Electrical Response and Growth of Olfactory Cilia of the Olfactory Epithelium of the Newt in Water and on Land

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ABSTRACT A correlation between the length of the olfactory cilia and the electrical activity of the olfactory epithelium was studied in newts living in water and on land. The olfactory cilia grew when newts were transferred onto land. The cilia in the olfactory bud became longest in 108 hours after the transfer and then became shorter, while those in the interstitium only gradually elongated. Slow potentials were evoked in the epithelium by the application of odorous fluids but not by odorous vapors for 20 hours after the transfer. Thereafter, the slow potential began to appear in response to odorous vapors and reached maximal magnitude between 60 and 70 hours after the transfer, while it was not evoked by odorous fluids in this period. In the later stage, the slow potential to odorous vapors decreased in magnitude and disappeared 120 hours after the transfer, while it began to reappear in response to odorous fluids. When these changes in the slow potential were compared with those in the cilium, a discrepancy was found between the period of maximal potential magnitude and that of maximal cilium length.

The slow potential evoked in the olfactory epithelium by the application of odorous vapors has been studied in some terrestrial animals, such as the dog, rabbit, and frog (Hosoya and Yoshida, 1937; Ottoson, 1954, 1956, 1958). It was later found that in the frog and toad there are three types of responses, namely on- , on-off- , and off-slow potentials (Takagi and Shibuya, 1959, 1960 *a*, *b*, *c*; Takagi, Shibuya, Higashino, and Arai, 1960; Higashino, Takagi, and Yajima, 1961). On the other hand, Shibuya (1960) reported that similar slow potentials of three types could also be recorded from the olfactory epithelium of some fishes when an odorous fluid was applied. However, when an odorous vapor was applied to the olfactory epithelium of a fish, or conversely when an odorous fluid was applied to the olfactory epithelium of a frog or toad, no slow potential generally appeared (Takagi, 1961). It seems that there is no striking microscopic difference between the olfactory epithelia of frogs and toads and those of fishes, and the cause of this functional difference is entirely unknown.

A newt generally lives in water. When food, such as small pieces of earthworm or other animal, is put on the bottom of an aquarium, a newt turns its head vertically downward, showing a posture suggesting smelling, reaches, and then eats it. This behavior persists after the eyes are destroyed, but not after the olfactory nerves are cut or after the nostrils are closed with small plugs. It reappears, however, when the plugs are removed. Consequently, it is concluded, in agreement with earlier workers, that behavior of the newt in the presence of food largely depends on olfactory sensitivity (Reese, 1912; Copeland, 1913; Matthes, 1924 *a*).

On the other hand, it is known that a newt on land can find prey by odor (Matthes, 1924 *b*) and moreover can locate a certain place by odor (Czeloth, 1931). Therefore, the newt can detect odor in both air and water. Matthes (1927) studied the structure of the olfactory epithelium at intervals of several hours after newts had been transferred to land, and found the olfactory cilia extending rapidly. He supposed that generation of sensibility to odorous vapors may be due to such extension of the olfactory cilia. It has already been shown that the olfactory epithelium of a newt on land produces slow potentials in response to stimulation with odorous vapors (Shibuya and Takagi, 1962).

In this paper, the changes of the slow potentials evoked by the application of both odorous fluids and vapors are studied in relation to the growth of the olfactory cilia, at successive intervals after newts are transferred from water to land. The above hypothesis by Matthes (1927) is discussed.

MATERIAL AND METHODS

Material Newts, *Triturus pyrrhogaster* Boie, of about 12 cm body length, were used. They were kept in an aquarium with water about 25 cm deep. They usually lived deep in the water and only occasionally put their heads partly out of water for respiration. Some of these newts were then transferred to a terrarium, similar to the aquarium in size but with about 2 cm of mud on the bottom, and were kept in a terrarium throughout the experiment. To study the changes in magnitude of the slow potentials produced by the application of odors and the changes in length of the olfactory cilia, three to five newts were taken out in each of the several successive periods of adaptation to land.

Olfactory Cilia and Their Growth Small balls of cotton wool moistened with ether were placed in the mouths of newts from water or land for 1 to 2 minutes (*cf.* Ai and Takagi, 1963). Thus, under narcosis, newts were decapitated, and the olfactory cavity was exposed by removing the dorsal skin and bones of the head.

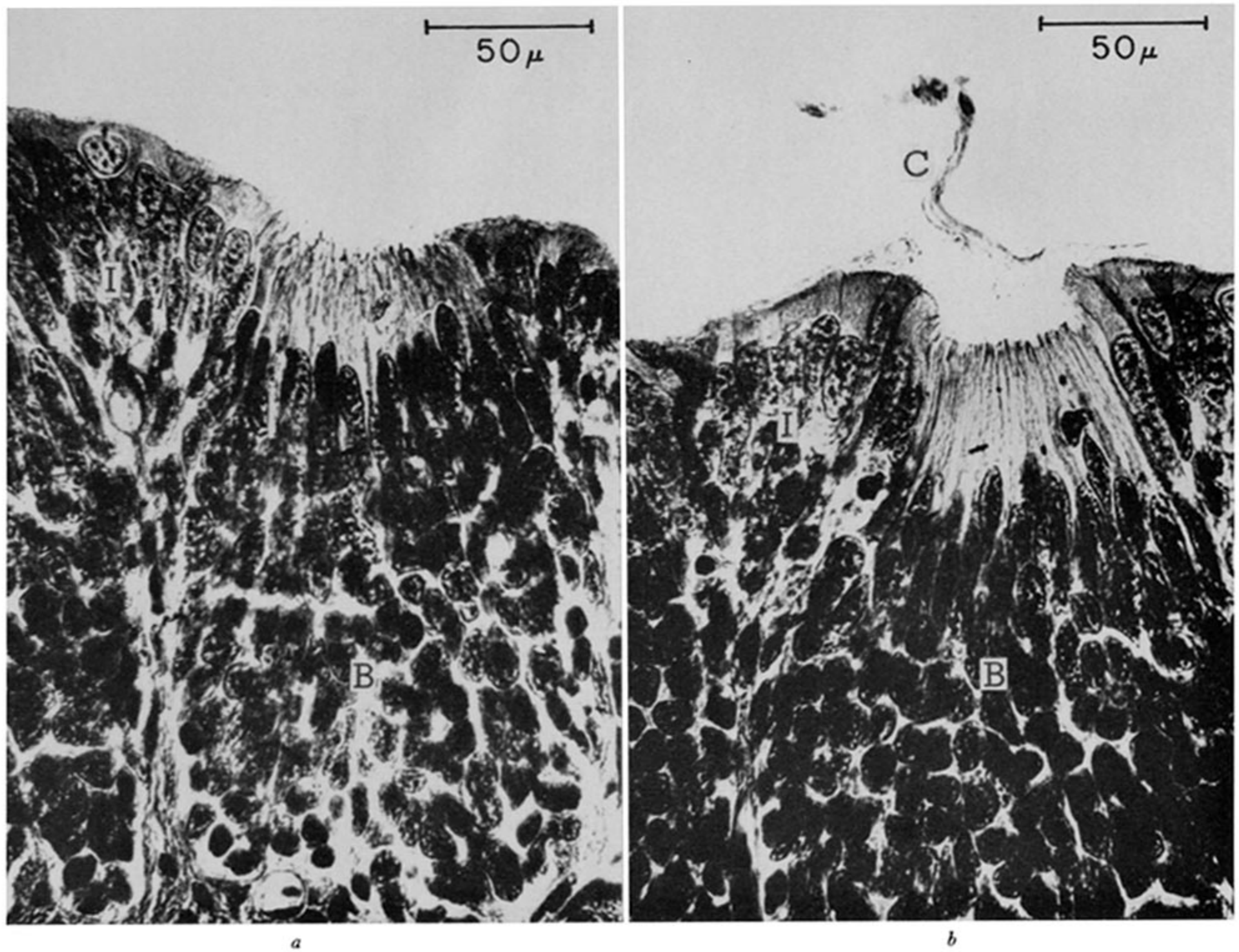

Figure 1. *a*, A stained section of the olfactory bud of a newt in water. *b*, A section showing elongated cilia of the olfactory bud of a newt on land about 100 hours (the olfactory cilia were gathered by fixation and dehydration of the tissue). *B*, olfactory bud. *I*, interstitium. *C*, olfactory cilia in a clump extended in length by accumulation of loosened interstitial cilia.

The olfactory sense organ of the newt is a slender tube composed of dorsal and ventral olfactory epithelia (Matthes, 1927) and lacks the olfactory eminentia found in the frog and toad. The olfactory epithelium of the newt used in this experiment is about 0.3 mm thick and light yellow-brown. When a stained section of the epithelium is observed under a microscope, two parts: the "olfactory bud" and the "interstitium between the olfactory buds" can easily be discriminated. They are arranged in juxtaposition. An olfactory bud is bottle-shaped, and the bud is composed of many olfactory and sustentacular cells (Fig. 1). The interstitium is also composed of many cells and occupies the space among the olfactory buds. The cilia are found on the surface of these two tissues.

To measure the length of the olfactory cilia, the ventral portion of the olfactory epithelium was excised in Ringer's solution, the tissue was taken to pieces on a slide glass, and the cilia length was measured with an ocular micrometer in the microscope. The movement of the olfactory cilia was observed in the newt just as in some other vertebrates (Hopkins, 1926; Le Gros Clark, 1957; Ai and Takagi, 1963). In Ringer's solution their movement was sometimes too rapid to permit measurement of their length. In these cases 0.5 per cent formol solution was run between the coverslip and slide and the length of the cilia was measured when the ciliary movement had slowed down.

After a newt was transferred onto land, the olfactory cilia were found to grow in length all alike. Although a small variation in length was found among the olfactory cilia of a newt, the length of the longest cilia, of which there were many, was measured in each newt. In many cases, the measurement was performed after the slow potential was recorded. The average of these measurements, one for each of three to five newts, was plotted. Each point in Figs. 2 and 7 was made in this way.

Recording of Electrical Activity After newts were decapitated under ether anesthesia, the head was pinned to a cork plate and the olfactory epithelium exposed. For recording the slow potential, a glass pipette with tip diameter of 200 to 300 μ and filled with Ringer's solution was put on the epithelium. The potential was recorded with an inkwriting recorder through DC amplifiers; the over-all band-pass width was from DC to approximately 50 CPS.

Slow potentials appeared reproducibly with the same magnitude when an odor of the same kind and of the same duration was applied repetitively, provided the interval between applications was long enough. The magnitudes of the slow potentials were measured in three to five newts in each stage and were averaged. Each point in Figs. 7 and 8 was plotted in this way.

Odorous Substances For stimulation with gaseous odors, saturated vapors of amyl acetate, amyl alcohol, ether, and chloroform were used. They were stored in 20 cc syringes, and were applied to the epithelium through a lucite or glass tube by mechanically pushing the inner tube of the syringe at various speeds.

For stimulation with liquid odors, extracts of dried pupae of silkworm and meats (10 gm in 100 cc of distilled water) were applied with an apparatus consisting of double glass tubes with slender tips and stopcocks (Shibuya, 1960). A stimulant was applied through the inner tube and, for washing, distilled water was applied through the outer tube.

These experiments were performed in October (room temperature 18–20°C).

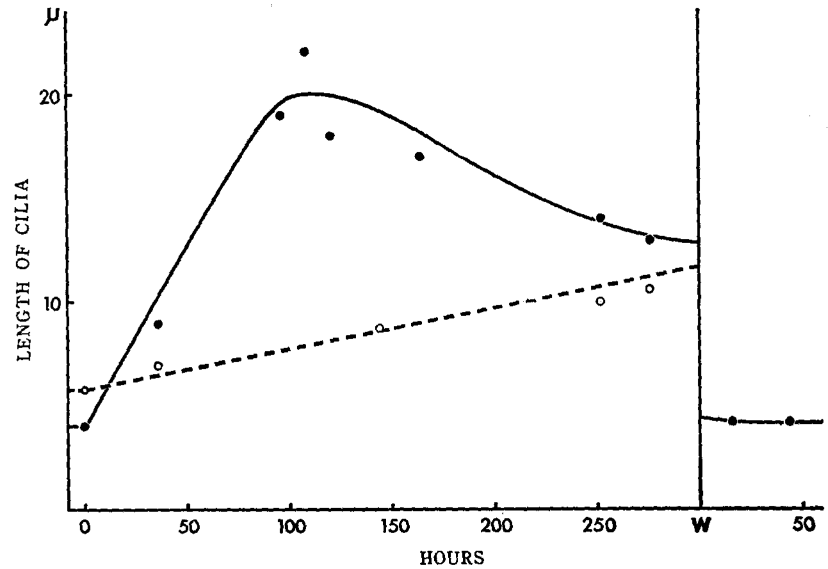

Figure 2. Curves to show the growth of olfactory cilia in the newt after the transfer from water onto land. The solid line indicates the length of the cilia in the olfactory bud. The dotted line shows the length of the cilia in the interstitium. *W*, indicates the time when a newt on land was returned to the water. It was then found that the elongated cilia became shorter and resumed their original length.

The same experiments on the slow epithelial potentials were performed in the newt from land in July (room temperature 26–28°C) as well.

RESULTS

I. *Growth of the Olfactory Cilia*

The cilia of the bud of a newt living in water are about 4 μ long and those of the interstitium are about 5 to 6 μ long. The lengths of these cilia do not change as long as a newt lives in water.

When a newt was transferred onto land, the olfactory cilia began to grow

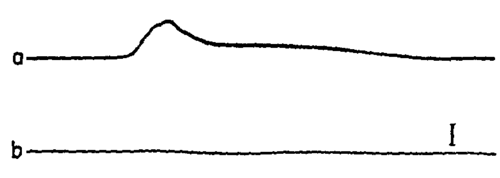

Figure 3. *a*, A slow potential evoked in the olfactory epithelium of a newt from water by the application of an odorous fluid (extracts of dried pupae of silkworm). *b*, No response was found in the epithelium by the application of amyl acetate vapor. A horizontal line under the records indicates olfactory stimulation. Voltage calibration, 200 μv. Time scale, 1 sec.

(Fig. 1 *b*). After 108 hours, the cilia of the olfactory bud had grown to 22 μ, which is more than five times as long as those of a newt in water, but later they gradually became shorter (Fig. 2).

The cilia of the interstitium also grew gradually in the newt transferred to land, reaching 8.8 μ after 144 hours and 13.0 μ after 348 hours. Thus, the cilia in the olfactory bud grew far more rapidly than in the interstitium (Fig. 2). After the newts had been kept on land 300 hours (about 12 days), they were returned to water. When the length of the olfactory cilia was measured in these newts after 10 or 30 hours, it was found that the cilia had resumed their original length (about 4 μ) (Fig. 2). It is not certain whether this shortening occurred because of breakage or whether the elongated cilia were replaced by new ones.

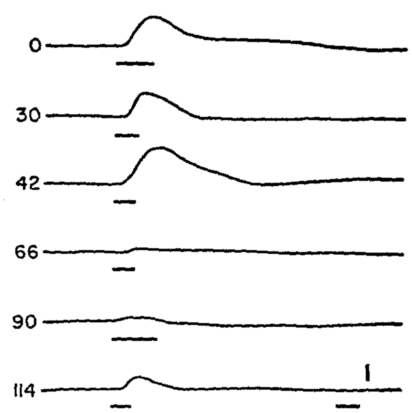

FIGURE 4. Slow potential evoked by the application of an odorous fluid (extracts of dried pupae of silkworm) to the olfactory epithelium of a newt transferred from water to land. Numerals on the left indicate hours after the transfer. The potential became largest after 42 hours but nearly disappeared after 66 hours. Reappearance of the potential was found after 114 hours. Further explanation in the text. Horizontal lines under the potentials indicate stimulation. Voltage calibration, 200 μv. Time scale, 1 sec.

II. *Olfactory Epithelial Potential of a Newt from Water*

When a fixed amount of an odorous fluid was flowed on the olfactory epithelia of newts which had lived long in water, slow potentials with magnitudes of 300 to 400 μv were usually evoked. They had a relatively steep rising phase and a slowly declining phase (Fig. 3*a*), resembling in shape the slow potentials found in *Channa argus* and a carp (Shibuya, 1960).

On the other hand, no response was observed when an odorous vapor was applied to the olfactory epithelia of these newts (Fig. 3*b*).

III. *Olfactory Epithelial Potential of a Newt from Land*

(a) RESPONSE TO AN ODOROUS FLUID

After newts in water were transferred onto land, the responses of the olfactory epithelia to an odorous fluid were recorded at successive intervals. Slow potentials which appeared immediately after the transfer continued to appear with the same magnitude for about 30 hours but then increased in magnitude to reach a maximum value of over 400 μv 30 to 40 hours after the transfer. After this, the potential rapidly decreased in magnitude and disappeared about 60 hours after the transfer (Fig. 4). These potentials closely resembled in shape those found in the newts from the water. It was found that the slow potential later began to reappear in response to odorous fluid (Fig. 7). The details of these changes will be described in a later paper.

(b) RESPONSE TO AN ODOROUS VAPOR

Although the olfactory epithelium of a newt from water did not respond to an odorous vapor at all, that of a newt from land began to respond to it about 30

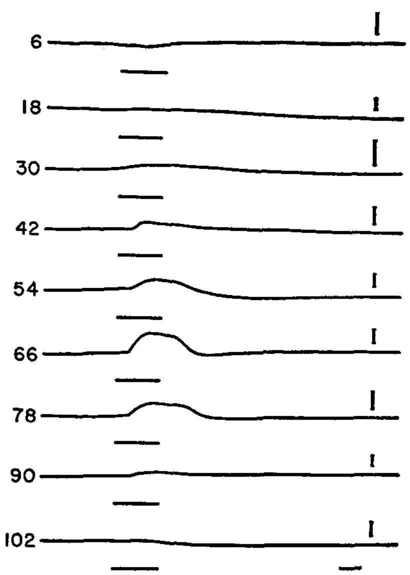

FIGURE 5. Slow potentials evoked by the application of amyl acetate vapor to the olfactory epithelium of newts from land. Numerals on the left indicate hours after the transfer. The potentials became largest after 66 hours, but disappeared after 102 hours. Horizontal lines under the potentials indicate stimulation. Voltage calibration, 100 μv. Time scale, 1 sec.

hours after the transfer from water to land. Small slow potentials appeared in this stage in response to amyl acetate (Fig. 5) and amyl alcohol vapors. When recorded at successive intervals, these potentials became larger and finally attained a maximum magnitude about 64 to 66 hours after the transfer. However, the potentials began to decrease in magnitude afterwards and nearly disappeared in about 120 hours after the transfer. Temporal changes of the magnitudes of the slow potentials were studied using several kinds of odorous vapors (Fig. 7).

An off-slow potential was found in the olfactory epithelium of the newt from land at the cessation of stimulation, especially with ether and chloroform vapors (Shibuya and Takagi, 1962). Off-slow potentials were also recorded at successive intervals after the transfer (Fig. 6). It was clear that the largest off-potential appeared when the on-slow potential became largest (64 to 66 hours after a newt was transferred onto land) and both disappeared together

(Fig. 7). The curves for the off-potentials closely resemble the curves for the on-potentials in shape but not in magnitude.

On the other hand, the above results, which were obtained in October (18–20°C), showed differences from those obtained in July (26–28°C). For instance, in response to amyl acetate vapor stimulation the slow potential began to appear in July as soon as 8 to 10 hours after a newt was transferred onto land, which was 15 to 20 hours earlier than the response observed in October. It attained a maximum magnitude 50 to 55 hours after the transfer, and then gradually decreased in magnitude. But such a rapid decrease as was

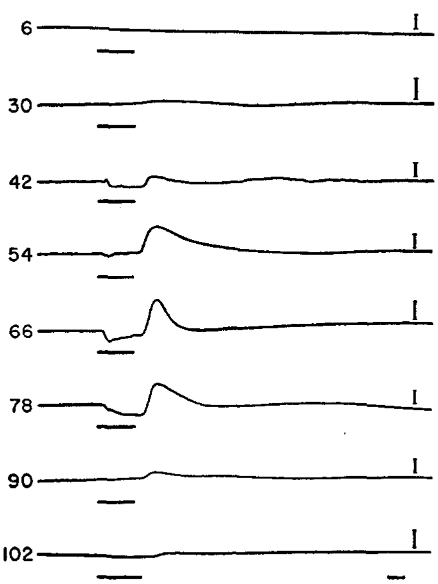

FIGURE 6. On-off-slow potentials evoked by the application of ether vapor. The potentials were recorded at the hours shown by the numerals on the left after the transfer. Horizontal lines under the potentials indicate stimulation. Voltage calibration, 200 μv. Time scale, 1 sec.

found in October was not observed in July. Small slow potentials were recorded even 264 hours (11 days) after a newt was transferred onto land (Fig. 8).

DISCUSSION

A newt on land can smell vapors (Matthes, 1924 *b*, 1927; Czeloth, 1931) and Matthes (1927) ascribed this function to the growth of the olfactory cilia. To test this hypothesis, an attempt was made in Fig. 7 to correlate the growth of the olfactory cilia which occurred after a newt was transferred onto land with the change in magnitude of the slow potentials evoked by odorous fluids. The slow potential maintained nearly the same magnitude for about 30 hours after the transfer, though the cilia elongated gradually but continuously. When the olfactory cilia became more than twice (about 10 μ) as long as those of the animal from water, the slow potential rapidly increased in magnitude. After reaching a maximum, the potential rapidly declined and finally disappeared in 65 to 75 hours after the transfer, though the cilia continued to elongate during this period.

Thus, it was found that the growth of the olfactory cilia does not parallel the changes in magnitude of the slow potentials. However, it is clear that a newt is not anosmic as Matthes (1927) presumed, but retains an ability to

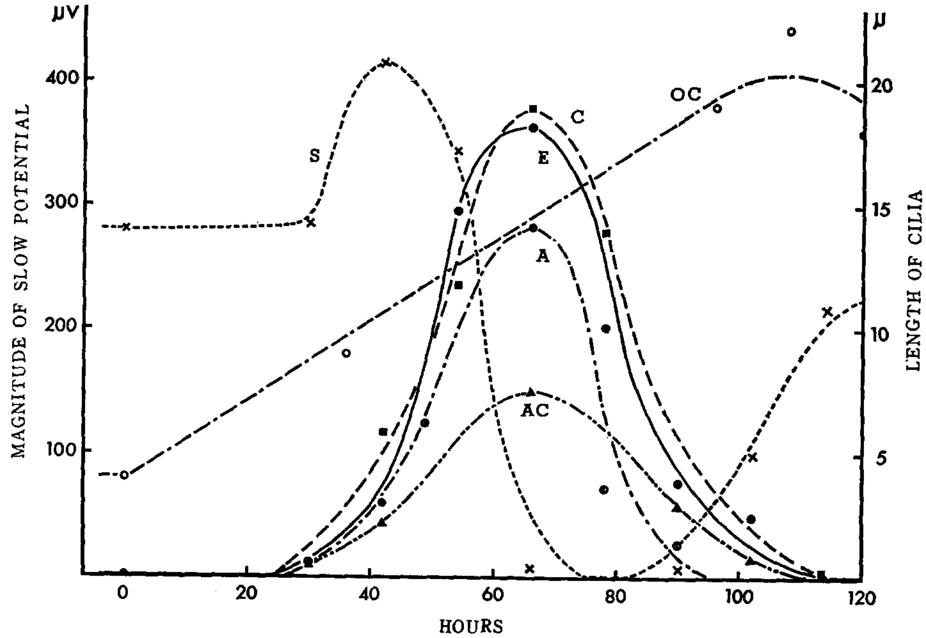

FIGURE 7. Development and disappearance of the slow potentials in relation to the growth of the olfactory cilia in the olfactory bud (in October). *S*, odorous fluid. *AC*, amyl acetate vapor. *A*, amyl alcohol vapor. *E*, ethyl ether vapor. *C*, chloroform vapor. The curves beside the last four symbols indicate respectively the magnitudes of the on-slow potentials evoked by amyl acetate and amyl alcohol vapors and of the off-slow potentials evoked by ether and chloroform vapors. *OC*, olfactory cilia; refer to scale at the right. An odorous fluid evokes a maximum slow potential when odorous vapors first become effective, and an odorous fluid is nearly ineffective when odorous vapors evoke maximum potentials, although the olfactory cilia are growing at a uniform rate during this time.

smell odorous fluids for some time after it is transferred onto land and that only later it becomes unable to respond to such stimuli. Although a correlation between the slow potential and the olfactory sensitivity is still unknown, the temporal increase in magnitude of the slow potential probably indicates a stage of temporal hypersensitivity.

In the case of stimulation with odorous vapors, the slow potential began to appear about 30 hours after the transfer to land. This corresponds to the period when the sensitivity to odorous fluids becomes maximum. Therefore, it is very probable that a newt in this stage detects both odorous fluids and vapors. Then, the slow potential increased in magnitude as time passed and became maximum when the olfactory cilia reached a length of 14 to 15 μ, which is about three times as long as the cilia of newts in water. Thereafter, the slow potential decreased in magnitude, disappearing when the olfactory cilia were longest. Thus, when the slow potential is largest, the olfactory cilia are not longest and when the latter are longest, the former is no more observed. Consequently, in stimulation with both odorous fluids and vapors, a correlation was not found between the length of the olfactory cilia and the magnitude of the slow potential.

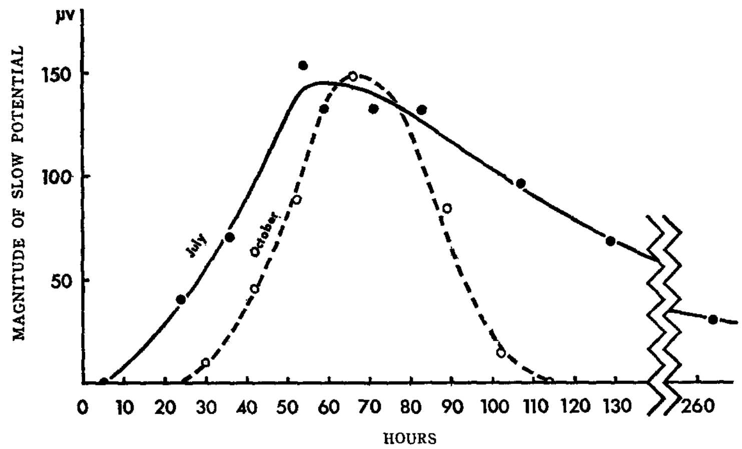

FIGURE 8. Comparison of the temporal changes in magnitude of the slow potentials after newts were transferred from water onto land in July and in October. The stimulant used was amyl acetate vapor.

In the light of these disagreements, Matthes' hypothesis (1927) that the development of sensitivity to odorous vapors in the newt depends on the growth of the olfactory cilia is not supported. On the contrary, our results strongly suggest that the growth of the olfactory cilia in the newt is a secondary phenomenon, for instance, due to the enhanced metabolism which may occur in the olfactory cells after the transfer in order to accommodate to living on land.

In Figs. 4 and 7, it is shown that in the land newt the response to odorous fluids reappears after about 100 hours. It seems that olfactory sensitivity to odorous fluids reappears in this period. Consequently, changes in the olfactory sensitivity of the newt to odorous fluids and vapors in this period are supposed to be an interesting subject of research and will be described in a later paper.

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