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Histology

**EFFECT OF ENVIRONMENTAL OSMOTIC PRESSURE
ON KIDNEY HISTOLOGY IN RAINBOW TROUT, *SALMO GAIIRDNERI* Richardson**

**WPŁYW OSMOTYCZNOŚCI ŚRODOWISKA NA BUDOWĘ HISTOLOGICZNĄ NERKI
PSTRĄGA TĘCZOWEGO *SALMO GAIIRDNERI* Richardson**

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Histological studies on kidney of rainbow trout individuals reared in freshwater ponds prior to their release into the Baltic as well as of those kept for several weeks in tanks filled with fresh and Baltic water revealed a reduction in number and size of Malpighi bodies, smaller lumen of renal tubules, and a lymphoid tissue proliferation in kidney parenchyma to have occurred in fishes dwelling in sea water.

INTRODUCTION

The ratio between osmotic pressure of body fluids and of the external environment is completely different in various groups of marine and freshwater fishes. Consequently, the osmoregulatory organs, gills and kidney in particular, have to perform two opposite functions: in freshwater fishes – not only to retain but also to collect the missing ions from the environment (Krogh, 1937), whereas in marine teleosts – to excrete the excessive ions. Freshwater fishes and elasmobranchs were found to have their glomerular filtratory apparatus better developed than sea-dwelling teleosts (Hickman and Trump, 1969).

Edwards (1928, 1929, 1935) classified kidneys of fishes from various families as glomerular and aglomerular. Marshall and Smith (1930), when classifying various species, made reference not only to the presence of glomeruli in kidney, but also to the degree of their development. Thus, those authors placed salmonids in the first group, i.e., among the species showing well-developed and large glomeruli, which in turn spurred further studies on renal morphology and function in various fish species carried out by, i.a., Grafflin (1931, 1937), Reichle (1959), Weatherley (1963), Thakur (1974), Kinter (1975).

Grafflin (1937) is of the opinion that there are environment-dependent differences in nephron morphology manifested through a lack (in freshwater, *Microphis boaja* Bleeker) or a presence (in euryhaline *Anguilla rostrata* Le Seur) of distal convoluted segment. Other workers such as Holmes (1961) and Virabhandrachari (1961), too, give some information on hyperosmotic environment-related changes in kidney microstructure. Those changes occurred always in those elements of kidney microstructure taking part in glomerular filtration. The latter, according to Caproeol and Sutherland (1968), Oguri and Sokabe (1974), and Sokabe and Ogawa (1974) can be rendered more efficient by juxtaglomerular cells present in teleosts.

The project reported herein was conceived in order to find out if there would be any changes in kidney microstructure of freshwater rainbow trout that could be related to the subsequent adaptation of the fishes to the Baltic water, and – if so – how soon and with what intensity those changes would occur.

MATERIAL AND METHODS

The studies were carried out in the years 1971–1974 in the Department of Fish Anatomy and Embryology, Institute of Ichthyology, Academy of Agriculture in Szczecin.

The material consisted of adult rainbow trout, *Salmo gairdneri* Rich. from various sources: individuals caught in the Baltic 18 months after their release into the sea; pond-reared individuals; and young fishes kept in concrete tanks filled respectively with Baltic and fresh running water.

There were two reasons for keeping the fishes in the tanks: firstly, it would not have been possible to catch one-year-old (1+) individuals in the sea only several weeks after their release, and secondly, the environmental conditions varying greatly in natural waters could have been kept maximally uniform in the culture. Water in the tanks was aerated, its temperature ranging within 11–13°C.

Scraps of tail-kidney were taken for the histological examinations and fixed in 10% buffered formalin, the paraffin technique being employed in mounting the blocks. 5–7 µm thick scraps were Mallory, haemalaun, and eosin stained.

The number of Malpighi bodies was ascertained from the histological mounts, only

those sections showing the central part* of a corpuscle being counted; the corpuscle diameter was measured as was the size of renal tubules lumen sections from three portions of a nephron (proximal and distal convoluted segments and collecting tubule); furthermore, the number of epithelial cells in the above-mentioned tubules was counted. All the measurements were taken exclusively on crosssections.

The scrap area examined was always the same: 8 and 4 mm² for large and small fishes, respectively. The numerical data obtained were subject to a statistical treatment.

RESULTS

The rainbow trout kidney, as is the case with other freshwater teleosts, consists of nephrons (= fundamental structural units) and intra-parenchymal lymphoid tissue.

Prior to the histological examinations, the fishes were measured and weighed, their kidneys and livers being additionally separately weighed after the dissection. This information was thought relevant for the final general considerations. The results obtained are summarised in Table 1.

The external medium of the fishes proved to bear some influence on kidney microscopic structure, the filtratory elements being primarily affected.

As seen from Table 2, both the large rainbow trout caught in the Baltic and the small ones kept in the seawater tanks showed a reduction in number and size of Malpighi bodies. The differences in numbers were more pronounced in young individuals; their number of glomeruli was by more than 36% lower than that of the freshwater individuals, the scrap areas examined being equal. Also the glomerulus diameters tend to be considerably reduced in a hyperosmotic medium. In the two experimental series the glomeruli were more than 24% smaller in the seawater individuals.

The fishes living in a hyperosmotic medium were found to have developed renal tubules with decreased lumen. It is true that the differences are not as pronounced as in the case of glomeruli; those results nevertheless, seem to correspond with numbers and sizes of Malpighi bodies (Table 3).

There were practically no differences between the numbers of cells lining the renal tubules as counted on cross-sections (Table 3), the few differences found being of no significance.

All the individuals dwelling in the Baltic waters showed a marked proliferation of renal lymphoid tissue (Table 1). Photomicrographs of kidney cross-sections (Figs. 1–4) provide a good illustration for the findings described above.

* It was assumed that, when producing scrap series, one third of 10–15 scraps (obtained in the central part) yielded by one renal corpuscle were only slightly differing in size; therefore, in order to have a stable reference point, the true number of Malpighi bodies was assumed to be 3–4 times lower (depending on the maximum diameter of the glomerulus) than the score obtained.

Table 1

Age, size, and weight (total value and values for weight of kidney and liver)
of fishes examined

| | Object of study | Age (months) | Length (cm) | | | | Height (cm) | | | | Weight (g) | | | | | |
|----|--|-----------------|-------------|-------|--------------|-------|-------------|-------|-----------|-------|------------|-------|-----------|-------|-----------|-------|
| | | | body (l.t.) | | trunk (l.c.) | | max. | | min. | | total | | kidney | | liver | |
| | | | \bar{x} | \pm | \bar{x} | \pm | \bar{x} | \pm | \bar{x} | \pm | \bar{x} | \pm | \bar{x} | \pm | \bar{x} | \pm |
| 1. | Rainbow trout from sea* | 30 | 46.5 | 12.6 | 42.6 | 11.3 | 12.1 | 2.3 | 4.36 | 2.1 | 1554 | 22.6 | 15.6 | 8.9 | 21.5 | 8.7 |
| 2. | Rainbow trout from pond | 30 | 42.6 | 9.6 | 35.9 | 8.5 | 11.5 | 1.5 | 4.2 | 1.8 | 1074 | 9.8 | 6.6 | 4.1 | 18.7 | 3.9 |
| 3. | Rainbow trout from seawater aquarium ** | 12 | 26.9 | 1.4 | 23.8 | 0.8 | 6.1 | 0.1 | 2.5 | 0.1 | 175 | 1.1 | 1.7 | 0.2 | 2.2 | 0.2 |
| 4. | Rainbow trout from freshwater aquarium | 12 | 25.2 | 1.2 | 22.6 | 0.7 | 5.2 | 0.1 | 2.4 | 0.1 | 165 | 1.1 | 1.3 | 0.1 | 1.6 | 0.1 |

* caught after 18 months in the sea

** caught after 16 weeks

Table 2

Numbers and sizes of Malpighi bodies in kidney of rainbow trout, *Salmo gairdneri* Rich.

| Object of study | Scrap area examin- ed (mm ²) | Malpighi bodies | | | | |
|-----------------------------------|---|-----------------|-------|------------------------------------|--------------------------|-------|
| | | Number | | Number per 1 mm ³ | Maximum diameter (μm) | |
| | | \bar{x} | \pm | | \bar{x} | \pm |
| 1. Large fish from sea | 8 | 24.8 | 14.1 | 172.08 | 62.28 | 10.3 |
| 2. Large fish from pond | 8 | 29.8 | 7.9 | 206.89 | 82.00 | 9.8 |
| 3. Small fish from sea water | 4 | 7.32 | 1.7 | 101.62 | 58.78 | 2.4 |
| 4. Small fish from fresh water | 4 | 11.00 | 2.5 | 152.70 | 77.50 | 5.3 |

DISCUSSION AND CONCLUSIONS

The studies on the renal microstructure described here prove unequivocally that rainbow trout, *Salmo gairdneri* Rich. belongs to the species provided with glomerular kidney, which is in accordance with previous opinions expressed by Marshall and Smith (1930) as well as by Hickman and Trump (1969).

The external environment, its osmotic pressure in particular, proved to exert a significant influence on the rainbow trout kidney microstructure. Considerable differences, related to the actual habitat of the fishes studied, were found between the sizes of glomeruli; particularly noteworthy is the fact that this differentiation is completed within a relatively short period of time (16 weeks). Young individuals living in the Baltic water showed a 24% reduction, on the average, in their glomeruli volume compared to the freshwater individuals (Table 2). Also the total number of glomeruli per renal scrap volume unit is lower in fishes living in the sea water. Those results and observations confirm earlier findings (Nash, 1931) of marine teleosts having lower numbers of poorly-developed glomeruli than freshwater fishes. Similarly, Virabhandrachari (1961) showed experimentally that the size of *Europlus maculatus* glomeruli was clearly reduced after several weeks in sea water. According to this author, such a change in the nephron structure is a response to the external conditions deviating from a state natural for the fish.

The results obtained here seem to correspond with data presented by Holmes and McBean (1963) and by Holmes and Steiner (1966) who found the glomerular filtration being reduced in rainbow trout kept in sea water.

As can be seen then, a reaction to a more hypertonic environment involves a restricted excretion of excessive water, there being a need for an active water retention. It is supposed that the lymphoid tissue proliferation found acts in favour of that, the lymphoid tissue performing the function of water resorption

Table 3

Changes in size of tubules and in number of epithelial cells lining renal tubules of rainbow trout (*Salmo gairdneri* Rich.) kidney

| Object of study | Tubule segment examined | Scrap area examined (mm ²) | Tubulus | | | | | |
|--------------------------------|-------------------------|--|-------------------|-------|----------------|-------|---------------------|-------|
| | | | external diameter | | lumen diameter | | no. of lining cells | |
| | | | \bar{x} | \pm | \bar{x} | \pm | \bar{x} | \pm |
| 1. Large fish from sea | proximal | 8 | 40.1 | 9.7 | 11.9 | 3.4 | 10.9 | 1.6 |
| | distal | | 42.7 | 6.4 | 15.5 | 4.1 | 14.3 | 1.7 |
| | collecting | | 58.7 | 5.6 | 22.3 | 4.6 | 18.2 | 2.5 |
| 2. Large fish from pond | proximal | 8 | 50.9 | 13.1 | 13.4 | 3.7 | 11.2 | 1.3 |
| | distal | | 48.0 | 8.2 | 19.5 | 6.3 | 16.2 | 2.2 |
| | collecting | | 64.2 | 12.3 | 22.6 | 6.4 | 21.2 | 2.6 |
| 3. Small fish from sea water | proximal | 4 | 34.1 | 6.1 | 11.3 | 1.6 | 11.3 | 1.4 |
| | distal | | 37.4 | 5.3 | 18.7 | 1.7 | 15.0 | 1.6 |
| | collecting | | 54.4 | 10.3 | 23.0 | 5.1 | 20.6 | 3.4 |
| 4. Small fish from fresh water | proximal | 4 | 40.3 | 4.1 | 12.7 | 3.6 | 11.9 | 1.6 |
| | distal | | 42.2 | 2.4 | 19.1 | 1.2 | 15.7 | 1.4 |
| | collecting | | 69.8 | 2.8 | 33.4 | 2.7 | 21.2 | 3.6 |

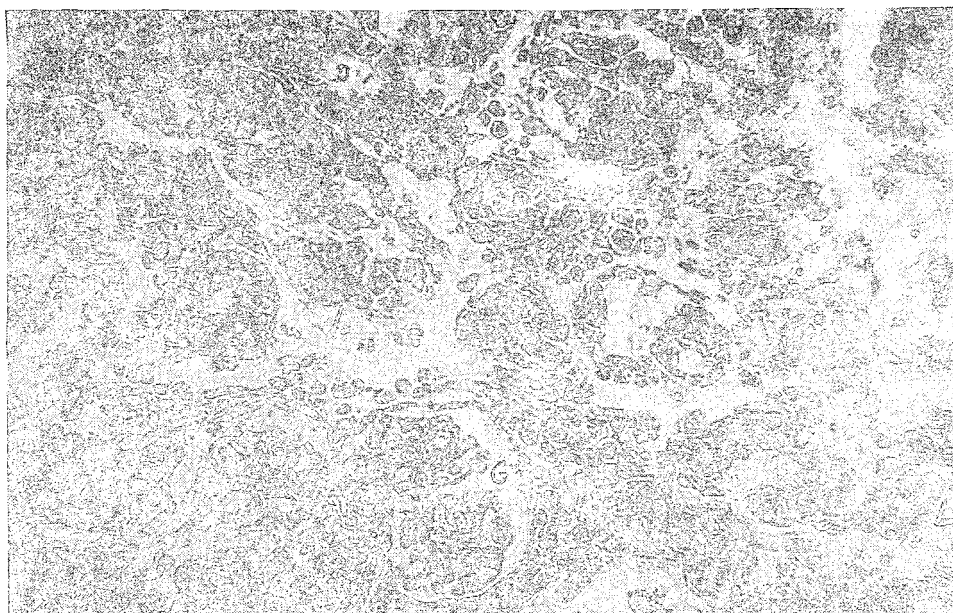


Fig. 1. A cross-section of kidney of a rainbow trout individual released to the sea. Mallory, 330x
G = glomerulus; P = proximal convoluted segment; D = distal convoluted segment; L = lymphoid tissue; C = collecting tubule

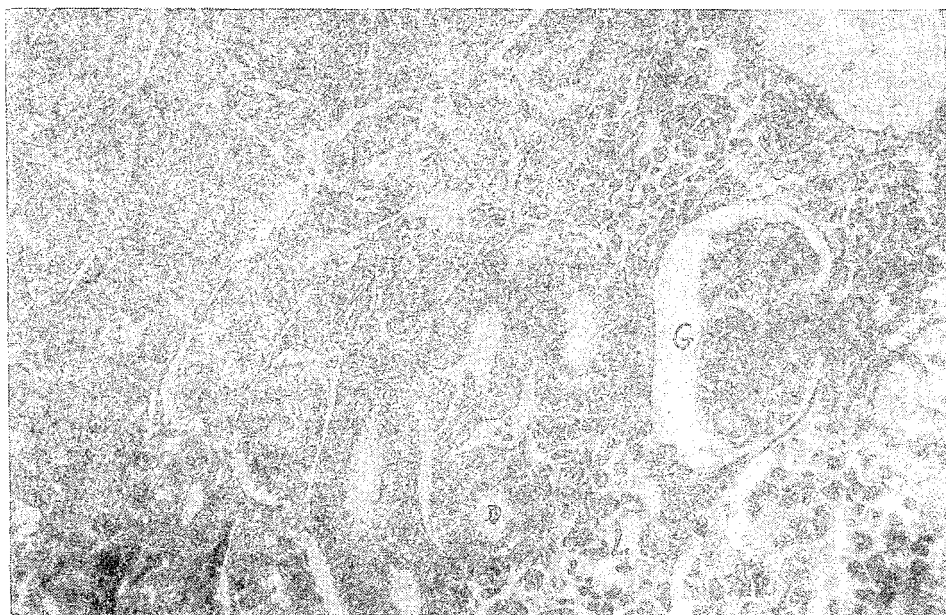


Fig. 2. A cross-section of kidney of a rainbow trout individual kept in a pond.
For explanations see Fig. 1. Mallory, 330x

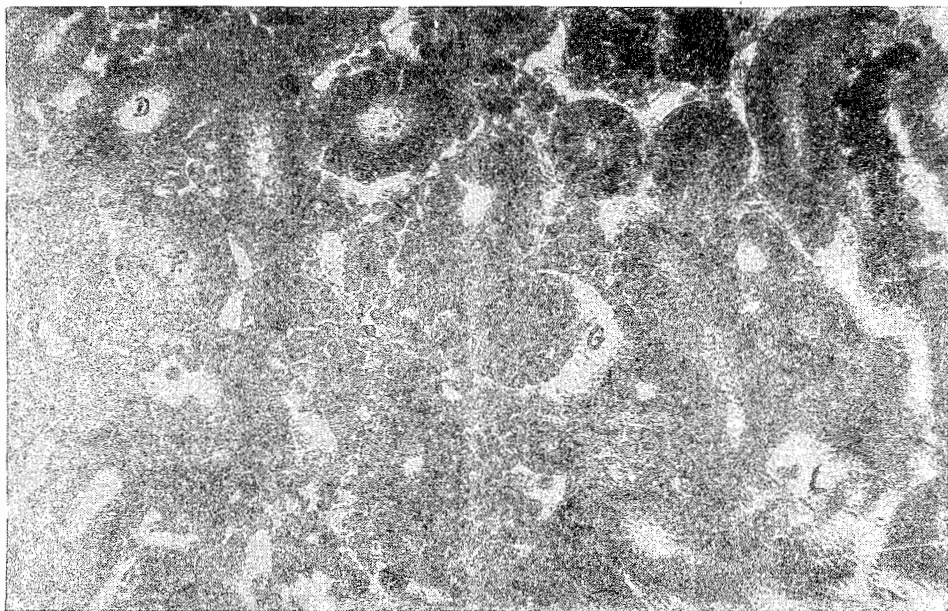


Fig. 3. A cross-section of kidney of a rainbow trout individual kept in a tank with Baltic water.
For explanations see Fig. 1. Mallory, 330x.

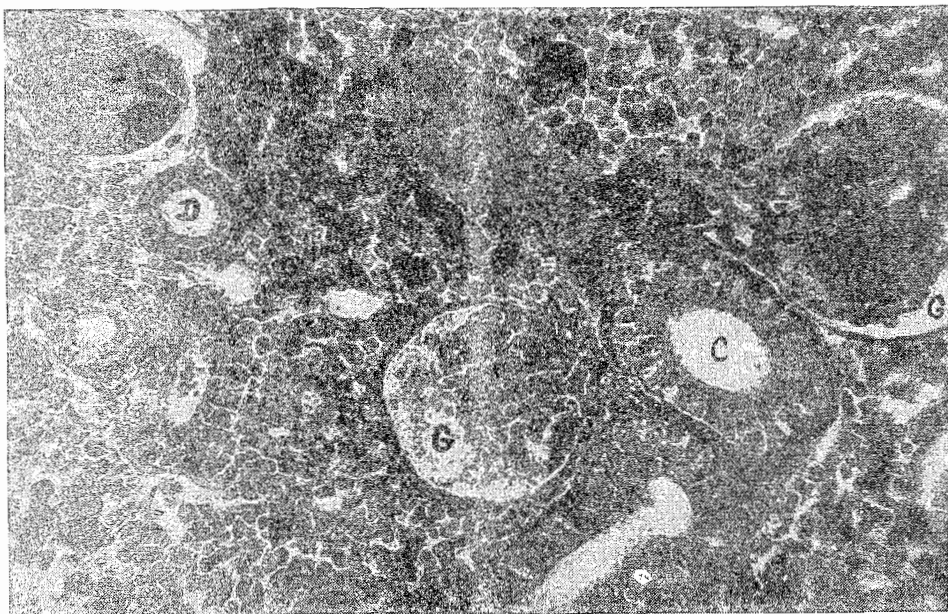


Fig. 4. A cross section of kidney of a rainbow trout individual kept in a tank with fresh water.
For explanations see Fig. 1. Mallory, 330x

In the light of the results obtained it should be also stressed that although no significant differences in the renal tubules lumen were found, there was a correlation between a decrease in the lumen diameter and in the size of Malpighi bodies in the saline water.

The problem discussed has not been fully elucidated as yet in view of the fact that Forster (1953, 1975) reports the presence, among the freshwater species, of ones with aglomerular kidney. In that case, he concludes, the osmoregulation presumably proceeds chiefly via gills and also via intestine.

The changes in the rainbow trout kidney microstructure described in the present paper confirm a well-known fact that kidney is an organ easily adapting to the actual needs of an organism. The process of structural changes in kidney, functioning mainly as a water-excreting organ, is reflected in the reduction of the filtratory apparatus accompanied by the simultaneous proliferation of lymphoid tissue.

The results obtained in the present work allow to draw the following conclusions:

1. The number of Malpighi bodies is reduced in sea-dwelling rainbow trout, the process being accompanied by a reduction in glomeruli size.
2. In young rainbow trout, even a relatively short period (16 weeks) in a saline medium (Baltic water) results in a clear decrease in size and number of their Malpighi bodies.
3. The reduction in Malpighi bodies size is accompanied by a decrease in renal tubules lumen diameter and in the number of cells lining the tubules.
4. There is a simultaneous growth of lymphoid tissue and an increase in its percentage in renal parenchyma.
5. The changes found in the kidney microstructure of rainbow trout confirm the species' extensive adaptability to a hyperosmotic environment.

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Streszczenie

Poddano badaniom metodami histologicznymi nerki dorosłych pstrągów tęczowych *Salmo gairdneri* Rich. wyłowionych z Bałtyku (pochodzących z zarybiania), hodowanych w stawie oraz pstrągów młodych hodowanych w basenach z wodą bałtycką i słodką bieżącą.

Okazało się, że zmiana środowiska na bardziej hipertoniczne w stosunku do wyjściowego spowodowała w nerkach pstrągów z wody morskiej redukcję ilości ciałek Malpighiego, zmniejszenie ich rozmiarów, nieznaczne zmniejszenie światła kanalików nerkowych i ilości komórek wyścielających te kanaliki, a także rozbudowę tkanki limfoidalnej.

Ц. Цыковска

ВЛИЯНИЕ ОСМОТИЧНОСТИ СРЕДЫ НА ГИСТОЛОГИЧЕСКОЕ СТРОЕНИЕ ПОЧКИ
РАДУЖНОЙ ФОРЕЛИ *SALMO GAIIRDNERI* RICHARDSON

Резюме

Гистологическими методами исследовали почки взрослых радужных форелей, выловленных в Балтике (выращенных искусственно и запущенных в море), выведенных в прудах, а также молодых форелей, выращенных в бассейнах:

- с проточной пресной водой,
- с водой из Балтики.

Оказалось, что изменение среды на более гипертоническую по отношению к начальной вызывало в почках форелей из морской воды снижение количества мальпигиевых клубочков, уменьшение их размеров, небольшое сокращение внутренних диаметров почечных протоков, снижение количества клеток, выстилающих эти протоки, а также развитие лимфоидной ткани.

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