rence in chemical composition. The inner layer stained blue with Azan (figure 1), Mallory's triple stain and aniline blue stain (figure 2). It gave a strong positive reaction with Luxol fast blue G in methanol and failed to react with aldehyde fuchsin. These results suggest that collagen may be involved in the composition of inner layer. The outer layer stained red with Azan (figure 1) and orange to red with Mallory's triple stain. This layer is intensely positive to aldehyde fuchsin (AF), Rhodamine B (figure 3) and potassium permanganate/alcian blue thus confirming the presence of keratin type of protein.

It could be concluded that the lining of the inner wall of the oesophagus in *C. annulata* is made up of an outer keratin and an inner collagen layers.

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PROLACTIN-DEPENDENT ACTIVATION OF MAMMALIAN SPERM METABOLISM

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PROLACTIN enhances the male fertility in many animal species[1,2]. Administration of OPRL stimulates testicular growth and spermatogenesis in mice and rats[3,4]. Bromocriptine, an ergot alkaloid, inhibits prolactin secretion and release by its direct action on pituitary acidophils[5]. Bromocriptine administration decreased prolactin levels in the serum of male albino rats[6]. Since information on the sperm metabolism and PRL concentration is meagre, an attempt was made to understand the effect of prolactin on sperm metabolism.

Adult male Wistar strain albino rats, 70±2 days old and 160±5 g body weight were administered with 20 μg of bromocriptine mesylate (Sandoz Pharmaceuticals, Switzerland) per rat per day for 15 days as suggested earlier[7]. The second group of six animals received subcutaneous injections of OPRL (NIH, Bethesda, USA) at a dose of 1 μg per g body weight per day for 5 days as described earlier[8]. Injections of vehicle (physiological saline) were given to the third group of animals (controls). The animals were maintained at laboratory conditions and fed a standard rat diet obtained from the Hindustan Lever Ltd., Bombay and water was supplied *ad libitum*. All the animals were sacrificed at 24 h after last injection.

Both control and experimental albino rats were sacrificed by cervical dislocation. The cauda epididymis was cut out and taken into the medium containing physiological saline. The tissue was minced and teased gently for the release of spermatozoa as suggested by Chinoy and Sanjeevan[9]. The sperm samples with motile sperm were taken for the analysis.

The testes were isolated with minimum mechanical stress. The adhering blood was blotted and used for biochemical analysis. The sperm motility was observed under the microscope and comparison was drawn between control and experimental groups of animals.

The cholesterol content[10], levels of ATPase[11], sorbitol dehydrogenase[12], glucose 6-phosphate dehydrogenase[13], acid phosphatase[14], alkaline phosphatase[14], 17β-hydroxy steroid dehydrogenase[15], and 3β-hydroxy steroid dehydrogenase[15] were estimated by the methods described earlier.

Prolactin levels have been modulated in adult male albino rats through the administration of exogenous prolactin and bromocriptine. The rats administered with exogenous PRL represented hyperprolactinemic conditions and those administered with bromocriptine recorded hypoprolactinemic conditions. This observation was in close consonance with the reports of earlier investigators[5,16-19]. The testicular cholesterol content decreased conspicuously in PRL-administered rats (table 1) indicating its active mobilization probably into androgenesis. The activity levels of 3β and 17β-hydroxy steroid dehydrogenases (HSD) were significantly elevated in the tissue indicating improved androgenesis in the testis. Since

Leydig cells contain receptor site for PRL[20-22], PRL-mediated activation of androgenesis and consequent mobilization of cholesterol can be expected under hyperprolactinemic conditions. This observation agreed with the reports on the increased serum testosterone after prolactin administration[23-26]. However, after the administration of bromocriptine and induction of hypoprolactinemia, the testicular cholesterol level was markedly elevated with significant inhibition in the activities of 3-β and 17-β HSD. This indicates inhibited testicular androgenesis with consequent accumulation of cholesterol in the tissue under hypoprolactinemic conditions.

The sperm of cauda epididymis in prolactin-administered rats had higher motility than the bromocriptine-administered rats. The sperm suspension of prolactin-administered rats had significantly higher ATPase activity (table 2) indicating activated energy metabolism in them. Sorbitol dehydrogenase activity which represents fructolysis was markedly increased indicating PRL-mediated activation of sperm metabolism. The activity levels of G 6-PDH, acid and alkaline phosphatases were conspicuously increased in the sperm of prolactin-administered rats. In contrast, in bromocriptine-administered rats the sperm showed inhibited ATPase activity, suggest-

Table 1 *Effect of prolactin and bromocriptine on the levels of cholesterol and the activity of 17β-hydroxy steroid dehydrogenase and 3β-hydroxy steroid dehydrogenase of testis*

Component	Control	Prolactin injected	Bromocriptine injected
Cholesterol (mg/g dry wt)	27.93 ± 2.63	19.87* ± 1.89	39.98* ± 3.73
17β-hydroxy steroid dehydrogenase (μmol NADPH oxidized/ mg protein/min)	0.31 ± 0.02	0.60* ± 0.05	0.19* ± 0.01
3β-hydroxy steroid dehydrogenase (μmol NAD reduced/ mg protein/min)	0.042 ± 0.003	0.09* ± 0.007	0.02* ± 0.002

Each value is an average of six individual observations; *$P < 0.001$ compared to controls.

Table 2 *Effect of prolactin and bromocriptine on the activity of ATPase, sorbitol dehydrogenase, glucose 6-phosphate dehydrogenase, acid phosphatase, alkaline phosphatase in the sperm suspension*

Parameter	Control	Prolactin injected	Bromocriptine injected
ATPase (μmol Pi formed/ml of sperm suspension/h)	0.82 ± 0.07	1.12* ± 0.1	0.35* ± 0.02
Sorbitol dehydrogenase (μmol of formazan formed/ml of sperm suspension/h)	0.40 ± 0.04	0.60* ± 0.05	0.19* ± 0.001
G 6-PDH (μmol of formazan formed/ml of sperm suspension/h)	0.30 ± 0.03	0.62* ± 0.06	0.17* ± 0.01
Acid phosphatase (μmol Pi formed/ml of sperm suspension/h)	0.21 ± 0.02	0.41* ± 0.04	0.19* ± 0.01
Alkaline phosphatase (μmol Pi formed/ml of sperm suspension/h)	0.18* ± 0.01	0.29* ± 0.02	0.11* ± 0.12

Each value is an average of six individual observations; *$P < 0.001$ compared to controls.

ing a general decrease in the sperm metabolism. The activity levels of sorbitol dehydrogenase, G 6-PDH, acid and alkaline phosphatases were markedly decreased in the sperm of these animals.

In general, it can be suggested that PRL concentration in circulation was responsible for testicular androgenesis and sperm metabolism.

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