

A morphological study in the kidney and spleen of gilthead, *Sparus aurata*, L. caused by sodium dodecyl sulphate

M. Rosety, A. Ribelles and C. Carrasco

Department of Morphological Sciences, Faculty of Medicine, University of Cádiz, Cádiz, Spain

Summary. This paper reports the morphological changes in the kidney and spleen of gilthead (*Sparus aurata*, L.), caused by acute action of the anionic detergent, sodium dodecyl sulphate (SDS). Twenty-five giltheads were exposed to SDS concentrations of 5, 8.5, 10 and 15 mg/l. Morphological changes depending on detergent concentrations and length of exposure were seen. Kidney shows loss of normal structure with tubular and renal corpuscle retraction; spleen shows tendency to damage the reticulæ structure and a progressive increase of leucocytes and red cells infiltration.

Key words: *Sparus aurata*, Sodium dodecyl sulphate, Detergent, Kidney, Spleen

Introduction

Marine pollution is intimately related to commercializing of domestic detergents, and their toxic effects can be demonstrated (Mishra et al., 1985, 1991; Zeni and Caligiuri, 1992; Bigliardi et al., 1994; Wilhelm et al., 1994). The high concentrations of detergents are important in the Bay of Cádiz because in it a important fishing activity is being developed.

The gilthead, *Sparus aurata*, L., is a common species of the Mediterranean and Western Atlantic and has been used to evaluate the biological effects of the marine fauna. Research on the influence of SDS on this species is particularly appropriate because of its importance in the fishing industry and in pisciculture (Arias, 1976; Arias et al., 1984; Varona, 1993).

Morphological and histochemical changes have been reported in gills, liver, pancreas and intestine of the gilthead (Ribelles et al., 1995a-c). This investigation shows some morphological changes caused by SDS in the kidney and spleen.

Materials and methods

The fish used in this investigation were twenty-five

juvenile giltheads, six months old, between 12 and 14 cm long and weighing from 30 to 40 g. All were born and raised on a fish farm. Five specimens were used as controls and the remainder divided into four groups, a, b, c and d, which were exposed to concentrations of 15, 10, 8.5 and 5 mg/L of SDS (Merck, Spain), respectively.

Each group was maintained in a PVC tank containing 200 litres of sea water, whose characteristics were: salinity 30‰, pH 7.4, temperature 16-18 °C, surface tension 72.7 mN/m, dissolved oxygen 8-8.6 mg/l, absent of heavy metals and contamination due to aerobe and anaerobe microorganisms. To avoid variations in detergent concentrations, test solutions were changed every 12 hours. The bio-degradation occurring in this time is less than 10% of the initial concentration (Flores et al., 1980).

After the animals had died from exposure to the detergent, their kidney and spleen were removed. Samples were fixed in 10% v/v formol buffered with 0.1M phosphate buffer, pH 7.2, dehydrated in increasing concentrations of alcohol, cleared with benzol and embedded in semisynthetic paraffin wax with a mean fusion point of 54-56 °C. Sections were cut at 5 µm.

Harris's haematoxylin and acetic eosin, Harris's Haematoxylin-VOF (Gutierrez, 1967) and Gridley's Reticulum Stain (Gridley, 1951) were employed as general stains.

Results

The time of death resulting at each concentration was: group a (5 mg/L) 250 hours; group b (8.5 mg/L) 12 hours; group c (10 mg/L) 6 hours; group d (15 mg/L) 30 minutes. The LC50 was 6.1 mg/L of SDS at 96 hours.

Kidney

Group control (Figs. 1A, 2A)

The most striking feature of the kidney cortex was the presence of numerous renal corpuscles. They appeared as spherical bodies surrounded by a small clear space. The proximal and distal tubules surrounded the renal corpuscles. These presented a variety of profiles in

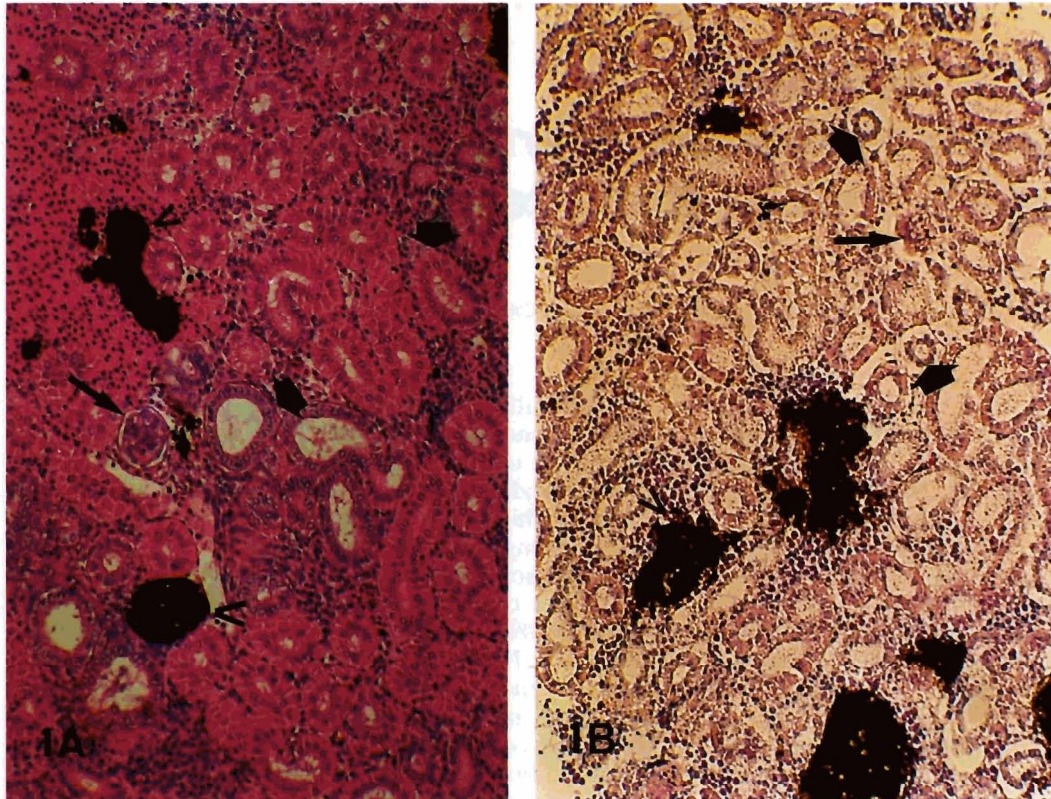


Fig. 1. Kidney of *Sparus aurata*, L. stained with H&E. **A.** Control. Renal corpuscle (thin arrow), tubule (thick arrow), black-brown pigment granules (arrowhead). x 250. **B.** Group C. Observe renal corpuscle retracted (thin arrow), tubules retracted (fat arrow), black-brown pigment granules (arrowhead). x 250

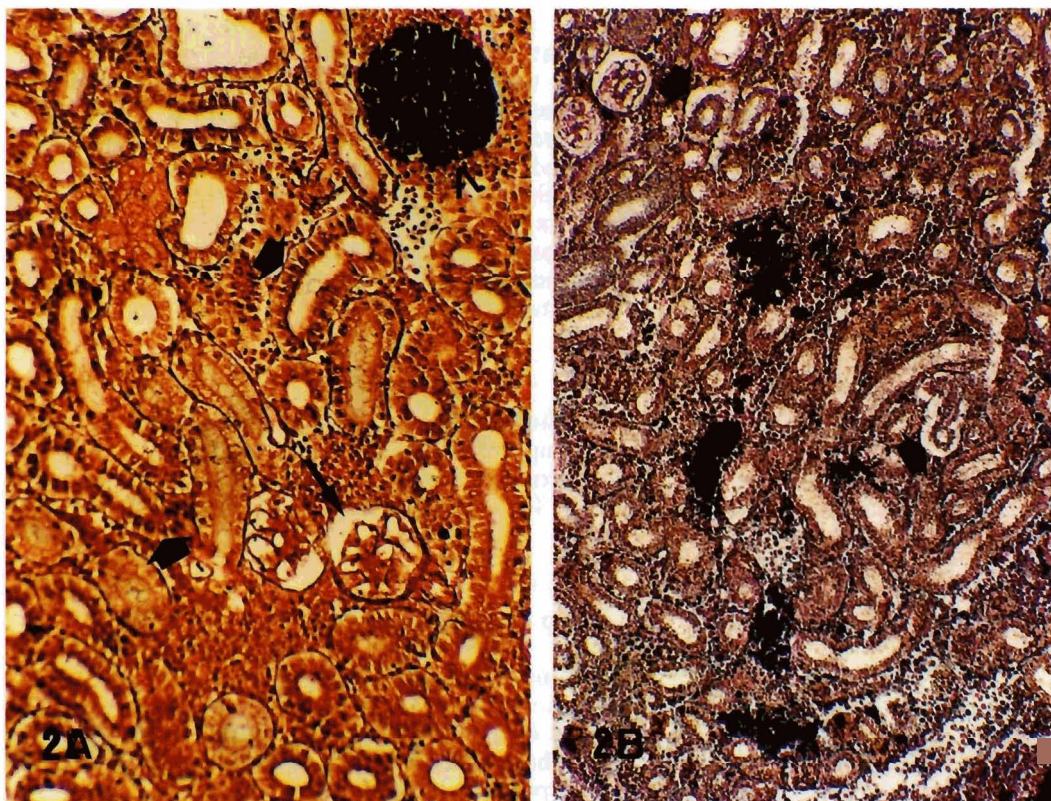


Fig. 2. Kidney of *Sparus aurata*, L. stained with Gridley's reticulum stain. **A.** Control. Renal corpuscle (thin arrow), tubule (thick arrow), black-brown pigment granules (arrowhead). x 312. **B.** Group d. Observe tubules retracted (thick arrow). x 250

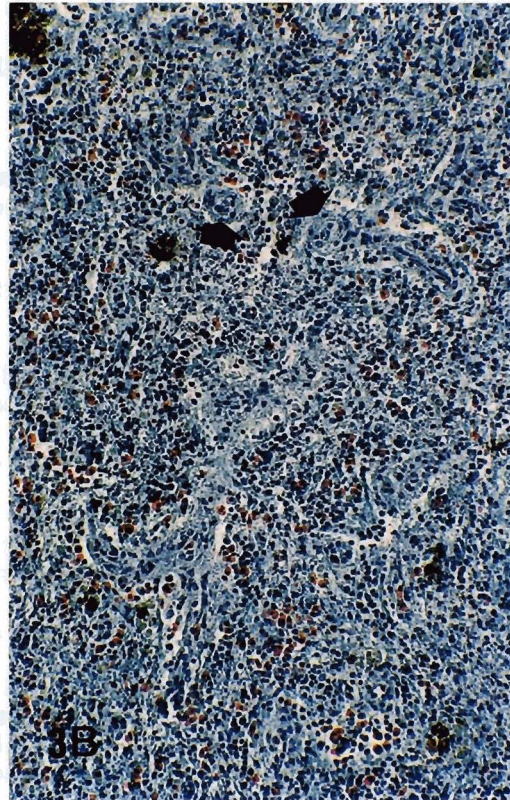
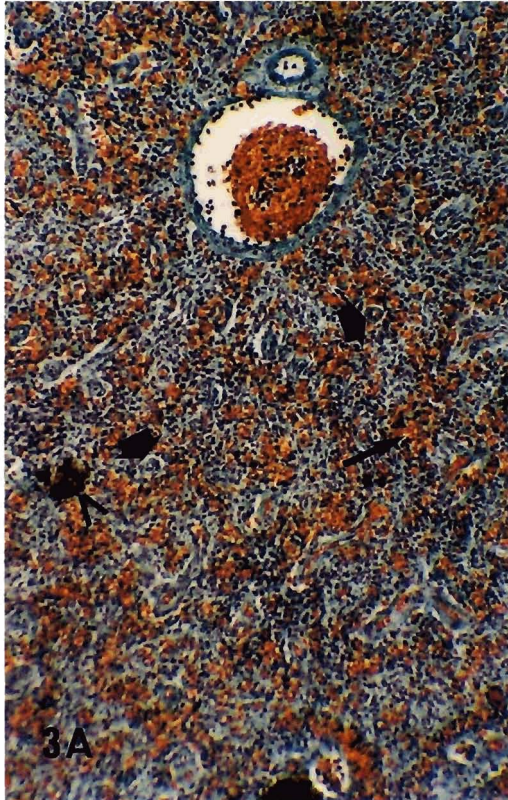


Fig. 3. Spleen of *Sparus aurata*, L. Stained with Haematoxylin-VOF. A. Control. Red pulp (thin arrow), splenic ellipsoids (thick arrow), black-brown pigment granules (arrowhead). x 312. B. Group b. Observe splenic ellipsoids closed (thick arrow) and the extensive increase in leucocytes. x 312

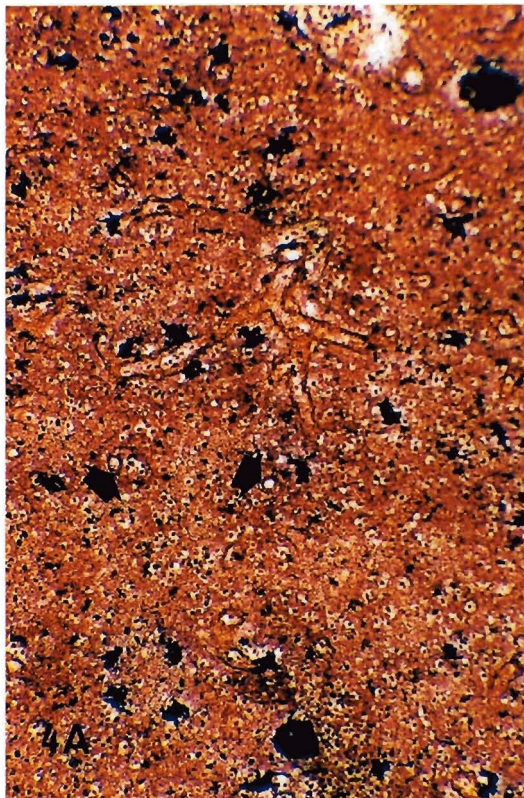


Fig. 4. Spleen of *Sparus aurata*, L. Stained with Gridley's reticulum stain. A. Control. Splenic ellipsoids (thick arrow). x 312. B. Group d. Observe splenic ellipsoids open (thick arrow) and the extensive separation of the cells belong parenchyma. x 312

Effect of SDS in kidney and spleen gilthead

sections, most of which appeared oval or spherical. However, because of their convolutions, it was also possible to find some whose profile resembled a U, J or even an S.

The bulk of the kidney consisted of parenchymal material, namely tubules, and only a small amount of stroma was present. The stroma consisted of reticular fibers that surrounded the various parenchymal elements.

Black-brown pigment granules appeared in the interstitial tissue; these pigment granules were identified as melanine by Sarasquete and Gutierrez (1984).

Morphological changes observed

Group a

At 5 mg/L tubules and renal corpuscles appeared with a light generalized retraction. There were accumulated leucocytes near renal corpuscles, which were slightly retracted.

Group b

At 8.5 mg/L tubular and glomerular retraction was extensive, and was complete in a lot of tubules. In the intersticie there was an increase of leucocytes.

Group c (Fig. 1B)

At 10 mg/L in some tubules there was a complete retraction and some renal corpuscles. In the intersticie morphological alterations did not appear.

Group d (Fig. 2B)

At 15 mg/L there was a retraction of the tubule cells in some tubules and retraction of some renal corpuscles, though the majority of them were normal. In the intersticie, morphological alterations did not appear.

Spleen

Group Control (Figs. 3A, 4A)

Two major territories were evident in a low-magnification view of a spleen section: white pulp and red pulp. The white pulp consisted of many closely-packed lymphocytes and appeared as islands of spotted blue in hematoxylin-eosin preparations. Their cells were grouped around an artery and surrounded it along its length. Therefore, in each island or bar of white pulp an artery, called the central artery could be seen. This was usually eccentrically localized.

The red pulp presented an overall red-staining response because of the large numbers of red cells that were present. The predominant components of the red pulp were the venous sinuses, and "cords" of splenic tissue, called Billroth's cords.

Black-brown pigment granules appeared in the

stroma; these pigment granules were described by Roberts (1975).

Morphological changes observed

Group a

At 5 mg/L the vessels and venous sinuses were normal and full of blood. The increase in leucocytes was extensive.

Group b (Fig. 3B)

At 8.5 mg/L the trabeculae were marked, infiltration by red cells was observed and the venous sinuses were closed. The increase in leucocytes was extensive.

Group c

At 10 mg/L there was infiltration by red cells. There was a slight increase in the leucocytes. The blood vessels were empty and the splenic ellipsoids were open.

Group d (Fig. 4B)

At 15 mg/l red cells were more numerous, fundamentally in the periphieria of the organ. The arteries and terminal arteries were empty and the splenic ellipsoids were open.

Discussion

The study about histological alterations in acuatic species induced by toxic action of the detergents are circumscribed to liver and kidney (Lang, 1967) and opercule (Roy, 1988), both in river species, and liver-pancreas, intestine and gills (Ribelles et al., 1995a-c) in sea species.

The mechanisms by which detergents exert their effects are not yet understood (Abel, 1974, 1976; Fukuda, 1982; Mishra et al., 1985; Kalmanzon et al., 1992). However, the damage to the tissues could be considered as cause of death. Everyone agrees that the damage to the gill is generalized, though the specific responsible cause of the toxic effect is yet unknown.

Kidney and spleen have no direct contact with the SDS dissolved in the water, but we have appreciated serious alterations. We do not know if molecules or ones derived from the toxin reach the internal organs, neither do we know the quantity; it may be supposed that it reaches them via the blood. Anyway, these lesions in the vital organs interfere with their functions, which can affect survival of the fishes.

The development of the lesions depends not only on concentration level but also on exposure time to the toxin. So those specimens that stayed longer under the toxic influences have suffered worse and more drastic damage to vital organs. Kidney shows tubular and renal

Effect of SDS in kidney and spleen gilthead

corpuscle retraction which depends on concentration and exposure time, and this causes loss of normal structure. Spleen shows a progressive increase in leucocytes and red cell infiltration, besides a tendency to damage the trabeculae structure related to dose and exposure time.

Acknowledgements. We are grateful to Instituto Sancti Petri (S. Fernando, Spain) for its assistance and for animals supplied. We thank M.T. Fernández-Llebrez and A. Mora for technical assistance.

References

- Abel P. (1974). Toxicity of synthetic detergents to fish, and aquatic invertebrates. *J. Fish Biol.* 6, 279-298.
- Abel P. (1976). Toxic action of several lethal concentrations of an anionic detergent on the gills of the brown trout (*Salmo trutta*, L.) *J. Fish Biol.* 9, 441-446.
- Arias A. (1976). Sobre la biología de la dorada (*Sparus aurata*, L.) de los esteros de la provincia de Cádiz. *Inv. Pesq.* 40, 201-222.
- Arias A.M., Drake P. and Rodríguez R.B. (1984). Los esteros de las salinas de San Fernando (Cádiz, España) y el cultivo extensivo de peces marinos. *L'aquaculture du Bar et des Sparidés*. Paris. INRA (Institut National de la Recherche Agronomique).
- Bigliardi P.L., Herron M.J., Nelson R.D. and Dahl M.V. (1994). Effects of detergents on proliferations and metabolism of human keratinocytes. *Exp. Dermatol.* 3, 89-94.
- Flores V., Sales D. and Establier R. (1980). Contaminación de las aguas de la Bahía de Cádiz (III). Ensayos de biodegradabilidad con dodecil-sulfato sódico. *Ingeniería Química* 130, 117-128.
- Fukuda Y. (1982). Specific reactions of goldfish gills exposed to linear alkylbenzene sulphonate. *Jpn. J. Ichthyol* 30, 268-274.
- Gutiérrez M. (1967). Coloración histológica para ovarios de peces, crustáceos y moluscos. *Invest. Pesq.* 31, 265-271.
- Gridley M.F. (1951). A method of reticulum stain. *Am. J. Clin. Pathol.* 21, 879-881.
- Kalmanzon E., Zlotkin E., Cohen R. and Barenholz Y. (1992). Liposomes as a model for the study of mechanism of fish toxicity of sodium dodecyl sulphate in sea water. *Biochim. Biophys. Acta* 1103, 148-156.
- Lang W. (1967). Untersuchungen zur Wirkungswiese von anionischen grenzflächenaktiven Stoffen auf histologische Beschaffenheit und Funktion verschiedener Organe bei (*Carassius auratus*). *Arch. Fish Wiss.* 18, 25-45.
- Mishra V., Lal H., Chawla G. and Viswanathan P.N. (1985). Pathomorphological changes in the gills of fish fingerlings (*Cirrhina mirgala*) by lineal alkylbenzene sulphonate. *Ecotoxicol. Environ. Saf.* 10, 302-308.
- Mishra V., Kumar V., Pandey S.D. and Wiswanathan P.N. (1991). Biochemical alterations in fish fingerlings (*Ciprinus carpio*) exposed to sublethal concentrations of linear alkyl benzene sulphonate. *Arch. Environ. Contam. Toxicol.* 21, 514-517.
- Ribelles A., Carrasco C. and Rosety M. (1995a). Morphological and histochemical changes caused by sodium dodecyl sulphate in the gills of gilthead (*Sparus aurata*, L.). *Eur. J. Histochem.* 39, 141-148.
- Ribelles A., Carrasco C., Rosety M. and Aldana M. (1995b). A histochemical study of the biological effects of sodium dodecyl sulphate on the intestine of gilthead seabream, *Sparus aurata* L. *Ecotoxicol. Environ. Saf.* 32, 131-138.
- Ribelles A., Carrasco C., Rosety M. and Aldana M. (1995c). Morphological and histochemical changes in the liver and pancreas of gilthead, *Sparus auratus* L., induced by acute action of the anionic detergent, sodium dodecyl sulphate. *Histol. Histopathol.* 10, 781-787.
- Roberts R. (1975). Pathology of fishes. Ribelin W.E. and Migaki G. (eds). Univ. Wisconsin Press. Madison. pp 399-428.
- Roy D. (1988). Impact of detergents on the proteins histochemistry of various cell types of the gill epithelium of *Rita rita*. *Ecotox. Environ. Saf.* 15, 206-211.
- Sarasquete M.C. and Gutiérrez M. (1984). Caracteres histoquímicos de los granos de pigmento marrón-negro del riñón de la dorada, *Sparus aurata*, L. *Inv. Pesq.* 48, 538-547.
- Varona M. (1993). Cultivos marinos, ganadería y futuro. *MAR* 304, 56-59.
- Wilhelm V., Kumar V., Pandey S.D. and Wiswanathan P.N. (1994). Quantitative in vitro assessment of N-alkyl sulphate-induced cytotoxicity in human keratinocytes (HaCaT). Comparison with in vivo human irritation tests. *Br. J. Dermatol.* 130, 18-23.
- Zeni C. and Caligiuri A.S. (1992). Morphological and ultrastructural changes induced by sublethal concentrations of an anionic detergent of Ictalurus species barbel taste buds. *Microbios* 69, 41-52.

Accepted March 4, 1997