In contrast with rapidly accumulating contradictory reports concerning the action of cyclic-AMP on phagocytic events, there are practically no reports about the influence of its counterpartner cyclic-GMP.

Other parameters of phagocytic activity are known to be influenced by cyclic-GMP. ESTENSEN et al.[10] reported recently that cyclic-GMP and cholinergic agents enhance degranulation of PMN and increase its leucotactic activity. Cyclic-AMP inhibits both leucotaxis[11] and degranulation[12]. These antagonistic activities of both nucleotides were also clearly demonstrated in our system. The effect of MB (a known stimulator of hexose monophosphate shunt) in increasing the NBT reduction was previously demonstrated[13]. It was further shown that ascorbic acid, another stimulator of hexose monophosphate shunt[14], also increases the NBT reduction ability of PMN[15].

In this investigation it is shown that when leucocytes are pre- incubated with cyclic-AMP and then endotoxin, ascorbic acid or MB are added, the stimulating effect of endotoxin and ascorbic acid is markedly reduced, while that of MB is unchanged. This might suggest that we are dealing with different mechanisms of stimulation of NBT reduction. Although our conclusions were made indirectly, they support the previous suggestions[6] about the influence of cyclic nucleotides on the intracellular mechanisms during phagocytosis and the antagonistic activities of cyclic-AMP and cyclic-GMP.

*Résumé.* On a étudié l'influence des nucléotides cycliques (AMP-cyclique et GMP-cyclique) sur la réduction du NBT. L'AMP-cyclique ainsi que la théophylline inhibent cette réduction, tandis que la GMP-cyclique l'active. La préincubation de leucocytes en présence de l'AMP-cyclique annule l'activation de la réduction par l'acide ascorbique mais ne l'influence pas par le bleu de méthylène. On peut en conclure que dans ce système, comme dans les autres, ces deux nucléotides jouent un rôle d'antagonistes.

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## Relative Oxygenase Activities in Juvenile Hormone Biosynthesis of Corpora Allata of an African Locust (*Schistocerca gregaria*) and American Cockroach (*Periplaneta americana*)

All known insect juvenile hormones contain a 10,11-oxirane ring in the sesquiterpenoid moiety of the molecule[1], and it is believed that this ring is introduced at the last enzymatic step in biosynthesis by mono-oxygenation of the corresponding sesquiterpenoid olefinic ester[2]. In this report we concern ourselves with the activity of this terminal oxygenase in corpora allata of 2 insect species, as revealed by the application of short-term in vitro radiolabelled assay procedures. Work in this laboratory[2] has shown that when corpora allata are taken from adult female *Schistocerca gregaria*, addition of sesquiterpenoid acid to the medium results in a large increase in the rate of biosynthesis of juvenile hormone, and that the corresponding olefinic ester is detectable within the glands. The kinetics of incorporation of (methyl-$^{14}$C) methionine and (C-2 $^{3}$H) *trans,trans*-farnesenic acid into both the olefinic ester (methyl farnesoate) and the 10,11 epoxy ester ($C_{16}$JH) confirm that methyl farnesoate is the immediate precursor of this juvenile hormone in *S. gregaria*[3]. Here we have compared the rate of epoxidation with the intracellular amount of methyl farnesoate over a wide range of epoxidation rates by utilizing both the natural variations in the biosynthetic capacity of the glands in reproductively active female locusts during the course of ovarian maturation (TOBE and PRATT, in preparation) and the effects of graded additions of farnesenic acid to the incubation medium[3]. For comparative purposes, we have also examined the relationship which obtains in corpora allata of reproductively active *Periplaneta americana*, whose principal juvenile hormone has also been identified as $C_{16}$JH (PRATT, unpublished data)[4]. We shall show that there is a large difference in the observed oxygenase activity of the glands from the 2 species and that in the case of the locust, there is no evidence that this is normally a rate-limiting step in juvenile hormone biosynthesis.

*Methods.* Animals were reared as previously described[2,5]; the female locusts employed were of known age between 5 and 20 days old, female cockroaches were of unknown age taken at different intervals throughout the oviposition cycle. The procedures for preparation of radio-labelled incubation media, incubation of the glands, extraction, separation and quantitation of the products by radio-TLC and liquid scintillation spectrometry were identical to, or minor modifications of, those described elsewhere[2,3]. In many of the experiments, tissue culture medium 199 (without glutamine, bicarbonate; with HEPES buffer 20 m*M*, pH 7.2) (Flow Control Laboratories Ltd.) served as the basis of the radio-labelled incubation medium. L-methionine was always present at a final concentration of 0.29 m*M* and (methyl-$^{14}$C) methionine (Amersham-Searle) was present at final specific radioactivities of 10.5 to 36.6 mCi/mmol in different experiments. When present, (C-2 $^{3}$H) *trans,trans* farne-

[1] $C_{16}$JH: methyl, 10,11-epoxy-3,7,11-trimethyl-*trans,trans*, 10R-2, 6-dodecadienoate; $C_{17}$JH: methyl 10, 11-epoxy-3, 7, 11-trimethyl-*trans, trans, cis* 10R, 11S-2,6-tridecadienoate; $C_{18}$JH: methyl 10, 11-epoxy-3,11-dimethyl-7-ethyl-*trans, trans, cis* 10R, 11S-2,6-tridecadienoate.
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senic acid was employed at a specific radioactivity of 25 mCi/mmol, in concentrations ranging from 4.8 to 24 $\mu M$. The identity of the radio-labelled hormone released from corpora allata of *P. americana*, was furtner confirmed by radio-GLC on 1.8 m columns of neutralized Carbowax 20M on Gas-Chrom Q, using isomeric mixtures of authentic non-radioactive juvenile hormones as internal reference compounds. The quantities of methyl farnesoate within the glands, and the rates of $C_{16}$JH biosynthesis were calculated from the incorporation of radio-labelled methionine, based upon its known specific radioactivity in the medium. All incubations were carried out for a period of 3 h. In some experiments the medium was separated from the glands at the end of the incubation prior to extraction; in these cases the total $C_{16}$JH present in glands and medium was used to calculate the average rate of biosynthesis of hormone.

*Results and discussion.* The corpora allata of *P. americana* resemble those of *S. gregaria* in that they are capable of efficiently incorporating exogenously supplied (C–2 $^3$H) *trans,trans*-farnesenic acid into double-labelled $C_{16}$JH when incubated in suitable media containing (methyl $^{14}$C) methionine. Moreover, in all experiments using corpora allata of similar de novo biosynthetic capacity, addition of farnesenic acid to the medium brought about an increase in the rate of hormone synthesis. However, there are marked quantitative differences between the glands of the 2 species. We have observed in the case of corpora allata from *S. gregaria* that the addition of optimum concentrations of farnesenic acid to the medium may, depending upon the physiological state of the glands, bring about increases of from 2-fold up to 100-fold in the rate of hormone synthesis. However, its addition to incubations of glands from *P. americana* rarely increases their rate of synthesis by more than 5-fold, and frequently by as little as 1.5-fold. This lower capacity of cockroach corpora allata to utilize exogenous farnesenic acid to promote very high rates of $C_{16}$JH synthesis cannot be due to restricted penetration by, or intra-cellular transport, of the acid as evidenced by the large quantities of methyl farnesoate which accumulate within the glands during incubation. This indicates that the exogenous intermediate has facile access to the biosynthetic pathway in the corpora allata of both species, and that it is rapidly esterified to the olefinic ester, presumably using S-adenosyl methionine[6]. Thus the observed differences can only be attributed to differences between the 2 species in the activities of the terminal oxygenase enzymes.

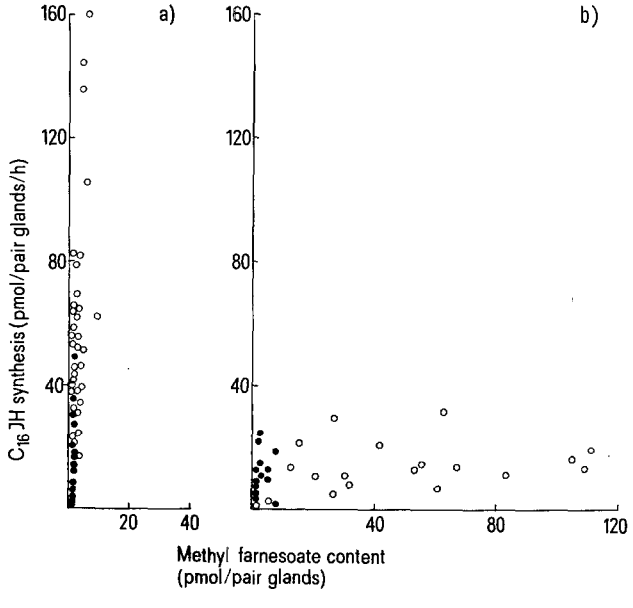


Fig. 1. Relationship between the rate of $C_{16}$JH synthesis and the intraglandular content of methyl farnesoate in corpora allata of a) *S. gregaria* and b) *P. americana*, as revealed by the incorporation of [methyl-$^{14}$C]methionine. ○, addition of farnesenic acid to the medium; ●, no addition of farnesenic acid.

In Figure 1 we correlate the observed rate of epoxidation to $C_{16}$ JH with the quantity of methyl farnesoate in the glands of these 2 insect species during both de novo biosynthesis and farnesenic acid stimulated synthesis. Since there is as yet no information on the identity or spatial dimensions of the intracellular compartment which contains the substrate for the oxygenase, it is not possible to express the amount of methyl farnesoate in terms of concentration sensu strictu. Indeed, much of the massive amounts of this material accumulating within the corpora allata of *P. americana*, when supplied with exogenous farnesenic acid, may well be dissolved in some lipoidal compartment of the cell which is not in direct contact with the enzyme. It is clear from Figure 1 that, despite the large biological variation, there is real difference between the oxygenase systems in these 2 insects. In corpora allata from *S. gregaria* there appears to be a general proportionality between the rate of epoxidation and the glandular content of methyl farnesoate after 3 h incubation. There is no evidence that the oxygenase becomes saturated with substrate, even at the highest rates achieved (up to 160 pmol/pair glands/h). Clearly the epoxidative capacity of these glands is normally much greater than their capacity for esterification. By contrast, the corpora allata of *P. americana* have a strictly limited expoxidative capacity and are rarely capable of exceeding a rate of 25 pmol/pair glands/h, despite having a very active esterification system which can lead to the accumulation of over 150 pmol of methyl farnesoate per pair glands after 3 h. As shown in Figure 1, these large accumulations of methyl farnesoate have not been observed under conditions of de novo synthesis thus far, which may be evidence of a feed-back mechanism which normally regulates the rate of synthesis of farnesenic acid so as to prevent total saturation of the oxygenase enzyme by methyl farnesoate.

During our in vitro incubation the medium surrounding the glands is kept in gaseous equilibrium with the atmosphere by gentle shaking. It is known that increasing the percentage of oxygen in the gas phase to 100% does not increase the rate of epoxidation by *S. gregaria* corpora allata[3]. In the intact animal, the corpora allata are supplied with oxygen via the tracheal system. When freshly dissected corpora allata are observed microscopically, the number of tracheoles entering the glands is easily visible: Figure 2 illustrates the contrast between typical specimens from each species. It is of interest to observe that the corpora allata of *S. gregaria* have a high level of oxygenase activity and are richly endowed with tracheolar end-cells, whereas those of *P. americana* have a limited oxygenase activity and are very poorly supplied with tracheoles. The corpora allata of *S. gregaria* have the appearance of a highly aerobic tissue, and this could reflect both the demands of the terminal oxygenase

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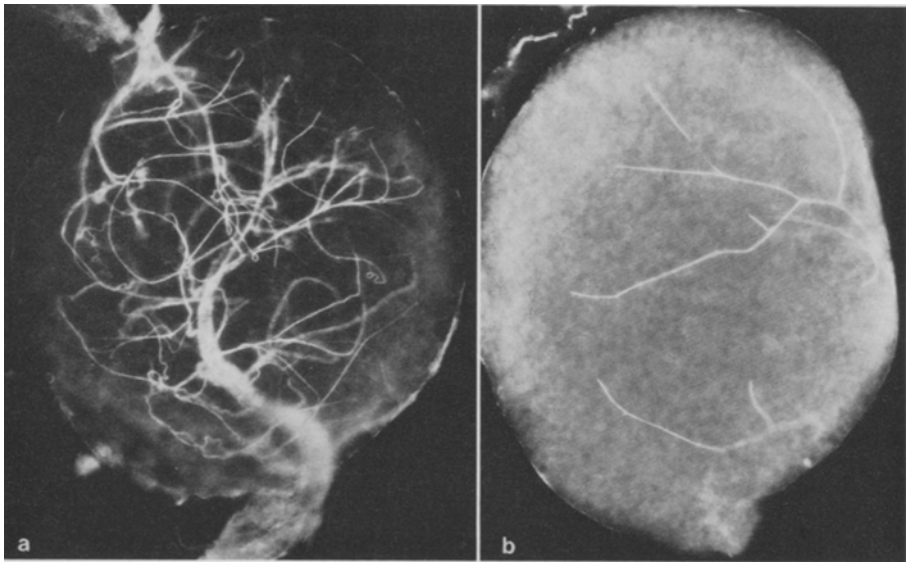


Fig. 2. Freshly dissected corpora allata from adult females of a) *S. gregaria*, and b) *P. americana*, viewed under dark-field illumination. The glands are approximately 300 µm in diameter. The tracheoles are easily visible.

system and of the aerobic respiration required to support high rates of de novo biosynthesis of juvenile hormone during periods of maximum glandular activity[7].

*Résumé.* Nous avons comparé in vitro dans les corpora allata de femelles adultes de sauterelles (*Schistocerca gregaria*) et de cafards (*Periplaneta americana*) le taux de composition de la méthyl farnesoate marquée et son epoxydation due à l'hormone juvénile $C_{16}$. Chez les sauterelles, un surcroît d'acide farnésique stimule l'estérification et l'époxydation, tandis que chez les cafards, une accumulation de méthyl farnesoate dans les glandes et l'indice d'une saturation de l'enzyme oxygénase. Cette contenance oxydative plus basse des glandes de cafard est en rapport avec leur trachéolation peu abondante.

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[7] We are grateful to Miss M. M. BLIGHT and Dr. A. F. WHITE for supplying reference compounds. S. S. TOBE acknowledges receipt of a Post-doctoral Fellowship from the National Research Council of Canada.

## Pinealectomy Inhibits Stimulation of Testicular Development by Long Photoperiods in a Hamster (*Phodopus sungorus*)

In mammals the pineal is generally assumed to have antigonadotrophic effects, which are increased by darkness or short photoperiods, and suppressed by light or long photoperiods[1,2]. REITER[2] has suggested that under long photoperiods 'all animals are effectively 'pinealectomized' since photic information is known to be inhibitory to pineal function'. Experiments in 2 species of hamsters corroborated this conjecture: in animals kept in long photoperiods, no effect of pinealectomy on testicular activity was observed, while the gonadal regression which is normally brought about by short photoperiods could be prevented by pinealectomy[3,4]. However, these observations were made in hamsters that had been kept in long photoperiods before pinealectomy, and had large and active gonads at that time. In sexually quiescent female ferrets, on the other hand, in which long photoperiods can induce estrus already in midwinter, this premature onset of estrus was delayed by pinealectomy[5]. In view of these discrepancies, the effect of pinealectomy under long and short photoperiods was tested in males of the Djungarian hamster *Phodopus sungorus*, a species in which marked photoperiodic reactions have been described[4,6–8]. The experiment was started in winter when the animals were sexually quiescent.

Males were used that had been kept under natural light conditions. Between January 4th and 8th, 22 hamsters were pinealectomized (P) following the method given by HOFFMAN and REITER[9]. Nine hamsters were sham-

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