

somes^{13,14} and mitochondria^{15,16}. Recently, selenite has been reported to react with glutathione forming seleno-intermediates, such as seleno-persulfide and -trisulfide¹⁷. Thus a seleno-intermediate may facilitate the transfer of electrons from glutathione to methemoglobin, causing reduction of the latter.

We found that the mean selenium level in rat plasma was 3.45×10^{-6} M. Thus the acceleration of methemoglobin reduction by selenium may be a physiological phenomenon. The oxidative damage of hemoglobin observed in selenium deficient animals may also be due to lack of selenium-catalyzed reduction of methemoglobin as well as deficiency of glutathione peroxidase.

From in vitro studies in the absence of selenium, Scott et al.¹⁸ estimated that methemoglobin reduction by glutathione represents 13% of the total reduction. How-

ever, our data suggests that under physiological conditions, the capacity of glutathione to reduce methemoglobin in the presence of selenium may be greater than this.

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Aggregation pheromones in 2 Australian hard ticks, *Ixodes holocyclus* and *Aponomma concolor*

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Summary. The presence of an aggregation pheromone has been demonstrated for the first time in indigenous Australian ticks. Filter paper discs exposed to either Australian paralysis ticks *Ixodes holocyclus* or echidna ticks *Aponomma concolor* showed inter-sex or intra-sex attraction for ticks of their own species. Nymphal exuviae of *Ap. concolor* were highly attractive to adult ticks. Discs were significantly attractive to *I. holocyclus* at distances up to 80 cm.

An aggregation pheromone has been demonstrated in argasid ticks³⁻⁵, in an ixodid tick⁶ and sex pheromones in a number of ixodid tick species⁷⁻¹⁰. *Amblyomma americanum* and *A. maculatum*^{5,7}, *Dermacentor variabilis* and *D. andersoni*^{7,10}, males are attracted by pheromones secreted by females while *A. maculatum* females^{8,9} and *A. hebraeum* nymphs⁷ are attracted to secretions of attached males. The principal hosts of the paralysis tick, *Ixodes holocyclus*, are 3 species of bandicoot¹¹ (omnivorous marsupials - *Perameles nasuta*, *Isodon obesulus* and *I. macrourus*) but it attaches to a large number of hosts including cattle and domestic pets, causing paralysis and some fatalities. The echidna tick, *Aponomma concolor*, appears to be found only on the 2 echidna¹¹ species (monotremes - *Tachyglossus aculeatus* and *T. setosus*) and its effect on the host is unknown. Both ticks are almost certainly confined to Australia.

Materials and methods. 1. General aggregation. Tests were carried out using unfed ticks by the 'petri-dish' method essentially as described by Leahy et al.³, except that in some experiments, 4 sectors were used instead of 8. *I. holocyclus* ticks were humidified during tests by attaching a moistened filter paper inside the lid and illuminating artificially but *Ap. concolor* ticks were neither humidified nor illuminated¹². Filter paper discs were held with adult *I. holocyclus*¹³ for either 7 days after ecdysis, or between days 7-21 or 42-63 after ecdysis, and then assayed as centres of aggregation with ticks aged 7, 21 or 63 days respectively. Discs were also held with adult *Ap. concolor*¹³ between days 36-42, days 70-126, days 166-168 or days 231-238 after ecdysis and assayed with ticks aged 42, 126, 168 or 238 days respectively. In addition, assays of saline extracted discs¹⁴ and of nymphal exuviae were made for *Ap. concolor*. Significance

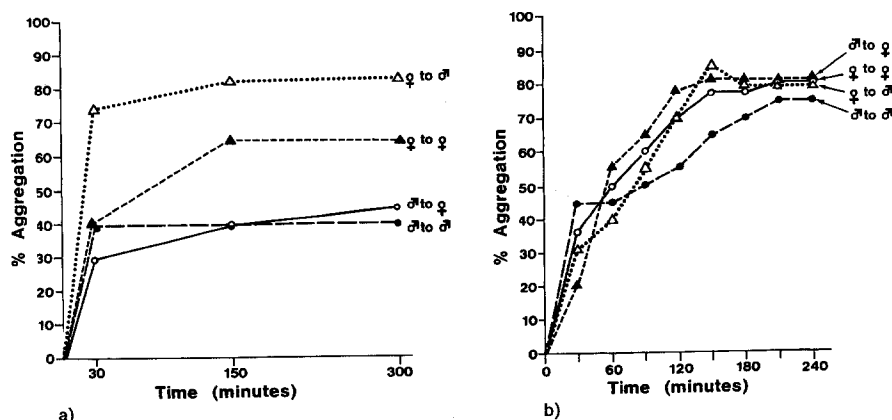


Fig. 1. Aggregation of unfed male or female ticks at discs previously treated by exposure to other ticks of the same-age (approximately 20 ticks per test). a *I. holocyclus* (7 days post ecdysis). b *Ap. concolor* (42 days post ecdysis).

of distribution in the treated and untreated areas was tested by chi-square with 1 df assuming that $\frac{1}{8}$ or $\frac{1}{4}$ of the ticks (according to the number of sectors) may be present by chance in each sector.

2. Distance test. Male and female *I. holocyclus* aged 42–56 days and *Ap. concolor* females, aged 238 days¹⁵, were distance tested in the dark by introducing 20–25 ticks centrally into a horizontal 1 cm diameter plastic and glass extensible tube closed off at one end by either a blank or treated disc contacted by ticks for 7 days.

Table 1. Percentage aggregation in 15 cm petri-dishes* of unfed, unmated ticks on filter paper discs (4 cm² area) contacted by conspecific ticks of that age (approximately 20 tick- per test)

Assay	<i>I. holocyclus</i>			<i>Ap. concolor</i>		
	Age: 7 (days post ecdysis)	21	63	42	126	168
♂ to ♂	40	80	25**	75	74	15**
♀ to ♀	64	10**	29	82	78	25**
♂ to ♀	45	67	55	82	45	—***
♀ to ♂	83	35	10**	80	40**	—***

*Method similar to that of Leahy et al.³. **The number aggregated not statistically significant ($\chi^2_{[1]}$ -test). ***Not tested.

Table 2. Percentage of exposed female ticks of each species moving towards and aggregating at discs in plastic and glass extensible* tubes

	<i>I. holocyclus</i>				<i>Ap. concolor</i>		
	Distance (cm)				Distance (cm)		
	10	20	60	80	10	20	40
	Percent aggregated				Percent aggregated		
On disc	92	86	95	82	90	85	47**
In disc end of tube	92	93	100	97	90	95	71**

Discs treated by contact with female ticks, over a range of distances, after 24 h exposure (20 ticks per test). *Varying lengths of glass tubing inserted into plastic centre piece. **The number aggregated not statistically significant ($\chi^2_{[1]}$ -test).

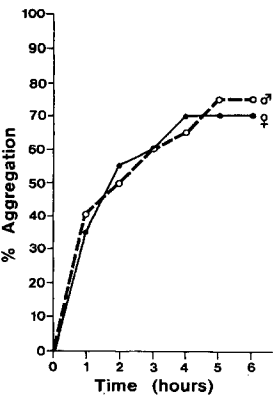


Fig. 2. Aggregation of unfed male or female *Ap. concolor* at discs treated with saline extracts of discs previously exposed to female ticks. Ticks (20 per test) were 35 days post ecdysis.

Results and discussion. Statistically significant aggregation ($p < 0.05$) at treated but not untreated discs occurred both within and between sexes and an apparent plateau was reached after 150 min for both species (figures 1a and 1b); it seems likely that both species liberate aggregation pheromones. Peak *I. holocyclus* aggregation occurred either at 7 or 21 days, tending to decline after 21 days (table 1). Aggregation of *Ap. concolor* was maintained at a similar level from 42 to 126 days for tests of within sexes attraction while it declined markedly between sexes but by 168 days all responses had apparently declined markedly. This may have been due to the short prior exposure of discs to ticks because the ticks remained active and were responsive up to 238 days in the short distance tests. Neither the *I. holocyclus* nor the *Ap. concolor* assays distinguished between the effects over time of declining secretion of the pheromone and declining responsiveness. The significant aggregation of *Ap. concolor* at discs treated with saline extracts of tick-

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- 2 Our thanks to Mr I. J. Lewis, Director, Cattle Tick Research Station, for initiating the study of possible pheromones in *I. holocyclus*, and for his continued encouragement throughout.
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- 12 *I. holocyclus* ticks are very sensitive to desiccation while *Ap. concolor* are relatively inactive in daylight.
- 13 The difference in ages was due to the initial experiments being conducted independently. *Ap. concolor* appears to have a much greater longevity than *I. holocyclus*.
- 14 4–6 discs were soaked in 1 ml of 1% NaCl solution for 24 h and the extract reapplied to fresh discs.
- 15 These ticks were derived from the original batch which was the only one available for use throughout this series of experiments but remained active at this advance age.

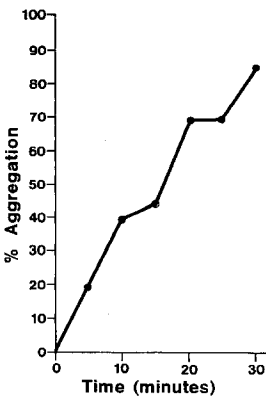


Fig. 3. Aggregation of female *Ap. concolor* at nymphal exuviae. Ticks (20 per test) were 35 days post ecdysis.

contacted discs (figure 2) was similar to aggregation at the original discs, demonstrating that pheromone can be successfully extracted in this medium. This is in agreement with findings for *Argas persicus*³.

Highly significant aggregation of female *Ap. concolor* on nymphal exuviae of that species ($p < 0.001$) occurred within 30 min (figures 3 and 4), and far exceeded the level of aggregation achieved with tick-contacted discs over the same period, although the final aggregation achieved with the disc assays was similar. It is not known whether the exuviae represented a more concentrated source of pheromone than the discs, or whether the more rapid aggregation was due to a different pheromone. Female-contacted discs produced significant aggregation of female ticks of *I. holocyclus* and *Ap. concolor* at

distances up to 80 cm and 40 cm respectively (table 2). Aggregation under natural conditions may assist both species in their host seeking success. In the case of *I. holocyclus*, where copulation between unfed adults has been reported¹⁶ and where, presumably, off-host mating may occur under natural conditions, aggregation of males and females could be an important factor in the fertilization of females. The attraction of nymphal cuticles for adults of *Ap. concolor* would certainly intensify the aggregation. However, there is no information available of the local distribution of adults of either species in an infested site.

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Natriuresis after water loading in rats bearing transplants of pars intermedia

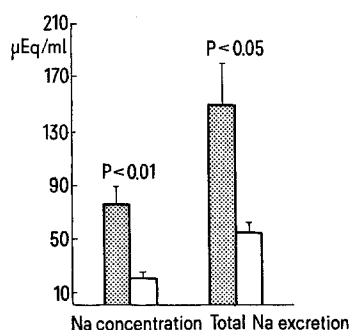
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Summary. After water loading, rats bearing ocular grafts of the pars intermedia of the pituitary showed that the content of sodium increased in urine, whereas potassium excretion and diuresis did not vary significantly. These results suggest that in the rat the pars intermedia is involved in the regulation of the electrolyte metabolism.

Orias and McCann^{2,3} showed that natriuresis rose in water loaded rats after the injection of melanocyte-stimulating hormone (MSH). Whether the natriuretic effect elicited by the dose level used by these authors evidenced an actual 'physiological' response, or whether it should be assigned to a 'pharmacological' one, is a point under discussion since little is known on the ability of the pars intermedia to secrete MSH at levels similar to the ones reached in blood after the exogenous treatment. Moreover, the question remains if the hormone secreted by the rat pituitary could evoke a natriuretic response in its own species.

Kastin and Ross⁴ were the first to show in rats what is nowadays a well accepted fact that the pars intermedia disconnected from the hypothalamus oversecretes MSH. This observation allows us to design a simple model for obtaining animals with continuous high levels of circulating endogenous MSH, offering a condition useful for clarifying the problem.



Urine sodium excretion in grafted and control rats. Values are the mean of 5 rats. Dotted bars correspond to grafted animals and clear bars to controls. p-values are at the top of each bar.

Materials and methods. Male Sprague-Dawley rats weighing 250–260 g at the moment of the transplantation were used. One neurointermediate lobe per receptor animal was grafted into the anterior chamber of the right eye, according to the technique of Olson and Malmfors⁵. Donors were rats of the same age and sex. After 70 days the grafts were observed under a stereomicroscope and those rats showing healthy, well growing transplants were selected together with an equal number of sham operated animals. From the moment of transplantation to the completion of the studies, the rats were maintained in a photoperiod of 12 h of darkness and 12 h of illumination, at a temperature 19–21 °C.

5 grafted rats and 5 controls were placed individually in metabolic cages and at the time of the experiment (09.00 a.m.) the rats were given by stomach tube 10 ml tap water. 1 h later they received a second gavage of the same volume. Urine was collected for 2 h from the moment of the second water loading. After the volume was measured, sodium and potassium content was estimated by flame spectrophotometry and expressed as μEq of cation excretion. During urine collection the animals were deprived of water and food intake.

After completion of the experiment, the rats were decapitated and blood collected in heparinized tubes. Plasma was obtained after centrifugation and it was diluted 2:1 with distilled water. Plasma MSH activity was tested in this material, semiquantitatively, using skins of the toad

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