

Endogenous  $O_2$ -consumption was slightly stimulated at a concentration of 1 mM  $NaN_3$  and the inhibitory effect of  $NaN_3$  was slightly reduced on glucose-supported respiration at the concentration of 10 mM as compared to lower  $NaN_3$  levels. These results are of interest for analyzing the principle of the uncoupling effect of this inhibitor<sup>13,14</sup>. Although NaCl enhanced the consumption of oxygen by *S. aureus* as shown in table 2, the salt did not overcome the inhibitory action of  $NaN_3$ . This observation seems to exclude a mechanism for the observed enhancement of bacterial  $O_2$ -uptake by salt that is based on a direct activation of enzymes constituting the electron transport system. As a working hypothesis, we assume that the activity of oxidative phosphorylation, depending on the membrane-associated  $K^+$ - or  $Na^+$ -stimulated ATPase<sup>15</sup>, may control indirectly the respiratory function of *S. aureus*.

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## The turnover of F-actin-bound ADP in vivo<sup>1</sup>

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**Summary.** A procedure for estimating the rate of turnover of F-actin-bound ADP in vivo is described. A turnover rate of  $0.88\ h^{-1}$  was determined for mouse muscle F-actin. The validity of the method when used to estimate the turnover rate of F-actin per se is discussed in relation to the possible exchange of F-actin-bound ADP.

The polymerization of actin has been extensively studied in vitro. However, relatively little is known about the polymerization process in vivo<sup>2</sup>. Actin exists in a monomeric state, G-actin, to which is bound one molecule of ATP and in a polymeric form, F-actin. The polymerization process involves the sequential addition of monomers with the concomitant dephosphorylation of the ATP to ADP which remains tightly bound to each actin subunit<sup>2</sup>. In earlier work<sup>3</sup> ADP bound to washed muscle homogenates has been used as a marker to estimate the actin concentration in muscle. The present report describes a procedure for estimating the rate of actin polymerization in vivo by determining the rate of turnover of [<sup>3</sup>H]-ADP bound to F-actin. F-actin-bound ADP may be conveniently radioactively-labelled by administration of [2-<sup>3</sup>H]-adenosine.

**Materials and methods.** Male adult mice, Quackenbush strain weighing 35–45 g, were injected s.c. with 200  $\mu$ Ci [2-<sup>3</sup>H]-adenosine (Radiochemical Centre, Amersham) in 0.2 ml-saline. In order to avoid leakage of the solution from the puncture site, injections were performed by passing the needle s.c. from the puncture site in the pelvic region to the mid-point on the dorsal surface of the thigh. In this manner each hind-leg received 0.1 ml of the isotope solution.

At various times up to 1 h after injection mice were anaesthetized with ether, the hind-limbs skinned and the muscle removed and placed in liquid nitrogen. This procedure was carried out as rapidly as possible. The frozen tissue was powdered using a percussion mortar. A sample of this frozen tissue powder was homogenized in 4.0 ml of ice-cold 0.5 M-perchloric acid in order to extract the tissue nucleotides. The perchloric acid extract was neutralized

with 5.0 M-KOH and the concentration of tissue nucleotides determined in the extract by ion-exchange chromatography<sup>4</sup>. The eluate fractions containing individual nucleotides were collected and the radioactivity in aliquots of these fractions determined by liquid scintillation spectrometry.

A 2nd sample of the tissue powder was homogenized in 20 mM-Tris buffer containing 100 mM-KCl and the precipitate washed repeatedly with further aliquots of buffer until no radioactivity above background was detectable in aliquots of the supernatants. The final washed precipitate was extracted twice with 2.0 ml 0.5 M-perchloric acid. The concentration of ADP in neutralized samples of this extract was determined enzymatically<sup>5</sup>. Further aliquots were used for liquid scintillation spectrometry. The presence of radioactivity in ADP only was confirmed by preparative electrophoresis<sup>6</sup>.

The precipitates remaining after extraction were dried and the concentration of protein in the dry residue determined according to the method of Lowry et al.<sup>7</sup>.

### Turnover rate of F-actin-bound ADP

Time t (h)	ATP:ADP sp. act. ratio ( <sup>3</sup> ADP/ <sup>3</sup> ATP)	Fractional turnover rate k (h <sup>-1</sup> )
0.25	0.11 ± 0.04 (3)	0.89 ± 0.36
0.50	0.23 ± 0.06 (3)	1.10 ± 0.34
0.75	0.22 ± 0.09 (2)*	0.75 ± 0.35
1.0	0.30 ± 0.08 (3)	0.76 ± 0.28

Mean ± SEM (number). \* Duplicates only, mean ± range given.

**Results and discussion.** The concentration of F-actin-bound ADP in muscle was  $2.44 \pm 0.46$  (11)  $\mu\text{mole g}^{-1}$  protein, a value in good agreement with published data<sup>3,8</sup>. The concentration of ATP in muscle was  $4.48 \pm 0.32$  (11)  $\mu\text{mole g}^{-1}$  muscle.

Preliminary experiments indicated that the specific-radioactivity of ATP increased linearly with time. This is characteristic of a labelling procedure in which the tracer is administered at a single localized site from which absorption may be slow and where the administered tracer has to be converted to the metabolite being measured<sup>9</sup>. Both conditions were present in the experiments described in this communication. Thus the sp. act. of ATP ( $S_{\text{ATP}}$ ) is proportional to time and the ratio of ATP sp. act. to F-actin-bound ADP sp. act. ( $S_{\text{ADP}}$ ) is described by the equation

$$\left(\frac{S_{\text{ADP}}}{S_{\text{ATP}}}\right)_t = \frac{1}{kt} (kt - 1 - e^{-kt})$$

(for derivation see Zilversmit<sup>9</sup>)

where  $k$  is the fractional turnover rate of F-actin-bound ADP ( $\text{h}^{-1}$ ) and  $t$  is time (h). The fractional turnover rate, calculated according to this formula, was  $0.88 \pm 0.13$  (11)  $\text{h}^{-1}$  (table). However, the validity of the method relies upon ADP being bound only to F-actin. This would seem probable since other components of muscle fibres which bind ADP exhibit considerably weaker binding<sup>3,10,11</sup>. Furthermore, the muscle homogenate is subjected to prolonged washing in the procedure reported here.

As discussed above the fractional turnover rate of F-actin-bound ADP should be directly proportional to the turnover

rate of F-actin. This assumes that the incorporation of bound  $^3\text{H}$ -ADP reflects the addition of monomers to F-actin rather than the exchange of the nucleotide on pre-existing polymers. Evidence both for and against exchange occurring in vitro and in vivo has been presented<sup>12</sup> and reviewed elsewhere<sup>12,13</sup> with the conclusion that exchange does not occur in vivo. The procedure described in the present report is currently being used to investigate further whether exchange of F-actin-bound ADP may indeed occur in vivo<sup>14</sup>.

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## Laboratory and field studies of the female sex pheromone of the olive moth, *Prays oleae*<sup>1</sup>

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**Summary.** Analysis by GC-EAG indicates that abdominal tip extracts of female *Prays oleae* contain a tetradecenal. Synthetic (Z)-7-tetradecenal elicits a strong EAG response from male *P. oleae* and field tests show it to be comparable in attractancy with the virgin female moth. (Z)-9-Tetradecenal also produces a strong EAG response but it is not an attractant and, when added to (Z)-7-tetradecenal, markedly reduces trap catches.

The olive moth, *Prays oleae* (Bern.) (Lepidoptera, Yponomeutidae) is an important pest of olives in the Mediterranean region, successive generations of larvae causing excessive flower drop in the spring and further damage in the summer by boring into the kernels of the developing fruits<sup>5</sup>. Following the identification of the female sex pheromone of *P. citri*<sup>6</sup>, an investigation of the pheromone of *P. oleae* was undertaken to provide a tool for monitoring and control of this pest. Attraction of the male moths to traps baited with virgin female *P. oleae* had already been demonstrated<sup>7</sup>, and the use of these traps to detect successive generations had been reported<sup>8</sup>.

**Materials and methods.** Larvae attacking olive flowers were collected in the field in the Evia region of Greece and allowed to pupate in the laboratory before being flown to London. Preparation of female moth abdominal tip extracts and examination of the extracts by gas chromatography (GC) linked to electroantennographic (EAG) recording from the male moth were carried out as for *P. citri*<sup>6</sup>, as were the preparation of synthetic chemicals and the record-

ing of the EAG response profile from the male moth to these compounds.

Field trials were carried out in the Chania region of Crete. Traps were of the delta type, having triangular cross-section (8 cm sides) and length 18 cm, with the inner basal surface coated with an adhesive. Potentially attractant synthetic chemicals were mixed with an equal amount of 2,6-di-tert-butyl-p-cresol (BHT) as antioxidant, and dispensed from sealed polyethylene vials (32 × 16 mm, 1.5 mm thick)<sup>9</sup>. Traps were hung from olive trees, normally 2–3 m above ground level and at least 50 m apart in a circular array. The traps comprising one replicate were rotated one position clockwise every 2–3 days, and the polyethylene vials were renewed every 30 days. Virgin female moths used to bait traps were obtained from field-collected larvae. Special care was taken to distinguish *P. oleae* and *P. citri* in all trap catches. Where appropriate, catch data was transformed to  $\log(10x+1)$  and subjected to analysis of variance, differences between treatment means being graded for significance at  $p=0.05$ .