up to 100 mM, whereas SGSL is strongly inhibited by both these sugars. Comparison with the *Trichosanthes cucumerina* seed lectin (TCSL), which exhibits antigenic similarity with SGSL, again shows that the relative trends remain the same[12]. Its binding to thiodigalactoside is however nearly 4 times weaker than to Gal and the lectin does not show a significantly higher affinity for sugars with hydrophobic substituents in the β-anomeric position. For example, the inhibition of lectin-activity by PNPβGal is only 1.33 times that by Gal, while the inhibitory potency of MUβGal is comparable to that shown by Gal.

Carbohydrate binding, particularly of mono- and disaccharides, is the defining feature of lectins and the basis of many methods of classifications of plant lectins[13]. Further, a good understanding of the primary binding site of lectins is critical in understanding the evolutionary patterns that members of the same lectin family might share. The haemagglutination-inhibition studies on the snake-gourd seed lectin suggest that like other cucurbit seed lectins, the lectin recognizes galactopyranosides, i.e. the axial hydroxyl at C-4 position is crucial for recognition by the lectin. The binding site of the lectin prefers the β-anomer of galactopyranosides compared to the α-anomer. Hydrophobic substituents at C-1 greatly improve the inhibitory potency of the ligands in these experiments, possibly due to favourable hydrophobic interactions at or close to the sugar-binding site of the lectin. The C-2 and the C-5 positions are not critical for binding, although reducing possible hydrogen bonding interactions by substituting the hydroxyls with –H, $-CH_3$, CH_3CONH- or $-NH_2$, can reduce the binding affinity. Comparison with reported agglutination-inhibition trends of various sugars for three other cucurbit seed lectins, *M. charantia* lectin, *T. kirilowii* seed lectin and *T. cucumerina* seed lectin, indicates that despite gross similarities in sugar binding displayed by them, fine variations exist among these four lectins, which could be found useful in investigations on glycoconjugates and in the study of cell-surface architecture.

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Effect of zinc deficiency on boundary layers of seminiferous tubules of testes of Wistar albino rat

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Dialogue between Sertoli cell and peritubular cell appeared to be disturbed, leading to various lesions in boundary layers of seminiferous tubules of zinc-deficient Wistar rats. Structural deformities included thickening of collagen fibres, accumulation of oedematous fluid, increased vacuolization in endothelial cells, several infoldings in lamina propria and extensive damage of boundary layers. Thus the arrest of spermatogenesis, a common feature of zinc deficiency, might be because of alternations in the microenvironment of seminiferous tubules owing to pertuberations in the boundary layers of seminiferous tubules.

ZINC deficiency leads to gonadal dysfunction, decreases testicular weight, causes shrinkages of seminiferous tubules, alters testicular steroidogenesis and defective capacitation of sperm[1–6]. The importance of the dialogue between peritubular myoid cells and Sertoli cells has been demonstrated in the fetal testis, the prepubertal testis and the adult testis[7–13]. Peritubular cells insure the structural cohesion, contraction of seminiferous tubule and are known to be one of the constituents of blood testis barrier[14–18]. In cooperation with Sertoli cells they

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produce the extracellular matrix (ECM) which in turn is responsible for cell migration, proliferation, differentiation, polarity stabilization of phenotypic expression, gene expression and responses to various paracrine and humoral factors including growth factors[19-21]. Various kinds of abnormalities in boundary tissues have been reported in subfertile and infertile humans[22].

The present study was designed to analyse the effect of dietary zinc deficiency on boundary layers of seminiferous tubules of Wistar albino rat, so as to evaluate the importance of zinc not only in maintaining the structural and functional integrity of various cell types of seminiferous tubules as revealed by many investigators but also to find out any change in boundary layers.

Weanling Wistar albino rats grouped as control (ZC), pairfed (ZPF) and zinc-deficient (ZD) were housed individually in polypropylene plastic cages with stainless steel grills. ZC and ZPF groups received a diet with 100 ppm zinc for 2, 4, 6 and 8 weeks. The ZPF group received 100 ppm zinc diet equivalent to that consumed by the ZD groups, whereas ZD groups were fed on zinc-deficient diets (1 ppm) for 2, 4, 6 and 8 weeks. The basal diet was prepared according to Wallace *et al.*[23] and all the groups received demineralized water *ad libitum*. The cages, grill and bottles were washed daily with tap water, twice with demineralized water and then with 10% EDTA (ethylene diamine tetraacetic acid) to prevent any contamination. Zinc was estimated by wet assay method on GBC-902 double beam atomic absorption spectrophotometer in air acetylene flame at 213.9 nm. Behavioural changes and other symptoms of zinc deficiency were also recorded. Daily consumption of diet, body weights of animals after every week and wet weight of tissues at the end of experiment were also recorded and evaluated statistically.

Animals were autopsied under mild anaesthesia after 2, 4, 6 and 8 weeks and small pieces of testes from each group were quickly fixed in ice cold Karnovsky's fixative (5% glutaraldehyde – 4% paraformaldehyde in 0.2 M cacodylate buffer, pH 7.2) for 18 h. The tissues were frequently washed with 0.2 M cacodylate buffer containing 5% sucrose, postfixed with 1% osmium tetroxide buffered with Caulifield's for 4 h at 4°C, dehydrated in graded series of alcohol and embedded in Spurr low viscosity resin. Ultrathin sections were cut on LKB-III ultratome, the sections were stained with uranyl acetate and lead citrate, then examined and photographed on Philips CM-100 electronmicroscope at AIIMS, Delhi.

Wistar albino rats fed on zinc deficient diet (1 ppm) for 4, 6 and 8 weeks exhibited several physiological changes like anorexia with a cyclical pattern of food intake and growth retardation.

Zinc concentration decreased insignificantly in 2ZD and 4ZD groups and significantly in 6ZD and 8ZD groups as compared to their respective ZC and PF groups (Table 1). The tunica propria (boundary tissue) of seminiferous tubules from control and pairfed groups comprised of elongated endothelial cells surrounded by extracellular material (ECM) consisting of basal lamina and thin collagen fibres (Figures 1–3). Parallel were the observations in 2ZD group (Figure 4) but as the period of experimental zinc deficiency was extended to 4, 6 and 8 weeks the insults to the region were more prominent. Further, the damages within the group and around the same tubule also varied, e.g. in 4ZD group at one place only few cellular debrises in the layers could be seen whereas at another region the endothelial cells exhibited loss of its characteristic spindle shape, several vacuoles and thickened layers of collagen fibres with accumulation of oedematous fluid (Figure 5). Tunica propria from 6ZD group displayed a few remnants of boundary tissue and the endothelial cells were completely damaged with very thin cytoplasmic processes. The intercellular connections were also lost. The lamina propria has lost its continuity and displayed many infoldings and thickening of the layer of collagen fibres (Figure 6). 8ZD group illustrated complete loss of the boundary tissues (Figure 7).

Growth retardation due to zinc deficiency observed in the present study is correlated to impaired enzyme activity, disturbed protein synthesis, anorexia and recurrent diarrhoea[24-35]. Bushwell and Levin[36] reported that zinc deficiency did not significantly affect relative organ weights, which also supports the present study (4ZD, 6ZD and 8ZD groups) (Table 2). Various abnormalities such as mature basal laminae with thickened and deep invaginations, decrease or increase in the number of peritubular cells/cross section, thickening in lamina propria due to alternations in nuclear volume of peritubular cells, increased deposition of collagen fibres and mucopolysaccharides have been reported from cryptorchid human testes[37-43]. Parallel are the observations in the boundary layers of 4ZD and 8ZD seminiferous tubules (Figures 5 and 7). These differences might be related to variable degree of lesions in both the Sertoli cells and peritubular myoid cells. Sertoli cells secrete collagen, laminin, chondroitin and keratan sulphates whereas the myoid cells synthesize collagen, fibronectin, chondroitin sulphates and these components lead to the formation of ECM of lamina propria[44,45]. It is possible that the cooperation between Sertoli cells and peritubular myoid cells might have been lost in various zinc-deficient groups (4ZD, 6ZD and 8ZD) (Figures 5–7) and as a result an abnormality in the synthesis of ECM could have occurred, leading to disruption in the boundary tissues of seminiferous tubules. Within tubules, the ECM is known to promote normal histotypes of both peritubular cells and Sertoli cells, the latter being connected to the basal lamina by hemidesmosomes[46,47]. ECM promotes and maintains cell polarity, Sertoli cell migration and tubule

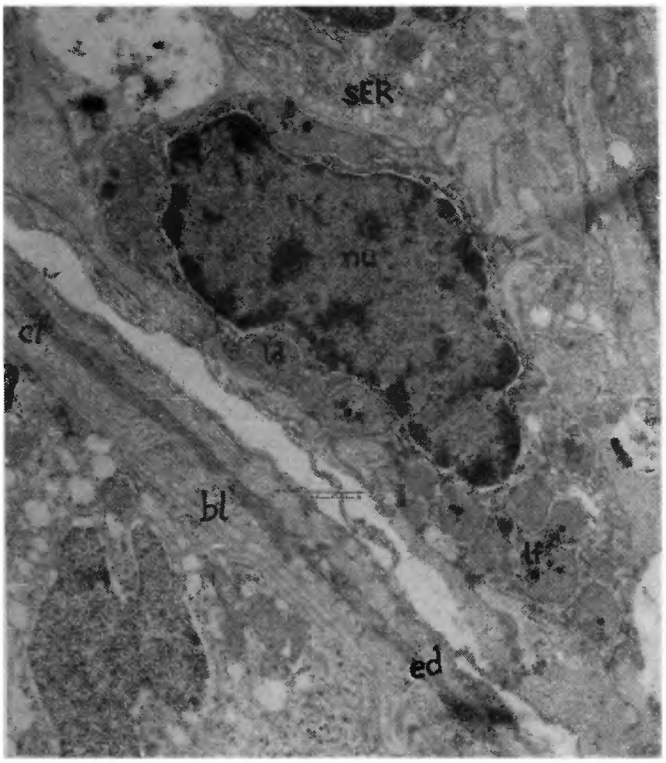

Figure 1. Electromicrograph of 2ZC group testis illustrating normal features of boundary tissue (bt) with layers of basal lamina (bl), collagen fibres (cf) and endothelial cells (ed.) (×10,000).

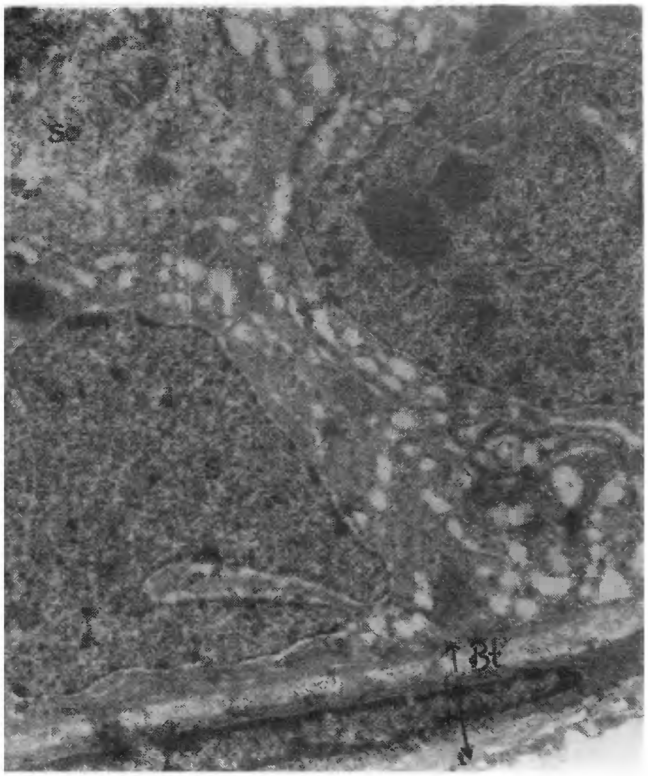

Figure 3. Ultrastructural details of seminiferous tubule of 8ZC group displaying Sertoli cell (S), spermatocyte (Sc) and boundary tissue (Bt) (× 12,650).

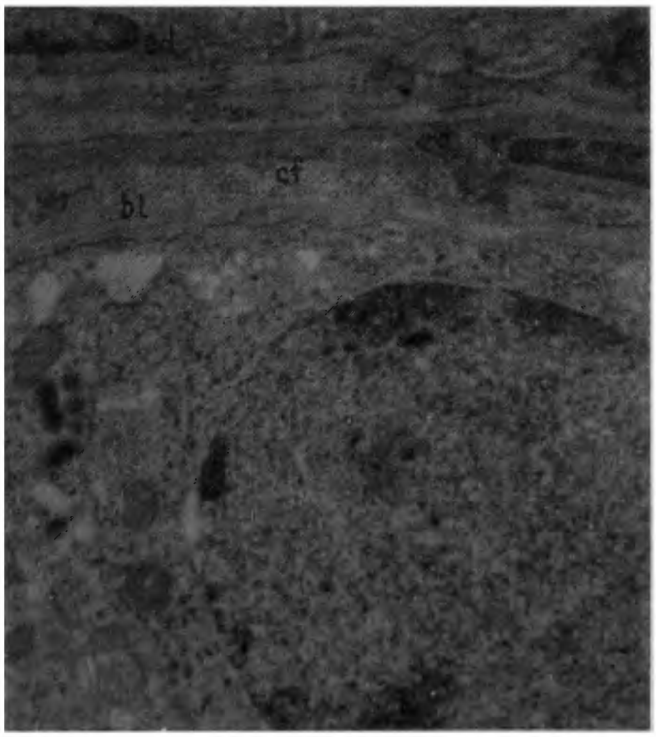

Figure 2. Boundary tissue of seminiferous tubule of 2ZPF group displaying basal lamina (bl), endothelial cell (ed) and collagen fibres (cf) (×20,000).

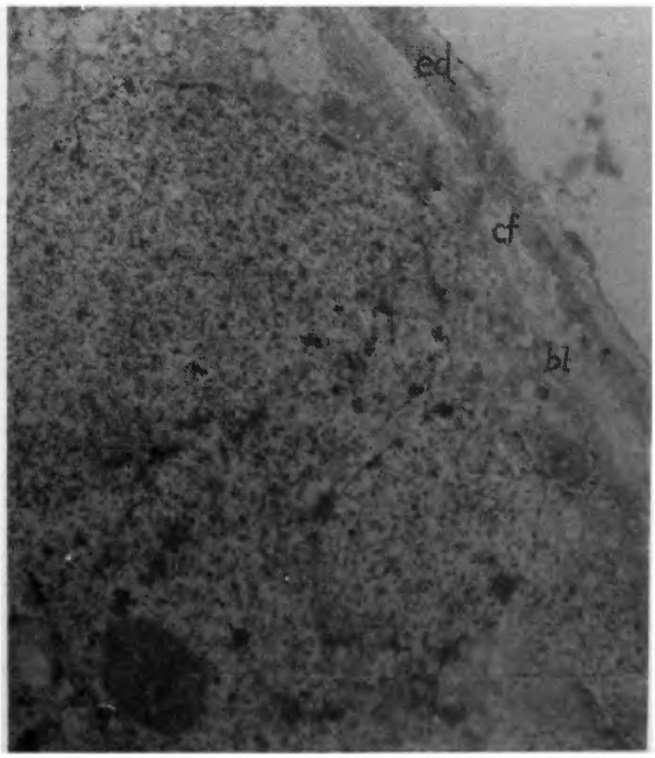

Figure 4. The boundary layers of seminiferous tubule of 2ZD group consists of basal lamina (bl), collagen fibres (cf) and endothelial cells (ed). Normal Sertoli cell is also lying adjacent to the layer (× 10,000).

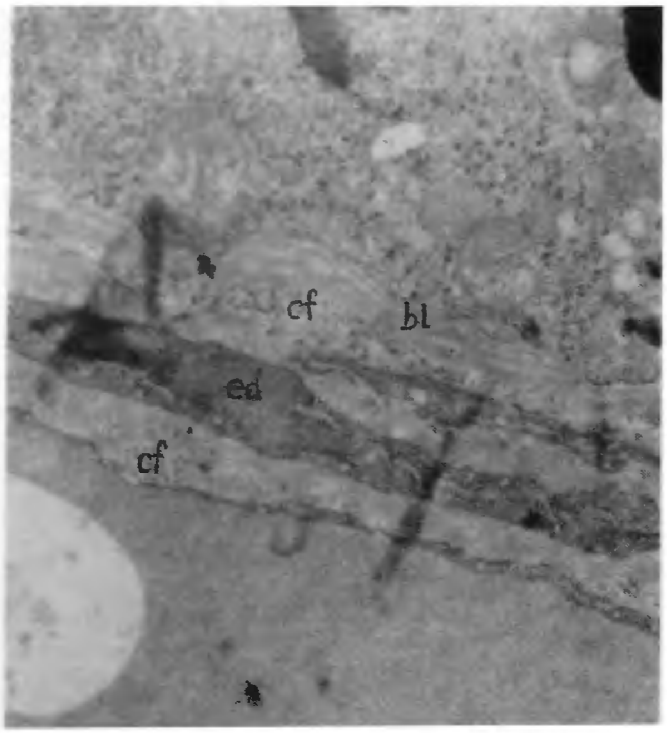

Figure 5. Boundary wall of seminiferous tubule of 4ZD group exhibiting layers of lamina (bl), layer of collagen fibres (cf) and extensions of endothelial cells (ed). Oedematous fluid is visible around endothelial cell (× 12,500).

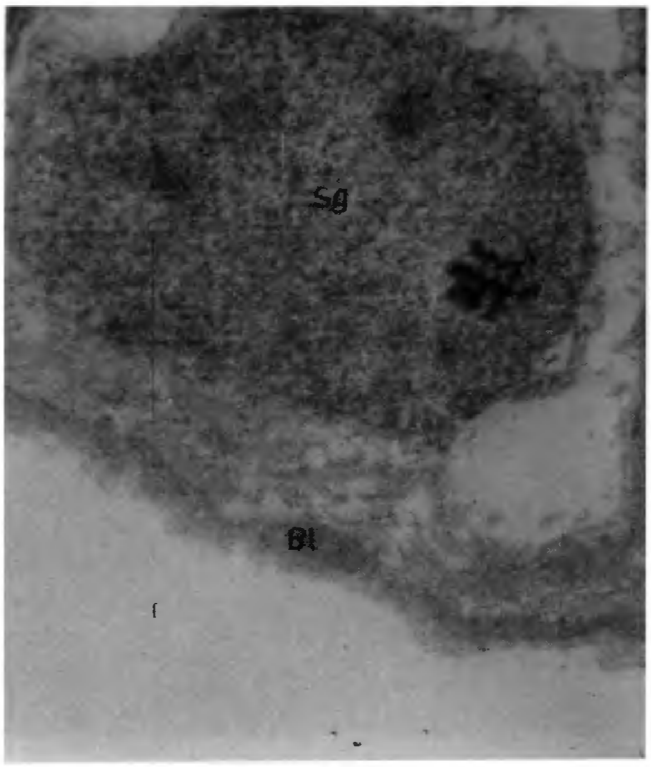

Figure 7. Boundary layers (Bl) of 8ZD group illustrating extensive damages. Spermatogonia (Sg) are present adjacent to layer (×8,200).

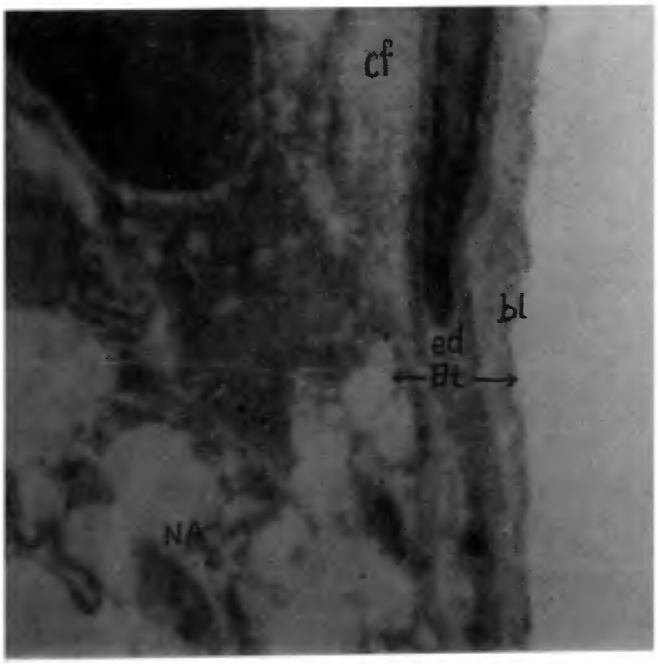

Figure 6. Electronmicrograph from 6ZD group displaying a large necrotic area (NA) in basal part of seminiferous tubules. The boundary tissue (Bt) consists of basal laminae (bl), endothelial cell (ed) and collagen fibres (cf) which appear thicker in some area. An accumulation of oedematous fluid is also present below the highly folded outer limiting membrane (× 12,500).

cord formation[12,48–55]. Cell polarity is a pre-requisite for normal Sertoli cell function which includes formation of the Sertoli cell barrier and vectorial secretion[56,57]. It also enhances the Sertoli cell–germ cell complex of adenylate cyclase, maintains the differentiated status of Sertoli cells morphology, function and decreases FSH-dependent aromatase activity[58–60].

Peritubular cells also produce a protease inhibitor involved in degradation of the basal lamina during early spermatocyte translocation and inactivate Sertoli cell plasminogen activator[61,62]. It also serves as a component of blood testis barrier[17,18]. The loss in structural integrity of myoid cells under zinc deficiency states might have led to impairment in the blood testis barrier functions of the tubule, hence to many destructive changes inside the seminiferous tubule. This has been well illustrated by the 8ZD group testis with complete loss in the boundary layers of the tubule and become very thin (Figure 7). Similar structural abnormality has been reported in sub-fertile and infertile humans[22].

Accumulation of oedematous fluids in different layers of boundary tissues in a zinc-deficient condition might be due to excess of histamine release as zinc is known to inhibit the redistribution of intracellular calcium which results in an impairment of histamine release, thus preventing oedema formation[63].

Table 1. Biochemical estimation of zinc (μg/mg of proteins)

	Two weeks exp.		Four weeks exp.		Six week exp.		Eight weeks exp.	
Group	Testes	Serum	Testes	Serum	Testes	Serum	Testes	Serum
ZC	2.259 ± 0.23979 a*	0.52755 ± 0.091052 a*	4.7337 ± 0.6004 a*	0.8631 ± 0.45829 a*	3.7255 ± 0.4899 a*	0.4799 ± 0.04908 a*	3.6448 ± 0.2256 a*	0.5974 ± 0.08696 a*
ZPF	2.227 ± 0.14884 b*c*	0.43418 ± 0.07879 b*c*	4.4898 ± 0.1677 b*c*	0.5288 ± 0.5691 b*c*	3.6137 ± 0.4317 b***c***	0.8663 ± 0.4472 b*c*	3.7935 ± 0.19069 b****c****	0.44005 ± 0.1058 b*c*
ZPF	2.3126 ± 0.19659	0.52216 ± 0.008534	3.9913 ± 0.191817	0.5484 ± 0.1005	1.64233 ± 0.1723	0.3378 ± 0.05808	1.5831 ± 0.13323	0.46185 ± 0.04994

Mean ± SE; a, ZC vs ZPF; b, ZC vs ZD; c, ZPF vs ZD. *P* values: $P > 0.1$ * Nonsignificant; $P < 0.05$ ** almost significant; $P < 0.01$ *** Significant; $P < 0.001$ **** Highly significant.

Table 2. Body weight (unit g)

Group	Initial	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
2ZC	71428 ± 6.424 a*	88.5714± 7.968 a*	120 ± 9.7184 a***						
2ZPF	71.4286 ± 6.424 b*c*	80.0 ± 4.7141 b*c*	87.142 ± 3.883 b***c*						
2ZD	70.00 ± 6.9985	78.75 ± 8.907	77.5 ± 9.2029						
4ZC	77 ± 4.4583 a*	100.0 ± 4.71404 a*	106 ± 4.2163 a****	126 ± 5.92129 a***	144 ± 5.4884 a****				
4ZPF	78 ± 3.7843 b*c*	93 ± 3.16227 b**c*	98 ± 1.4054 b****c*	103 ± 3.31476 b****c***	111 ± 3.16227 b****c****				
4ZD	78 ± 3.7843	86 ± 3.9126	93 ±4.1722	88 ± 3.784	90 ± 4.1573				
6ZC	95.55 ± 6.478 a*	95.55 ± 5.6211 a*	121.111 ± 5.9802 a*	144.44 ± 6.4009 a*	183.33 ± 5.00 a***	203.33 ± 3.962 a****	216.66 ± 4.3301 a****		
6ZPF	94.44 ± 4.4606 b*c*	94.44 ± 5.0345 b*c*	107.77 ± 6.561 b*c*	136.66 ± 4.677 b****c***	151.11 ± 3.2808 b****c***	161.11 ± 4.487 b****c***	154.44 ± 6.152 b****c**		
6ZD	90.00 ± 6.5486	103.75 ± 7.5423	105 ± 5.71428	110 ± 8.0812	125 ± 7.8246	131.25 ± 8.907	125 ± 10.3016		
8ZC	75.555 ± 1.863 a*	101.11 ± 1.1785 a*	126.66 ± 3.0618 a****	142.22 ± 6.319 a****	171.111 ± 5.4326 a****	181.111 ± 6.718 a****	190.00 ± 7.5 a****	207.777 ± 8.6200 a****	233.33 ± 10.458 a****
8ZPF	75.00 ± 1.513 b*c*	96.00 ± 5.0184 b**c*	102.00 ± 5.6218 b****c*	108.00 ± 4.388 b****c*	120.00 ± 4.157 b****c***	123.00 ± 4.727 b****c**	135.00 ± 7.243 b****c***	134.00 ± 4.7661 b****c*	139.00 ± 5.973 b****c*
8ZD	75.00± 3.239	91.00± 3.3147	94.0± 4.2163	97.00± 4.727	100.00± 4.157	107.00± 4.727	111.00± 4.288	121.00± 6.564	129.00± 5.544

Mean ± SE: a, ZC vs ZPF; b, ZC vs ZD; c, ZPF vs ZD. *P* values: $P > 0.1$ * Nonsignificant; $P < 0.05$ ** almost significant; $P < 0.01$ *** Significant; $P < 0.001$ **** Highly significant.

Although molecular mechanism of the interaction of Sertoli cell with peritubular cells is not well understood, experiments have shown that disruption of Sertoli cell function, resulting from a depletion in the number of late spermatids is followed by dramatic structural alternations in peritubular tissues and peritubular cell morphology[64,65].

The testis is known to require a well-controlled mechanism of efficient coordination of the function of the different cell types and the zinc deficiency leads to many structural anomalies in boundary layers of seminiferous tubules, which might lead to destructive changes inside the tubule as a result of loss of blood testis barrier and damages in extracellular matrix. This might have resulted in impaired Sertoli cell polarity, differentiation, migration, proliferation and destabilization of phenotypic expression as a consequence of defective response to various paracrine and humoral factors.

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Biological reworking of sediments by crabs: A cause for erosion of the Digha beach, West Bengal

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Beach erosion in and around Digha, West Bengal, a perennial problem for developmental work, is related to exceptionally high rate of organic reworkings of backshore to upper foreshore soft sediments. Extensive burrowing activities, especially by typical sandy beach crab *Ocypode* spp., produce a characteristic bioturbated top layer (1–1.5 m thick) having very high concentration (55–80 m^{-2} area) of interconnected complex burrow cavities, which weaken and divide the coherent layer into numerous small sediment blocks amenable to quick erosion by wave and tidal actions. Repetition of this process over the years has been significantly enhancing long-term erosion of the Digha beach.

EROSION of the Digha beach, West Bengal, has been a matter of serious concern over the years for developmental work of the area including its tourism industry. The erosional process has so far been interpreted to be solely related to some critical physical and hydrodynamic preconditions[1-3] such as beach orientation, angle of wave approachability, tidal current velocity and so on. Construction of gravel barriers and mangrove plantation has been used as remedial measure to check coastal decay which, however, remains unabated. After a brief pause of three to four years, severe erosion of coastal tract by no less than 20 m in width and 10 km in length was recorded in 1997 at Digha.

Bioerosion[4] is an important process in marine sedimentation and benthic ecology[5]. No less than 12 animal phyla, several plant groups and protozoans are important agents in marine bioerosion. Of these, fungi, algae, sponges, worms, gastropods, crustaceans, echinoids and fishes are most active. Vast supratidal and intertidal flats of the Digha coast harbour a myriad of burrowers and borers. A majority of the crustaceans and gastropods are found to be burrowers. Relative importance of bioeroders may in some cases be greater than other erosive agencies[5]. The Digha beach like many other tropical to subtropical soft beaches, is inhabited by decapods in huge numbers. Most of them possess sharp hard parts like pincers, walking legs, chela and mouth for excavation of sediments. Decapod bioerosion of California tidal flats[6] is comparable to that of the Digha beach.

The present neoichnological study has focused attention on the burrowing activities of the predominant decapod crustaceans along the coastal profiles developed at Digha, Shankarpur, Junput and Bakkhali areas from west to east on the Bay of Bengal coast (Figure 1 *a*). Of these, the Digha beach is being gradually eroded while others are stable. Size, shape and concentration of burrows *vis-à-vis* their environmental zonation have been studied and compared. The burrow cavities are replicated by white paraffin wax casting method[7].

The selected four areas exhibit development of comparable geomorphic profiles which include wide back swamps with salt marshes (clays and silts), mobile beach dunes (medium to fine sands), supratidal backshore (intercalated sands, silts and clays) and intertidal foreshore (sands and silts with little clays, MZ 2.75 phi to 1.4 phi) from land (north) to sea (south). The width of the beaches varies from 750 m at Junput to 1.25 km at Shankarpur. The statistical beach orientation is azimuthal 75°–255°.

The areas experience maximum 40°C to minimum 22°C annual range of temperature, 1480 cm to 2400 cm of annual rainfall, 25% to 38% of salinity range, pH variation 7.6–8.5, maximum spring tidal range 6.5–7.6 m, maximum neap tidal range 2–2.5 m, north-south both ways wind actions, seasonal premonsoon cyclonic storms (southeast to northwest) and wave action approaching the beach at 70°–85°.

Crabs, namely *Ocypode ceratophthalma* (pallas), *O. cardimana* (Desmarest), *O. macrocera* (Edwards), *O. stimpsoni* (Ortmann), *Uca marionis* (Desmarest) and *Ilyoplax pusillus* (De Hann), are found to burrow habitually in immense numbers all over the coastal profiles of the studied areas. *Ocypode* spp., being the most abundant in number, produces characteristic I, J, U, Y, multibranched Y and complex network burrow systems on the backshore and foreshore areas defining a conspicuous burrow zone running all along the Bay of Bengal coast. The old individuals produce short and thick (45 cm long and 10–25 cm circumference) burrows selectively in the backshore depressions. The burrows eventually join together, forming huge and complex