(10 µmole/insect) at the time of ecdysis and the start of cuticle tanning as was shown in previous studies[13,14]. During the time of tyrosine accumulation in the pharate adult, tyrosine hydroxylase activity remained very low (< 1 nmole/min/insect) its activity was not significantly greater than during the nymphal period (figure, B). Total tyrosine hydroxylase activity increased over 30-fold at the time of ecdysis suggesting enzyme activation or synthesis perhaps linked to the release of bursicon during ecdysis[3]. Tyrosine hydroxylase activity then continued to rise to a peak at 6 h after ecdysis (18 nmoles/min/insect), whereas tyrosine titers rapidly decreased during the post ecdysial period of intense cuticular tanning. Both enzyme activity and substrate titers decreased rapidly during the next 18 h, then more gradually the next few days.

Previous studies have shown that dopa decarboxylase increases during the pharate adult period concomitant with the rise in free tyrosine[5]. However, little or no metabolism of tyrosine occurs because the amino acid is not a good substrate for that enzyme[5,6]. In vivo studies have also shown very little decarboxylation prior to ecdysis but high levels thereafter[7,8]. Therefore, tyrosine is not metabolized to any appreciable extent prior to the activation or synthesis of tyrosine hydroxylase, a critical factor in the build-up of a large substrate pool[15]. Tyrosine hydroxylase which arises during or shortly after ecdysis appears to be the controlling enzyme system for initiating tanning substrate biosynthesis in the cockroach, *P. americana.*

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The effect of camphor on mitochondrial respiration

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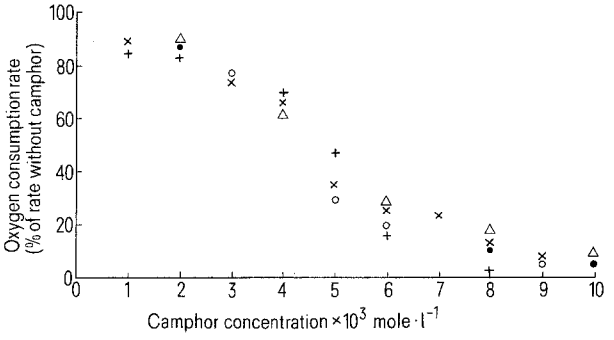
Summary. Camphor at < 8 µmoles/mg protein reduced the rate of oxygen consumption by rat liver mitochondria. The effect occurs only with NAD^+-linked substrates. Succinate linked respiration was inhibited but this appears to be caused by some conversion of succinate to malate. At higher levels, camphor increases oxygen consumption with succinate substrate, by uncoupling at site II.

Camphor has been shown to reduce oxygen consumption in *E. coli*[4,5], and mammalian mitochondria (rat kidney)[6]. The work with *E. coli* suggested that camphor seems to have some effect on the cell membrane. While camphor is not as effective as many other respiration inhibitors, there has been no previous study of the mechanism by which camphor reduces oxygen consumption. This mechanism is the subject of the present study.

A large number of chemicals have been shown to inhibit oxygen consumption of isolated mitochondrial preparations by either blocking electron transport at specific points in the respiratory chain or energy transfer at sites leading to ATP production[7-13]. In the study reported here, inhibitors blocking at different points in the electron transport chain have been used to locate the site of camphor interaction with the respiratory chain.

Materials and methods. Liver mitochondria from 170–220 g male Wistar rats were prepared by a method similar to that of Schneider[14] in a solution containing 0.25 M sucrose, 10 mM HEPES buffer at pH 7.4 and 1 mM ethylenediaminetetraacetic acid.

The mitochondria were washed twice and resuspended in a medium containing only 0.25 M sucrose and 10 mM HEPES. The protein content of each preparation was determined by the biuret reaction. The rate of mitochondrial oxygen consumption was determined by measuring the concentration of oxygen in solution as a function of time with a Clark type electrode. The mitochondria were resuspended in the incubation medium at a concentration of 1 mg protein/ml. Acceptor control ratios at 20 °C were routinely measured for each preparation. The rate of ox-



Rate of oxygen consumption expressed as a percentage of the rate without camphor present. Several determinations were done using different substrates: △ and X malate plus glutamate; + glutamate plus pyruvate; O pyruvate plus malate; ● glutamate.

ygen consumption in stage 4 respiration was measured in a medium containing 50 mM KCl; 25 mM Tris-HCl at pH 7.4; 10 mM KH_2PO_4/Na_2HPO_4 buffer at pH 7.4; 8 mM $MgCl_2$; 70 mM sucrose and 0.05% bovine serum albumin. The following NAD^+-linked substrates were used: 5 mM pyruvate; 5 mM pyruvate plus 5 mM malate; 10 mM glutamate; 10 mM glutamate plus 5 mM malate, 10 mM glutamate plus 5 mM pyruvate. Succinate at a concentration of 5 mM or with 3 $\mu mole \cdot l^{-1}$ rotenone was used to study succinate: ubiquinone reductase linked oxygen uptake. Finally 5 mM ascorbate plus 0.25 mM tetramethylphenylenediamine (TMPD) and 1 $\mu g \cdot mg^{-1}$ protein of antimycin A was used to study the effect of camphor on cytochrome c linked respiration.

Synthetic grade d,l-camphor supplied by BDH was used. Stock solutions of camphor were prepared in 30% ethanol/water solvent to overcome the low solubility of camphor in water. The camphor solution was added in aliquots of 25–50 µl. Runs where equivalent amounts of the solvent alone were added were done as blanks. No detectable changes in the rates of oxygen consumption were observed in these blank runs.

Results and discussion. The rate of oxygen uptake with the NAD^+-linked substrates, was decreased by the addition of camphor. Inhibition as a function of camphor concentration is shown in the figure. The rates were approximately halved by the addition of 4–5 mM camphor which is equivalent to 4–5 $\mu mole \cdot mg^{-1}$ protein.

When succinate without rotenone was employed as the substrate, maximum inhibition corresponding to a 50% decrease in the rate of oxygen consumption occurred at a camphor concentration of about 8 mM. The results were the same as those reported previously for rat kidney mitochondria[6]. When rotenone was present with succinate as substrate, camphor, at concentrations < 8 mM, did not produce any decrease in the rate of oxygen consumption suggesting that at these concentrations, camphor specifically inhibits NAD^+-linked respiration. The decrease in oxygen consumption observed for the succinate substrate in the absence of rotenone may result from some initial conversion of succinate to malate in the citric acid cycle with the subsequent effect of camphor on malate linked oxygen uptake.

With succinate substrate, concentrations of camphor > 8 mM caused a large increase in the rate of oxygen consumption. A similar effect was observed when 5 mM of succinate was added to mitochondrial suspensions which had been respiring on NAD^+-linked substrates and which contained > 8 mM camphor. Oxidation of NAD^+-linked substrates was not enhanced by these concentrations of camphor. These results may be explained in terms of currently accepted pathways of electron transport and energy transfer[7–13].

Camphor at concentrations < 8 mM is postulated to block electron transport from NADH to ubiquinone or block energy transfer at site I and this is consistent with the results of the experiments with camphor at concentrations < 8 mM. If higher concentrations of camphor uncouple at site II, an increased rate of oxygen uptake will result with the succinate substrate, but not with NAD^+-linked substrates whose electron transport path to site II has been inhibited.

When ascorbate plus TMPD were used as the substrate in the presence of antimycin A, camphor caused a relatively small decrease, $< 25\%$, in the rate of oxygen consumption at concentrations > 10 mM. This indicates that camphor partially blocks electron transport from reduced cytochrome c or energy transfer from site III. High camphor concentrations (> 8 mM) are required to observe measurable decreases in oxygen consumption. Therefore, the result of this blockage of electron transport or energy transfer is masked by uncoupling by camphor at site II when the succinate substrate is employed.

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The antennae and mating behaviour of *Drosophila* females[1]

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Summary. The females of *Drosophila bipectinata* and *D. malerkotliana* are able to discriminate between their own and alien males in the absence of antennae. Thus mate recognition seems to depend on contact chemoreceptors in these 2 species.

The role of antennae in the mating behaviour of females has been studied in many species of *Drosophila*. Mayr[2] found that the removal of the antennae of *D. melanogaster* females reduced their receptivity to courtship, which led him to suggest that the antennae of females act as receptors in the chain of stimuli-response courtship reactions. The removal of the antennae of females not only reduces their receptivity to courtship but it also removes sexual isolation between *D. pseudoobscura* and *D. persimilis*[2,3]. However, Manning[4] has found that the removal of antennae has no effect on sexual isolation between *D. melanogaster* and *D. simulans* which suggests that the females of these species discriminate between their own and alien males through contact chemoreceptors. An interesting case has been