Table II. Catches of male *Choristoneura rosaceana* with various *cis*-TDAC/*cis*-TDAL mixtures

Bait (100 µg)		Mile 56 July 4–July 17	
cis-TDAC		8	(2)
cis-TDAC/*cis*-TDAL	(100/1)	64	(2)
	(10/1)	35.5	(2)
	(1/1)	16	(2)
	(1/10)	4.5	(2)
	(1/100)	15	(2)
cis-TDAL		0	(2)

Figures indicate ♂♂/trap; figures in brackets = number of traps.

Table III. Catches of male *Choristoneura fumiferana* in traps baited with various isomer mixtures of TDAL

Bait (100 µg)		Mile 56 July 7–July 17		Thessalon July 2–July 12	
trans-TDAL		16.3	(3)	—	
trans-TDAL/*cis*-TDAL	(100/1)	107	(2)	50	(2)
	(10/1)	147.5	(2)	58	(2)
	(1/1)	22	(2)	7	(2)
	(1/10)	5.5	(2)	0.5	(2)
	(1/100)	1	(2)	1	(2)
cis-TDAL		0	(2)	—	

Figures indicate ♂♂/trap; figures in brackets = number of traps.

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At this time it is concluded that *cis*-11-tetradecenal is a potent attractant for male *Choristoneura conflictana*. Verification of this compound as the primary component of the sex pheromone of this insect, and elucidation of any secondary components, must await the results of laboratory studies now in progress.

From the field tests conducted at Thessalon and Mile 56 several interesting inferences may be made regarding the sex pheromone systems of 2 other *Choristoneura* species, the oblique banded leafroller *Choristoneura rosaceana* and the spruce budworm *Choristoneura fumiferana*. Roelofs and Arn[6] identified *cis*-TDAC as the sex attractant of the red banded leafroller, *Argyrotaenia velutinana*. The same compound was later shown to be the sex attractant of the oblique banded leafroller[7]. These species have overlapping seasonal and diurnal cycles, and share the same host plants, and the authors were uncertain as to how specificity was effected. A reinvestigation of the sex pheromone system of the red banded leafroller has revealed that dodecyl acetate and a small amount of *trans*-TDAC are secondary components in the blend[8]. As shown in Table II the addition of approximately 1% of *cis*-TDAL to the primary *C. rosaceana* pheromone dramatically increases attractancy. It is postulated that a reinvestigation of extracts from female oblique banded leafrollers would reveal the presence of *cis*-TDAL as a secondary component, and a further means of effecting specificity (Table II).

The spruce budworm *Choristoneura fumiferana* was the first lepidopteran species shown to possess an aldehyde as a female produced sex attractant. The elucidation of the pheromone structure as *trans*-TDAL[5] was followed by a report that *trans*-TDOL and *trans*-TDAC exhibited significant inhibitory activity in the field when mixed with the budworm pheromone[9]. Results (Table III) from Mile 56 dispute the isomeric integrity of the budworm pheromone, and place the optimum amount of *cis* isomer between 1% and 9%. The trend, also borne out by the Thessalon data, indicates that greater than 9% of the *cis* compound reduces the effectiveness of the attractant.

Adenyl Cyclase Activity of Mouse Liver Membranes after Incubation with Endotoxin and Epinephrine

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Summary. Adenyl cyclase activity in isolated mouse liver cell membranes was stimulated two-fold by endotoxin. Furthermore, endotoxin inhibited epinephrine induction of adenyl cyclase activity, apparently through interruption of the phospholipid moiety of the enzyme complex.

Endotoxin, a lipopolysaccharide (LPS) extracted from gram-negative bacteria[1], has an affinity for the phospholipid portion of cell membranes[2–4]. In the liver, the interaction of endotoxin with cell membrane phospholipids could interfere with the synthesis of adenosine 3′,5′-monophosphate (cyclic-AMP), a compound essential in liver carbohydrate metabolism. Indeed, in vivo, endotoxin decreases total body carbohydrate by 80%[5,6] and a marked depletion of liver glycogen has been shown to be a contributing factor in the lethality of endotoxemia[7].

It was hypothesized that if endotoxin interacts with cell membranes, it may alter the activity of adenyl cyclase, an enzyme located within the plasma membrane. To determine if endotoxin altered the activity of liver cell adenyl cyclase, the amount of cyclic AMP formed was ascertained after the in vitro conversion of ^{14}C-adenosine labeled ATP to ^{14}C-labeled cyclic-AMP by adenyl cyclase. Additionally, the modifying action of LPS on epinephrine

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activation of adenyl cyclase was measured. This hormone was shown by SUTHERLAND[8] to mediate glyconeogenesis via cyclic-AMP.

Materials and methods. Liver preparations. Livers were removed from male (C57B1/6×CBA) F_1 hybrid mice obtained from Cumberland View Farms, Clinton, Tennessee. When adenyl cyclase activity of hepatic cell homogenates was determined, the livers were homogenized in chilled 50 m*M* *tris*(hydroxymethyl)aminomethane (*TRIS*) buffer pH 7.6.

Membrane purifications. Plasma membranes were purified from 6 g of mouse liver. This was accomplished on an aqueous two-phase polymer system as previously described[9].

Measurement of adenyl cyclase activity. Adenyl cyclase activity was assayed by the conversion of ATP to cyclic-AMP by that enzyme. The reaction was carried out in a system containing 2.0–2.5 (0.2–0.3) mg protein equivalents of either whole liver cells or purified plasma membranes in a total volume of 0.25 ml. This volume contained concentrations of 25 m*M* *Tris*-HCl of pH 7.5, 2 m*M* 8-C-14 ATP (1 μCi/mole, Amersham-Searle), 1 m*M* unlabeled cyclic AMP (Sigma), 5 m*M* $MgCl_2$, 10 m*M* theophylline, and, when added: 0.1 m*M* L-epinephrine bitartrate (Sigma) solubilized immediately before use, 10 m*M* NaF, or 0.5 mg of *Salmonella typhosa* endotoxin (Difco). The reaction was terminated after a 10 min incubation at 37 °C by immersing the tubes in boiling water for 3 min.

After cooling and centrifugation, 50–100 μl of the supernatant were spotted on Whatman No. 3 paper. ATP, ADP, AMP, adenosine and adenine were separated from cyclic AMP by descending chromatography in isopropanol-formic acid-water (70:5:30, v/v) solvent. 5 to 15 μg of each nucleotide, nucleoside or base were also spotted on the chromatogram to allow for visualization of the spots under UV-light. The separated cyclic AMP was identified, cut from the chromatogram and counted in a Nuclear-Chicago scintillation counter in 10 ml of Spectrafluor (Amersham-Searle) scintillation cocktail.

Results. Adenyl cyclase response to endotoxin. The amounts of cyclic AMP formed in vitro when liver homogenate was incubated with endotoxin and/or epinephrine are presented in Figure 1. Incubation of whole liver cell homogenates with LPS or epinephrine stimulated approximately 2- and 3-fold increases, respectively, in cyclic AMP formation. Epinephrine stimulation of adenyl cyclase activity was reduced in the presence of endotoxin. When purified plasma membranes were used, responses observed in the homogenates were similar but smaller in magnitude. The epinephrine response has been shown to be decreased in purified membranes, and it is generally assumed that the epinephrine receptor may be partially inactivated during the plasma membrane purification procedure[10–12].

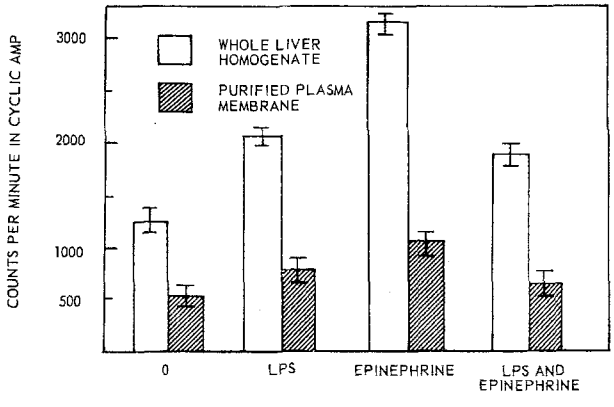

Fig. 1. Cyclic AMP formation in liver homogenate and purified liver cell membranes after incubation with endotoxin and epinephrine.

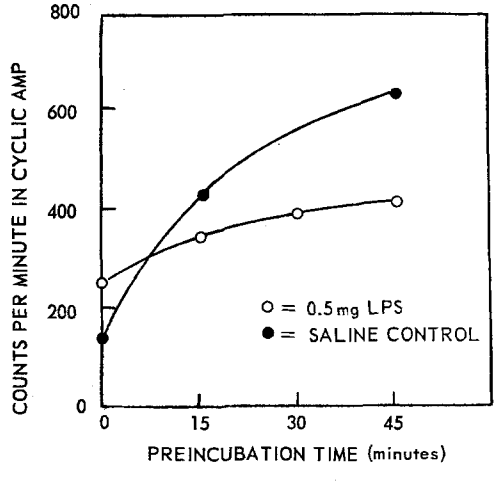

Fig. 2. Cyclic AMP formation in purified liver cell membranes activated by NaF with or without incubation in LPS.

Sodium fluoride activation. Stimulation of membrane adenyl cyclase by sodium fluoride resulted in an enzyme activation curve (Figure 2) in purified liver membranes similar to that reported by others[13] for heart and brain adenyl cyclase. Membranes were incubated with endotoxin and NaF for the specified time periods, then labeled substrate was added and incubation was continued for an additional 10 min. Endotoxin interfered with the sodium fluoride activation of purified mouse liver plasma membranes. After 45 min of incubation with endotoxin, sodium fluoride activation of adenyl cyclase was only 60% of that seen in preparations to which endotoxin was not added.

Discussion. The data presented in this report support our hypothesis that endotoxin can directly stimulate adenyl cyclase activity in mouse liver cell membranes in vitro. This finding is in agreement with those of GIMPEL et al.[14] who also demonstrated that injection of *Escherichia coli* endotoxin caused hepatic adenyl cyclase to increase. This increase in enzyme activity did not alter phosphodiesterase activity in liver tissue[15] implying a direct interaction between endotoxin and the adenyl

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cyclase-membrane complex. In addition, we found that epinephrine stimulation of adenyl cyclase activity was inhibited by endotoxin.

Others[16] have obtained results which conflict with those presented here. They found no increase in cyclic-AMP with endotoxin alone, but observed an augmentation of the epinephrine response. In their model, however, endotoxin was injected in vivo and homogenates made. Our in vitro system, in contrast, permits the endotoxin to interact in a controlled amount directly with the membrane.

It is possible that endotoxin could block epinephrine binding to the receptor unit. This appears unlikely, however, because endotoxin, even at 1 mg/ml concentrations, does not interfere with the binding of norepinephrine to isolated fat cell membranes (K. KORITZ, unpublished data). It was not possible to perform this experiment with epinephrine, but a similar lack of interference with epinephrine binding may be inferred.

A possible explanation for endotoxin inhibition of adenyl cyclase responsiveness to epinephrine is an interaction of endotoxin with membrane phospholipids. The lipid portion of the membrane is necessary for adenyl cyclase activity since solubilized preparations of adenyl cyclase are unresponsive to hormonal stimulation[8,17]. Furthermore, hormone responsiveness in these solubilized preparations can be restored by the addition of specific phospholipids[11,17].

Endotoxin-phospholipid interaction may be explained by the three-component model of the adenyl cyclase system[13]. In this model, an intermediate phospholipid moiety (transducer) functions between the hormone receptor on the external surface of the plasma membrane and the catalytic unit (adenyl cyclase) on the internal surface of the plasma membrane. Interruption of hormonal induction through transducer alteration may explain how endotoxin stimulates adenyl cyclase activity and blocks epinephrine stimulation of that enzyme.

Evidence for an interaction of endotoxin with the transducer segment of the membrane-enzyme complex comes from our study of endotoxin inhibition of sodium fluoride activation of adenyl cyclase. This activation is postulated to occur at the transducer (phospholipid moiety) level[18]. As previously stated, endotoxin has an affinity for the phospholipid portion of cell membranes[3,4]. Since endotoxin interfered with the sodium fluoride activation of purified mouse liver membrane adenyl cyclase, it is probable that the toxin could act at the transducer level, which may be the phospholipid moiety of the membrane-enzyme complex.

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The Choice of Oviposition Sites by the Lady Bird Beetle *Adalia bipunctata* (L.)

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Summary. Adalia bipunctata females lay eggs on brussels sprout leaves in presence or absence of aphids, but the latter furnish an additional stimulus. The glass walls of the jar attracted the adults to lay eggs on it to some extent. Presence of cemented eggs did not hinder the oviposition of the adults on the brussels sprouts leaves.

It was previously known that the odour of aphids was the most important component of the stimulus pattern for the oviposition of many aphidophagous insects[1–3]. Other authors[4,5] reported that many coccinellid egg batches occurred on uninfested plant stems.

The aim of the present work is to assess the role of the host plant as well as other substrates on the oviposition behaviour of *Adalia bipunctata*, in the presence or absence of aphid stimulus.

Methods. Single pair of newly emerged *A. bipunctata* adults was confined in a glass jar, 1 kg capacity with muslin cloth roof. A brussels sprouts leaf cemented with 100 aphids (*Myzus persicae*) was offered daily to the adults in the cage. Another cage containing one pair of adults offered 2 plant leaves, one of them cemented with 100 aphids and the other leaf was free from aphids. The petioles of all leaves offered were wrapped with moistened cotton to prevent their wilting. The cages were illuminated from above with fluorescent lamps (2400 Lux) for 16 h day length.

In another experiment 80 *A. bipunctata* newly laid eggs were marked with red colour, cemented on a brussels sprouts leaf and offered daily to a pair of adults (40 eggs/individual[6]). In another cage, 1 pair of adults were offered daily 2 plant leaves, one of them cemented with 80 eggs and the other leaf free from eggs.

These experiments were repeated 10 times. The number of eggs laid on the plant leaves as well as of those laid elsewhere in the jar were recorded throughout the life of each female. The relative suitability of the different substrates for oviposition was compared with that of the brussels leaves which were taken as the standard. This was calculated as $\frac{T-S}{T+S} \times 100$ where T and S being the number of eggs laid on the other substrates and the standard substrate respectively[7]. Values above or below zero reflected greater or lesser preference for the other materials with regard to the standard.

Results. 1. Oviposition site selection in the presence of aphids. The relative suitability of the different substrates for oviposition was compared with that of the brussels sprouts plant leaves. The data in Table I showed that the plant leaves elicited a higher ovipositional response,

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