Erling Andechs (Germany) bunker but the experiment in that case was not continued long enough to test the relationship of these rhythms to the menstrual cycle[1]. The impressive dissociation of the SW rhythm and the Temp rhythm in humans has not been reported in the case of other mammals investigated under comparable circumstances. In humans the nocturnal peak of the hormone melatonin has been shown to be highest during the pre-menstrual period, including the first day of bleeding[8]. In the present study, the subject had the longest duration of sleep during the pre-menstrual period, which may be correlated to higher levels of melatonin.

The multiple-oscillator model of Wever suggests the presence of two oscillators, one controlling SW rhythm and the other the Temp rhythm, which are coupled and freerun together during states of internal synchronization. Eventually these oscillators uncouple and freerun more or less independently, revealing their own natural periods, leading to spontaneous internal desynchronization. While desynchronized, the two oscillators assume a 'compromise period' closer to the natural period length of the stronger oscillator, which happens to be the temperature oscillator. The temperature oscillator in nearly all subjects has a period close to 25 h, ranging from 24 to 27 h. Those subjects with SW oscillator in the circadian range, between 20 h and 30 h, usually do not show desynchronization. Only subjects who possess a SW oscillator on the fringes of this range (below 20 h or beyond 30 h) eventually show desynchronization[9]. There are other models that attempt to explain the phase relationship between various circadian rhythms in humans. The data from the present study reinforce the multiple-oscillator theory. In addition to the temperature minimum that occurs during sleep phase there is yet another temperature minimum that indicates the point at which sleep onset would have occurred if the SW rhythm had remained synchronized with the Temp rhythm. The freerunning Temp rhythm, over a few days, shows modulation of frequency in the manner of 'relative coordination', suggesting that there may still exist some coupling forces between the two oscillators.

Our study on a young human female subject has provided interesting information on the state of the different circadian rhythms and the menstrual cycle under conditions of desynchronization. Further studies of the interaction between menstrual cycle and other circadian physiological parameters, especially neuroendocrine rhythms, promise interesting and significant results.

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Influence of nutrient stress on pyrethrin production by cultured cells of pyrethrum (*Chrysanthemum cinerariaefolium*)

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Pyrethrum (*Chrysanthemum cinerariaefolium*) produces the insecticidal pyrethrins. Callus cultures of pyrethrum were subjected to nutrient stress to study its effect on growth and pyrethrin production. Nitrogen stress induced two-fold increase in pyrethrin level in two weeks, sugar stress resulted in reduction of pyrethrin level, and phosphate stress did not alter pyrethrin production. We discuss here the implications of these results.

PYRETHRIN production by tissue cultures has been reported in pyrethrum and *Tagetes*[1,2]. We have recently reported the production of high-pyrethrin-yielding callus cultures derived from high-yielding plants[3]. With a view to enhancing the yield potential of pyrethrum callus cultures, we studied the influence of nutrient stress on pyrethrin production.

Callus culture was initiated from leaf explants of elite plants of *C. cinerariaefolium* by the method reported earlier[3]. The callus was maintained on Murashige and

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Skoog's medium[4] supplemented with 2.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), 5.0 mg/l kinetin, 3% sucrose and 1% agar (callus maintenance medium) by regular subcultures every 45 days. Cell-suspension cultures were obtained by transferring portions of callus pieces into liquid medium of the same composition as maintenance medium but without agar. The cultures were agitated at 80 rpm on a rotary shaker at 25 ± 2°C in continuous light of 2000 lux. Cells were separated and inoculated into various experimental media. In these media, nitrates (potassium nitrate and ammonium nitrate), phosphate (potassium dihydrogen orthophosphate) or sugar (sucrose) were eliminated; all other nutrients and hormones were present.

The cells were harvested at weekly intervals and fresh weight as well as dry weight were recorded. Dry weight was determined by drying the cell mass at 60°C in a hot-air oven for 48 h.

Fresh cells were ground in a glass mortor with acid-washed sand and one-fourth the volume of anhydrous sodium sulphate. The ground cells were further homogenized in petroleum ether (40–60) at 4°C. The extract was centrifuged at 10,000 *g* for 5 min. The clear supernatant was used for pyrethrin analysis.

The pyrethrin extract was subjected to TLC in the solvent system, petroleum ether and ethyl acetate 9:1 (v/v). The pyrethrin I and II spots were pooled and eluted in ethyl acetate. Absorption at 227 nm was measured and pyrethrins quantified as described earlier[3]. HPLC analysis was done as reported earlier[3] using HPLC column μ porosil (10 m) and run at isocratic conditions using the solvent mixtures hexane: ethyl acetate (90:10) with flow rate of 2 ml min^{-1} and pump pressure of 400 psi. Detector was set at 254 nm. The area of pyrethrin peaks was measured and compared with standard values obtained for authentic pyrethrins, and the pyrethrins quantified.

Growth of the cells was measured over a period of three weeks at weekly intervals in control and nutrient-stress media (minus nitrate, minus phosphate or minus sugar). The growth increment was nearly eight fold in control medium, whereas in nutrient-stress media it was only 1.5- to 2.0-fold (Figure 1).

Cells in control medium and nutrient-stress media were analysed at weekly intervals for three weeks for pyrethrin content. After two weeks nitrogen-stress medium showed over 2.0-fold increase in percentage of pyrethrin over the control (Figure 2). We have earlier reported similar increase in capsaicin production in nitrogen- and phosphorus-stress media[5]. Sugar stress resulted in lower pyrethrin content at three weeks, whereas in phosphate-stress medium the percentage of pyrethrin was comparable with that in control. Pyrethrin production measured as μg per culture was much lower in the nutrient stress media than in control. This was due to reduction in growth of cells in nutrient-

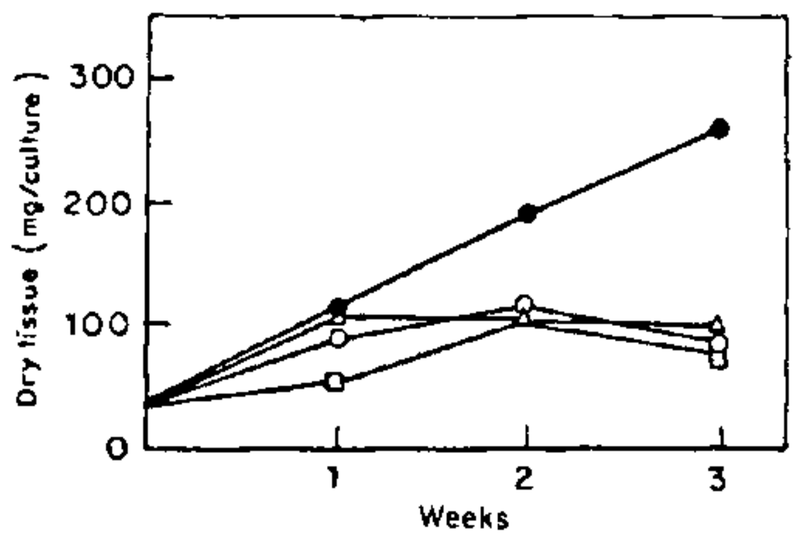

Figure 1. Growth of pyrethrum cells in culture: ●, Control; △, phosphorus-stress medium; ○, nitrogen-stress medium; □, sugar-stress medium.

stress media. The depletion of essential nutrients is known to enhance production of secondary metabolites by restricting the growth of cells, which enhances the utilization of precursors for secondary metabolism[6]. In the present experiments nitrate stress enhanced pyrethrin production. Since nitrogen is not a constituent of pyrethrins it is envisaged that nitrogen depletion affects only primary metabolism and growth, thereby channelling the available primary metabolites [remote precursors] to pyrethrin biosynthesis. Phosphate or sugar stress did not enhance pyrethrin content. This may indicate that pyrethrin production is a relatively high-energy-

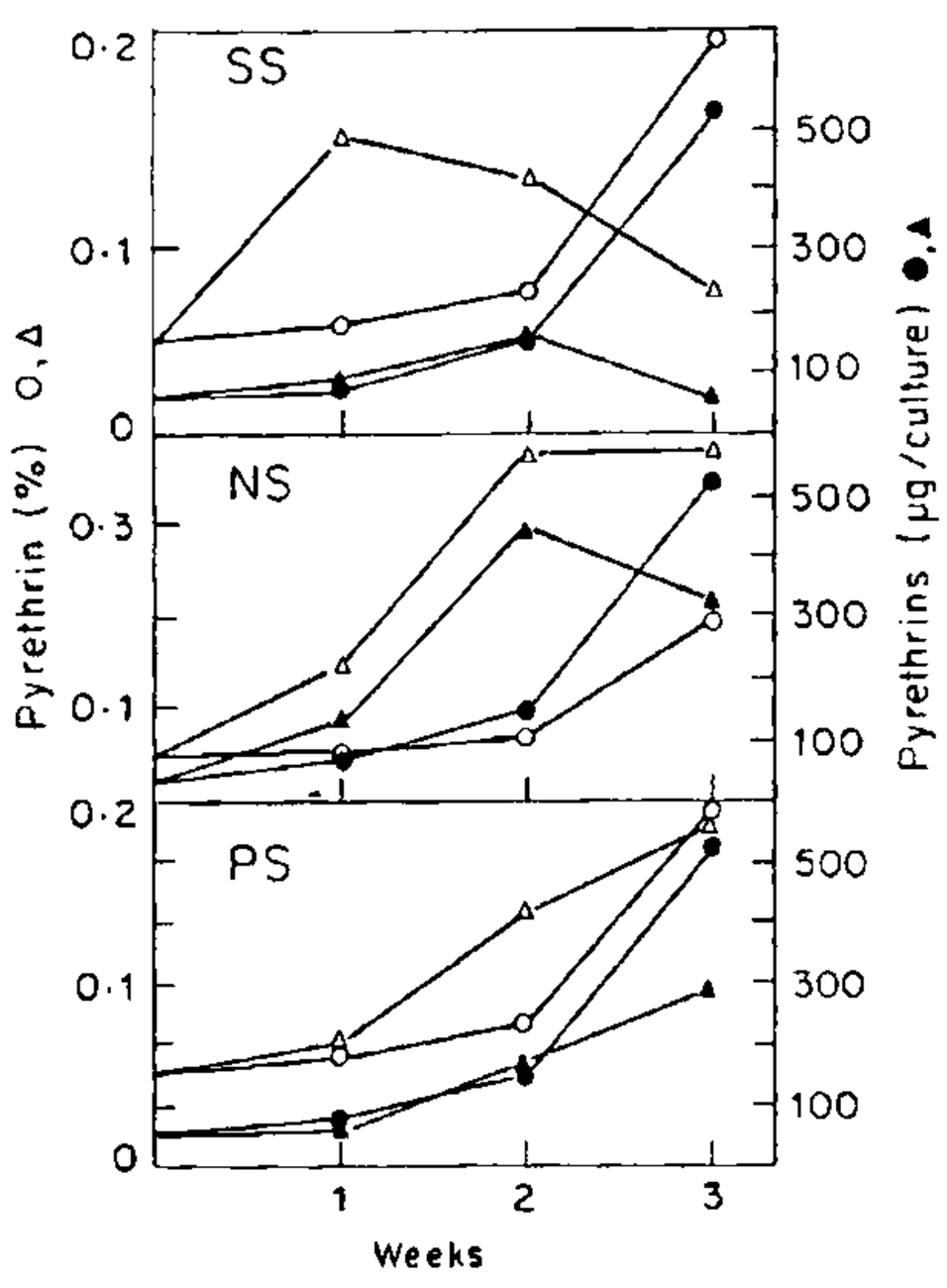

Figure 2. Pyrethrin production by pyrethrum cells in culture: ○, pyrethrin percentage in control; △, pyrethrin percentage in nutrient-stress medium; ●, μg pyrethrin per culture in control; ▲, μg pyrethrin per culture in nutrient-stress medium. SS, sugar-stress medium; NS, nitrogen-stress medium; PS, phosphorus-stress medium.

requiring process, and hence its biosynthesis may be affected by lowered ATP metabolism as a result of phosphate stress and reduced glycolysis due to sugar depletion.

The increase in pyrethrin content in nitrogen-stress medium will find application in scaling up cell cultures for pyrethrin production using a two-stage culture method. Further work on scaling up of cell culture in airlift bioreactor is in progress.

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Malathion-induced changes in biogenic-amine levels and acetylcholinesterase activity in the cockroach *Periplaneta americana*

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Acetylcholine is the major neurotransmitter in insects. Many insecticides inhibit the enzyme acetylcholinesterase, which degrades acetylcholine and prevents cholinergic toxicity. Other substances whose neurotransmitter function has been demonstrated in mammals have been reported in insects. Here we show that sublethal doses of malathion, which has high anticholinesterase activity in the cockroach *Periplaneta americana*, cause a decrease in endogenous levels of the putative neurotransmitters 5-hydroxytryptamine, dopamine and noradrenaline and an increase in the level of 5-hydroxyindoleacetic acid.

SYNAPTIC transmission via acetylcholine is an accepted mechanism of nerve transmission in insects[1-4]. A large number of acetylcholinesterase (AChE) blockers are therefore used as insecticides. Even though their role is far from clear, other, putative neurotransmitters such as 5-hydroxytryptamine (5-HT), dopamine (DA) and noradrenaline (NA) have also been reported in insects. Presence of 5-HT was reported in *Manduca sexta*[5] and *Periplaneta americana*[6,7], DA as reported in *Aedes togoi*[8], both 5-HT and DA were reported in *Drosophila melanogaster*[9], while 5-HT, DA as well as NE were found in *P. americana*[10]. Flangan and Allan[11] demonstrated that 5-HT may, in fact be a neurohormone in *Rhodnius prolixus.*

Butcher[12] showed that there was interaction between cholinergic, tryptaminergic and adrenergic nerves in rats. According to him, AChE facilitates the release of dopamine, which subsequently acts as a neurotransmitter. It appears logical that, if, in fact, there is a functional interaction between cholinergic and monoaminergic nerves in insects, levels of 5-HT, DA and NA would be affected by anti-AChE pesticides.

Here we report the effects of the anti-AChE pesticide malathion on activity of AChE and levels of 5-HT, DA and NA in the common cockroach *P. americana.*

Adult *Periplaneta americana* (2.75 ± 0.087 cm length, 0.86 ± 0.017 g weight) were treated with 20% (0.21 μg per insect) and 80% (0.84 μg per insect) LD_{50} of malathion (LD_{50} was determined for *P. americana* $LD_{50} = 1.05$ μg per insect). The pesticide (98% purity) was dissolved in acetone and 0.05 μl of the solution containing appropriate dose of pesticide was applied to the dorsal thoracic surface of each insect using a microsyringe; controls received acetone alone. Biochemical estimations were made 24 h and 96 h after treatment.

AChE activity was measured by the method of Ellman *et al.*[13] Brain, along with ventral nerve cord, was removed and homogenized in 0.1 M phosphate buffer, pH 8 (50 mg per ml of buffer), for 5 min in an ice bath. The homogenates were centrifuged at 1000 g for 30 min at -4°C and the supernatants used as enzyme source. Enzyme activity was measured at 25°C in a 10-mm-path-length cuvette using an incubation mixture consisting of 0.01 ml of 5×10^{-4} M freshly prepared acetylthiocholine iodide as substrate, 0.05 ml of 12.5×10^{-4} M DTNB (5,5′-dithiobis (2-nitrobenzoate)) solution dissolved in 0.1 M phosphate buffer (pH 7), 0.05 ml of the enzyme-containing supernatant and 1.45 ml of 0.1 M phosphate buffer (pH 8). Blanks contained 0.01 ml of buffer instead of substrate. Change in absorbance at 412 nm was monitored for 3 min. Enzyme activity was expressed as micromoles of substrate hydrolysed by comparing absorbance curves with a glutathione standard curve. Protein was estimated according to Lowry *et al.*[14]

Biogenic-amine levels in nervous tissue of treated and control insects were measured fluorimetrically. Twelve milligrams of nervous tissue pooled from two insects was taken for each replicate. Tissue was homogenized in 3.0 ml of acidified butanol for 5 min and the homogenate centrifuged at 2000 *g* for 10 min. Supernatants were shaken in capped tubes for 10 min with 5.0 ml of heptane and 1.0 ml of 0.1 N HCl. The mixture was centrifuged for 5 min at 2000 *g*. The aqueous layer was used for measuring 5-HT, NA and DA. NA and