INTRINSIC ANTIBIOTIC RESISTANCE PATTERNS OF SOME RHIZOBIAL STRAINS ISOLATED FROM TREE LEGUMES

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Rhizobia survive better in sterile than in non-sterile soil, suggesting that biological agents are responsible for the fall in numbers of rhizobia. Secretion of antibiotics by microorganisms and the resultant biological inhibition of other susceptible microorganisms like rhizobia can be demonstrated in soil as well as in pure cultures. Antibiotics have been used in the rhizobial study for finding out the resistance patterns, for identifying in both pure cultures and in field experiments and for developing antibiotic-resistant mutants. Rhizobia show variation in the susceptibility to antibiotic substances. The present communication deals with the minimum inhibitory concentration (MIC) values of five antibiotics for eight rhizobial strains and their resistance patterns.

Eight rhizobial strains Ep, Ev-1, Ev-2, Ev-3, G-1, G-2, L-1 and L-2 were isolated from four tree legumes by using standard techniques. The plants from which rhizobial strains isolated are: Erythrina parcellii Hort. Bull., (Strain, Ep), E. variegata Linn (Syn. E. indica Lam.) (strains Ev-1, Ev-2, and Ev-3), Gliricidia sepium (Jacq.) Steudel. (strains G-1 and G-2) and Leucaena leucocephalum (Lam.) de Wit. (strains L-1 and L-2).

A rapid screening method of assessing MIC of five different antibiotics was performed by serial dilution tube technique. The antibiotics used were: benzyl penicillin (pen), gentamycin sulphate (gen), kanamycin acid sulphate (kan), rifampicin (rif) and streptomycin sulphate (str). MIC values and the patterns of intrinsic antibiotic resistance were studied for all the rhizobial strains.

As a group, the MIC values of benzyl penicillin were higher (25–100 μgml⁻¹) and of gentamycin were less (0.125–20 μgml⁻¹) for all the strains.

The MIC values were comparatively high for the strains Ev-3 (slow-growing, dry type) and Ep (slow-growing, wet type) (i.e., ranging from 10–80 μgml⁻¹). The strains Ev-1, Ev-2, G-1 and G-2 (fast-growing, wet type) were resistant to four (pen, str, rif and kan) out of five antibiotics tested. The strains L-1 and L-2 possessed very low MIC values for all antibiotics tried and L-1 was the most sensitive of all. Thus associations between resistance patterns were random within the strains of the same host and among different hosts. Each strain was diverse, showing different patterns of resistance to different antibiotics.

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EFFECT OF LUTEINIZING HORMONE-RELEASING HORMONE ON THE TESTIS AND EPIDIDYMIS OF THE DEVELOPING PIGEON, COLUMBA LIVIA

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It has been shown that the treatment with luteinizing hormone-releasing hormone (LH-RH) induces ovulation in hen, causes rapid rise of plasma LH when injected into chicken and turkey hen and induces testicular growth in Coturnix coturnix maintained on daily 15 hr light period and birds treated with a drug to depress testicular growth. Most of these studies have been carried out on adult birds and very little attention has been paid on the effect of LH-RH on the gonads of newly-hatched birds. The present study was aimed at finding out whether LH-RH releases gonadotropins stimulating the growth of the testis in newly-hatched pigeons. In earlier studies, maturation of seminiferous tubule elements and the increased plasma androgen levels have been taken as criteria in FSH and LH bioassays, respectively, in newly-hatched birds.

In the present work, the effect of LH-RH on the release of gonadotropins is determined by studying the corresponding stimulatory changes in the seminiferous tubules, Sertoli cells, spermatagonia, Leydig cells and...
the androgen-dependent epididymis in recently hatched pigeon, *Columba livia*.

Five-day-old pigeon hatchlings were divided into three groups. A group of five hatchlings was autopsied on the first day of the experiment to serve as initial controls. Of the remaining two groups, one group received a daily subcutaneous injection of 200 ng LH-RH in the wing web (obtained from NIAMDD, Bethesda, Maryland, USA: Batch No. 2) in 0.2 ml saline for 25 days and the other group received a daily subcutaneous injection of 0.2 ml saline for 25 days and served as final control. At autopsy the relative weights of the testes were recorded and the representative testis and epididymis were fixed in Bouin’s fluid for histological and histometrical studies. The remaining testes from each group were used for histochemical assay of the enzyme \( \Delta^3-3\beta \)-hydroxysteroid dehydrogenase (\( \Delta^3-3\beta \)-HSDH) as described earlier.\(^{10}\)

The diameter of the testis and seminiferous tubules, the number of spermatogonia per tubule, number of Sertoli cells per tubule, Sertoli cell nuclear diameter and Leydig cell nuclear diameter were recorded from at least 25 sections of the testis from each of the birds from final controls and LH-RH treated. Histometrical studies on the testis in the initial control group were not carried out as the testis exhibited only seminiferous cords instead of seminiferous tubules and the interstitial had only juvenile spindle-shaped Leydig cells along with fibroblasts. The data were analyzed using student’s t test.

In the testis of pigeon from the initial controls, the seminiferous cords were lined by a large number of Sertoli cells and a few spermatogonia. The tubular nature of the seminiferous tubules was yet to be formed. The interstitial area was made up of juvenile spindle-shaped Leydig cells which were difficult to distinguish from fibroblasts whereas in the testis from the final control and LH-RH treated groups, there was the formation of seminiferous tubules and the round Leydig cells could be identified. In these birds, Sertoli cells still outnumbered spermatogonia (table 1). However, there was no significant difference between the number of spermatogonia per tubule in the testis of LH-RH treated and final control birds (table 1). Also there was no significant change in the diameter of the testis, seminiferous tubules and Sertoli cell nuclei, and the number of the Sertoli cells per tubule between LH-RH treated and final controls. The interstitium of LH-RH treated birds showed large hypertrophied Leydig cells with large vesicular nuclei. The Leydig cell nuclear diameter in LH-RH treated birds registered a significant increase when compared to those in final controls (table 1, figures 1 and 2). Histochemical studies revealed an increased \( \Delta^3-3\beta \)-HSDH activity in the

<table>
<thead>
<tr>
<th>Table 1: Effect of LH-RH on the testis and epididymis in <em>Columba livia</em></th>
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<tbody>
<tr>
<td><strong>Control</strong></td>
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<tr>
<td>Testis wt/100 g body wt ± SE (mg)</td>
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<td>Testis diameter ± SE (µm)</td>
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<tr>
<td>Seminiferous tubule diameter ± SE (µm)</td>
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<td>No. of germ cells/C. S. of tubule ± SE</td>
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<tr>
<td>No. of Sertoli cells/C. S. of tubule ± SE</td>
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<tr>
<td>Sertoli cell nuclear diameter ± SE (µm)</td>
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<td>Leydig cell nuclear diameter ± SE (µm)</td>
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<td>( \Delta^3-3\beta )-HSDH activity* in Leydig cells</td>
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<td>Diameter of epididymal tubules ± SE (µm)</td>
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*P* values calculated by Student’s *t*-test between control and LH-RH treated group; NS—Nonsignificant; *+*—Intensity of enzyme reaction is visually graded.
Leydig cells of LH-RH treated birds when compared with that in controls (figures 3 and 4). Histometric data on epididymes showed a significant increase in the epididymal tubule diameter in LH-RH treated birds when compared to those of controls (table 1).

The hypertrophy of the Leydig cells following LH-RH treatment in developing pigeons suggests an increased LH release. Further, the large round vesicular Leydig cell nuclei, and increased Δ5-3β-HSDH enzyme activity in the Leydig cells accompanied with the increased diameter of epididymal tubules in LH-RH treated pigeons suggests an increased secretion of androgen, albeit indirect, increased LH release. Increased plasma LH after LH-RH treatment have been observed in domestic cock and hen. Similarly, LH-RH agonist has been shown to increase plasma testosterone levels in cocks. However, these studies do not mention about the release levels of FSH after LH-RH treatment. This is probably because, RIA for FSH in birds has been developed in recent years and is available to a limited number of investigators. In the present study, administration of LH-RH did not affect the seminiferous tubule elements like Sertoli cells and germ cells. This observation indicates (i) the absence of LH-RH-induced release of FSH or (ii) the failure to release enough FSH to stimulate the seminiferous tubule elements after LH-RH treatment for 25 days at the given dose level.

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EFFECT OF 25-AZACPROSTANE ON THE GROWTH AND REPRODUCTION OF THE BUG DISYDERCUS SIMILIS

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Studies based on the structure and biological activity of certain compounds which inhibit insect growth and development permitted the design and synthesis of a number of azasteroids which considerably enhance the growth and the development inhibitory activity. One of these compounds, 25-azacprostane, was tested on the insect Dysdercus similis for its growth-regulating activity.

The bug Dysdercus similis was reared at 27° ± 1 C and RH of 65 ± 5%, and fed on soaked cotton seeds. The freshly eclosed fifth instar nymphs were topically treated on the abdominal tergum with 1 μl/insect of varying concentrations of the compound 25-azacprostane (5 β-chol-24-dimethylamine). The concentrations ranged from 500 to 2000 ppm. One μl of 1000 ppm/insect was the concentration at which the activity was maximum. Controls were treated with 1 μl/insect of the carrier solvent acetone. The experiments were repeated thrice. The abnormalities after ecdysis in the treated insects and their gonads were recorded.

The freshly eclosed fifth instar nymphs when treated with 1 μl/insect of 1000 ppm of 25-azacprostanone, moulled after 12 to 15 days into abnormal forms while the controls moulted into adults after 7 to 8 days. This prolongation in the life span of the final instar larva was also observed by Chippendale and Reddy. Sixty per cent of the treated nymphs moulled into adults with malformed wings. The wings were short, less melanized, crumpled and often varied in length. (figure 1). The testes of these adults were oval-shaped, and the vas deferens was thin tube-like or bulbous and short. The accessory glands also varied in size in most of the treated adults (figure 5). Wing malformation in the adults as a result of the steroid was reported. The ovaries of these malformed adults had 5 to 6 oocytes in each ovariole. They showed yolk deposition but the size of the oocytes was much less than the oocytes of control insects (figure 3). Ovarian development and egg viability hindered by the steroids in the adults with malformed wing was reported earlier. Thirty per cent of the treated nymphs moulted into adulthood having short wings, two to three segmented antennae, with curved appendages of varied lengths. The tarsus was unsegmented in most cases (figure 2). The male reproductive system was like that of the normal adult but the females showed abnormalities. The number of oocytes was reduced to 2 to 3 in most cases. Vitellogenesis was hindered. In certain cases the oocytes varied in size in an ovariole, the larger occupying the distal position (figure 4). These oocytes showed that the yolk deposition was drastically affected. Fecundity and hatchability were considerably reduced. A small per cent (10%) of these were unable to extricate properly and the exuviae was found attached to the appendages or to the abdomen. Such insects died in a