Development of the auditory tympana in the cricket *Teleogryllus commodus* (Walker): Experiments on regeneration and transplantation

E.E. Ball[1]

Department of Neurobiology, Research School of Biological Science, Australian National University, P.O. Box 475, Canberra City, A.C.T. 2601 (Australia), 17 July 1978

Summary. Grafting and regeneration experiments on prothoracic legs of the cricket *Teleogryllus commodus* (Walker) demonstrate: a) that the legs retain their identity, as indicated by formation of tympanal cuticle in the adult, when transplanted to the site of a mesothoracic leg in immature animals, and b) that the presence of tympanal cuticle on a regenerate leg depends on the instar of amputation.

During an investigation of the development of the cricket auditory system[2-5] prothoracic leg transplants were performed to determine the feasibility of using this system to examine the specificity of reconnection of the auditory neurons. These transplants were seldom successful but those which were, and some regeneration experiments, provide some insights into the control of differentiation of the auditory tympana.

For transplant experiments pairs of animals of approximately equal sizes were anesthetized with CO_2. A mesothoracic leg was then severed from the host at the base of the coxa and a prothoracic leg from the same side of the donor was severed at the same level and placed into the stump of the host leg. The transplanted limb was held in place briefly until the blood had begun to coagulate enough to support the limb. The container holding the animal was then set on ice for approximately 30 min after which the animals were allowed to move about freely. These transplants were carried out on animals in instars 3–6 as determined by metathoracic femur length[2]. No attempt was made to stage animals within an instar except for elimination of animals which were obviously about to moult.

In 4 animals (out of approximately 170) a recognizable major (i.e. posterior) tympanum was present in the adult on prothoracic legs transplanted to the mesothorax (figure 1, A and B). The low success rate was due to loss of transplanted legs and in some cases to the death of the host animal. A higher success rate would presumably have been obtained had it been possible to get synchronous moulting of the experimental animals, thus allowing all operations to be done on animals early in the intermoult period.

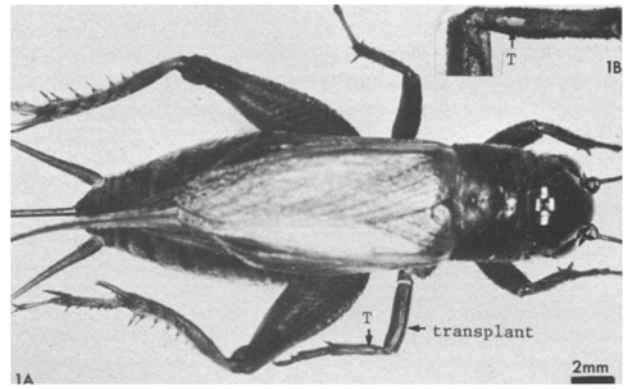
The positive results indicate that the prothoracic nature of the leg has already been determined by the 3rd instar and thereafter is unaffected by its position on the thorax. This is in agreement with findings on the cockroach[6].

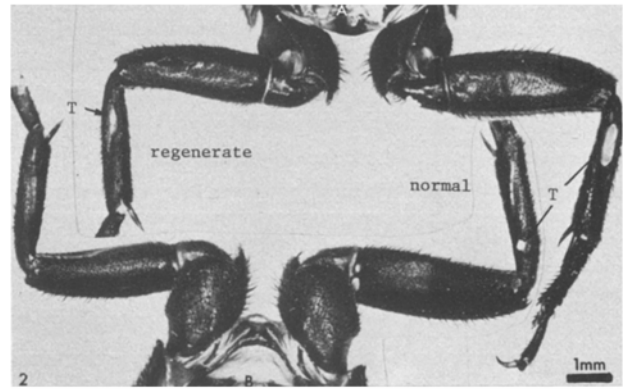
Regeneration experiments were also performed to see whether an ear, as recognized by formation of tympanal cuticle, would be present on the regenerate leg and, if it was, over how few instars this could occur. For these experiments a prothoracic leg of 5 2nd and 5 7th instar crickets was amputated at the trochanter with scissors.

Regeneration following amputation during the 2nd instar yielded an almost full-sized adult leg with at least some tympanal cuticle clearly present in the area where the larger posterior tympanum would normally form in all 5 animals and in 1 case a small area of tympanal cuticle where the smaller anterior tympanum would normally form (figure 2).

Amputation during 7th instar resulted in production of an adult leg which was in some cases almost full size (figure 3). In no instance was tympanal tissue formed, although in most cases a depression with a reduced density of cuticular sensilla was present in the tympanal region.

Since regenerate legs of 7th instar amputees are nearly full size it appears unlikely that tibial size alone determines whether or not tympanal cuticle will form. However, it is possible that the tibia must first reach a certain size and





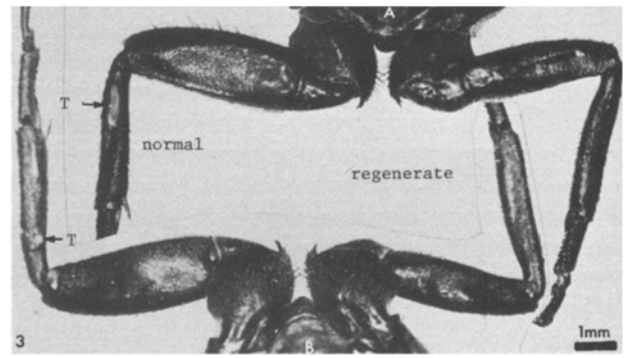


Fig. 1. *A* Adult animal which has developed tympanal cuticle (T) on a prothoracic leg (transplant) transplanted to the mesothorax at 5th instar. *B* Another transplanted prothoracic leg which has developed tympanal cuticle (T). Fig. 2. Regenerated prothoracic leg in an adult *T. commodus* following amputation at the trochanter during the 2nd instar. *A* Posterior view of the legs, note regenerated tympanal cuticle (T). *B* Anterior view of the legs, tympanal cuticle absent from regenerate leg. Fig. 3. Regenerated prothoracic leg in an adult *T. commodus* following amputation at the trochanter during the 7th instar. *A* Posterior view of the legs. *B* Anterior view of the legs. T indicates adult tympanal tissue.

then go through a specific sequence of differentiation before tympanal cuticle is produced. This hypothesis would be consistent with the presence of the depressed areas and reduced density of sensilla in the tympanal region of regenerates following amputation at 7th instar. During normal development the tympanal areas gradually lose their sensilla over the last 3 immature instars before the distinctive adult tympanal cuticle is produced at the imaginal moult. Another possible explanation of the results is that cells are committed to form tympanal tissue at a certain critical time in the life of the insect. If this time is missed the signal is no longer present or the presumptive tympanal cells are no longer capable of responding.

1 I thank Mike Bate for the helpful discussion throughout the project and Carmen Vilcins for excellent technical assistance.
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The fasciolicidal activity of a halogenated benzenesulfonanilide

T.J. Hayes[1] and M. Mitrovic

Animal Health Research Department, Hoffmann-La Roche Inc., Nutley (New Jersey 07110, USA), 12 May 1978

Summary. Halogenated benzenesulfonanilides have potent fasciolicidal activity. The efficacy of 1 such compound in mice, sheep, and cattle is reported.

Certain orthosubstituted halogenated benzenesulfonanilides have been known to possess antimicrobial[2] and molluscicidal[3] activity. Activity against the common liver fluke, *Fasciola hepatica,* has not previously been reported.

Compounds were detected as part of a routine screening program for chemicals with fasciolicidal activity. The screening method has been described[4]. Numerous 2-hydroxy as well as 2-acetoxy and 2-propionoxy substituted benzenesulfonanilides with various halogen constituents have been found[5] to possess marked fasciolicidal activity. Data from studies with a potent compound of this type is presented here to illustrate such activity.

The compound, 2-hydroxy-2′,3,4′,5,5′,6-hexachlorobenzenesulfonanilide, was 100% effective against *F. hepatica* in mice when administered for a 7-day period (14–21 days post infection) in the diet at 0.0125%; an effective concentration that compares very favorably with known fasciolicides[6]. As a single oral dose, the $ED_{>90}$ in mice against 14-day-old flukes was 30 mg/kg and the LD_{50} in normal mice was 179 mg/kg.

Established fasciolicides are known to exhibit different degrees of efficacy depending on the age of the parasite at the time of treatment. Thus in sheep the compound was tested against experimentally induced fascioliasis at 4, 6, and 14 weeks after infection (table 1). Against a 4-week-old *F. hepatica* infection a single oral dose of 5, 10 or 20 mg/kg b.wt was 98, 100, and 100% effective, respectively. Against 6-week-old flukes, 3, 4 and 5 mg/kg were highly effective giving reductions of 96, 99, and 100%, respectively, while 2 mg/kg resulted in an apparent reduction of 42%. Treat-

Table 1. Efficacy of 2-hydroxy-2′,3,4′,5,5′,6-hexachlorobenzenesulfonanilide against experimental *Fasciola hepatica* infections of various durations in sheep.

Experiment	Age of infection at treatment	Dose (mg/kg)	Mean number of live flukes recovered	% Reduction
1	4 weeks	0	154	0
		20	0	100
		10	0	100
		5	3.0	98
2	6 weeks	0	57	0
		5	0	100
		4	0.5	99
		3	2.5	96
		2	33	42
3	14 weeks	0	141	0
		5	0	100
		2	1.5	99
		1	63	55
		0.5	121	14

Experimental conditions: 28 mixed-breed young sheep were used in 3 separate experiments. The animals were approximately 12–14 weeks old when exposed orally to 400 (experiment 1), 100 (experiment 2), or 200 (experiment 3) selected metacercariae of *Fasciola hepatica.* At the time shown for each experiment, the animals (weight ca. 30 kg experiments 1 and 2; 40 kg experiment 3) in groups of 2 were each given a single oral dose of drug suspended in Methocel vehicle (experiments 1–1.75% Methocel, 20 ml/sheep; experiment 2–1.25% Methocel at a volume of 1 ml/kg; experiment 3–1.25% Methocel, 0.5 ml/kg). In each experiment 1 group received vehicle without drug (0 dose group = placebo). The animals were necropsied 13 or 14 days after medication and flukes recovered by dissection of the liver.

$$\% \text{ Reduction} = \frac{\text{placebo count} - \text{test count}}{\text{placebo count}} \times 100$$