tubes of freeze-dried material stored for different durations showed dermonerotic activity in rabbit skin ranging from  $10.0 \times 10.0$  mm to  $20.0 \times 10.0$  mm. Thus, these cultures were found to maintain not only viability, but also there was no alteration in their antigenic composition. No difference in dermonecrotic activity between the older and freshly freeze-dried cultures was observed. Tubes under the atmo-

sphere of gaseous nitrogen, however, showed scanty growth immediately after freeze-drying and no growth in older cultures. Since these tubes did not yield growth at the time of final testing their antigenic composition and dermonecrotic activity could not be determined. In our experience both Lomodex and aqueous gum acacia work very well as suspending fluids for freeze-drying.

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## In vitro characterization of adrenergic receptors mediating extrusion of preformed sebum from preputial gland of rat<sup>1</sup>

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Summary. Extrusion of preformed sebum from the preputial glands of rat after phenylephrine and adrenaline treatment, and its inhibition by a-receptor blocking agents under in vitro conditions, shows that secretory response of the glands is influenced by a-adrenergic receptors, and isoproterenol – a  $\beta$ -agonist – is not effective in elicitation of exudation of secretion from the preputial gland.

There is neurohistochemical evidence that the preputial gland, a sebaceous analogue, of rat is supplied by adrenergic as well as cholinergic nerves<sup>2</sup>. It is not definitely known to what extent the sebaceous gland is under the control of nervous system. Neurohistological studies have yielded no evidence of secretory innervation for the sebaceous gland. Serrati<sup>3</sup> thought that vegetative nervous system regulates sebaceous secretion, basing his conclusion on the observations on patients with various neuronal disorders. Savill<sup>4</sup> stated that the sebaceous glands of the skin are under the control of autonomic nervous system. Nexmand<sup>5</sup> observed seborrhea following complete trans-section of the facial nerve, while Hodgson-Jones et al.<sup>6</sup> noted no change in sebaceous secretion in denervated area of skin. Starling<sup>7</sup> stated that sebum is squeezed out by intradermal injections of epinephrine, but Kligman and Shelley8 believed that Starling's observations were erroneous as they could not find any expulsion of preformed sebum after either epinephrine or acetylcholine administration. Present experiment is an attempt to observe the effects of adrenergic agents under in vitro conditions, so as to probe deeper into the study of involvement of neurotransmitters in the release of preformed sebum from the preputial glands of rat.

Material and methods. Wild rats (Rattus rattus) were used in the present study. The amount of preformed sebum is greater in wild rats than that in albino rats. When the amount of preformed sebum is greater, it is easy to detect its extrusion visually. For this purpose wild rats were selected. The preputial glands were removed and immersed in 20 ml of oxygenated Ringer's solution. After 20 min of exposure to Ringer's solution; adrenaline, isoproterenol or phenylephrine were added to Ringer's solution separately at concentrations of  $1 \times 10^{-5}$  moles/20 ml in each case. Extrusion of the sebum was considered as the positive response. In another set of experiments, 3 different blocking agents viz., propranolol ( $\beta$ -blocking agent), dibenamine and phentolamine ( $\alpha$ -blocking agents) at  $1 \times 10^{-4}$  moles/20 ml concentration were added. Preputial glands

were exposed to blocking agents for 30 min. After 30 min, adrenaline was added and extrusion of secretion that followed was considered as the positive response.

Results. The table represents the pattern of responses of preputial glands to various drugs employed during the course of present study. Addition of adrenaline or phenylephrine to the Ringer solution caused quick extrusion of secretion from the duct and the response was apparent over a considerable period of time. Isoproterenol addition did not elicit such a positive response.

Later, the glands were preincubated for 30 min with blocking agents viz., dibenamine, phenotolamine or propranolol, with a view to differentiate more precisely between a- and  $\beta$ -receptor-mediated responses. At the end of 30 min of preincubation, adrenaline was added in each case. It was observed that the glands preincubated with  $\alpha$ -receptor blocking agents – dibenamine or phentolamine – did not respond, while those preincubated with  $\beta$ -receptor blocking agent – proprenolol – still showed a positive response to addition of adrenaline.

Discussion. Skin glands of lower vertebrates (anurans) are reported to have  $\alpha$ -adrenergic receptor-mediated secretory response<sup>9</sup>; while cutaneous and hedonic glands of the redspotted newt are reported to respond to cholinergic stimu-

In vitro response of the preputial gland of rat to adrenergic agonists and antagonists

Experimental group treated with	Response	
Adrenaline	+	
Phenylephrine	+	
Isoproterenol		
Dibenamine + adrenaline	_	
Phentolamine + adrenaline		
Propranolol+adrenaline	+	

<sup>+</sup> Sign in the column of response indicates positive response to the drug whereas - sign indicates negative response.

li<sup>10,11</sup>. Among the skin glands of mammals, sweat glands are known to be influenced by catecholamines.

There are species-specific differences in adrenergic receptor sites, for example,  $\beta$ -receptor agonists cause sweating in bovides, whereas  $\alpha$ -receptor agonists are effective in equides<sup>12</sup>. From the results (table), it becomes clearly evident that the extrusion of preformed secretion from the preputial gland can be effectively brought about by  $\alpha$ -adrenergic agonist-phenylephrine, while the  $\beta$ -adrenergic agonist (isoproterenol) is ineffective in eliciting a positive response. Thus it is clear that the extrusion of preformed

- sebum from the preputial glands of rats may be regulated through the  $\alpha$ -adrenergic receptors.
- Further, it was observed that the glands preincubated with  $\alpha$ -receptor blocking agents did not respond to added adrenaline while those preincubated with  $\beta$ -receptor blocking agent still showed a positive response. This suggests that  $\alpha$ -blocking agents are effective in blocking the response of the glands to adrenaline, while  $\beta$ -blocking agents are without such effect. This can be said to confirm the contention that extrusion of sebum from the preputial glands of rat is mediated via  $\alpha$ -adrenergic receptors.
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## Cannibalism in Anopheles pharoensis Theo.1

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Summary. A single 4th instar larva of Anopheles pharoensis could consume within 24 h an average number of 5.3-11.6 larvae of the 1st instar. The number consumed differed according to crowdedness and the presence of other mature larvae. Dissection of the midgut of these larvae revealed the presence of various undigested parts of young larvae.

The cannibalistic behaviour of the 4th and 3rd instar larvae of Anopheles pharoensis was early noticed by the present author<sup>2</sup>. In a recent study on the genetic control of A. pharoensis<sup>3</sup>, close observations of laboratory reared larvae revealed some unexpected mortality in the late larval instars which was probably a result of such cannibalistic behaviour. Larvae were observed trying to seize each other, which would probably result in injury of the body wall. allowing microorganisms to invade the hemocoel and probably causing death or inactivation of the injured larvae. These inactive larvae then become an easy prey to other predaceous ones, and partially eaten larvae were commonly seen in the breeding pans. Old skins from moulting larvae could be observed in the breeding pans only for a short time after moulting, and then were most probably eaten by the other larvae.

Cannibalism was early recorded in Aedes aegyptt<sup>4</sup> and Anopheles stephensi<sup>5</sup> but was not considered to be a usual feature in mosquitoes<sup>6</sup> until it was recently investigated in Anopheles stephensi<sup>7</sup>. The present paper provides some quantitative studies of this cannibalistic behaviour in A. pharoensis.

Material and methods. Larvae of A. pharoensis used in the present investigation were obtained from the standard colony maintained in the Institute of Genetics, Mainz University, FRG.<sup>3</sup>. Larvae were reared at a normal density of 0.6 L/cm<sup>2</sup> during the first 2 instars and reaching 0.4 L/cm<sup>2</sup> during the latter instars. Small plastic cups 7 cm in diameter and 5 cm in height were used in the present investigation. Laboratory temperature was kept constant at 27 °C. 1, 10 and 20 4th instar larvae were confined with 10, 20 and 40 larvae of the 1st instar. 3 replicates of each combination were carried out. Larvae were kept in fresh

tap water and given no food. The number of larvae of the 1st instar consumed, and the number of the 4th pupating, were recorded after 24 and 48 h. Some of the remaining 4th instar larvae were dissected and the contents of the midgut were examined.

Results and discussions. Results shown in the table indicate that 1 single 4th instar larva could consume 5.3-11.6 young larvae in 24 h when they were present together at a density from 0.3 to 1.1 L/cm<sup>2</sup>. With the presence of more mature larvae (10 or 20), the consumption rate also increased with crowdedness but not at such high rates. Competition between mature larvae would reduce their predaceous capacity and give the young larvae a chance to escape. With a

Cannibalism in Anopheles pharoensis

Larval density (L/cm)	Density of 4th: 1st at:			Average No. of consumed lst instar larvae after:	
	0 h	24 h	48 h	24 h	48 h
0.3	1:10	0.3; 2.3	0.3:2,3	5.3	1.0
0.6	1:20	0.3:11.0	0.0:10.0	9.0	3.0
1.1	1:40	0.0;28.3	-	11.6	-
0.5	10:10	6.3: 0.0	_	1.5	_
0.8	10:20	6.3: 0.0	_	3.1	_
1.3	10:40	5.3: 1.6	3.6:0.0	7.2	0.4
0.8	20:10	12.3: 0.0	_	0.8	_
1.1	20:20	13.3; 1.0	13.0*:0.6	1.4	0.1
1.6	20:40	13.6: 2.3	13.3*:2.0	2.8	0.1

<sup>\*</sup> Reduction in 4th instar density was due to mortality not pupation.