

Effect of age on the percent distribution of cells in peritoneal fluid of women between 20 and 40 years old

Cells	Age (years)			
	20-25	26-30	31-35	36-40
No. of women	11	32	11	16
Mesothelial cells	64.1 ± 5.2*	60.2 ± 3.9	44.9 ± 7.2	44.9 ± 6.4
Lymphocytes	15.1 ± 2.3	17.4 ± 2.1	11.6 ± 2.7	16.0 ± 2.4
Polymorphonuclear leukocytes	7.5 ± 2.6	12.0 ± 2.7	18.5 ± 5.5	9.3 ± 2.3
Histiocytes	6.9 ± 2.5	4.6 ± 0.8	2.7 ± 1.0	4.9 ± 1.5
Erythrocytes	3.2 ± —	2.9 ± —	18.6 ± 7.6	21.8 ± 7.5
Squamous cells	1.7 ± 1.5	2.9 ± 1.3	4.8 ± 3.7	3.0 ± 1.7

*Mean ± standard error.

consecutive cells were counted and grouped as mesothelial cells, lymphocytes, polymorphonuclear leukocytes, histiocytes, erythrocytes, and squamous cells. All specimens contained mesothelial cells which we interpreted as proof that the peritoneal cavity had been entered and that a sample of peritoneal fluid had been obtained. The standard error for each mean cell count was calculated from the formula $SE = \sqrt{\Sigma d^2 / N(N-1)}$ and Student's *t*-test was used to obtain probability values for significant differences between the various means (Table).

Mesothelial cell proportions were significantly reduced from 64.1 ± 5.2% in women 20-25 years old to 44.9 ± 6.4 % in women 36-40 years (*p* < 0.05). Lymphocyte distributions were not significantly altered by age. The increase in % polymorphonuclear leukocytes as well as the decrease in % histiocytes between 20-25 and 31-35 years were not significant at the 95% confidence limits. Erythrocytes and squamous cells were sometimes absent from cytologic specimens, especially in younger women,

which accounts for the larger recorded variation. No 'daisy cells' were observed nor did we see gross morphologic changes in the peritoneal fluid observed. In these women, age appeared to have no influence on the volume of fluid aspirated.

Peritoneal fluid in women appears to be in a state of equilibrium in regard to the formation of new cells and the destruction of old cells¹⁰. Possibly, mesothelial cell renewal was decreased in women of the old age groups so that fewer cells exfoliated into peritoneal fluid. Increased cellular destruction probably was not responsible for the decrease in mesothelial cell counts of older women because we did not observe 'daisy cells' (degenerate mesothelial cells) or bare nuclei. If the turnover of mesothelial cells was altered by age, then cytodifferential counts of peritoneal fluid may provide an index of physiologic age in women.

¹⁰ F. D. BERTALANFFY, Am. J. Obstet. Gynec. 85, 389 (1963).

Loss of Fecundity in *Dysdercus koenigii* F. due to Vapours of *Acorus calamus* L. Oil

B. P. SAXENA and A. C. MATHUR¹

Regional Research Laboratory, Council of Scientific and Industrial Research, Jammu-Tawi 180001 (India), 15 July 1975.

Summary. The vapours of *Acorus calamus* L. oil have profound influence on *Dysdercus koenigii* F. Higher concentration of vapours impedes copulation, whereas slightly lower doses hamper the maturation of ova resulting in partial loss of fecundity/infecundity – even the chorionized eggs get stuck in common oviduct.

Oviposition and vitellogenesis in *Thermobia domestica* (Packard)² are impaired by *Acorus calamus* L. oil. Under the influence of the oil, the normal activity of prefollicular cells is antagonized resulting in resorbtion of matured oocytes followed by destruction of all cellular parts previtellarium and vitellarium³. The present communication deals with the changes induced in the ovarioles of *Dysdercus koenigii* F. by *Acorus calamus* L. oil vapours.

Material and methods. Circular filter papers (11 cm diam.) impregnated with 0.05, 0.1, 0.15 ml of calamus oil were fixed to the under surface of the covers of glass troughs (9×7 cm) containing laboratory reared⁴, freshly moulted *Dysdercus* adults. This allows considerable surface for the evaporation of the oil. Dry cotton seeds and a water siphon were placed in every container. The bugs were dissected after 6, 8, 10, 12 days in Ringer's solution, fixed either in alcoholic Bouin's fluid or Carnoy's fixative, and processed for permanent mounts as described previously².

Results. No copulation was observed with higher doses, but with 0.05 ml dose some insects did copulate and laid a few fertilized eggs. In controls, the mating starts on 3rd or 4th day after adult emergence.

The bugs have telotrophic ovaries with 7 + 7 ovarioles. Usually a normal ovariole on 6th day possesses 8 matured eggs of identical size filled with yolk (Figure A). The first batch of eggs is laid on the same day or next day, i.e.

¹ We wish to thank Dr. C. K. ATAL, Director, for facilities and interest in the work, and Dr. E. B. ROHDENDORF of Institute of Entomology, Czech. Acad. of Sciences, Praha, for suggestions and critically going through the manuscript.

² B. P. SAXENA and E. B. ROHDENDORF, *Experientia* 30, 1298 (1974).

³ E. B. ROHDENDORF and B. P. SAXENA, *Regulation of Insect Reproduction* (Liblice Institute of Entomology, Czechoslovak Academy of Sciences, Praha 1974).

⁴ B. P. SAXENA and J. B. SRIVASTAVA, *Experientia* 28, 112 (1972).

6th or 7th day. In contrast, the vapour affected ovarioles exhibited several structural abnormalities. The damage starts from the distal end of the ovariole where the first egg is almost reabsorbed (Figure B). As the severity of the damage increases, either 1 or 2 oocytes with yolk are observed and the rest are very small, if at all present, but their arrangement continues to be linear (Figure C, D and E). Such a linear arrangement is lost in ovarioles with the 0.1 ml dose (Figure F and G). An extreme case was observed where a mature egg hangs on the side of the germarium (Figure G). At the higher dose of 0.15 ml, previtellarium and vitellarium are reduced to a small tube with one chorionized egg (Figure H and I) or tubes with remnants of ova. At this stage, the fat reserves of the body are almost exhausted. The fat reserves decrease day by day in treated insects, though their stomachs are always full of food.



Ovarioles of *Dysdercus koenigii* F. A. Normal B to J affected by *Acorus calamus* oil vapours. A, normal with 8 identical ova in vitellarium and 5-6 oocytes in previtellarium; B, 0.05 ml dose, 2 basal ova reduced, on 7th day of exposure; C, 0.05 ml dose, only 2 ova with yolk after 15 days; D, 0.05 ml dose after 17 days; E, 0.1 ml after 10 days; F and G, 0.1 ml after 7 days, eggs with yellow egg-shell could be seen. H, I and J, 0.15 ml dose, egg with yellow egg-shell in common oviduct and in vitellarium; H, single or many ova with or without yolk (F, J). Double line shows the eggs with yellow egg shell.

Discussion. The effect of *A. calamus* oil vapours on *D. koenigii* and *T. domestica* are almost similar. In addition, some more observations have been made in the case of bugs, such as loss of linear arrangement, occurring in chorionized eggs in the vitellarium and on the side of the germarium, and sticking of eggs in the common oviduct. In both cases, the degeneration process begins from the distal end. Similarly prefollicular cells in this case appear to be affected. These cells receive a wrong code and perform wrong functions at wrong place and time³. Besides, the preceding case can be compared with *D. cingulatus* ovaries affected by tepa³. However, to mention calamus oil as a chemosterilant is a subject for debate. Usually antimetabolic chemosterilants affect females only, particularly when administered to adults. But when they are administered in the larval stage (that is at the initiation of vigorous synthesis of nucleic acids in many different tissues), basic effects with general characteristics are usually produced rather than the specific ones⁶. In one of our experiments, treated 5th instar nymphs moulted into normal adults, but their ovaries failed to develop⁷. In other words, the effect is specific rather than general.

The consumption of fat reserves could be due to inhibition of digestion, thus indirectly affecting oogenesis, as in the case of the antimetabolite 5-fluorouracil⁸. The validity of this assumption has to be investigated in the case of *Acorus* oil. It is known that oogenesis is not an autonomous process; it is possible that the sterility is achieved due to interference with regulatory functions rather than the function of the ovarian tissue. We found the corpora allata is greatly reduced (almost $\frac{1}{3}$ in comparison to normal individuals) in *Pyrrhocoris apterus* nymphs treated with *A. calamus* oil vapours⁹. However, the possibility cannot be ruled out that this vital organ, acting as messenger for triggering vitellogenesis in insect ovaries, is the cause of sterility.

The *A. calamus* oil vapours also effectively derail the normal functioning of ovaries of *Callosobruchus chinensis* L.; *Trogoderma granarium* Evarts; *Tribolium castaneum* Herbst and *Sitophilus oryzae* L. which are important pests of food products, and *Anthrenus vorax* Waterhouse pest of woolen cloth. The oil of *A. calamus* is accordingly a versatile, non-toxic pest-controlling agent¹⁰ for stored grain.

⁵ K. SUKUMAR and M. B. NAIDU, J. econ. Entomol. 66, 20 (1973).

⁶ G. C. LABRECQUE, P. H. ADCOCK and C. N. SMITH, J. econ. Entomol. 35, 802 (1960).

⁷ Unpublished observations.

⁸ S. AKOV, Biol. Bull 129, 439 (1965).

⁹ B. P. SAXENA, Unpublished observations at Inst. of Entom., Praha.

¹⁰ B. P. SAXENA and A. C. MATHUR, in press (1975).

Structure of the Foregut Cuticle of *Periplaneta americana*

R. C. MURTHY¹

Zoology Department, University of Lucknow, Lucknow (U.P., India), 12 August 1975.

Summary. The cuticular lining of the foregut of *Periplaneta americana* did not contain either pore canals or the openings of dermal glands, and the length of the cuticular spines decreased posteriorwards.

Cuticle of arthropods, specially that of insects, has been studied by many workers and the studies have been reviewed by WIGGLESWORTH² and RICHARDS³. However, the structure of the foregut cuticle of insects is not well known. The present study gives an account of the structure of the cuticle of the foregut of *Periplaneta americana*.

Adult cockroaches were starved for 3-4 days to empty their gut. The foregut was dissected out, slit opened longitudinally, washed thoroughly with water to remove adhering particles and then left in water for 1 h. It was then possible to separate the cuticle lining of the foregut from the epithelium and muscles. Foregut was fixed in