records showed positivity from the first moment of recording and the positivity increased with dark adaptation in a typical manner. This sequence of change covered by these four periods was recorded with striking regularity in all four batches of embryos. In addition, it was also duplicated in a study of embryos of the bullfrog (*Rana catesbiana*).

The visual cells of the frog retina develop, typically as in other animals, after the neural layers have formed (*7*). Nilsson (*8*) observed the first appearance of the outer segment discs in *Rana pipiens* to be at the age of 5 days and 20 hours. The first electrical activity which was recorded was therefore about 1 day later than this. This early electrical activity was characteristically different from that elicited from the fully developed retina. This is in agreement with the results of some previous workers (*1–3*) but not with those of others (*4–6, 9*). The disagreement is only an apparent one in some cases. Kennedy (*9*), for example, employed tadpoles of *Rana pipiens* which were collected in the field and which were not staged. In a letter to us, Kennedy explained that the animals "were as near to the point of metamorphosis as possible, simply for size reasons." Thus his animals were well outside what we have here called period III. With respect to Muntz's results (*6*), we have a letter from him which states that the spawn was released in the laboratory on 20 March 1963 and that it was 2 April before an ERG was recorded. This would place his embryos from which he first recorded an ERG at the 13-day-old stage, well into the period of *b*-wave development. There is no difference, therefore, between our results and those obtained by Muntz. We have no explanation for the failure of Müller-Limmroth and Andrée (*5*) to record a unique ERG in the early stages of development. Their animals, when first tested, appear to have been young enough for the early, negative responses to have shown themselves. We conclude, therefore, that our results are in essential agreement with those who assert the existence of a change in form of the ERG during development of the retina.

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Cutaneous Water Loss in Reptiles

Abstract. *Cutaneous and respiratory evaporation were compared in five species of reptiles at 23°C. There seemed to be a clear correlation between water loss and aridity of the animal's habitat, total evaporation from the desert lizard* Sauromalus obesus *being about 5 percent of that from the crocodilian* Caiman sclerops. *Cutaneous evaporation was the major avenue of water loss in all animals examined. This is contrary to the common belief that reptilian skin is practically impermeable to water.*

Reptiles phylogenetically represent the first vertebrates to become truly terrestrial. As opposed to the Amphibia they are considered to have an integument quite impermeable to water, and it has often been emphasized that this was important in their adaptation to terrestrial conditions. Recently, however, we have shown that the crocodilian *Caiman sclerops* loses water by evaporation at a surprisingly high rate, one-half to one-third as fast as amphibians (*1*). It seems probable that this reflects a greater diversity in permeability of the integument in different reptile groups than was formerly believed.

In our study we have compared cutaneous and respiratory water loss in two species of turtles, two species of lizards, and one crocodilian, which represent three different evolutionary lines. We found large differences in both cutaneous and respiratory water losses which seem correlated with the environments in which these animals usually live.

Evaporation was estimated from weight loss of the animal with a correction for weight loss due to metabolic loss of carbon. Urine and fecal losses were either prevented by taping or measured by cannulating the cloaca. Weight loss from the head (principally respiratory tract) and from the rest of the body (skin) was measured separately by keeping the body in a polyethylene bag (thickness, 0.1 mm), containing a desiccant to prevent possible diffusion loss of water, which was sealed around the neck with adhesive tape. Water loss from the head was obtained by placing the animal in a plastic chamber, passing dry air slowly over the animal, and then determining the weight loss. By subtracting the loss