ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY 32, 131–138 (1995)

A Histochemical Study of the Biological Effects of Sodium Dodecyl Sulfate on the Intestine of the Gilthead Seabream, *Sparus aurata* L.

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Received May 26, 1994

This paper reports the morphological and histochemical changes caused by acute action of the detergent sodium dodecyl sulfate (SDS) on the intestine of the gilthead (*Sparus aurata* L.). Sixty-five giltheads were exposed to SDS concentrations of 5, 8.5, 10, and 15 mg/liter. Surface tensions were determined and the LC_{50} values calculated. The effects of SDS on the intestine were more pronounced in those specimens exposed to higher concentrations and for longer periods. Three causes of death were determined: (i) decrease in surface tension, (ii) destruction of tissue, and (iii) alteration of biomacromolecules. Both (ii) and (iii) were determined by histochemical techniques.

INTRODUCTION

Detergents pollute the environment and their toxic effects can be demonstrated. This investigation demonstrates some morphological and histochemical changes produced in *Sparus aurata* L. by the action of a common detergent, sodium dodecyl sulfate (SDS), and their effect on survival. Research on the influence of SDS on this species is particularly appropriate because of its importance in the fishing industry and in pisciculture (Arias *et al.*, 1976; Varona, 1993).

SDS is one of the most widely commercialized domestic detergents in the United States, Japan, and Western Europe; more than 70 million kilograms are sold per year. Primary biodegradation of SDS is rapid; it disappears in less than a day in cultures or in river water test (Swisher, 1970). However, although it loses its original identity, the effects of the intermediate breakdown products persist until biodegradation is complete (Bukema *et al.*, 1982; Kimerle and Swisher, 1977; Oba *et al.*, 1977).

The mechanisms by which detergents produce their effects are not well understood (Helenius and Simmons, 1975). It is thought that the decrease in surface tension that they induce is the main cause of death (Prat and Giraud, 1964; Bock, 1965; Mann, 1972); in some places in the Bay of Cádiz surface tensions as low as 42.3 mN/m have been recorded (Flores *et al.*, 1979). However, gilthead tissues also absorb this detergent and its high affinity for lipid membranes provokes changes in the phospholipid bilayer.

METHODS

The fish used in this investigation were 65 juvenile giltheads, 6 months old, between 12 and 14 cm 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 lots, A, B, C, and D, which were exposed to concentrations of 5, 8.5, 10, and 15 mg/liter of SDS (Merck, Spain), respectively, until 50% of fish in each treatment had died. The LC_{50} causing death in a period of 96 hr was determined according to Sprague (1976) and Loomis (1982).

Each lot was maintained in a PVC tank containing 200 liters of seawater with the characteristics of 30‰ salinity, pH 7.4, 16–18°C, 8–8.6 mg/liter dissolved oxygen, and the absence of heavy metals and contamination due to aerobe and anaerobe microorganisms. To avoid variations in detergent concentration, test solutions were changed every 12 hr. The biodegradation occurring in this time is less than 10% of the initial concentration (Flores *et al.*, 1980).

Surface tension was measured using a Lauda TE 1 C/2 with SAE+KM3 tensiometer. An initial reading was taken for all lots. Lot B was further measured at 6 hr and lots C and D at 12 hr.

After the animals had died from exposure to the detergent, their intestines were removed. For practical purposes the intestines were divided into three sections, proximal, middle, and distal. Samples were fixed in 10% (v/v) formol buffered with 0.1 *M* phosphate buffer, pH 7.2, dehydrated in increasing concentrations of alcohol, cleared with benzol, and embedded in semisynthetic paraffin was with a mean fusion point of 54–56°C. Sections were cut at 5 μm.

Harris' hematoxylin and acetic eosin and Harris' Hematoxylin–VOF (Gutierrez, 1967) were employed as general stains. The histochemical techniques used are provided in Tables 1 and 2.

RESULTS

When the results are extrapolated on semilogarithmic paper and the time by which 50% of fish in each lot had died is noted at 96 hr for *S. aurata* L. the LC_{50} is 6.1 mg/liter. Table 3

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TABLE 1
Histochemical Reactions of Carbohydrates

Reactions	Functions and/or compound shown
Schiff reagent (Pearse, 1960)	Free aldehydes
Periodic acid–Schiff (PAS) (McManus, 1948)	Glycogen, neutral mucopolysaccharides, and/or glycoproteins
Diastase–Pas (Lillie and Greco, 1947)	Glycogen, neutral mucopolysaccharides
α-Amilase–PAS (Lillie and Greco, 1947)	Glycogen
Clorhydric hydrolysis–PAS (Martoja and Martoja-Pierson, 1970)	Sialic acid
Colloidal iron–potassium ferrocyanide (Hale, 1957)	Acid mucopolysaccharides
Alcian blue, pH 2.5 (Martoja and Martoja-Pierson, 1970)	Carboxyl-rich glycoconjugates (sulfated or not)
Methylation–alcian blue, pH 2.5 (Lillie, 1958)	Block of mucopolysaccharide acidic groups
Methylation–saponification–alcian blue, pH 2.5 (Lillie, 1958)	Carboxylated mucopolysaccharides
Alcian blue, pH 2.5–PAS (Pearse, 1960)	Neutral mucopolysaccharides and acid mucopolysaccharides
Hyaluronidase–alcian blue; pH 2.5 (Pearse, 1960)	Hyaluronic acid
Alcian blue, pH 1 (Martoja and Martoja-Pierson, 1970)	Acid glycoconjugates (sulfated)
Alcian blue, pH 0.4 (Martoja and Martoja-Pierson, 1970)	Very sulfated glycoconjugates
Hyaluronidase–alcian blue, pH 0.4 (Pearse, 1960)	Chondroitin B sulfate
Toluidine blue (Martoja and Martoja-Pierson, 1970)	Metachromasia, acid mucopolysaccharides

TABLE 2
Histochemical Reactions of Proteins

Reactions	Functions and/or compound shown
Bromophenol blue–Hg (Chapman, 1971)	Proteins in general
Hartig Zacharias method (Martoja and Martoja-Pierson, 1970)	Siderophile proteins
Nihydrin–Schiff (Pearse, 1960)	Proteins rich in $-NH_2$ groups (lysine)
1,2-Naphthoquinone-4-sulfonic acid, sodium salt (NQS) (Lillie *et al.*, 1971)	Proteins rich in arginine
p-Dimethylaminobenzaldehyde (Barka and Anderson, 1963)	Proteins rich in tryptophan
Ferric ferricyanide–Fe(III) (Chevremont and Frederic, 1943)	Proteins rich in SH groups (cysteine) and other reductor groups
Thioglicolate–potassium ferricyanide–Fe(III) (Chevremont and Frederic, 1943)	Proteins rich in S–S groups (cystine)

indicates time to death and the surface tension resulting at each concentration.

The histochemical behavior of the proteins and carbohydrates studied is summarized in Tables 4–7.

Lot A. At 15 mg/liter there is great retraction of the intestinal villi. Whole blocks of epithelia containing calciform cells become detached. This is more pronounced in the proximal intestine. In the distal intestine the muscle layer is thickened (Figs. 1–4). Sialic acid disappears from the muscle in the proximal intestine but remains in the distal intestine. Levels of acidic mucopolysaccharides and carboxylated acidic mucopolysaccharides decrease slightly in the calciform cells (Figs. 2 and 5). Sulfated acidic mucopolysaccharides disappear. There is a generalized small loss of proteins in epithelial cells of the proximal intestine and a complete loss in the submucosa. Siderophile protein levels fall slightly in the epithelial cells.

Lot B. At 10 mg/liter there is complete detachment of the epithelium in some areas. The submucosa is hypertrophied and the muscle layer thickened. All these features are more prominent in the proximal intestine (Fig. 6). Neutral mucopolysaccharides disappear from the submucosa of the proximal intestine. Sialic acid also disappears from both the submucosa and the muscle layer of the proximal intestine. Levels of acidic mucopolysaccharides and carboxylated acidic mucopolysaccharides fall slightly in the calciform cells. Sulfated acidic mucopolysaccharides are no longer seen. There is a generalized small loss of proteins in epithelial cells and a complete loss of protein in the submucosa. Siderophile protein levels fall slightly in the epithelial cells and submucosa.

Lot C. At 8.5 mg/liter there is complete detachment of the epithelium especially in the proximal intestine. The submucosa is infiltrated by lymphocytes and the muscle layer is thickened. Neutral mucopolysaccharides disappear from the submucosa as does sialic acid, which is also absent in the muscle layer. Levels of acidic mucopolysaccharides and carboxylated acidic mucopolysaccharides fall moderately in the calciform cells (Fig. 7). Sulfated acidic mucopolysaccharides disappear. There is a generalized small loss of proteins in epithelial cells and muscle layer and a complete loss of protein in the submucosa. Siderophile protein levels fall slightly in the epithelial cells and submucosa.

Lot D. At 5 mg/liter there is retraction of the intestinal villi and fusion of their tips. The submucosa displays hypertrophy

TABLE 3
Time to Death of 50% of Animals and the Surface Tension Resulting at Each Concentration

SDS concentration (mg/liter)	Time to death	Surface tension (mN/m)		
		Initial	6 hr	12 hr
15	30 min	49.9	—	—
10	6 hr	53.3	53.9	—
8.5	12 hr	54.3	—	56.1
5	250 hr	56.0	—	58.2

TABLE 4

Histochemical Reactions of Carbohydrates in the Intestine of *Sparus aurata* L. against Different Concentrations of SDS

	Goblet cells										Epithelial cells									
	Ctrl		15		10		8.5		5		Ctrl		15		10		8.5		5	
Reaction	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI
Schiff	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PAS	2	2	2	2	2	2	2	2	2	2	0	0	0	0	0	0	0	0	0	0
Diastase–PAS	2	2	2	2	2	2	2	2	2	2	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
α-Amilase–PAS	2	2	2	2	2	2	2	2	2	2	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Clorhydric hydrolisis–PAS	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Colloidal iron–potassium ferrocyanide	3	3	3	2	2	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Alcian blue, pH 2.5	3	3	3	2	2	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
Methylation–alcian blue, pH 2.5	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Methylation–saponification–alcian blue, pH 2.5	3	2	2	2	1	1	1	1	1	1	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Hyaluronidase–alcian blue, pH 2.5	0	0	0	0	0	0	0	0	0	0	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Alcian blue, pH 1	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alcian blue, pH 0.4	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyaluronidase–alcian blue, pH 0.4	0	0	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Toluidine blue (metachromasia)	Y	Y	Y	Y	Y	N	Y	N	Y	N	N	N	N	N	N	N	N	N	N	N

Note. 0, negative; 1, weak; 2, moderate; 3, strong. Ctrl, control; 15, 10, 8.5, 5 are concentrations used in mg/liter. NT, not tested. Y, yes; N, no. PI, proximal intestine; DI, distal intestine.

and there is infiltration by lymphocytes, though this is variable, even within the same section. The muscle layer is thickened. Neutral mucopolysaccharides are no longer found in the submucosa. Sialic acid disappears from both the submucosa and the muscle layer. Levels of acidic mucopolysaccharides and carboxylated acidic mucopolysaccharides fall moderately in the calciform cells. Sulfated acidic mucopolysaccharides disappear. There is a generalized small loss of proteins in the muscle layer, and siderophile protein levels fall slightly in the epithelial cells, submucosa, and muscle layer (Fig. 8).

DISCUSSION

The LC_{50} determined is higher than that cited by authors working with other fish (Marchetti, 1964; Laroche *et al.*, 1972; Quevedo *et al.*, 1984; Aguilera and Huq, 1982). The gilthead may therefore be considered relatively resistant to SDS, perhaps, as suggested by Gomez *et al.* (1984), due to its pelagic nature, but perhaps also due to the idiosyncrasy of the species with respect to this agent. This is of great importance because fish that have been exposed to toxic concentrations of detergent are eaten.

Mann (1972) reached the conclusion that surface tension values of less than 40 mN/m are lethal for the majority of species. These values are reached with 2 to 2.5 mg/liter of SDS. Surface tension values of 50 mN/m are dangerous to the normal development of marine fauna (Bock, 1965). Marchetti (1964), however, suggests that there is not always a simple relationship between surface tension and toxicity.

In agreement with Sprague (1976) and Mallat (1985), this work does not confirm a single cause of death. Rather, the decrease in surface tension, destruction of tissue, and effects at the organ level are cocontributors to the death of giltheads exposed to SDS. The loss of epithelial integrity allows detergent to enter the blood, thereby reaching the organs of the fish.

These observations demonstrate that the development of the lesions depends on the concentration of and length of exposure to the agent. Lesion pathology along the intestine varies from greater epithelial destruction in the proximal intestine to inflammatory reactions with lower intensity in the more distal region. The constant swallowing of water and its passage along the gut of the fish explain the greater severity of lesions in the proximal intestine.

CONCLUSIONS

In summary, effects of SDS on the intestinal epithelium depend on concentration and exposure time, with a greater

TABLE 5

Histochemical Reactions of Carbohydrates in the Intestine of *Sparus aurata* L. against Different Concentrations of SDS

	Submucosa										Muscular layer									
	Ctrl		15		10		8.5		5		Ctrl		15		10		8.5		5	
Reaction	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI
Schiff	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
PAS	1	1	1	1	0	1	0	0	0	0	1	1	1	1	1	1	0	0	1	0
Diastase–PAS	1	1	1	1	NT	1	NT	NT	NT	NT	1	1	1	1	1	1	NT	NT	1	NT
α-Amilase–PAS	0	0	0	0	NT	0	NT	NT	NT	NT	0	0	0	0	0	0	NT	NT	0	NT
Clorhydric hydrolisis–PAS	1	1	1	1	0	1	0	0	0	0	1	1	0	1	0	1	0	0	0	0
Colloidal iron–potassium ferrocyanide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alcian blue, pH 2.5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Methylation–alcian blue, pH 2.5	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Methylation–saponification–alcian blue, pH 2.5	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Hyaluronidase–alcian blue, pH 2.5	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Alcian blue, pH 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Alcian blue, pH 0.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hyaluronidase–alcian blue, pH 0.4	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Toluidine blue (metachromasia)	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

Note. 0, negative; 1, weak; 2, moderate; 3, strong. Ctrl, control; 15, 10, 8.5, 5 are concentrations used in mg/liter. NT, not tested. Y, yes; N, no. PI, proximal intestine; DI, distal intestine.

variation in those specimens subjected to higher concentrations and longer exposure times. The development of the lesions begins with a retraction of the intestinal villi and fusion of their tips; later, the epithelium is completely detached, and the submucosa is hypertrophied.

In addition to the levels of acidic mucopolysaccharides and carboxylated acidic mucopolysaccharides, the levels of proteins in general and siderophil proteins decrease due to the observed development of the lesions along the proximal, middle, and distal intestine, depending on the concentration of and time of exposure to the toxin, the proximal intestine being the most affected.

TABLE 6

Histochemical Reactions of Proteins in the Intestine of *Sparus aurata* L. against Different Concentrations of SDS

	Goblet cells										Epithelial cells									
	Ctrl		15		10		8.5		5		Ctrl		15		10		8.5		5	
Reaction	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI
Mercury–bromophenol blue	0	0	0	0	0	0	0	0	0	0	2	2	1	1	1	1	1	1	2	2
Hartig Zacharias method	0	0	0	0	0	0	0	0	0	0	2	2	1	2	1	1	1	1	1	1
Ninhydrin–Schiff	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2-Naphthoquinone-4-sulfonic acid, sodium salt (NQS)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
p-Dimethylaminobenzaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ferric ferricyanide–Fe(III)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thioglicolate–potassium ferricyanide–Fe(III)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Note. 0, negative; 1, weak; 2, moderate; 3, strong. Ctrl, control; 15, 10, 8.5, 5 are concentrations used in mg/liter. PI, proximal intestine; DI, distal intestine.

TABLE 7

Histochemical Reactions of Proteins in the Intestine of *Sparus aurata* L. against Different Concentrations of SDS

	Submucosa										Muscular layer									
	Ctrl		15		10		8.5		5		Ctrl		15		10		8.5		5	
Reaction	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI	PI	DI
Mercury–bromophenol blue	1	1	0	1	0	0	0	0	1	0	2	2	2	1	2	1	1	1	1	1
Hartig Zacharias method	2	1	2	1	1	1	1	1	1	1	2	1	2	1	2	1	2	1	1	1
Ninhydrin–Schiff	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1,2-Naphthoquinone-4-sulfonic acid, sodium salt (NQS)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
p-Dimethylaminobenzaldehyde	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ferric ferricyanide–Fe(III)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thioglicolate–potassium ferricyanide–Fe(III)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Note. 0, negative; 1, weak; 2, moderate; 3, strong. Ctrl, control; 15, 10, 8.5, 5 are concentrations used in mg/liter. PI, proximal intestine; DI, distal intestine.

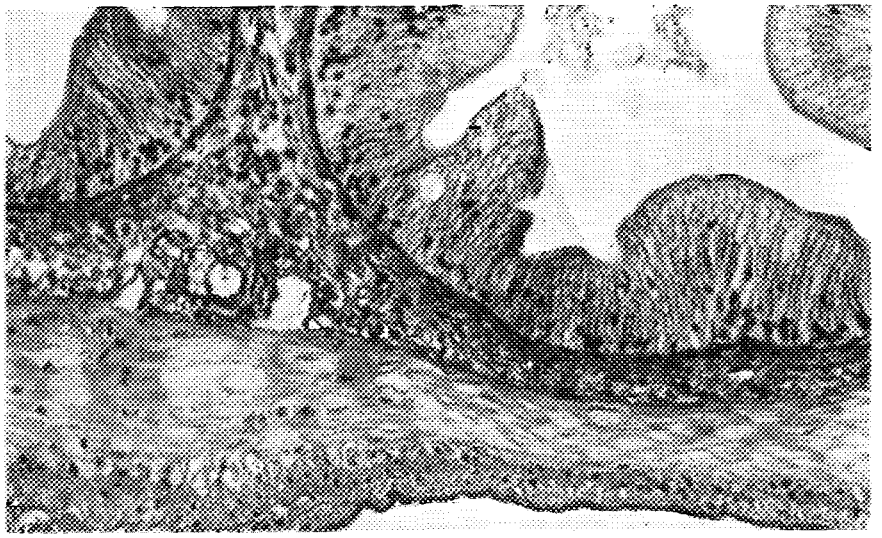

FIG. 1. Distal intestine of *S. aurata* L. VOF–hematoxylin, ×400.

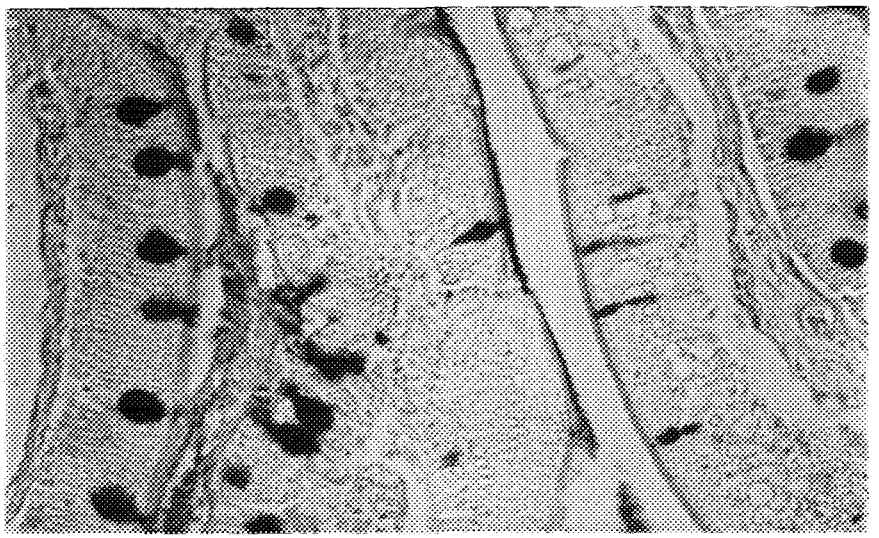

FIG. 2. Proximal intestine of *S. aurata* L. Alcian blue, pH 2.5, ×400.

FIG. 3. Distal intestine of *S. aurata* L. Siderophile proteins, ×400.

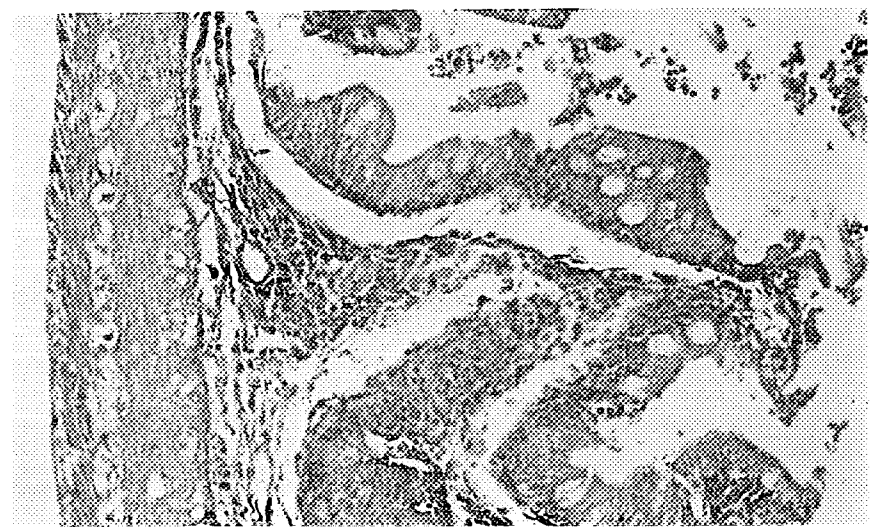

FIG. 4. Proximal intestine of *S. aurata* L. exposed to 15 mg/liter SDS. VOF–hematoxylin, ×400.

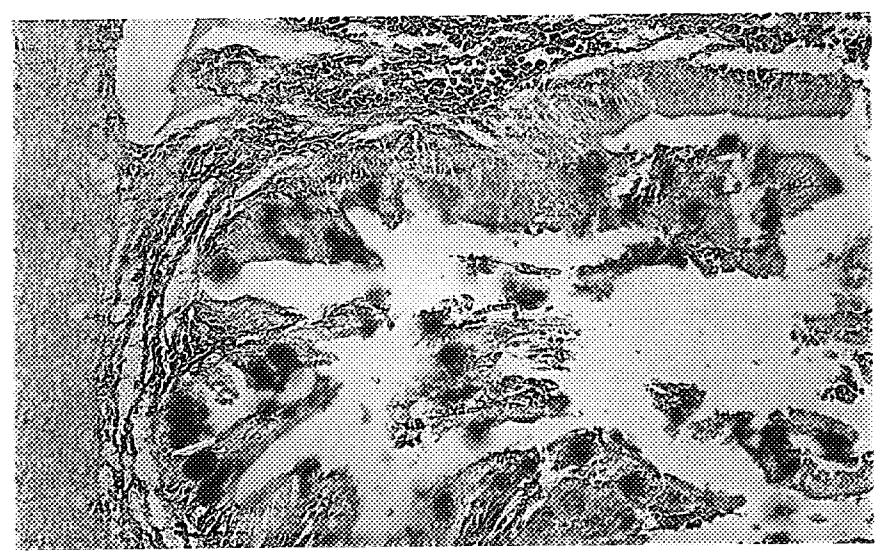

FIG. 5. Distal intestine of *S. aurata* L. exposed to 15 mg/liter SDS. Colloidal iron–potassium ferrocyanide, ×400.

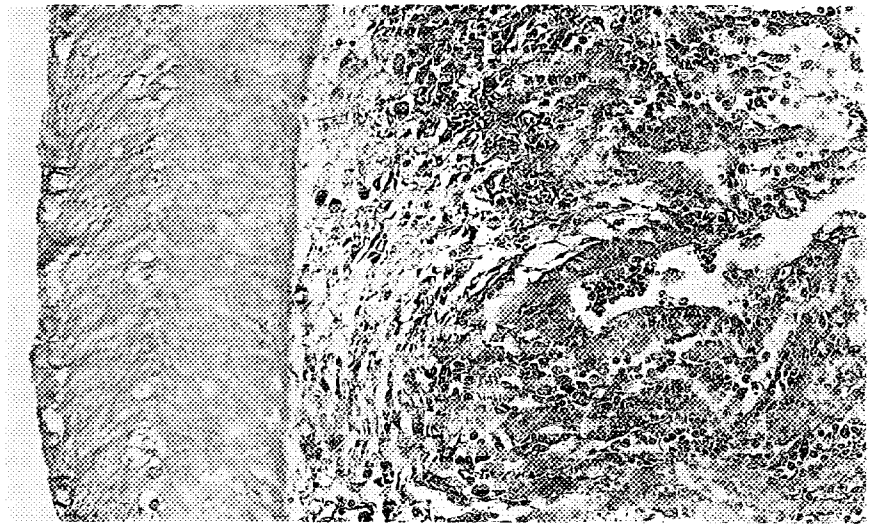

FIG. 6. Proximal intestine of *S. aurata* L. exposed to 10 mg/liter SDS. VOF–hematoxylin, ×400.

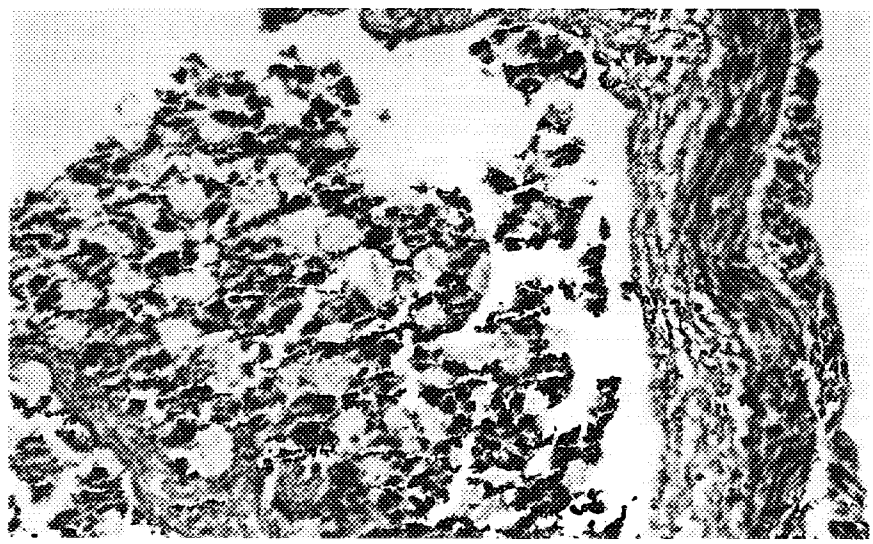

FIG. 7. Proximal intestine of *S. aurata* L. exposed to 8.5 mg/liter SDS. Toluidine blue, ×400.

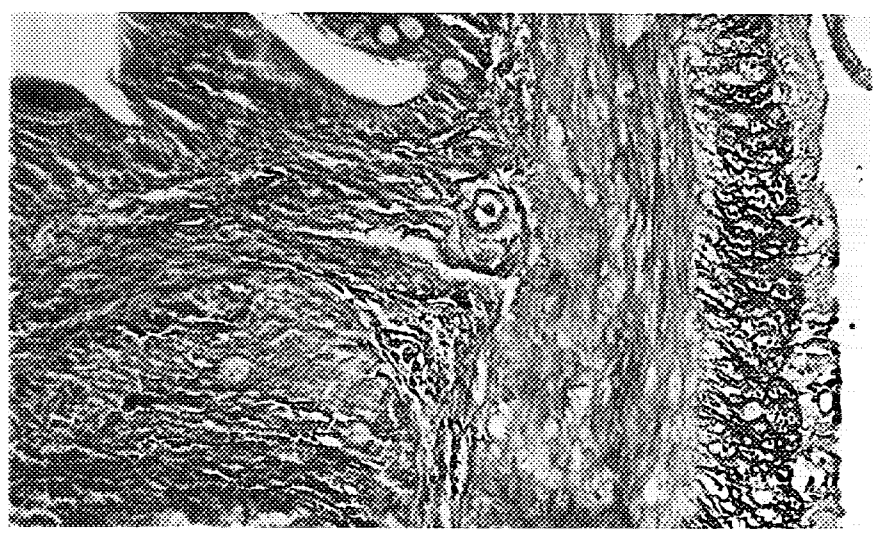

FIG. 8. Proximal intestine of *S. aurata* L. exposed to 5 mg/liter SDS. Siderophile proteins, ×400.

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