Table II. Transplantation of SV40 transformed gecko cells into autogeneic and allogeneic hosts

Animal no.	Cell line inoc.	No. transformed cells inoc. ($\times 10^7$)	Period transformed in vitro before inoc.	Route of inoc.	No. nontransformed cells inoc. ($\times 10^7$)	Route of inoc.	Results
			Autografts				
1	T1	5.9	20 passages in 143 days	s.c.[a]	1.0	s.c.	No tumor after 24 months
4	T4	1.3	17 passages in 126 days	s.c.	1.0	s.c.	No tumor at death after 13 months
5	T5	3.0	16 passages in 115 days	i.p.[b]	0		No tumor after 29 months
8	T8	2.1	11 passages in 83 days	s.c.	2.1	s.c.	No tumor after 29 months
			Allografts				
2	GE-1	0.03	9 passages in 95 days	s.c.	0.02	s.c.	No tumor at death after 11 months
3	GE-1	0.04	9 passages in 95 days	s.c.	0		No tumor after 36 months
7	GE-1	7.9	39 passages in 311 days	i.p.	0		No tumor at death after 8 months

[a] Subcutaneous; [b] Intraperitoneal.

antigen by the indirect immunofluorescence method. Morphological transformation took place in about 7–11 weeks (1–6) passages after infection at 30 °C, and was accompanied by a rise in the percentage of T antigen positive cells (Table I). The critera used to evaluate transformation were 1. change in morphology from fibroblast-like to epithelial-like, 2. change in growth pattern to a much higher saturation density, and 3. a rise in the percentage of T antigen positive cells. SV40 virus could not be detected in the culture medium after transformation had occurred.

Four animals were inoculated with their own SV40 transformed tail cells, and of these 3 were simultaneously inoculated in the opposite leg with non transformed cells of the same passage level as a control. (Table II). 3 animals were inoculated with the SV40 transformed gecko cell line of embryonic origin, GE-1, and of these one was simultaneously inoculated in the opposite leg with non transformed cells at the same passage level as a control. After 36 months 3 animals have died due to nutritional deficiencies, but no tumors were observed.

Discussion. Cell lines were easily started from the tails of geckos and transformed by SV40 virus, but tumors were not induced in geckos by either autografts or allografts of SV40 transformed cells. These results agree with the general conclusion reached by PONTEN[6] that SV40 transformed cells usually have a weak tumorigenic capacity after animal implantation, the exception being hamster cells. It has been shown in man[7], calves[8], rats[9], and mice[10] that autografts of SV40 transformed cells caused either no visible growth in the host or only a small nodule that invariably regressed. One explanation given for these results is that rejection may be due to the altered antigenicity of SV40 transformed cells. In a previous study of SV40 transformed gecko cells[3], evidence was found for a SV40 tumor specific transplantation antigen. This may explain why tumor growth was not achieved in these experiments. Also since these animals represent the highest group of vertebrates retaining a considerable measure of regenerative ability, neoplastic growth may have been controlled by regenerative power, as the work of Seilern-Aspang and Kratochwil[11] suggest is possible. Finally the number of cell inoculated may have been less than the critical minimum needed for tumor growth, or the cells may not have been passaged long enough at the time of inoculation to have become capable of unlimited growth. A definitive explanation must await further experimentation.

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Ecdysterone-Induced Mortality and Inhibition of Feeding in Diapausing *Rhodnius prolixus* Stal

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Summary. Administration of ecdysterone was fatal to diapausing *Rhodnius prolixus;* the male adults and 19-week-old male fifth instar larvae were more susceptible than the 3-week-old larvae. The hormone also inhibited the feeding activity of the bugs.

Like other diapausing insects, growth and moulting in the diapausing *Rhodnius prolixus* is also initiated by ecdysone produced after blood meal[1]; the general physiology of the dormant bugs is also similar to that of other diapausing insects[2,3]. Administration of synthetic ecdysone has terminated diapause in some species of insects[4,5] suggesting the possibility of similar effects on *R. prolixus*. The present paper deals with the actions of synthetic ecdysterone on the survival and feeding behaviour of larval and adult *R. prolixus*.

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Ecdysterone (Rhoto Pharmaceuticals, Osaka) doses, ranging from 0.5 to 8 μg in 2 μl of 10% ethanol, were injected into the previously chilled bugs through the 2nd abdominal inter-segmental membrane; the controls received only alcohol. 30 to 40 individuals in 3 replicates were used in each experiment conducted at 30 °C and 70% RH. The mortality data were recorded daily and subjected to probit analysis.

Administration of ecdysterone inflicted heavy mortality in the diapausing male 5th instar larvae and male adults. The treated insects showed gradually increasing signs of lethargy and metabolic exhaustion and became completely inactive a couple of days before death. The Lt_{50} (time required for 50% mortality) values presented in Table I show a positive correlation between the dose of hormone and the mortality in 3- or 19-week-old diapausing larvae, but not in the adults. Compared with the 3-week-old larvae, the 19-week ones were more sensitive to the adverse effect of ecdysterone, as most of them died within a week of treatment.

To eliminate the possibility of sexual differences in the action of ecdysterone, 40 fifth instar female larvae (3 weeks old) and 20 female adults (2 weeks old) were injected with 2 μg of the hormone. The Lt_{50} values being 16.1 and 5.4 days respectively are not significantly different from those of the males (Table I).

Table I. Lt_{50} values for various doses of ecdysterone on 3- and 19-week-old diapausing male 5th instar larvae and 2-week-old male adults of *R. prolixus*

Dose (μg)	Lt_{50} values (days)		
	3-week-old larvae	19-week-old larvae	Male adult
Control	No mortality	No mortality	No mortality
0.5	21.2[a]	6.4[c]	—
1.0	18.6[b]	3.5[f]	6.9[e]
2.0	15.2[c]	3.4[f]	6.1[e]
4.0	13.11[d]	2.8[f]	6.1[e]
8.0	11.53[d]	0.9[g]	5.1[e]

Lt_{50}, time required for 50% mortality during the 7 week (for 3-week-old larvae only) or 3-weeks experimental period–Lt_{50} values not followed by the same letter, are significantly different at 5% level.

It was considered probable that the hormone-treated larvae might have completed growth and development and moulted to the next instar had there been more energy to sustain the hormonally triggered biochemical processes. An attempt was therefore made to replenish the nutrient reserves in the 3-week-old larvae by allowing them to feed on a rabbit at various times before and after hormone treatment. The feeding response of the larvae immediately after treatment was rather irregular probably due to the physical injury; later on, (6 12 or 26 h after treatment) most of the larvae showed partial feeding activity and increased their weights by about 50–70% of the control. Yet they failed to complete metamorphosis, though the increased nutrients did help extend the life-span from about 17 to over 40 days (Table II). The eventual mortality of these larvae is another indication of severe physiological derangement caused by the hormone before the insects could complete feeding a few hours later. For instance, WIGGLESWORTH[2] has already demonstrated the extreme sensitivity of fat body cells to even minute amounts of ecdysone produced during the feeding process of the bug.

Administration of hormone after feeding did not interfere with the developmental physiology of most larvae, enabling about 80% to metamorphose to adults (Table II). However, about 25% of these adults died within a week of ecdysis while several others had incompletely stretched wings or part of exuviae attached to the tip of the abdomen. Such juvenile hormone-like effects, which have also been reported on other insects[6], may be due to the unregulated acceleration of biosynthetic processes by ecdysterone, leading to imaginal moult.

The reduced ingestion of blood by the treated larvae suggested an inhibition of feeding activity by the hormonal action. Figure A shows that the administration of 2 μg of ecdysterone inhibited feeding activity; the inhibitory effect increased gradually and most of the adults stopped feeding within 2 days and larvae within 4 days of treatment. Almost all the treated larvae and half as many adults were fairly active on the warm rabbit skin, probing constantly with the proboscis. However, the amount of blood ingested was always significantly less than the controls: the treated larvae which actually fed, ingested only about 0.02 to 0.05 g of blood while the amount was negligible in the adults (Figure B). Similar results were obtained when a group of 35 larvae were allowed to re-feed on rabbit, 6, 12 or 18 days after the

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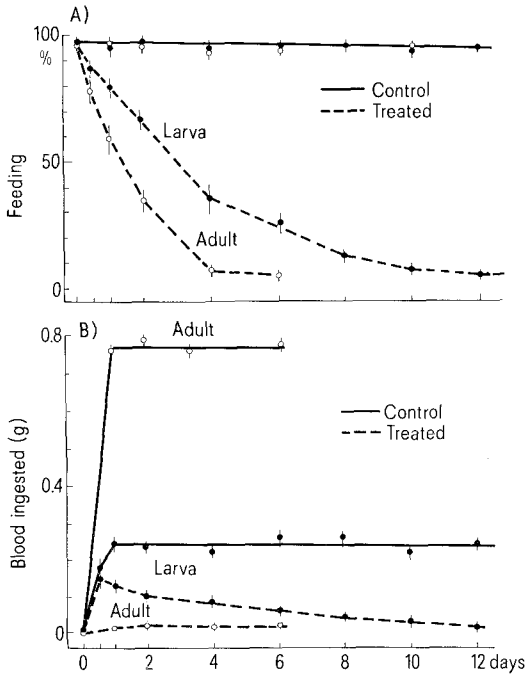
Table II. Effect of ecdysterone (2 μg/larva) and feeding on the survival and moulting of 3-week-old male diapausing 5th instar larvae of *R. prolixus*

Time of feeding	Mortality (%)		Lt_{50} treated	Adults obtained (%)	
	Treated	Control		Treated	Control
6 h after hormone administration	35	10	40	0	90
12 h after hormone administration	30	12	60	0	88
1 day after hormone administration	35	8	60	0	92
6 days after hormone administration	80	15	18	0	85
12 days after hormone administration	100	8	17	0	92
1 day before hormone administration	30	20	—	70	80
3 days before hormone administration	25	16	—	75	82
6 days before hormone administration	20	15	—	80	85

30 larvae/experiment. Lt_{50}, time required for 50% mortality. Data were recorded for 9 weeks of experimental period.

first blood meal. The inhibition of feeding in the treated larvae suggests that, rather than the amount of nutrients ingested by the bug, the level of hormonally initiated biochemical activities which are usually associated with post-diapause development and apolysis regulate the feeding activity in *R. prolixus*. In nature, of course, the rate of production of hormone and the initiation of development processes are synchronized with the ingestion of sufficient amount of blood meal and stretching of abdominal receptors.



Effect of ecdysterone (2 μg/insect) on the feeding activity of the 3-week-old diapausing 5th instar larvae (circles) and male adults (square) of *R. prolixus*, at various times after the treatment. A) Percent inhibition of feeding (± SE) in the treated individuals. B) The amount of blood ingested (in g ± SE) by those individuals which showed feeding activity.

The precise role of ecdysterone in causing mortality and inhibition of feeding activity cannot be explained satisfactorily by the present data. The apparent lethargy and exhaustion in the treated larvae indicated the inability of the diapausing individuals with limited nutrient reserves to sustain various endergonic biosynthetic activities which are usually initiated by ecdysone. For instance, ecdysone stimulates RNA and protein synthesis in the fat body and epidermal cells, leading to moulting in the bugs[2]. Indeed, the 19-week-old larvae, which were in a state of starvation and contained only minute reserves of glycogen, lipids and protein[1,2] succumbed to hormonal treatment much earlier than the 3-week larvae. The mortality in the larvae may thus be attributed to the probable physiological derangement created by the hormone.

The adult mortality was probably due to the opposing actions of exogenous ecdysone and endogenous juvenile hormone; simultaneous administration of the two hormones was fatal to *Tenebrio molitor*[7]. Juvenile hormone regulates ovarian development in the female *R. prolixus*[8]; presumably the males also contain the hormone.

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On the Mineralocorticoid and Hypertensogenic Properties of 16β-Hydroxy-Dehydroepiandrosterone[1]

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Summary. Dosages of either 1 or 2 mg daily of 16β-hydroxy-dehydroepiandrosterone, given to mononephrectomized, salt-loaded female rats, had no detectable effect upon saline consumption, blood pressure, kallikrein excretion or heart and kidney weight. Its alleged mineralocorticoid properties, as judged by these criteria, were not demonstrable.

A recent article synopsizing evidence that low renin essential hypertension is caused by a mineralocorticoid (mc) hormone, reported and identified a possible culprit[2]. The C_{19} steroid, 16β-hydroxy-dehydroepiandrosterone (16β-OH-DHEA), provided the preponderant biologic mc activity in urine of patients with the disorder, in marked contrast to the urine of normotensives, or normal renin essential hypertensives, where the biologic mc potency was due to the aggregate activities of aldosterone, DOC, cortisol and corticosterone. 16β-OH-DHEA was found to have 1/40th the sodium-retaining potency of aldosterone in adrenalectomized rats. With an mc activity of that order, the quantities of 16β-OH-DHEA present in urine might well account for hypertensive disease.

All of the C_{21} mc's capable of causing hypertension in man do so in mononephrectomized, salt-loaded rats – including aldosterone[3,4], deoxycorticosterone[5,6], 18-hydro-

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