primed host cells. However, in the system that is described in this paper, the hosts had a paralyzed immune system, as a result of irradiation. In these animals priming can hardly be expected. Moreover, priming of host cells is not a prerequisite as immune spleen cells, injected simultaneously with tumor cells in normal mice, are able to exert an in vivo antitumor effect[10].

Natural killer cells among the spleen cells may be responsible for the in vivo antitumor effect; these cells can be affected by silica[13]. This option is, however, unlikely, as a substantial antitumor activity of the immune spleen cells remains after in vitro culture during 24 h (to be published), whereas natural killer cells lose their activity within 4 h of culture[14].

Macrophages among the spleen cells do not seem to cause the lengthening of survival time, as elimination of macrophages did not abrogate the effect of the immune spleen cells[10,15]. This shows that the injected syngeneic spleen macrophages cannot replace host macrophages. This could be due to the inability of i.p. injected spleen macrophages to invade the tumor. Another explanation could be that spleen macrophages are not the relevant macrophages. An example of such a difference in relevance is that irritant-induced peritoneal macrophages could give a local antitumor effect in co-operation with sensitized lymphocytes, in contrast to resident macrophages[10].

Macrophages among the injected bone marrow cells can differentiate into mature macrophages, but it is clear that the number of macrophages is smaller in silica treated mice than in control mice. We conclude that host macrophages are involved in the antitumor effect of transferred immune spleen cells.

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Effect of indole-3-acetic acid on the histology of gonads and their development in *Dacus dorsalis* Hendel

J.N. Thakur and S.K. Mann[1]

Department of Bio-Sciences, Himachal Pradesh University, Simla-171005 (India), 5 June 1981

Summary. The paper deals with the effects of indole-3 acetic acid on the histology of the gonads and their development in *Dacus dorsalis* Hendel (Diptera; Tephritidae). Flies were topically treated with solutions of different concentrations. This treatment effectively reduced the size of gonads when applied to newly-emerged flies. Most severely affected parts were the germinal regions of the gonads. The number of sperm bundles was much reduced in the treated testes as compared to controlled ones.

The possibility of using chemicals for sterilizing an insect population has interested every individual learning of the effects of chemosterilants on the reproductive organs. *Dacus dorsalis* Hendel was taken as an experimental insect for the present investigations, since it is a serious pest of most of the fruits and vegetables throughout India. In our present experiment, we tested indole-3-acetic acid as a chemosterilant as an alternative to tepa, metepa, apholate, and tretamine, which have been used to sterilize fruit flies such as the oriental fruit fly, *D. dorsalis,* the melon fruit fly, *Dacus cucurbitae* Cocquillett and the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann[2]. Indole-3-acetic acid has been added to the list of chemosterilants as a compound affecting reproduction in insects[3]. Since then, according to available evidence, no work has been done on the effects of this chemical on the histology of the gonads and their development.

Materials and methods. Laboratory-reared 0-24-h-old adults of either sex of *D. dorsalis* were anesthetized by keeping them in a freezer for 2-5 min and then treated topically with 1 µl of solutions of different concentrations of indole-3-acetic acid in acetone, with the aid of a micrometer controlled calibrated syringe. The chilled flies were easy to handle and mortality was negligible. The concentrations tested were 0.3, 0.5, 2 and 5%. For control purposes, flies were treated with the same amount of acetone alone. 6 replicates of 50 flies each per concentration were studied. Following the treatment on alternative days, 2-5 flies from each replicate were dissected in physiological saline solution. Their reproductive organs were taken out for histopathological study. Testes and ovaries were measured before being permanently mounted, using a monocular microscope equipped with a vernier scale. Measurements included greatest length, width and length of the anterior curved portion, and diameters of ovaries. The average sizes of testes and ovaries was calculated and tabulated (table 1). For histopathological studies, ovaries and testes were fixed in Bouin's fluid (water solution) for 12-24 h. Several sections were taken for histopathological study. Sections were stained in haematoxylin and eosin and examined by a research microscope.

Results. The data obtained showed the effect of indole-3-acetic acid on the size of gonads. Results were statistically analyzed by applying F and Student's t-tests at 5% and 1% levels of significance (tables 1 and 2). It was observed that until 4-6 days after treatment there were no measureable effects on the size of gonads, but thereafter up to maturity a statistically significant reduction in size was observed.

There was no linear correlation between the dose and the reduction in size of gonads, as evidenced by statistical analysis. Therefore, it is advantageous to use this chemical as a chemosterilant because it caused sterility without inducing any deleterious effects on flies even at higher concentrations. The only histological changes which could be correlated with the treatment were those observed in the gonads.

Histology of testes: IAA at all concentrations tested significantly reduced the size of the gonads (table 1). More effect was observed on the reduction of length than of width. In order to measure the overall effects on testes, their lengths, widths and lengths of anterior curved portions were added (table 1). In severely affected testes (fig. 2) the germinal regions were partly collapsed and devoid of spermatogonia. In several testes, peripheries were wrinkled due to shrinkage of inner cell contents resulting in the appearance of a gap between the testicular sheath and the inner part (fig. 2). In treated testes a few sperm bundles were observed, while in control testes large numbers of such bundles were found (fig. 1 and 2). In control flies, testes showed a clear differentiation of germ cells into spermatogonia, spermatocytes, spermatids and sperm bundles, whereas no such differentiation was observed in the testes of treated flies. It was also observed that those cells that had reached the pre-spermatid or spermatid stage at the time of treatment were apparently differentiated into mature spermatozoa, while cells in primordial stages degenerated, appearing amorphous and granular.

Histopathology of ovaries. Statistically analyzed data showed that in treated flies reduction in the size of ovaries occurred within a specific period of time (8–12 days) when the development of ovaries was at its peak. Before and after this period reduction in the size was found to be statistically insignificant (table 2). Treatment of mature flies did not show any effect even at higher concentrations. In ovaries linear correlation existed between the 2 concentrations (0.3 and 0.5%) at 12 days post treatment. In some of the affected flies primary follicles showed no oocyte differentiation (fig. 4), while in others, oocytes occupied 75% of the follicles. In control flies ovarioles contained primary follicles fully occupied by oocytes and well developed nurse cell nuclei (fig. 3). Unlike the follicles of control flies of the same age, treated follicles appeared to lack nurse cells. Follicles in more advanced stages of development did not appear to be severely affected. It was observed that the germarium of treated ovarioles was the most severely affected region and was much reduced in size.

Discussion. The results of our experiments demonstrated that the response of *D. dorsalis* to IAA was stronger in newly emerged flies than in mature flies. Our observations regarding the reduction in size of gonads are in accordance with the results obtained by Schwartz[4], who observed a significant reduction in size of gonads of *Hippelates pusio* Loew, when fed with tepa, metepa, and apholate. He also reported the destruction of germinal regions in treated testes and ovarioles. Similar reduction in size was observed by Smittle et al.[5] in *Blattella germanica* L. with tepa, and by Dutt and Ghatak[6] in *Apion chorchori* Marsh. with apholate and metepa. Grover et al.[7] observed reduction in size of testes and ovaries of *Culex pipiens fatigans* Wiedemann with apholate, metepa, tepa and hempa along with a deficiency of immature sex-cells and mature sperm, although sperm production continued at a lower rate. Reduction in testis size may be due to a decrease in cell contents and synthetic processes of testicular constituents, disturbances of cell division and destruction of cells. Saxena and Aditya[8] and Madhukar et al.[9] showed that treatment of insects with chemosterilants reduced the content and inhibited the synthesis of deoxyribonucleic acid in testes. In contrast to our results, Rai[10] did not find any reduction in

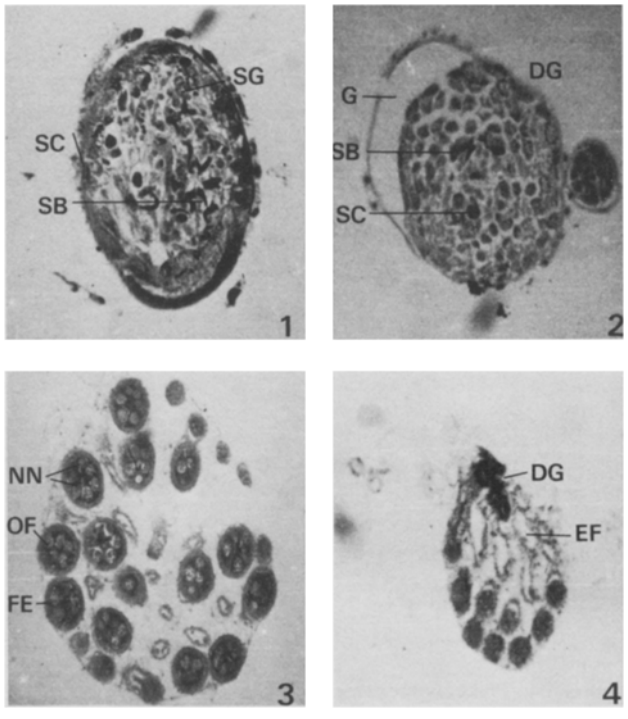

Figure 1. Section of control testis showing different types of germ cells and sperm bundles.

Figure 2. Section of treated testis (concentration 0.5%) showing less differentiation in germ cells, one sperm bundle, wrinkled periphery, gap between testicular sheath and inner part and reduced size.

Figure 3. Section of normal ovary showing developing oocytes, occupying 100% of follicles, and well developed nurse cell nuclei.

Figure 4. Section of treated ovary (concentration 0.5%) showing reduction in size, a few empty follicles, reduced germarium, no differentiation in nurse cell nuclei and oocytes.
Abbreviations: DF, Degeneration of germinal region; EF, empty follicle; FE, follicular epithelium; G, gap (appeared to be due to shrinkage of inner contents); NN, nurse cell nuclei; OF, ovarian follicle or egg chamber; SB, sperm bundle; SC, spermatocyte; SG, spermatogonia.

Table 1. Effect of indole-3-acetic acid on the overall size of testes (mm) of *Dacus dorsalis* Hendel treated topically with 1 µl of solution of different concentrations (mean of 6 replicates)

Post-treatment days	0.3% concentration	0.5% concentration	2% concentration	5% concentration	Control
8	1.947a*	1.986a	x	x	2.069
12	1.801a**	1.847a**	x	x	1.132
16	1.746a**	1.793a**	2.114b*	1.736a**	2.070

size of testes of *Aedes aegypti* L. treated with apholate. These controversial results may be due to specificity in the action of chemicals and need confirmation by further experiments. Reduction in the testis size and collapse of the testicular envelope might be correlated with deterioration of testicular contents as observed by Schwartz[4] in *H. pusio*. In contrast to our findings, hempa and hemel did not produce any abnormalitites in ovaries of *Achoea janta* L. even at very high doses, while tepa and metepa significantly reduced the size of the ovary[11]. Morgan[12], working with house flies, reported extensive damage to the ovary after treatment with hempa. According to our findings reduction in size of the ovary may be due to the direct effect of the chemical on cell division in the follicle cells as observed by Beattie[13], in *Lucilia cuprina* Wied. Secondly, reduction in ovary size may be due to damage of nurse cells which feed ova, and degeneration of follicle cells, which in turn leads to inhibition of yolk formation as evidenced by Nath and Sharma[14] who studied the effects of apholate and tepa on *Locusta migratoria migratorioides* Reiche & Farmaire.

Table 2. Effect of indole-3-acetic acid on the size of ovaries (mm) of *Dacus dorsalis* Hendel

Post-treatment days	0.3% concentration	0.5% concentration	Control
8	0.435a	0.429a	0.45
12	0.762a	0.595b**	0.743
16	1.570a	1.598a	1.629

Student's t-tests were performed to determine the significance of the difference between IAA treated values and control values and among the different concentrations. Figures in the same row with same letter are not statistically significantly different from one another at 5% level. * $p < 0.05$; ** $p < 0.01$.

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Centromeric heterochromatin in the karyotype of the male Greater Kudu *(Tragelaphus strepsiceros)*

Anne Dain and H.M. Dott

Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge CB2 4AT (England), 17 July 1981

Summary. The chromosomes of a male Kudu *(Tragelaphus strepsiceros)* have been studied by C-banding and H^3 thymidine labelling. It is suggested that heterochromatin may have accumulated on the 14th pair of autosomes before its translocation to the Y-chromosome.

The chromosomes of the Kudu *(Tragelaphus strepsiceros)* were described by Wallace and Fairall[1] who found in the male an extra-large Y-chromosome, a slightly smaller, unpaired autosome and at meiosis a large quadrivalent, which they took to be an end to end association of the X- and Y-chromosomes with an autosomal bivalent. This demonstrated the association of the Y-chromosome with one homologue of an autosomal pair. Wurster et al.[2] discussed the observations by Schmid et al.[3] of the aggregation of heterochromatin on the sex chromosomes in species with extra-large sex chromosomes. They proposed the translocation of this material from autosomes to sex chromosomes during Robertsonian fusions which reduced the chromosome numbers. 2 species with almost complete and complete reduction, the Sitatunga *(Tragelaphus spekei)* and the Blackbuck *(Antilope cervicapra)* have 29 and 30 pairs respectively, with fundamental numbers of 58 and 60. Their late-labelling heterochromatin is mostly on the extra-large sex chromosomes. Wurster[4] demonstrated by measurements and autoradiographic studies that the original large acrocentric X-chromosome of the Sitatunga underwent inversion, breakage and fusion with the homologue of the autosome to which the Y is attached. Our study concerns the C-banding and autoradiographic characteristics of the male Kudu ($2n = 31$), another species with extra-large sex chromosomes and almost complete reduction; but only the Y-chromosome is translocated to one homologue of an autosomal pair, which is complete and unattached in the female Kudu ($2n = 32^{1,5}$).

Animals and methods. Tissues were obtained post mortem from an adult male Kudu *(Tragelaphus strepsiceros)* at Marwell Park Zoo, Winchester, Hampshire, England.

Chromosome spreads were prepared from the monolayer cultures of kidney fibroblasts by a modification of the method of Tucker et al.[6] and C-banding was done by a modification of the method of Sumner[7]. H^3-thymidine pulse labelling was carried out by the treatment of 4 monolayer cultures with culture medium containing methyl ^3H-thymidine for 30 min. Photographic prints were enlarged approximately $\times 4000$ and linear measurements of the H^3-thymidine labelled chromosomes X, Y and 14 from 5 cells were made using vernier calipers. An image analyzing computer (Quantimet 720) was used to measure total chromosome and centromeric C-band areas on karyotypes prepared from 5 C-banded cells ranked according to Wallace and Fairall[8]. In the X-chromosomes the 2 strong proximal C-bands were also measured.

Results. The C-bands (fig. 1, a and b) and the H^3-thymidine labelling patterns (fig. 2) define 2 distinct regions which show the partial homology of the Y-chromosome and the