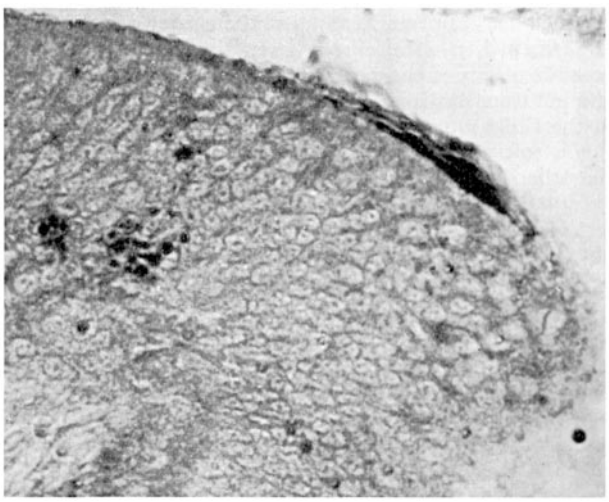
of the membranous labyrinth of the chick embryo, noting the presence of sulphated mucopolysaccharides at the level of the cupula, and acid and not sulphated ones at the level of the tectorial and otolithic membranes.

In this note we report the results of our cytochemical research on the inner ear bud of the chick embryo with special reference to the earliest stages of morphogenesis.

The observations were made on chick embryos from the second to the 13th day of incubation. The embryos were fixed in Helly, Gendre, Carnoy and Bouin fluids, in formalin and in acetone. The various tests for the identification of glucide substances (PAS, Alcian-PAS[7], chromotropic research with toluidine blue 1:5000 at various pH) were carried out, as well as extraction with acetone and with pyridine in hot medium for the lipids.

The data obtained from our observations can be summed up as follows:

(A) In embryos of the 5th, 6th and 7th day of development (the moment when the morphogenesis of the cristae with the cupulae begins and that of the maculae with the otolithic membrane), a characteristic localization of PAS-positive material could be noted in the cytoplasm of some cells of the bud of the crista ampullaris. This material, localized at the level of some cell groups situated in the central part of the bud, appears in the form of granules of a considerable size in coincidence with the cells of the basal part of the crista, while in the more superficial one (towards the lumen of the ampullar bud) it is seen as small granules. It is not chromotropic to toluidine blue or alcianophile, and is resistant to digestion with saliva (on material fixed in Gendre) and to extraction with acetone or pyridine under heat.



Crista ampullaris of chick embryo around the 6th day of incubation. PAS reaction (× 404). Note the presence of the PAS positive cupula and granules in some of the cells of the central part of the bud of the crista.

These findings make one think that it is a material formed of neutral mucopolysaccharides.

(B) As development progresses, that is to say on the 7th and 8th day of incubation (the period when the morphogenesis of the structures in question is almost complete), a decrease of the intracellular PAS positive material described above can be noted, a decrease which is even more accentuated with the further development. Around the 13th day of incubation, only small and scattered PAS-positive granules can be observed in the cytoplasm of support cells of the sensory epithelium of the crista.

A similar picture to that observed in the ampullar bud is also found in the buds of the otolithic maculae at approximately the same period of development. This picture, however, is less evident in the latter, the cells of which only show fine and scattered granules of PAS-positive material.

We wish to report the new finding of the appearance of cells with a neutral mucopolysaccharide content, in the epithelium of the buds in question, immediately preceding and concomitant with the early period of the morphogenesis of the cupula and the otolithic membrane. This finding might justify one in advancing the hypothesis that this material could present the precursor of the acid mucopolysaccharides that characterize the cupula ampullaris and the otolithic membrane.

Riassunto. Viene segnalata la presenza di materiale mucopolisaccaridico neutro in alcune cellule dell'epitelio del labirinto membranoso dell'embrione di pollo durante i primi periodi dello sviluppo. Viene avanzata l'ipotesi che tale materiale possa essere connesso con la genesi delle cupole ampollari e delle membrane otolitiche.

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Localization of Non-Specific Phosphatases in the Testes of *Meriones hurrianae* Jerdon, the Indian Desert Gerbil, and *Suncus murinus sindensis* Anderson, the House Shrew

Meriones hurrianae, inhabitant of the Rajasthan desert, and *Suncus murinus sindensis*, the common house shrew, were used to determine the localization and distribution of acid and alkaline phosphatases in the testes. The animals were killed by rapid exsanguination and the testes quickly removed and chilled at 4°C before fixing in previously cooled absolute acetone for 24 h or 85% cold alcohol, and embedded in paraffin after rapid dehydration. Sections were incubated at 37°C using sodium β-glycerophosphate as the substrate. GOMORI's[1] metal precipitate techniques were used to demonstrate these

[1] G. W. GOMORI, *Microscopic Histochemistry: Principles and Practice* (University of Chicago Press, 1952).

enzymes. Good results were obtained when the sections were incubated from 15 to 30 min for the demonstration of alkaline phosphatase and from 5 to 10 h for the localization of acid phosphatase; beyond this period of incubation, diffusion was noted in both cases. The pH of the incubating solution for alkaline phosphatase was maintained at 9.2, whereas for acid phosphatase it was 5 throughout the experiment.

In the testes of the gerbil and the shrew, the basement membrane revealed intense alkaline phosphatase activity (Figures 1 and 2). The positive reaction for acid phosphatase, however, was more pronounced in the shrew (Figure 3) as compared to the gerbil (Figure 4). This is in conformity with recent observations in the rat[2]. Positive reactions were also observed in Sertoli cells, where the activity was associated with the dense cytoplasmic bodies. The surfaces of the spermatogenetic cells were positive to alkaline phosphatase but relatively less reactive to acid phosphatase activity in both animals. In either case the nuclear membrane, the nucleolus, the chromatin material, but not the nucleoplasm of the various spermatogenic cells were found to be positive for both the non-specific phosphatases. The post-nuclear cap in the late spermatids of the shrew exhibited intense activity to both acid and alkaline phosphatases (Figures 2 and 3), but in contrast, a very weak reaction was observed in the gerbil (Figures 1 and 4). The Golgi bodies of the different spermatogenic cells, particularly of spermiogenic cells, gave a strong positive reaction to glycerophosphate, which has also been shown in the spermatogenic cells of the rat testis[3,4]. Moreover, the acrosome cap of the spermatids did not indicate any activity and gave a negative reaction for both the phosphatases. A very faint localized activity could be observed in the mitochondrial element of various cells. In both animals, the spermatids showed intense activity to the non-specific phosphatases, as compared to spermatogonia and the spermatocytes. The sperm heads of the gerbil (Figure 1) and the shrew (Figure 2) displayed more activity to alkaline phosphatase than to acid phosphatase. The sperm tails, however, gave uniformly weak reaction for both the phosphatases. Alkaline phosphatase reactivity inside the seminiferous tubule was uniform as compared to acid phosphatase activity, which to some extent showed cyclic variations. The interstitial cells of the gerbil gave a very weak reaction to both acid and alkaline phosphatases, whereas in the shrew a positive reaction was observed in the nuclear membrane and the nucleolus; nucleoplasm and the chromatin material being negative.

The observations thus indicate a general disposition of non-specific testicular phosphatases. Although no attempt has been made in this preliminary communication to describe their enzyme constitution and in particular their ultimate fate in the various cytoplasmic inclusions, it is, however, interesting to mention the similarity of reactions between the nucleus and the post-nuclear cap in both animals. This appears to support the earlier observations of MATHUR[5] in *Meriones*, wherein he has suggested a possible origin of the post-nuclear cap from the nucleus and not from the Golgi body[6]. Further, the strong activity in the Golgi apparatus indicates, on the one hand, that this is solely due to non-specific phosphatases, while, on the other hand, it may also be due to the other specific phosphatases as reported by TICE and BARNETT[4] in the testis of rat. A detailed study of the non-specific phosphatases, their reactivity with other substrates at various pH levels, and quantitative analysis will be published elsewhere[7].

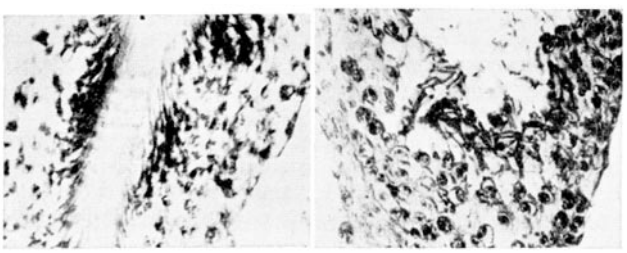

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Fig. 1. Section of testis of *Meriones* showing the distribution of alkaline phosphatase. Note the intense reaction in the sperm head. The post-nuclear cap is weakly positive. × 400.

Fig. 2. Section of testis of *Suncus* showing a strong alkaline phosphatase reaction in the post-nuclear cap and the Golgi apparatus. × 400.

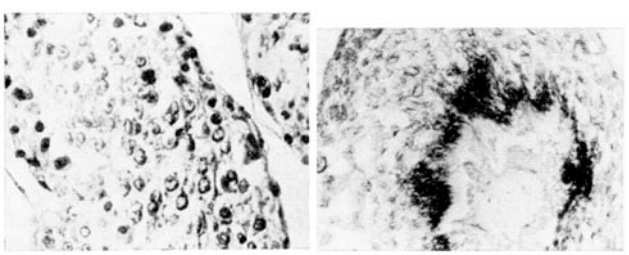

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Fig. 3. Section of testis of *Suncus* showing reactivity of acid phosphatase in various regions, and in particular the post-nuclear cap and the Golgi apparatus.

Fig. 4. Acid phosphatase activity in *Meriones* testis. Note a weak reaction in the post-nuclear cap. The sperm heads are positive.

Zusammenfassung. Der Sitz der Aktivität alkalischer und saurer Phosphatase in den Hoden von *Meriones* und *Suncus* war meist identisch, doch wurde bei *Suncus* auch festgestellt, dass da wo die hintere Kernkappe in verschiedenen Keimzellen auftrat, die Kernmembranen und der Nucleolus des Zellkerns der Zwischenzellen stark positiv reagierten.

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