The initial population density on different trees varied between 70 and 283 larvae per kg of branches, the mean values of the plots between 98 and 190 larvae per kg (Table II). In spite of these large variations, the relative population reductions on plots treated the same way were so similar (Table II) that the results of the replicates could be computed (values of Table I).

The results show that without treatment the populations were reduced by about 50% within 3 weeks (natural mortality). In order to estimate the values of the mortalities induced by the different treatments, the experimental values had to be corrected by applying Abbott's formula. The corrected results in the last column of Table I show that, at the concentrations used, both Thuricide 90T alone and DDT alone induced an additional larval mortality of about 55%, whereas the supernatant (ET) alone had almost no toxic effect. However, in combination with Thuricide, both DDT and ET caused more than 80% mortality. The result of the combination with DDT corresponds to independent action of the chemical insecticide and the biopreparation. This is not true for the combination of Thuricide with ET, where independent action would account for 59% mortality only. Therefore supplemental synergism[15] has to be postulated for this combination.

The controls of the glue tables revealed no differences between the control plots and the plots treated with ET alone. Large numbers of larvae of *Z. diniana*, and relative large numbers of other insect species, descended or fell only from the trees in plots treated with DDT.

Biopreparations of *B. thuringiensis* are used against many lepidopterous insect pests. However, even against susceptible species, as in the case of *Z. diniana*, their use may be limited since sometimes control is not fully satisfactory. Our experiments indicate that this handicap might be overcome by the addition of ET.

Except for one Russian preparation, all commercial preparations of *B. thuringiensis* produced at present are devoid of ET which, in conformation with the rules of biological control, is not wanted because of lack of specificity and its toxicity for vertebrates[16]. The producers therefore either use strains of the serotype H3 which does not synthetize ET or, if strains of the serotype H1 are used, the spore/crystal complex is separated from the culture medium which contains the ET and which is discarded. According to present knowledge, biopreparations based on the spore/crystal complex only are practically devoid of substances which are poisonous for organisms other than larvae of Lepidoptera.

The production of highly specific biopreparations is certainly commendable. However, because of their limited usefulness, the production of more potent preparations containing relatively high amounts of ET should also be considered and studied carefully. Although ET has an oral toxicity for mice similar or slightly higher than DDT[17], the amount of ET needed to produce the same combination effect against *Z. diniana* is at least 10 times less than that of DDT (assuming 50 mg of ET per liter of supernatant[16]). Low doses of ET are probably harmless for insects and other organisms which are not susceptible for the spore/endotoxin complex of *B. thuringiensis*, as suggested by the results of the glue table controls and by the finding that repeated injections of sublethal doses of ET in mice did not lead to toxic effects, i.e. to accumulation[18]. We may therefore expect that the specificity of bacterial preparations is not unduely reduced by the addition of β-exotoxin, whereas the effect of the endotoxin in susceptible species might be considerably enhanced. This has recently been confirmed by ALYOSHINA, though no further proofs have been offered[19].

Summary. Addition of either DDT or the supernatant of a centrifuged liquid culture of *Bacillus thuringiensis*, serotype H1, containing β-exotoxin, enhanced the action of the bacterial preparation Thuricide 90T against larvae of the larch pest *Zeiraphera diniana*, increasing mortality from 53% to more than 80%. Since DDT alone produced 57% mortality, its combined action corresponds to independent action. The preparation of β-exotoxin, on the other hand, had only little effect alone but synergized the action of the bacterial preparation considerably.

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[16] R. P. M. BOND, C. B. C. BOYCE, M. H. ROGOFF and T. R. SHIEH, in *Microbial Control of Insects and Mites* (Academic Press, 1971), p. 275.
[17] R. P. M. BOND, oral communication.
[18] H. DE BARJAC and J.-Y. RIOU, Rev. Path. comp. Méd. exp. *6*, 367 (1969).
[19] O. A. ALYOSHINA, Proc. 8th Int. Congr. Plant Prot., Moscow 1975, *3*, 19 (1975).

Development of the Electric Discharge in Mormyrid and Gymnotid Fish (*Marcusenius sp.* and *Eigenmannia virescens*)

The recent identification of the environmental factors leading to gonad growth in several species of the two major groups of weakly electric fish (the Mormyriformes of Africa, and the Gymnotoidei of South America) and the repeated successful reproduction of two of these species in captivity[1], has enabled us to carry out a longitudinal study of the development of the electric organ, electroreceptors[2] and the discharge itself. In this paper we present the first results of a detailed study of the ontogeny of electric discharge in the gymnotid *Eigenmannia virescens* and the mormyrid *Marcusenius sp.*

Method. Electrical recording was carried out in a specially constructed glass cell of low capacity (0.90 ml), filled with water of constant conductivity (650 μmho. cm^{-1}) and maintained at constant temperature.

Results. Eigenmannia. The first discharges (Figure 1) were detected on Day 8 and were of very low amplitude (20–30 μV in all specimens) rizing to about 150 μV within 20 min (Figure 2a). The discharge was discontinuous at first and occurred in short bursts which became longer and longer until the fish was continuously discharging after 12 min. Discharge frequency was very low on Day 1

[1] F. KIRSCHBAUM, Experientia *31* in press (1975).
[2] F. KIRSCHBAUM and J.-P. DENIZOT, C. r. Acad. Sci., Paris in press (1975).

(150 to 200 Hz at 25 °C) reaching "adult" frequencies of 350 to 400 Hz by about Day 20 (Figure 2b). From this point onwards, each fish had acquired its unvarying individual frequency. The form of the discharge was found to be similar to that of the adult from the beginning (Figure 1), but the pulse duration was longer with respect to the interpulse interval. Pulse width was comparable to that of the adult by about Day 100.

Marcusenius. The yolk sack was still present and the fish practically immobile on Day 8, when the first discharges were recorded. The discharge frequency was exceedingly low at first, with several minutes between each

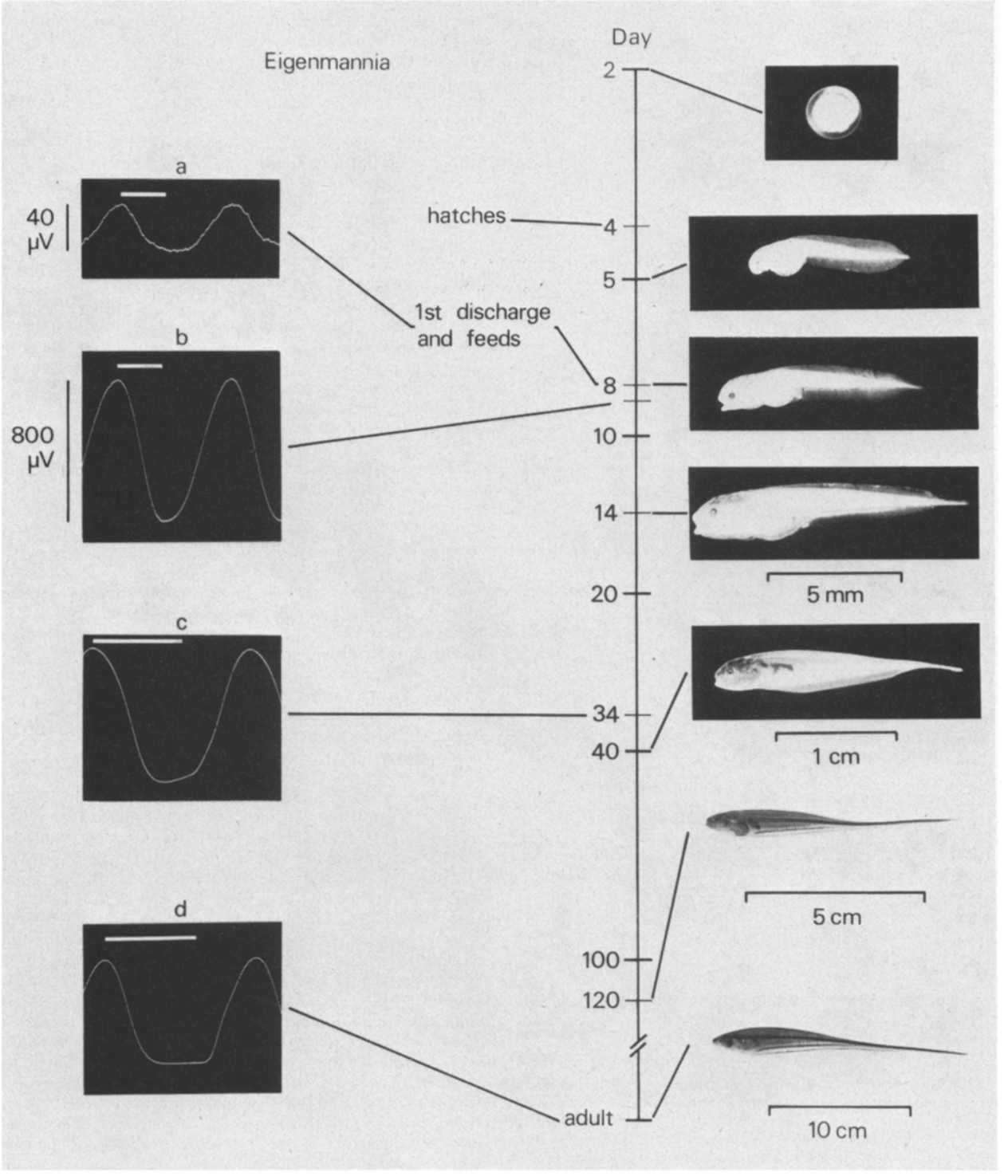

Fig. 1. *Eigenmannia*. At right, various stages in the morphological development from egg to adult at 27 °C. At left, the electric discharge (head positivity upwards). a) The appearance of the discharge during the 6th min of electrical activity (discharge still discontinuous). b) The discharge of the same specimen 12 h later. Note low frequency of about 160 Hz in a) and b) (2 msec bar) and gradual narrowing of the pulse (traces a) to d)) with respect to the interpulse interval. Amplitudes are those measured in the standard recording cell.

pulse. The first discharge produced by a specimen of *Marcusenius* under continuous recording is shown in Figure 3a (i). Within 2 h the amplitude had doubled from the initial value of 50 μV. The duration of the first pulse was approximately 50 times that of the adult, developing after 12 h into a form (1) typical for the larva which remained relatively unchanged for the first 40 days (Figure 3b (i)).

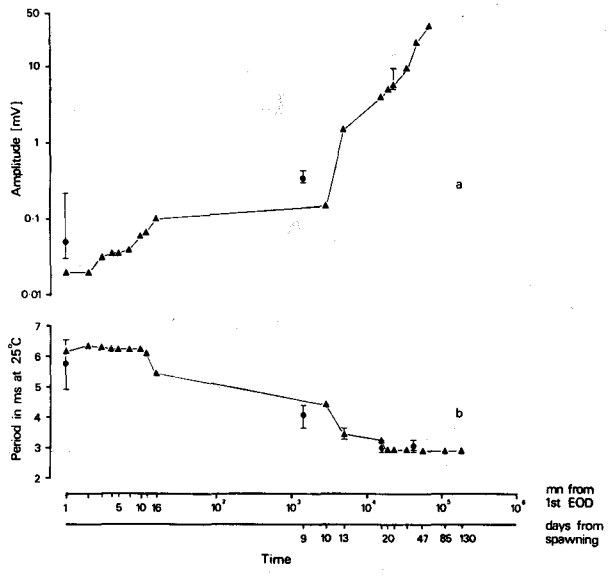

Fig. 2. *Eigenmannia*. a) Peak to peak discharge amplitude measured between electrodes in the standard recording cell. b) Discharge period corrected to 25 °C. Triangles represent the development of 1 individual from the first spawning, while the circles and bars represent the median and range of specimens taken from all 3 spawnings at representative stages (N varies between 18 and 4).

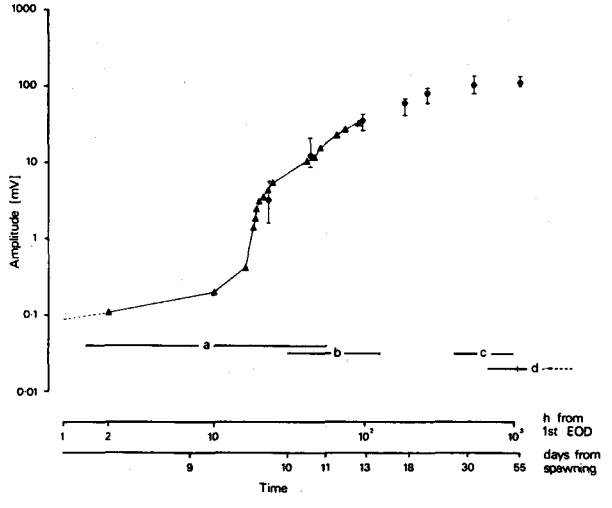

Fig. 4. *Marcusenius*. Peak to peak discharge amplitudes measured between electrodes in the standard recording cell for 1 individual from the second spawning (triangles). Circles and vertical bars represent the median and range of a group of 8, and later 6, fish from the same spawning. Note the close synchrony in the development of the individuals (c.f. Figure 2). Horizontal bars refer to the onset in the first member and the disappearance in the last member of the group, of the stages referred to by the same letter in the Results section. For stage (d) the vertical line is its time of appearance in the last fish of the group.

A small head-positive phase (p) occurs 1.7 msec before the "main" discharge peak and remains constant at 500–800 μV, while the main pulse continues to increase in amplitude (Figure 4). A smaller head-negative after potential follows the "main" discharge. The total duration of the discharge is approximately 3 msec compared with only 100 μsec in the adult (Figure 3f (i)) and the main phase is of the opposite polarity.

Four distinct developmental stages can be recognized:

a) Days 9 and 10: Multiple discharging. A large proportion but not all of the discharges are followed by one or more smaller discharges. These are mainly head-positive and bear little resemblance to the "main" discharge. They also tend to occur very reproducibly at 6 or 12 msec latencies (Figure 3b (ii)). Up to 35 subsequent small pulses have been recorded in trains lasting up to 200 msec and filling the whole inter-pulse interval.

b) Days 11 to 14: Double discharging. Usually only one, but sometimes two pulses follow the "main" discharge at 7 to 12 msec in a very small proportion of all the discharges emitted. The "main" and secondary discharges have the same form and approximately the same amplitude (Figure 3c).

c) Days 25 to 50: Variable preceding pulses. The preceding, fixed-amplitude, head-positive pulse (p) (Figure 3b (i)) suddenly displays a second highly variable component (vp) about 0.3 msec from its peak (Figure 3d (i)). The amplitude of this component varies from zero to several millivolts, sometimes approaching or even exceeding the amplitude of the main pulse itself (Figure 3d (ii)). Its amplitude is inversely related to the discharge frequency and there is often a reciprocal amplitude variation between it and the main discharge.

d) Days 37–53: Head-negative component. About Day 40 a very narrow head-negative component develops 0.4 to 0.8 msec from the head-negative peak of the larval discharge, its amplitude rapidly increases (Figure 3e (i)) while the larval pulse shows no further increase in size. Within 15 days, its amplitude is 10 times that of the larval pulse. It is this head-negative discharge which becomes the adult pulse. When examined on expanded time base (Figure 3e (ii)), it can be seen that it is very similar in form to that of the adult fish (Figure 3f (i)). The larval discharge seems to remain and can still be seen at high gain in the adult (Figure 3f (ii)) as can the preceding head-positive pulse. Fish of 65 to 85 days show the characteristic adult discharge.

Discussion. Both *Eigenmannia* and *Marcusenius* hatch on Day 4 and start discharging on Day 8 when maintained at 27 °C. It is interesting to note that *Eigenmannia* starts discharging and feeding on the same day. The lack of discharge before this time should enable the larvae to hide effectively from any predator capable of detecting their discharges. *Marcusenius*, however, starts discharging 4 days before the yolk sack is completely absorbed and feeding commences. This difference can probably be accounted for by the extensive parental care shown by *Marcusenius* compared with its apparent absence in *Eigenmannia*. Our observations indicate that a nest is constructed by the male *Marcusenius* in which both the eggs are placed and the larvae of several spawnings are guarded. The form of the discharge in *Eigenmannia* is very similar to that of the adult and shows little modification throughout the fish's development. In *Marcusenius* the situation is much more complicated. The presence of a larval discharge of long duration (20 to 50 times that of the adult) and opposite polarity could serve a function, for example, in the nest maintenance of this species. The multiple discharges which appeared during the first week of electrical activity occurred at latencies

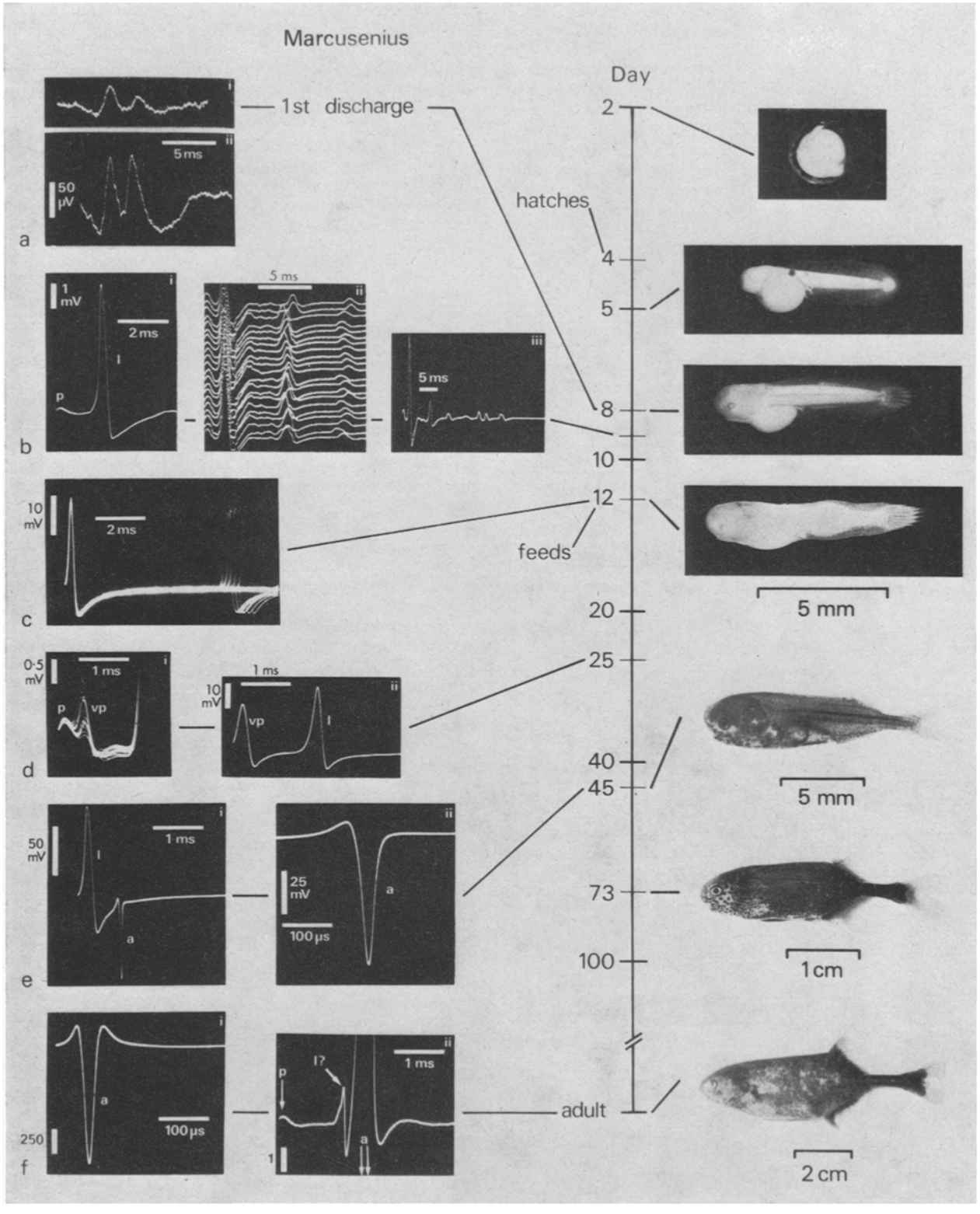

Fig. 3. *Marcusenius*. At right, various stages in the morphological development from egg to adult at 27 °C. At left, the main events in the ontogeny of the electric organ discharge (head positivity upwards). a) (i) the first discharge produced by a fish under continuous recording. (ii) the discharge of the same specimen 2 h later. b) (i) the characteristic larval discharge (1) on Day 9, (ii) and (iii) multiple discharging. c) double discharging (superimposition). d) (i) and (ii) variable amplitude component (vp) on preceding pulse (p) (superimposition during frequency increase, see text). e) (i) the adult head-negative spike (a), 2 days after its first appearance, (ii) as (i) but on expanded time-base. f) (i) the characteristic adult discharge (c. f. e(ii)). f) (ii) as (i) but × 250 gain – only relative amplitudes are given due to different recording conditions. The preceding pulse (p) is still visible as is a diphasic pulse considered to be the vestige of the larval discharge. Only the rising and falling edges of the head-positive components of the main discharge are visible. The major head-negative spike is too fast to be seen.

which suggest that the electrosensory system is "oscillating"[3]. Supplementary discharges at fixed latency could be the fish "echoing" to its own discharge[4,5], and in particular the very long trains of pulses at intervals of 11 to 12 msec. The tuberous electroreceptors are present and probably functional at the occurrence of the first discharge[2], but it is possible that the inhibitory path ways normally blocking response to the fish's own emission[6] are not yet established. Recordings made in large tanks showed that multiple discharging was not an artifact due to the very small volume of the recording cell.

[3] G. W. M. WESTBY and F. KIRSCHBAUM, in preparation.
[4] C. J. RUSSELL, J. P. MYERS, C. C. BELL, J. comp. Physiol. *92*, 181 (1974).
[5] B. KRAMER, J. comp. Physiol. *93*, 203 (1974).
[6] B. ZIPSER, Ph. D. Thesis, Yeshiva University, New York (1971).
[7] Recipient of fellowship provided by the Deutsche Forschungsgemeinschaft.

Preliminary histological results suggest that, unlike *Eigenmannia*, no recognisable electric organ exists in *Marcusenius* until several weeks after the first discharge. The appearance of the characteristic head-negative adult discharge on about Day 40 probably corresponds to the appearance of the first electroplaques.

Summary. Larvae of both species start discharging at 8 days. *Eigenmannia* immediately produces pulses similar to those of the adult. *Marcusenius* however, possesses a characteristic larval discharge 20 times longer and of opposite polarity to the adult discharge which appears on Day 40.

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Post-Tetanic Changes of Bilateral Dorsal Root Potentials Evoked by Stimulation of the Cutaneous Afferents

Long-lasting tetanization of the spinal afferents profoundly affects transmission at their synaptic terminals. After conditioning tetanus, the size of the testing monosynaptic reflex evoked by stimulation of the tetanized afferent nerve is increased. This post-tetanic potentiation is most probably caused by prolonged hyperpolarization of the presynaptic fibres[1]. The same explanation was applied to the potentiation of presynaptic inhibition recorded as the dorsal root potential (DRP) in the root by which an afferent volley enters the spinal cord[2,3]. It is known that the DRP is produced not only at the point of entry of an afferent volley but spreads to the opposite side of the cord. Since contralateral depolarization in many respects differs from the ipsilateral potential[4], in the present investigation the post-tetanic changes of bilateral DRPs evoked by stimulation of the cutaneous afferents were studied.

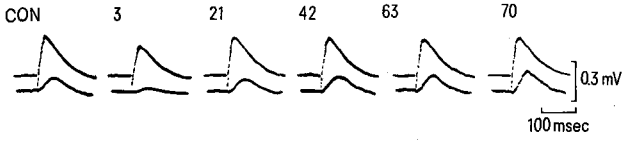

Fig. 1. Changes of bilateral dorsal root potentials produced by stimulation of the superficial peroneal nerve after conditioning tetanus of the same afferents. Upper traces of each record show ipsilateral and lower traces contralateral DRPs. Negativity is signaled by an upward deflection. The strength of afferent stimulation was 1.18 times the threshold strength for the ipsilateral potential. After the first record which shows the control DRPs, a conditioning tetanus of 350 c/sec for 15 sec was given. The next records illustrate changes in the size of the DRPs. The numbers indicate time in sec after termination of the tetanus.

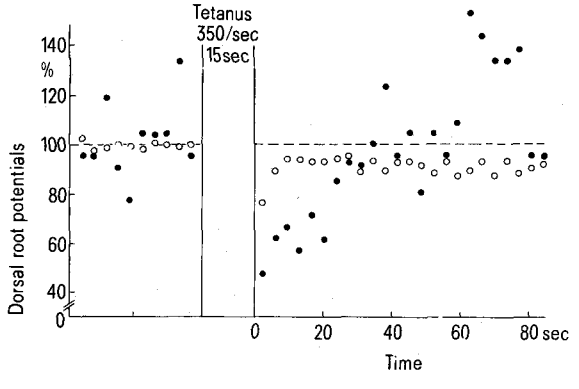

Fig. 2. Post-tetanic changes of ipsilateral and contralateral dorsal root potentials. The DRPs were produced by stimulation of the superficial peroneal nerve at 1.22 threshold strength. At this intensity of stimulation ipsilateral DRP attained about 65% of its maximal size. Abscissa, time in sec. Ordinate, size of the DRPs calculated as percentages of the controls evoked just before tetanus. Open circles represent ipsilateral and close circles contralateral DRPs.

Methods. The experiments were performed on 24 spinal cats lightly anaesthetized with thiamylal sodium (30 mg/kg i.p.). The testing DRPs were evoked by stimulation of the superficial peroneal or posterior tibial nerves every 3–5 sec and led off bilaterally from the most caudal rootlets of the L7 dorsal roots. The conditioning stimulation of the same nerve lasted 15 sec and its frequency ranged from 100 to 450 c/sec.

Results and discussion. Prolonged tetanization of the cutaneous nerve produces differentiated changes in the size of the DRPs on both sides of the spinal cord. They are most easily observed when the strength of an afferent stimulation is adjusted to produce ipsilateral testing DRPs which attain no more than 60–70% of their maximal size. Figure 1 and 2 show the most frequently encountered changes of bilateral DRPs. It may be seen that, just after terminating the tetanus, the ipsilateral DRP is decreased to about 70% of the initial value. Then the depolarization increases, but during several tens of seconds it does not fully recover, attaining 92–95% of the control level. The initial depression of the contralateral DRP is much deeper (up to 45–48% of the control) but its duration is shorter. The size of the potential rapidly increases to regain after about 25 sec its control value and then it augments further, displaying a significant delayed post-tetanic potentiation to about 150% of the initial level. Post-tetanic potentiation of the ipsilateral DRP was observed only in 2 out of 24 preparations. When present, it was small (up to 118% of the control), variable and of short duration.

Post-tetanic depression of bilateral DRPs depended on their initial amplitude. On the ipsilateral side of the cord, the most conspicuous decrease of the DRPs occurred when they were produced by a just above threshold stimulation. With the increase of the DRPs,