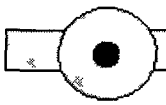
CONSOLIDATED DISCUSSION



Consolidated Discussion

The investigations presented in the Thesis have yielded some new information pertaining to the chemical characterization of mouse urine with special reference to estrus. The urinary volatiles of mouse have been identified and have confirmed certain compounds as signalling pheromones. Further, odour preference test, biochemical and bioassay have helped to strengthen some of the hypotheses put forward by other workers.

The odour preference test revealed that female produces different types of odours as per the reproductive phases. It is well established that almost all male mammals are more attracted to the estrous females than to diestrus or non -estrus females. Based on the behaviour of the male which exhibited more preference towards proestrous and estrous urine by frequently visiting and spending more time in investigating the urinary sample. It reveals that the estrus phase has got specific volatiles that are actually involved in attraction. The increase in attractiveness of female odours during estrus is consistent with earlier findings (Zeigler *et al.*, 1993; Lai *et al.*, 1996). Male mice exhibited more time in self-grooming activity in response to the odours of opposite sex and particularly to estrus. It is known that grooming initiates secretions from preputial gland, penis or other glands and spread them around the anogenital area (Thiessen, 1977; Harriman and Thiessen, 1985; Sprujit *et al.*, 1992; Moffatt and Nelson, 1995). The male is involved in self-grooming activity in broadcasting scents to attract potential mates and increasing willingness to mate. It is consistent with the

hypothesis that self-grooming rates vary in response to urinary odours of different reproductive state.

Castration is reported to influence odour preference and production by altering aromatase activity (Powers *et al*, 1985; Yahr and Stephen 1987). Gonadectomy in gerbils, rabbits, musk shrews, golden hamsters and Colombian squirrels reduces the behavioural activities (Gonazelez-Mariscal *et al.*, 1992). Similarly in the present study castrated animals failed to discriminate the female urinary odours and showed a decline in grooming activity. However, the ability of castrated male mice was restored in odour discrimination and grooming activity by administration of testosterone. The present observation lends support to the view that the testosterone interferes with the behaviour and preference of odours (Gregory *et al.*, 1975; Merkx, 1984).

In rodents, the role of main olfactory system (MOS) is evaluated using $ZnSO_4$-irrigation, which destroys sensory cells in the olfactory epithelium and the accessory olfactory system (AOS) by VNO ablation that is the peripheral receptor of AOS (Archunan and Dominic, 1990c). Accordingly, in this investigation the frequency of visit by the $ZnSO_4$-irrigated mice towards urine samples was affected, while the duration of visit and grooming behaviour was unaltered. These findings suggest that the main olfactory system is probably involved in attraction, whereas, other behavioural activities such as body rubbing, grooming etc, may depend on vomeronasal system. The present data are also consistent with the previous result that the MOS rather than AOS mediate the transportation of attractant odours from female to male (Powers, *et al.*, 1979). The present study suggests that the substance produced from estrus urine that is perceived by the male may be volatile in nature.

The biochemical study revealed that the presence of the highest amount of excretory protein in male urine is nothing but major urinary protein, which is reported in several mammalian species (Clarke *et al.*, 1984; Mucignat-Caretta *et al.*, 1995). Castration reduced the urinary protein production, while on testosterone replacement there was a considerable increase in urinary protein. Experimental evidence demonstrates that these urinary proteins act as carriers for ligands and in certain cases MUP itself act as pheromones (Mucignat- Caretta *et al.*, 1995). In addition, proestrus and estrus phase excreted higher concentration of protein than that of all other stages. Estrus appears to be important in releasing the specific volatiles, hence the result suggests that the urinary protein in estrus may carry more volatile which are believed as putative pheromones and probably involved in attraction of the males. Clancy *et al.*, (1988) provide evidence that the female urinary pheromone is of low molecular weight and bind with urinary protein.

The present study also reveals that there is a significant relationship between testosterone and lipid excretion. In the normal male mice the serum testosterone and urinary lipids were in higher concentration than those in castrated mice. However, administration of testosterone is found to increase in lipid excretion. This result is consistent with the report of Rasmussen and Perrin (1999) that there is a significant correlation between serum testosterone and urinary triglycerides concentration in elephant *Elephas maximus*.. Further, the present results clearly indicate that there is a positive relationship between lipids and estrogen. This can be justified with the reports of Hansel and Convey (1983) in which phospholipids are broken down by methylation process for the production of progesterone.

The fatty acid(s) observed in the present study during estrus phase individually or combined may act as sex attractant towards males. Fatty acids have been reported to act as sex attractant in certain mammalian species (Mattinal et al; 1991) as well as individual identification (Poddar -Sarkar and Brahmachary, 1999). The difference in the fatty acid composition may be due to the alterations of lipid metabolism especially of metabolic events affected due to the absence of estrogen. Interestingly, in estrogen replacement there was a reappearance of certain fatty acids namely Pentadecanoic, henecosanoic, lignoceric, mysteric and elaidic. The gonadal hormones not only play role in excretion of biomolecules, they also influence in manifesting several behaviours. Higher level of estradiol concentration was reported during the proestrus and estrus phase. In the present study the hormones estrogen and progesterone varied significantly during various reproductive phases.

One of the striking features of identified volatile profiles in the present study is the relatively large number of alkanes (both male and female) more than other constituents such as alcohols, ketones etc. Further certain alkanes were present in almost all the reproductive phases of female urine suggesting that these compounds probably are common metabolic end products in mice. However, certain compounds namely isocroctylamine, 4 -methyl-2-heptanone and azulene of proestrus stage and the compounds, 1 -H-cyclopop-e.auzulene, caryophyllene, copanene of estrus stage have been identified in the present study, and these volatiles are not present in other phases. The compound 1-methyl iodoundecane was observed both in proestrus and estrus but was absent in all other phases. As estrus and proestrus urine are believed to contain sex -attracting compounds, these periods may be considered as behaviourally important in releasing the effective chemical signals. This is in contrast to the report of Andreolini, *et al.*, (1987) that

urinary volatiles across estrous cycle of mice differed only quantitatively and not qualitatively and suggested that are no estrus specific compounds.

It is important to note that, the estrus and proestrus -specific compounds were lost due to castration. However, estrogen administration into ca strated females showed reappearance of certain compounds namely 1 -iodo2-methyl undecane and 4-methyl 2-heptanone, which are detected in estrus and proestrus stages. An interesting aspect is that the compound, 4 -methyl 2- heptanone, observed in proestrus ur ine of mice, which is also estrogen dependent, is reported in many other mammals with slight variation in structure. 2 - heptanone is identified in the urine of male *A.Stuartii* (Toffegaurds, *et al.*, 1999) male wolf (Raymer et al., 1986), copine voles (Boyer *et al.*, 1989) and deer mare (Ma *et al.*, 1999). Furthermore, the 2- heptanone has been identified as honeybee pheromone (Lensky and Cassier, 1995) and as sex attractant in wasps (Weston *et al.*, 1997).

In the present investigation five male specific compou nds have been identified in which three compounds are characteristically shown as sex attractants. Further, both the earlier reports and the present one show that the 4-ethyl phenol acts as an attractant for opposite sex and aversion towards the same sex. Strikingly, the compounds identified in the present study do not correlate with the chemical compounds identified in the urine of ICR strain male mice (Nishimura, *et al.,* 1989). It, therefore, appears that chemicals involved in pheromonal communication dif fer according to their genetic background.

It is also interesting to note that the compound II, the 3 -amino-s-triazole, has a close similarity with thiazoline, which is reported in male mice urine (Liebech, *et a.,l* 1977; Novotny, *et al.,* 1985a). The comp ound 3-amino-s-triazole,

contains a pentagonal structure along with a nitrogen compound similar to thiazoline (Liebech, *et al.,* 1977). In fact, the compound II has aromatic nature but lacks sulphur while thiazoline contains sulphur without aromatic nature. Since 3-amino-s- triazole is aromatic in nature, it is possible to act as sex attractant.

The present study reveals that the compounds identified in the male urine were abolished by castration. Besides, the only volatile ie. 3-ethyl 2, 7dimethyl octane (IV), reappeared in the testosterone treatment, which was absent during castration. This report is consistent with the report of Novotny et al., (1984) that a testosterone dependent unique volatile constituent of male mice urine namely, 2,3 dehydro–exo brevicomin, which displays a strong dependence on testosterone levels in the male mouse.

The present results show that the responders lead to many kinds of behavioural moments including licking behaviours. The compound 4 -methyl 2-heptanone and 1-iodomethyl u ndecane significantly enhanced licking behaviour when compared with other compounds. Similarly, Selvaraj and Archunan, (2002) report that two estrus specific urinary compounds namely hydroperoxide and 4-azidopentane are involved in the attraction of the op posite sex in house rat. The estrus specific compounds 1 -iodo-2-methylundecane, azulene significantly enhanced the grooming response when exposed individually. Experiments on voles, rats and ground squirrels show that these animals are actively involved in more self-grooming when exposed to odours of conspecifics than the same sex (Witt *et al.*, 1988; Sprujit *et al.*, 1992; Ferkin *et al.*, 1996). These reports conclude that self-grooming may be a tactic used by animals to communicate sexual interest by broad casting scents that are specifically important for reproduction (Ferkin *et al.*, 1996, 2001). In the present study the compound namely 1 -iodomethyl

undecane identified in both the proestrus and estrus phase seems to be involved in the enhancement of most of the behaviours such as sniffing, licking and grooming. Similarly, another compound azulene which is present only in the estrus urine, seems to be involved in the grooming behaviours of mice. Further, the proestrus specific compound 4-methyl 2-heptanone is involved almost all-behavioural activities.

Urinary compounds that are identified in the present study are unique to male mouse, which had molecular weight less than 300 carbon atoms less than 20. Air-borne pheromones usually contain 5 -20 carbon atoms and must be volatile to reach the receiver; the molecular weights of pheromones are less than 300 (Dominic 1991). The compounds identified in the present study have physical properties necessary for consideration of pheromones. The bioassay provides additional support for considering the identified compounds as pheromones.

A century of experiments in olfaction has recently yielded results of such fundamental importance that they will become the foundation of a pervasive new chemosensory technology (Bell, 1996). An important finding in the present study is that the identification of specific volatile compounds in estrus urine and confirmation of the identified compounds with male mice by behavioural studies. Moreover, the technology used in this study is mostly non-invasive i.e. the collection of samples without making any disturbance to the animals, and it also provides evidence that excretory products are good indicators for understanding the internal physiology. This finding would help to develop eco -safe pheromonal trap in rodent pest management (RPM). The future use of pheromones in wildlife biology as attractants or repellents and reproduction of zoo animals as an indicator in female reproduction and aphrodisiacs would be possible.