respectively, were 0.11, 0.46 and 0.56 μM $C_2H_4$/plant × time (time = days needed till the final level of ethylene accumulation was attained). Older tomato plants, which were inoculated at an age of 31 days, showed no further increase of enzyme activity. This failure, however, could be due to the deteriorated status of the plants which were growing on increasingly more exhausted media.

In transfilter associations the tomato plants were inoculated at an age of 5 days. The bacteria concentrated along the separating membrane just as in transfilter associations of *Portulaca* callus and *Rhizobium*[6], and nitrogenase activity was induced (fig. 2). 0.26 μM $C_2H_4$/plant × time were produced. A correct comparison of associations and transfilter associations was not feasible, because both systems differed in several details. Nevertheless, the nitrogenase activity induced in transfilter associations was in the range obtained in associations without separation of plants and bacteria. These data indicate that tomato plants are able to induce easily nitrogenase activity even through a membrane which was impermeable for bacteria.

Thus, the tomato plant, which yields our most important canned fruit[13], is able to induce nitrogenase activity in rhizobia. However, before discussing, the possible practical use of tomato/*Rhizobium* associations, one should clarify the mechanism of this induction. One possibility would be the production of inducing factors. Another, less probable, possibility would involve the removal of inhibiting substances from the medium by the growing plants and subsequent derepression of the rhizobial nif-operon. Transfilter inductions could be explained best by inducing substances. They were essential for further experiments in which we are trying to get direct evidence for the existence or non-existence of inducing factors.


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## Influence of oestradiol on tissue respiration of the Indian garden lizard, *Calotes versicolor*


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*Summary.* Ovariectomy reduced the respiratory rates of liver and skeletal muscle homogenates of the Indian garden lizard, *Calotes versicolor*. Administration of oestradiol dipropionate elevated the rate of tissue respiration of ovariectomized animals. This finding lends support to the view that oestradiol, like testosterone, is able to stimulate the oxidative metabolism of this animal.


It has recently been shown that orchidectomy reduces, and testosterone administration increases, the rate of respiration of tissues of *Calotes versicolor*[2,3]. Since oestrogens in general are known to have physiological actions opposite to those of testosterone[4], the effects of ovariectomy and oestrogen therapy on tissue respiration were studied in this species.

Adult female lizards were collected from nature and acclimated to laboratory conditions[3]. After a fortnight 25 animals were bilaterally ovariectomized and 10 sham-operated under open ether anesthesia. 1 month later the ovariectomized animals were divided into 3 groups of 6 animals each. Groups I and II received 20 μg and 40 μg of oestradiol dipropionate (Ovocyclin®, Ciba India Ltd.) in olive oil respectively and group III received olive oil alone. One group of sham-operated animals served as control. 5 intramuscular injections were given on alternate days in 0.1 ml of vehicle. 24 h after the last injection all animals were killed by decapitation and respiratory rates of liver and skeletal muscle measured manometrically[3]. During the course of experimentation all animals were provided with maggots and water ad libitum. Statistical analysis of the data was done using Student's t-test[5].

*Results.* Ovariectomy significantly reduced the respiration of liver ($p < 0.01$) and of skeletal muscle ($p < 0.05$). 20 μg of oestradiol increased the respiratory rate of liver and skeletal muscle ($p < 0.02$ and $p < 0.05$ respectively) of ovariectomized animals and 40 μg of the steroid when injected into ovariectomized animals increased the respiratory rate of liver only ($p < 0.01$, see table 1).

*Discussion.* Results indicate that ovariectomy significantly reduced the respiratory rates of tissues of *Calotes versicolor* and that oestradiol was able to stimulate tissue respiration of ovariectomized animals. This effect is similar to that of orchidectomy and testosterone administration on the respiratory rates of liver and skeletal muscle of *Calotes versicolor*[3], *Hemidactylus flaviviridis*[6] and *Natrix piscator*[7]. The present study shows that oestradiol is as effective as testos-

Effect of ovariectomy and oestradiol on respiratory rate of liver and skeletal muscle of *Calotes versicolor*

| Status* | Oxygen consumption (μl $O_2$/mg wet tissue/h) | |
|---|---|---|
| | Liver | Skeletal muscle |
| Sham-operated + oil | 0.725 ± 0.068 | 0.877 ± 0.381 |
| Ovariectomized + oil | 0.315 ± 0.045[a] | 0.411 ± 0.071[b] |
| Ovariectomized + 20 μg oestradiol | 1.144 ± 0.232[c] | 1.174 ± 0.204[d] |
| Ovariectomized + 40 μg oestradiol | 1.133 ± 0.172[e] | |

* 6 animals per group. Compared to sham-operated + oil: [a] $p < 0.01$; [b] $p < 0.05$. Compared to ovariectomized + oil: [c] $p < 0.02$; [d] $p < 0.05$; [e] $p < 0.01$.

terone in stimulating reptilian oxidative metabolism. This is interesting since oestrogen has many important biological actions opposite to those of testosterone.

There are only a few reports on the effects of oestrogens on oxidative metabolism of lower vertebrates. Hoar[8] injected goldfish with stilbesterol and observed a marked stimulation of oxidative metabolism. He offered the suggestion that the steroid may increase in some way the reactivity of neuromuscular mechanisms thus promoting locomotor activity which indirectly results in an increased demand for oxygen. But this possibility has not been explored further. It is possible that the action of oestrogen on reptilian oxidative metabolism may be brought about in several ways viz, by stimulating metabolic pathways thereby increasing the substrate concentration or by acting on the respiratory chain.


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## Avermectins: novel insecticides, acaricides and nematicides from a soil microorganism


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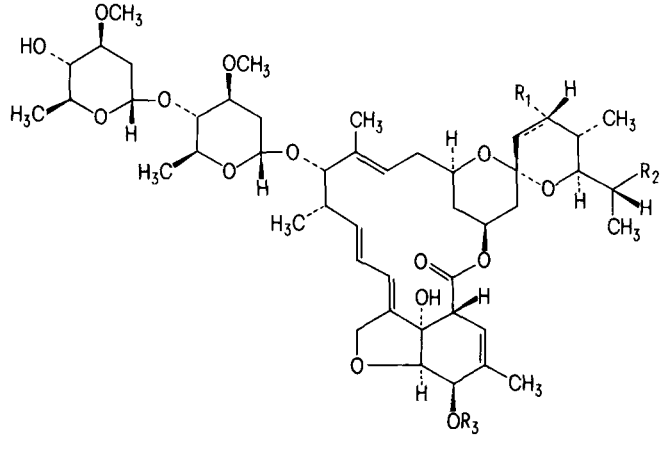
*Summary.* The avermectins, streptomycete-derived macrocyclic lactones originally isolated as antiparasitic agents, have also demonstrated high potencies in laboratory evaluations against insect pests in several orders, phytophagous mites and the plant-parasitic nematode, *Meloidogyne*. Studies suggest that the avermectins' mechanism of toxicity is fundamentally different from those associated with current natural and synthetic pesticides.


We report here the broad spectrum agricultural pesticidal activity of the avermectins, a new class of macrocyclic lactones isolated from the soil organism *Streptomyces avermitilis*[3]. These compounds were originally discovered in the MSDRL screening programs as anthelmintic agents, demonstrating potencies in the 10-300 parts per billion range (μg/kg b.wt) when administered to sheep, cattle, dogs and poultry infected with a spectrum of common gastrointestinal parasites[4]. Initial indications of insecticidal activity were shown in tests against the confused flour beetle[5] and the ectoparasitic larvae of the sheep blowfly[6]. Subsequent work has also demonstrated potent activity against other veterinary ectoparasites including mites and ticks[7]. In recent laboratory and field evaluations, we have extended these observations to include agricultural and household insect pests of several orders, phytophagous mites and the plant-parasitic nematode, *Meloidogyne incognita*.

The structures of the 8 major components (designated $A_{1a}$, $A_{1b}$, $B_{1a}$, $B_{1b}$, $A_{2a}$, $A_{2b}$, $B_{2a}$, $B_{2b}$) of the avermectin complex are represented below[8]. Of these, avermectins $B_{1a}$ and $B_{2a}$ are the most promising candidates as agricultural pesticides.

In laboratory studies, avermectin $B_{1a}$ has demonstrated high toxicity for the 2-spotted spider mite *(Tetranychus urticae)* on bean plants compared to commercially used acaricides. When applied in solution directly onto adult and nymphal populations on foliage, avermectin $B_{1a}$ was 50-200 times as potent as these materials, with an $LD_{90}$ of 0.02-0.03 ppm (table). Avermectin $B_{1a}$ has also shown high activity against several other tetranychid and eriophyid mites, including the citrus rust mite *(Phyllocoptruta oleivora)*, citrus red mite *(Panonychus citri)* and the strawberry spider mite *(Tetranychus turkestani)*.




| Avermectin | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| $A_{1a}$ | | $C_2H_5$ | $CH_3$ |
| $A_{1b}$ | | $CH_3$ | $CH_3$ |
| $A_{2a}$ | OH | $C_2H_5$ | $CH_3$ |
| $A_{2b}$ | OH | $CH_3$ | $CH_3$ |
| $B_{1a}$ | | $C_2H_5$ | H |
| $B_{1b}$ | | $CH_3$ | H |
| $B_{2a}$ | OH | $C_2H_5$ | H |
| $B_{2b}$ | OH | $CH_3$ | H |

Where $R_1$ is absent, the double bond (=) is present. Both sugars are α-L-oleandrose.