Structural Changes in the Gills, Intestine, and Kidney of *Etroplus maculatus* (Teleostei) adapted to different Salinities

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SUMMARY

There is an increase in the number of the so-called 'chloride cells' in the gill epithelium with increasing salinity of the medium. In 75% and 100% sea-water the 'chloride cells' exhibit a change in structure, in that they become more granular and acquire an apical vacuole. There is also a general decrease in the number of mucous cells with increasing salinity of the medium of adaptation.

The glomerulus tends to become smaller in the fishes adapted to high salinity as compared to the fresh-water fish. There is also a deposition of pigment around the nucleus and an indication of some changes in the nucleus of the kidney tubule cells in fishes adapted to 100% sea-water.

In the intestine, adaptation to high salinities results in an increase in the nuclear size of the columnar epithelial cells, an increase in the number of flask-shaped cells, and a considerable increase in the thickness occupied by the tunica propria.

INTRODUCTION

IT has been shown by Pampapathi Rao (1958) that the fresh-water teleost, *Etroplus maculatus*, can be adapted to 100% sea-water by gradual transfer from fresh water through intermediate salinities. It is well known that the fresh-water fishes keep up the hypertonicity of their blood mainly by the help of the water-excreting kidney. But some loss of salts through urine cannot be avoided, and this is usually made good either through food or through the active absorption of chlorides through the gills against a gradient, and also to a certain extent through resorption of salts in the distal segment of the kidney. But the whole condition is reversed in the case of marine teleosts, where the water-excreting kidney becomes a liability, since the fish has got to maintain its internal environment hypotonic to the medium. In marine teleosts water lost through osmotic conditions is compensated by swallowing sea-water. The sea-water contains considerable amount of salts which the fish has to get rid of, and Smith (1930) showed that the ion balance is kept up by the continuous extrarenal excretion of salt in addition to renal excretion. Keys and Willmer (1932) first discovered the large acidophil cells on the gill-tissue of the eel, which they named 'chloride cells'. They suggested that these cells have an important role to play in salt excretion. Similar cells were observed by Copeland (1948) in the gills of *Fundulus*, and by Getman (1950) in the gill epithelium of *Anguilla*. Morris (1957) described similar cells in *Lampetra fluviatilis*, before entry into fresh water.

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In fish, therefore, osmotic regulation involves water-excretion by the kidney and salt-uptake by the gills in hypotonic media, while in hypertonic media there is water-retention by the kidney, salt-excretion by the kidney as well as the gills, and water-uptake through the agency of the intestine.

While there are some studies on the structural changes in the gill epithelium with change in the osmotic concentration of the medium (see Morris, 1957, for a review), there are few studies along similar lines with regard to the changes in the kidney and scarcely any studies on the changes in the intestine. The structure of the kidney of fishes has been extensively studied in relation to the natural habitat of the organism (Smith, 1930, 1932; Grafflin, 1937; Tampi, 1959).

It will, therefore, be of interest to know the structural changes that these three principal organs (namely the gill epithelium, the kidney, and the intestinal epithelium) undergo in a fish such as *E. maculatus*, which normally lives in fresh water (hypotonic medium), when it is adapted to sea-water (hypertonic medium).

The present paper records the results of such a study.

Materials and Methods

E. maculatus was collected from local fresh-water ponds, and stocked in a large tank in the laboratory. To begin with, 16 fish were put in 30% sea-water. After 4 days 12 of them were transferred to 50% sea-water, and the remaining 4 left in 30% sea-water for 3 weeks. Again after 4 days 8 of the fish from 50% sea-water were transferred to 75% sea-water, and the remaining 4 were adapted to 50% sea-water for 3 weeks; of the 8 fish put in 75% sea-water, 4 were transferred to 100% sea-water after 4 days, and the remaining 4 adapted to 75% sea-water for 3 weeks. The 4 fish transferred to 100% sea-water were left in that medium for 3 weeks, as in the other media.

All the fish which were kept in different media were fed every day with rice and bits of earthworm.

Simultaneously, some fish were maintained as controls in the fresh-water medium. The gills, kidneys, and intestine of these were fixed in Bouin's fluid, dehydrated in alcohol, embedded in paraffin, and sectioned at 6 μ, and stained with Mallory's triple stain or iron haematoxylin and eosin. Similarly, gills, kidneys, and intestine of fish adapted to various salinities were fixed in Bouin's fluid made up in sea-water, sectioned, and stained separately. The glomerular diameter was measured in freshly mounted tubules from macerated kidneys of fish adapted to fresh water and to 100% sea-water. Several such tubules were measured and the average taken.

Results

Gill epithelium. Each gill filament is supported by a skeletal axis. The gill epithelium is primarily made up of basal and platelet cells, mucous cells, and 'chloride cells' (fig. 1, A). Mucous cells are scattered throughout the normal gill epithelium of fresh-water fishes, mainly towards the tip and base of the

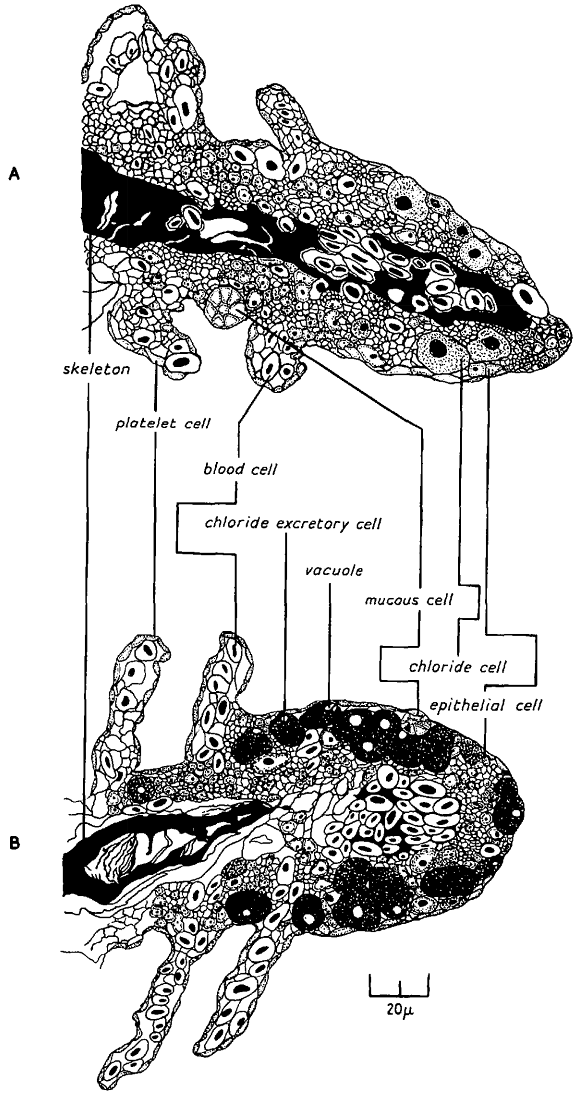

Fig. 1. Sections showing the gill epithelia of *E. maculatus* from fresh water (A) and from 100% sea-water (B).

filaments. They stain light blue in Mallory's triple stain and appear vesicular. In addition to these cells there are other cells with the nucleus stained red, and the cytoplasm blue and granulated in appearance. Some of these cells are oval and some are round or irregular in shape. The nucleus of the cell is situated towards the centre of the cell. They resemble similar cells described by earlier workers in various fresh-water teleosts and also recently in the lampern by Morris (1957). It is thought that these cells are responsible for the active uptake of salt from the hypotonic medium and have been named 'chloride cells'. Whether they have this function is not known, but the name will be used in this paper. These cells are present over most parts of the gill epithelium, usually in the interplatelet areas.

With adaptation to increased salinity (i.e. 30% sea-water) interesting changes in the structure of the gill epithelium are noticed. An increase in the size (table 1) and number of the chloride cells has been observed. Interestingly enough the size of the mucous cells is also seen to be increasing. No change has been observed in the cytoplasmic granulations of the chloride cells.

In the sections of the gills of the fish adapted to 50% sea-water it has been noted that the number and size of the chloride cells increased further. Against this the mucous cells showed a decrease in their numbers.

In 75% sea-water it has been noted that the number and size of the chloride cells is the same as in 50% sea-water. However, towards their periphery a vacuole was seen and their granulation was found to be increasing. It has been observed that the chloride cells present in the interfilamentar junctions were slightly enlarged. The mucous cells showed a steady increase in their size and a decrease in numbers.

In the fish adapted to 100% sea-water there was a decrease in size of the chloride cells, the diameter varying between 3·6 μ to 5·4 μ. But the chloride cells present in the interfilamentar junctions were found to have increased in size. The granulation of these cells was much increased. A vacuole was found towards their periphery (fig. 1, B). The mucous cells had been reduced to negligible numbers.

Intestine. The following layers are seen in transverse sections of the intestine of *E. maculatus* adapted to fresh water (fig. 2, A). The outermost layer of the intestine is the serosa. Then follows the muscular layer consisting of the circular and longitudinal muscle-fibres. Just beneath this is the stratum granulosum. It is followed by a thin tunica propria, stained deep blue; this extends into the spaces of the rugae. Then comes the last layer, the epithelial layer, with characteristic columnar cells with their nuclei stained red with Mallory. The luminal ends of the columnar epithelial cells of the intestine are densely stained. There are a few goblet cells in the columnar epithelial layer, opening into the lumen of the intestine. They stain light blue. Most probably these are mucous cells.

One interesting feature observed in the structure of the intestine is that with the increase in salinity from 30% to 100% sea-water the tunica propria

TABLE I

Changes in the size of cells in the gill epithelium and kidney of E. maculatus *on adaptation to media of increasing salinity.*
(All figures are in μ.)

	Gill Epithelium				Kidney							
	Diam. of chloride cell		Diam. of mucous cell		Outer diam. of tubule		Diam. of lumen		Diam. of tubule cell		Maximal diam. of glomerulus	
Medium to which adapted	*Mean*	*S.D. ±*	*Mean*	*S.D. ±*	*Mean*	*S.D. ±*	*Mean*	*S.D. ±*	*Mean*	*S.D. ±*	*Mean*	*S.D. ±*
Fresh water	2·9	1·3	5·6	1·3	17·6	4·1	5·2	1·5	13·5	1·2	90·0	22·4
30% sea-water	4·4	1·5	7·5	1·8	10·4	4·2	3·6	1·8	9·9	7·3	—	—
50% sea-water	6·6	1·2	7·9	1·4	16·6	4·3	4·4	1·9	12·2	3·2	—	—
75% sea-water	6·4	1·0	8·4	1·7	16·9	6·0	4·0	1·8	13·0	3·7	—	—
100% sea-water	4·5	0·9	—	—	14·0	5·7	4·9	2·3	9·2	2·6	71·4	20·3

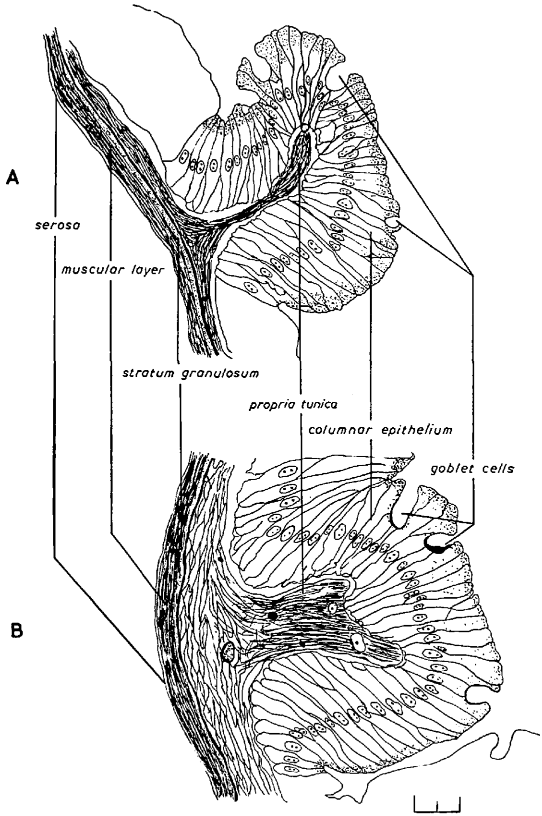

FIG. 2. Transverse section of the intestine of *E. maculatus* adapted to fresh water (A) and to 50% sea-water (B).

becomes much wider (figs. 2, 3). Further, it was also found that with increasing salinity there was an increase in nuclear size, and an increase in numbers of goblet cells present in the columnar epithelial layer.

Kidney. The following structure was noticed in the sections of the kidney of fish kept in fresh water. The kidney cells have a uniformly stained cytoplasm with nuclei towards the centre. The lumen is intercellular and the diameter of the lumen ranges from 3·6 μ to 7·2 μ. The external diameter of the tubule varies from 12·6 μ to 23·4 μ, and the size of the tubule cells ranges from 9·9 μ to 14·4 μ. The kidney cells are narrow towards the lumen and broad towards their outer side. The glomerular diameter of the fish adapted to fresh water is about 90 μ.

Several changes are noticed in the structure of the kidney as a result of adaptation to changes in salinity. In fish adapted to 30% sea-water the lumen

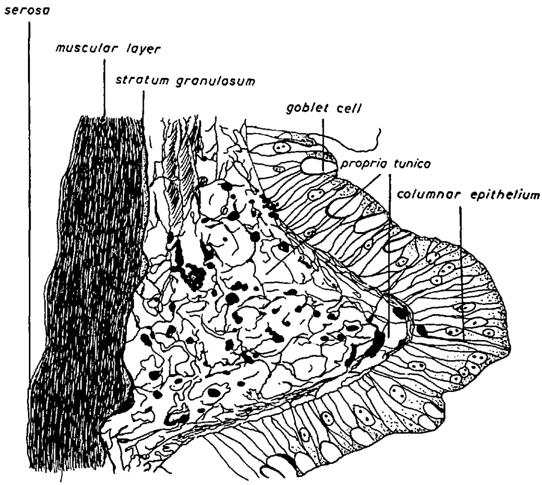

FIG. 3. Transverse section of the intestine of *E. maculatus* adapted to 100% sea-water.

is very much reduced in many tubules, but quite open in some. The cytoplasm present towards the lumen of the cell is densely stained and that towards the periphery is clear. The nucleus is at the centre of the cell. A decrease in the diameter of the lumen is observed. The diameter ranges from 1·8 μ to 5·4 μ. The external diameter as well as the cell size are reduced (table 1).

In sections of the kidney of fish adapted to 50% sea-water a large granule of black pigment was found to have accumulated inside almost all the cells, and the nucleus could not be distinguished in these cells. In some cells numerous black granules were found, probably in the process of formation of the big black pigment granule inside the cell. The outer diameter of the tubule, the lumen diameter, and the cell size are like those of fishes in fresh water (table 1).

In 75% sea-water the accumulation of black pigment inside the cells was increased. There are no significant differences in other respects from what is seen in the kidneys of fishes adapted to 50% sea-water.

In 100% sea-water a vacuole was noticed inside the cell in some cells, and wherever there was a vacuole the nucleus was absent. The formation of the vacuole is a very peculiar phenomenon. There was a decrease in the cell size. The glomerular diameter showed a decrease to about 71·4 μ. Sometimes the tubule did not appear to end in a glomerulus.

Discussion

The results presented above show that adaptation of *E. maculatus* to increasing grades of sea-water results in changes in two main features of the gill epithelium. One important change concerns the size and structure of the granulated pink-staining cells. When the fish is adapted to 30% sea-water there is a noticeable increase in the size of the granulated cells, and they also appear to be more abundant. In this medium there would not be a need for active uptake of salt from the medium. It is not clear what function they perform. It might be that they are the precursors of the chloride excretory cells, and are derived from the chloride cells of the fresh-water fish, since they are not fundamentally different in structure, although they are larger and a little more densely granulated. The same trends are increasingly seen in fish adapted to 50% sea-water. It is possible that in this medium they are already concerned with excretion of salt. In fish adapted to 75% and 100% sea-water the chloride cells exhibit structural changes, the most conspicuous being the appearance of a vacuole at the apical end of the cell. The granulation is denser. The vacuolated cell does not appear to be a new cell-type, but rather a derivative of the granulated chloride cell. Such cells bearing apical vacuoles have already been described in marine *Fundulus heteroclitus* (Copeland, 1948). It has been suggested by Copeland that these cells are concerned with salt excretion and that they are derived from the chloride cells of fish adapted to fresh water. There is no evidence against such a transformation and the present results support Copeland's view. Instead of secreting inwards, the cells would now secrete outwards, thus reversing their physiological polarity. However, Parry, Holliday, and Blaxter (1959) have recently obtained some evidence which indicates that those acidophil cells may not be very important in salt regulation.

There is a considerable increase in the size of the individual mucous cell of the gill epithelium with increase in the salinity of the medium. But the number of mucous cells decreases conspicuously with increase in salinity. It might be that mucous requirements in fresh water are more important (in reducing inward permeability to water) than in sea-water.

It is likely that when the fresh-water *E. maculatus* is transferred to sea-water the intestine would take up the osmotic functions that are well known in marine teleosts. The results reported above indicate that there is some change in the structure of the intestine on adaptation to hypertonic media. The main changes are noticed in the columnar epithelium and the tunica propria, both of which increase in thickness. The nuclear size of the columnar cells also increases. Whether all these changes reflect any increase in the water-absorptive function of the intestine is not established. The significance of the increase in the number of the goblet cells is also not clear.

The changes in the kidney are noteworthy. The most conspicuous change is the reduction in glomerular size with adaptation to sea-water. It might be that this reduction in glomerular size is a direct result of the decreased need for filtration. Such a view would be in agreement with Smith (1932). However, it has been shown that filtration does not depend much on the osmotic gradient, but that the hydrostatic pressure in the capillaries and the total filtering surface are the important factors influencing filtration. In the present study no estimate of the total filtering surface is made. Hence it could not be said at present whether the decrease in glomerular size in fish adapted to sea-water results in a reduction in the total filtering area. But under the conditions in which the glomerular size changed, it is logical to assume that the total filtering surface is decreased. The changes in the sizes of cells and lumen are also to be looked upon as consequences of changes in kidney function. That the physiology of the tubule cells is affected may be noticed from the nuclear changes described above.

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