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Toxicological Examinations of Oxolinic Acid and Nalidixic Acid in Yellowtail

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Toxicological examinations of oxolinic acid (OA) and nalidixic acid (NA) were performed at overdoses six and twenty times the standard dose in yellowtail, *Seriola quenqueradiata*. Examined doses of OA were 60mg and 120mg / Kg / day and doses of NA were 120mg and 400mg / Kg / day. The dosing period was seven days and the recovery period which followed was fourteen days.

The side-effects in fish dosed with OA and NA were loss of appetite and dark body discoloration. The effects of OA and NA on the hematology were appearance of macrocytic erythrocytes and occurrence of macrocytic anemia. The effects of OA and NA on the histopathology were fat reduction of hepatocytes and nephrosis, which were severer in fish dosed with NA than OA. The hematological data indicate that both OA and NA inhibit DNA synthesis of erthroblasts of the dosed fish.

Key words: toxity, oxolinic acid, nalidixic acid, yellowtail

Oxolinic acid (OA) and nalidixic acid (NA) are synthetic antibacterial agents having a pyridopyrimidine ring and are effective against various Gram-negative bacteria pathogenic to cultured fishes (EGUSA 1981). Both OA and NA cause antibacterial actions to R factor-carrying bacteria, threrfore they are recommended as second selecting drugs. They have propensities to be involved in problems of overdose because they evoke drug resistant mutants soon during or after the medication.

In this study, the toxicity of OA was examined at overdoses six and twenty times the standard dose in yellowtail, *Seriola quinqueradiata* because OA is of use against infection with *Pasteurella piscicida* in cultured yellowtail. The toxicity of NA was also examined at overdoses as a reference drug because NA is not permitted to be used for treatment of any diseases in yellowtail. The present paper descrides the side-effects of the drugs and the hematological abnormalities and histopathology of the dosed fish.

Materials and Methods

Drugs and Fish

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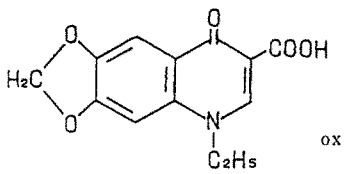
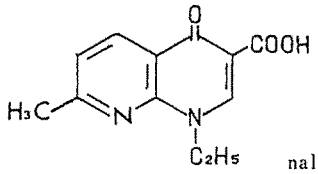
Examined drugs were oxolinic acid(OA) (Tanabe Pharmaceutical Co., Ltd) and nalidixic acid (NA) (Daiichi Pharmaceutical Co., Ltd) as a reference drug (Fig. 1). In order to examine their toxicity, doses were set at six and twenty times the standard dose. Doses of OA were 60mg (OA－6) and 120mg (OA－20) per Kg of body weight per day. Doses of NA were 120mg (NA－6) and 400mg (NA－20) per Kg of body weight per day. 200 yellowtail (average body weight 450g) were kept in separate net cages for each dose of drug and for control. The drug was mixed in minced Japanese anchovy with 0.5% of dinder (Meiji Confectionery Co., Ltd). Drug administration was performed daily by free feeding at a feeding ratio of 15%. The administration period was seven days and the recovery period of fourteen days followed (December 6 to 26, 1983). Sampling was performed on the first and fourteenth day after the dosing. Side-effects were daily observed throughout the experimental period. Water temperature was gradually decreased from 24°C to 20.5°C during the experimental period.



oxlinic acid



nalidixic acid

Fig. 1 Chemical structure of oxolinic acid and nalidixic acid.

Hematological and Histological Examinations

Each 10 fish were sampled from the dosed groups and the control group. On hematological examinations, red blood cell count (R. B. C.) (Clay Adams Co., Ltd, Blood Cell Counter), hematocrit (Ht), hemoglobin (Hb) (Clay Adams Co., Ltd, Accu-Stat System) and serum protein (T. P.) (Clay Adams Co., Ltd, Accu-Stat System) were measured. Then, the mean corpuscular volume (M. C. V.) and mean corpuscular hemoglobin concentration (M. C. H. C.) were calculated. On histopathological examinations, the liver, spleen, kidney, digestive tracts, heart, brain, gills and lateral musculature were observed with routine histological procedures.

Results

I. Oxolinic acid

1. Side-effects

Fish dosed with OA－6 and OA－20 suffered a slight loss of appetite, accompanied by dark body discoloration on the seventh day of dosing. These phenomena recovered soon in the early recovery period (Table 1). Weight gain of fish dosed with OA－6 and OA－20 was comparadle to that of the control (Table 2).

2. Hematology

Control: R. B. C. was constant on both the first and fourteenth days samples. The volume of erythrocytes was greater on the fourteenth day than on the first day, and the amount of T. P. was significantly increased on the fourteenth day (Tadle 2).

OA−6: On the first day after dosing, R. B. C. was quite comparable to the control while erythrocytes became significantly macrocytic. T. P. was slightly reduced, compared with the control. On the fourteenth day of the recovery period, R. B. C. markedly decreased in number and erythrocytes became normocytic and normochromic. T. P. was significantly reduced, compared with the control (Table 2).

OA−20: On the first day after dosing, R. B. C. was relatively reduced and erythrocytes became markedly macrocytic. T. P. was greater in amount than the control. On the fourteenth day of the recovery period, R. B. C. decreased significantly indicating macrocytic hyperchromic anemia. T. P. was significantly reduced, compared with the control (Table 2).

3. Histopathology

OA−6: On the first and fourteenth days after dosing, fat reduction with atrophy of hepatocytes occurred to a slight degree in the liver of most dosed fish. Hemosiderin deposition in macrophages was observed in the spleen of all dosed fish. The magnitude of hemosiderosis was comparable to the control. There was no obvious pathological change resulting from the toxic effects of OA in the kidney, digestive tracts, heart, brain, gills and lateral musculature (Table 4).

OA−20: On the first and fourteenth days, the liver mostly showed slight fat reduction with atrophy of hepatocytes (Plate I−4, II−1). The kidney of a few dosed fish showed nephrosis characterized by hyaline droplet degeneration but the rest did not show any obvious indication of OA toxic effect (Plate II−2). The spleen of all fish showed hemosiderosis in comparable degrees to the control (Plate II−3). No toxic effect was observed in any other organs examined (Table 4).

II. Nalidixic acid

1. Side-effects

Fish dosed with both NA−6 and NA−20 lost appetite on the third day of dosing. This became more severe day by day to complete loss on the second day and lasted to the sixth day in the recovery period, after which the appetite recovered. Dark body coloration accompanied the loss of appetite (Table 1). Weght gain of fish dosed with both NA−6 and NA−20 was less than that of the control but weight reduction did not occur (Table 3). On internal examination, the liver of most fish showed pale coloration on the first and fourteenth days after dosing.

2. Hematology

NA−6: On the first day after dosing, R. B. C. was significantly reduced, indicating occurrence of macrocytic normochromic anemia. The amount of T. P. was greater than

that of the control. On the fourteenth day of the recovery period, R. B. C. became comparable to the control while erythrocytes became significantly microcytic. T. P. was significantly reduced, compared with the control (Table 2).

NA−20: On the first day, R. B. C. was significantly reduced, indicating occurrence of macrocytic normochromic anemia. The amount of T. P. was greater than that of the control. On the fourteenth day, R. B. C. was reduced, indicating normocytic hyperchromic anemia. T. P. was markedly decreased (Table 3).

3. Histopathology

NA−6: On the first day after dosing, the liver of all fish showed slight to severe fat reduction with atrophy of hepatocytes (Plate II−4). All kidneys observed showed nephrosis characterized by hyaline droplet degeneration from a slight to moderate degree (Plate III−1). All spleens observed showed hemosiderosis in degrees comparable to the control. There was no obvious change indicating toxic effects in the any other organs examined (Table 5). On the fourteenth day, fat reduction of hepatocytes became slight to moderate in the liver of all fish. The kidney of some fish still showed slight nephrosis. All spleens observed showed hemosiderosis in degrees comparable to the control. There was no toxic effect in the other organs examined (Table 5).

NA−20: On the first day, the liver of all fish showed slight to severe fat reduction with atrophy of hepatocytes (Plate III−2). Most kidneys showed slight to moderate nephrosis (Plate III−3). All spleens showed hemosiderosis from a slight to severe degree (Plate III−4). On the fourteenth day, the severity of degeneration of hepatocytes and nephrosis was reduced in most fish. All spleens showed hemosiderosis in degrees comparable to the control. There was no toxic effect in the other organs examined (Table 5).

Discussion

Both oxolinic acid (OA) and nalidixic acid (NA) have a pyridopyrimidine ring and inhibit multiplication of Gram-negative bacteria. OA is more active in inhibiting bacterial multiplication than NA. NA is more toxic to animals than OA. It is recognized that both OA and NA inhibit DNA replication of bacteria (ATLAS *et al.* 1969). Because the pyridopyrimidine ring of the drug disturbs DNA synthesis, teratological examinations of NA have been performed in mammals (NISHIURA *et al.* 1971, SATO *et al.* 1980) as well as studies made on the mutagenic activity (SHIRATORI *et al.* 1980). The teratogenesis and mutagenic activity of NA in mammals were not obvious enough to be evaluated.

As to the toxicological study of OA and NA in yellowtail, dosing fish with NA 400 mg / Kg / day resulted in macrocytic normochromic anemia after seven-day dosing. Dosing fish with NA 120mg / Kg / day also showed a similar abnormality. Dosing fish with OA 60 and 120mg / Kg / day did not exhibit an anemic condition though the erythrocytes were markedly macrocytic after seven-day dosing. Fish dosed with OA 120mg / Kg underwent macrocytic anemia on the fourteenth day of the recovery period. Appearance of

macrocytic erythrocytes accompanied by anemia is hematologically due to deficiencies of vitamin B_{12} and folic acid, and due to drugs inhiditing DNA synthesis (TAKAHISA 1978). It can not be ignored that increased demand of vitamins to metabolite the drug would evoke vitamin deficiencies. But OA and NA were acturally distributed at high concentions in the kidney of dosed yellowtail (UENO *et al.* umpublished data). Pyrimidine biochemically associates with DNA synthesis (MARTIN *et al.* 1983). These facts indicate that both OA and NA can inhibit DNA synthesis in erythroblasts of fish. Thus, the toxic effects of OA and NA would result in macrocytic anemia. Especially in fish dosed with OA, hematological disturbances were worse on the fourteenth day than on the first day after dosing. This indicates that the elimination of OA is very slow in yellowtail. Therefore, disturbances of DNA synthesis by OA are assumed to occur in organs with active DNA synthesis, the same as hemopoietic tissue. The toxicological effects of OA and NA on spermatogenesis and oogenesis will need to be examined in a further study.

On histopathological examinations, degeneration of hepatocytes and nephrosis occurred more severely in fish dosed with NA than OA. These pathological changes were recognized as manifestations of toxic effects of the drug on cells. NA was found to be more toxic to cells than OA, comparing fish administered with the same dose of the drugs. NA and OA have a propensity to evoke resistant mutants during therapy (ATLAS *et al.* 1969). This propensity would give rise to a problem of overdose during the medication. But overdose must be avoided to prevent toxic effects of the drugs.

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Table 1. Side-effects on feeding of dosed fish

	Dosing							Recovery period												
Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Control	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	
OA − 6	−	−	−	−	−	−	+		−	−	−	−	−	−	−	−	−	−	−	−	
OA − 20	−	−	−	−	−	−	+		−	−	−	−	−	−	−	−	−	−	−	−	
NA − 6	−	−	±	±	±	+	++		++	++	+	±	−	−	−	−	−	−	−	−	
NA− 20	−	−	±	±	+	+	++		+++	++	+	+	±	−	−	−	−	−	−	−	

−: normal, ±: slightly reduced, +: moderately reduced, ++: reduced, +++: markedly reduced,
Eighth day and twenty-first day: no feeding for sampling,
OA − 6: oxolinic acid 60mg/Kg B. W./day,
OA − 20: 120 mg/Kg B. W./day,
NA − 6: nalidixic acid 120mg/Kg B. W./day,
NA − 20: 400mg/Kg B. W./day.

Table 2. Effect of oxolinic acid on hematology

Dose	Control		OA − 6		OA − 20	
Date	1st day	14th day	1st day	14th day	1st day	14th day
No.	10	10	10	10	10	10
B. W.	483	528	502	550	527	562
B. L.	302	310	307	318	307	319
R. B. C.	497±32.4	491±30.4	487±3.2	449±28.4	475±3.0	427±19.1*
Ht	53.5±3.3	58.4±3.8	57.4±3.2	53.1±3.5	57.1±3.0	53.0±0.4
Hb	12.7±0.8	14.5±1.1	13.0±0.8	13.5±1.6	13.2±0.8	14.0±0.7
M. C. V.	107.9±4.6	118.9±1.8	118.2±6.0*	118.7±5.6	121.8±7.5*	124.1±6.5
M. C. H. C.	23.8±1.3	24.8±0.6	22.7±0.9	24.5±0.9	23.1±1.5	26.6±1.6
T. P.	4.2±0.2	4.8±0.1	4.1±0.2	4.1±0.3*	4.4±0.2	4.2±0.2*

Data: mean ± S. D., *: significantly different from control data (p less than 0.05)
OA−6: 60mg/Kg B. W./day, OA−20: 120mg/Kg B. W./day, B. W.: body weight (g),
B. L.: body length (mm), R. B. C.: red blood cell count ($X10^4/mm^3$), Ht: hematocrit (%),
Hb: hemoglobin (g/dl), M. C. V.: mean corpuscular volume (μ^3),
M. C. H. C.: mean corpuscular hemoglobin concentration (%),
T. P.: total protein in serum (g/dl).

Table 3. Effect of nalidixic acid on hematology

Dose	NA− 6		NA− 20	
Date	1st day	14th day	1st day	14th day
No.	10	10	10	10
B. W.	499	537	486	516
B. L.	303	312	304	314
R. B. C.	417±16.5*	475±21.9	429±27.1*	423±38.4*
Ht	46.3±2.3	52.8±0.3	59.2±2.7	49.9±4.7*
Hb	11.1±0.7*	13.6±0.6	11.4±0.5*	13.9±1.5
M. C. V.	111.0±3.0	111.3±6.1*	115.0 ±4.3	118.1±4.0
M. C. H. C.	24.0±0.5	26.0±0.9	23.1±1.0	27.7±0.9*
T. P.	4.4±0.3	4.2±0.3*	4.6±0.4	4.1±0.3*

Data: mean ± S. D.,
*: signficantly different fron control data (p less than 0.05),
NA−6: 120 mg/Kg B. W./day,
NA−20: 400 mg/Kg B. W./day.

Table 4. Effect of oxolinic acid on histopathilogy

	Control					Oxolinic acid 60mg/Kg B. W.										Oxolinic acid 120mg/Kg B. W.									
Date	1st day					1st day										1st day									
Findings / No.	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Hepatocytes																									
Atrophy	−	−	−	−	−	+	±	−	±	±	±	−	−	−	±	−	±	±	±	±	+	±	+	−	±
Fat reduction	−	−	±	±	±	++	±	−	±	±	+	±	±	±	+	±	±	±	±	±	±	+	++	±	+
Kidney																									
Nephrosis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	++	−	−
Spleen																									
Hemosiderosis	++	++	+	+	+	+	++	+	+	++	++	++	+	+	++	++	++	+	+	++	+	+	+	+	+
Heart	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	K	−	−
Brain	−	−	−	−	−	−	−	−	−	M	−	−	−	−	−	−	−	−	−	−	−	−	M	M	−
Date	14th day					14th day										14th day									
Findings / No.	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Hepatocytes																									
Atrophy	−	−	−	−	−	±	−	±	±	−	±	+	−	−	−	+	±	−	+	+	−	+	−	−	±
Fat reduction	±	±	−	+	−	+	−	+	+	−	+	+++	−	±	±	+	±	±	++	+	−	±	−	±	+
Kidney																									
Nephrosis	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Spleen																									
Hemosiderosis	+	++	++	+	++	+	±	+	+	++	+	±	++	++	++	+	+	+	+	+++	++	++	+	+	++
Heart	−	−	−	−	−	−	−	−	−	−	−	−	=	−	−	−	−	−	−	−	−	−	−	−	−
Brain	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	M	M	−	−	−	−	M	−	−	M

−: no change, ±: very slight, +: slight, ++: moderate, +++: severe,
K: *Kudoa pericardialis*,
M: *Myxobolus buri*.

Table 5. Effect of nalidixic acid on histopathology

	Nalidixic acid 120 mg / Kg B. W.										Nalidixic acid 400mg / Kg B. W.									
Date	1st day										1st day									
Findings / No.	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Hepatocytes																				
Atrophy	±	+	±	+	±	±	+	−	++	++	+	+	++	+	+	+	±	±	+	++
Fat reduction	+	++	+	++	+	+	++	±	+++	+++	++	+	+++	++	++	+	+	±	++	+++
Kidney																				
Nephrosis	±	±	+	+	±	±	++	+	+	±	++	++	−	−	++	+	+	±	±	++
Spleen																				
Hemosiderosis	+	++	+	++	++	+	++	+	+	+	+	++	++	++	+++	+	++	+	+++	++
Heart	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Brain	−	−	−	M	−	−	M	−	M	−	M	−	−	M	−	−	−	−	−	−
Date	14th day										14th day									
Findings / No.	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Hepatocytes																				
Atrophy	++	+	±	±	±	±	+	±	+	+	−	+	+	+	+	+	−	+	+	±
Fat reduction	++	+	−	±	+	+	++	±	+	++	±	+	±	+	+	−	+	++	++	+
Kidney																				
Nephrosis	−	±	±	−	−	−	±	±	±	−	±	−	−	+	+	+	+	±	−	−
Spleen																				
Hemosiderosis	+	+	±	±	+	+	+	+++	+	++	++	++	+	+	++	++	++	++	++	+
Heart	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Barin	−	M	M	−	−	−	−	−	M	−	M	−	−	−	−	M	M	−	−	−

−: no change, ±: very slight, +: slight, ++: moderate, +++: severe,
M: *Myobolus buri*.

Explanation of Plate I

Fig. 1 Liver of control fish. H−E, X400.

Fig. 2 Kidney of control fish. H−E, X320.

Fig. 3 Spleen of control fish. Berlin blue, X320.

Fig. 3 Liver of fish dosed with OA−20, on first day after dosing. Hepatocytes show fat reduction with atrophy. H−E, X320.

Explanation of Plate II

Fig. 1 Liver of fish dosed with OA−20, on fourteenth day of recoverery period. Hepatocytes show fat reduction with atrophy. H−E, X320.

Fig. 2 Kidney of fish dosed with OA−20, on first day after dosing. Nephrons and hemopoietic tissue do not show any change indicating toxic effect of OA. H−E, X320.

Fig. 3 Spleen of fish dosed with OA−20, on first day after dosing. Hemosiderin (displayed black) deposition is comparable to the control. Berlin blue, X320.

Fig. 4 Liver of fish dosed with NA−6, on first day after dosing. Atrophy of hepatocytes is severe. H−E, X320.

Explanation of Plate III

Fig. 1 Kidney of fish dosed with NA−6, on first day after doring. Hyaline droplet degeneration(H) in tubular epithelial cells. H−E, X320.

Fig. 2 Liver of fish dosed with NA−20, on first day after dosing. Hepatocytes show fat reduction with atrophy. H−E, X320.

Fig. 3 Kidney of fish dosed with NA−20, on first day after dosing. Hyaline droplet degeneration(H) in tubular epithelial cells. H−E, X320.

Fig. 4 Spleen of fish dosed with NA−20, on first day after dosing. Hemosiderosis is comparable to the control(Plate I −3). Berlin blue, X320.

Plate I

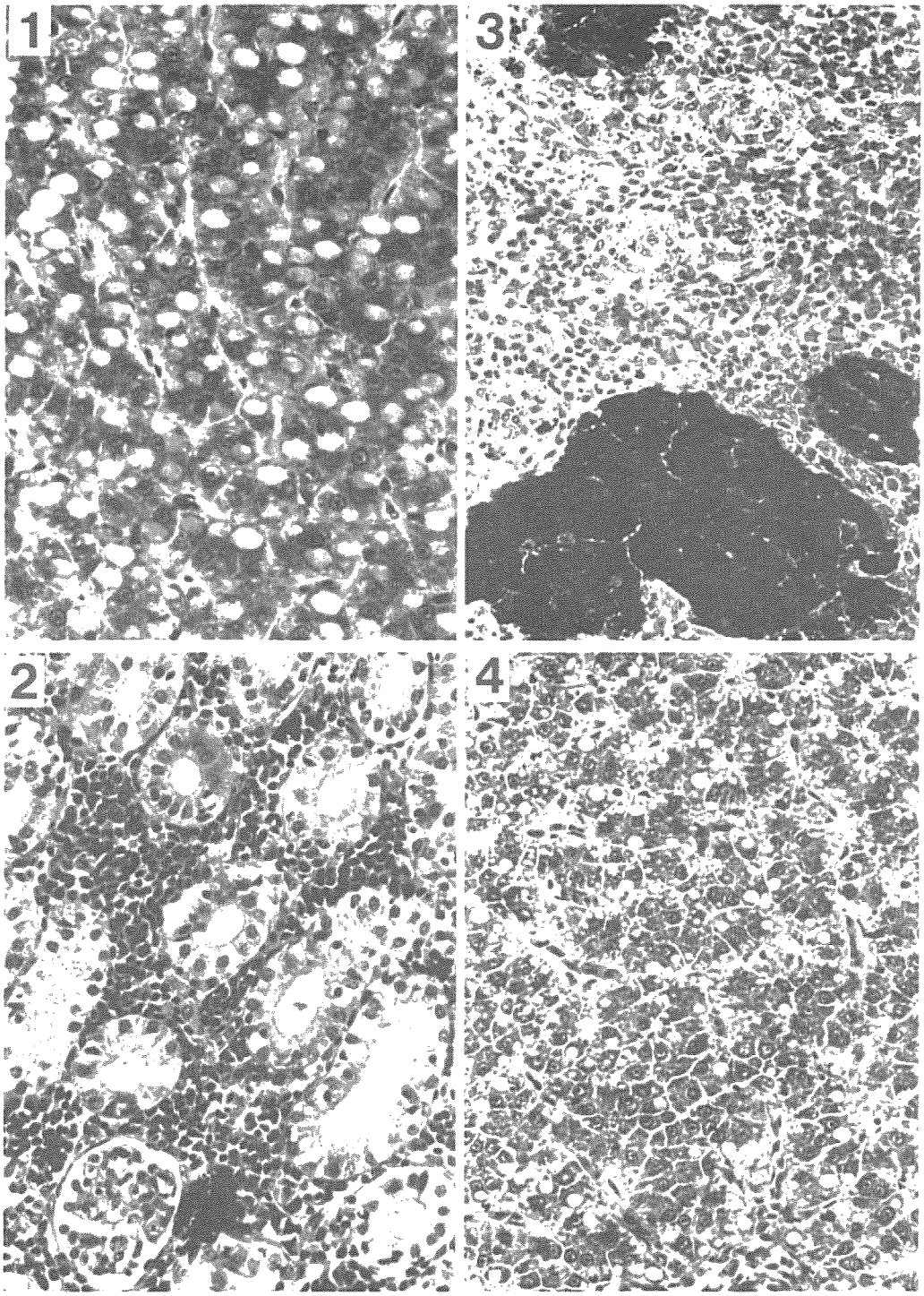

Plate II

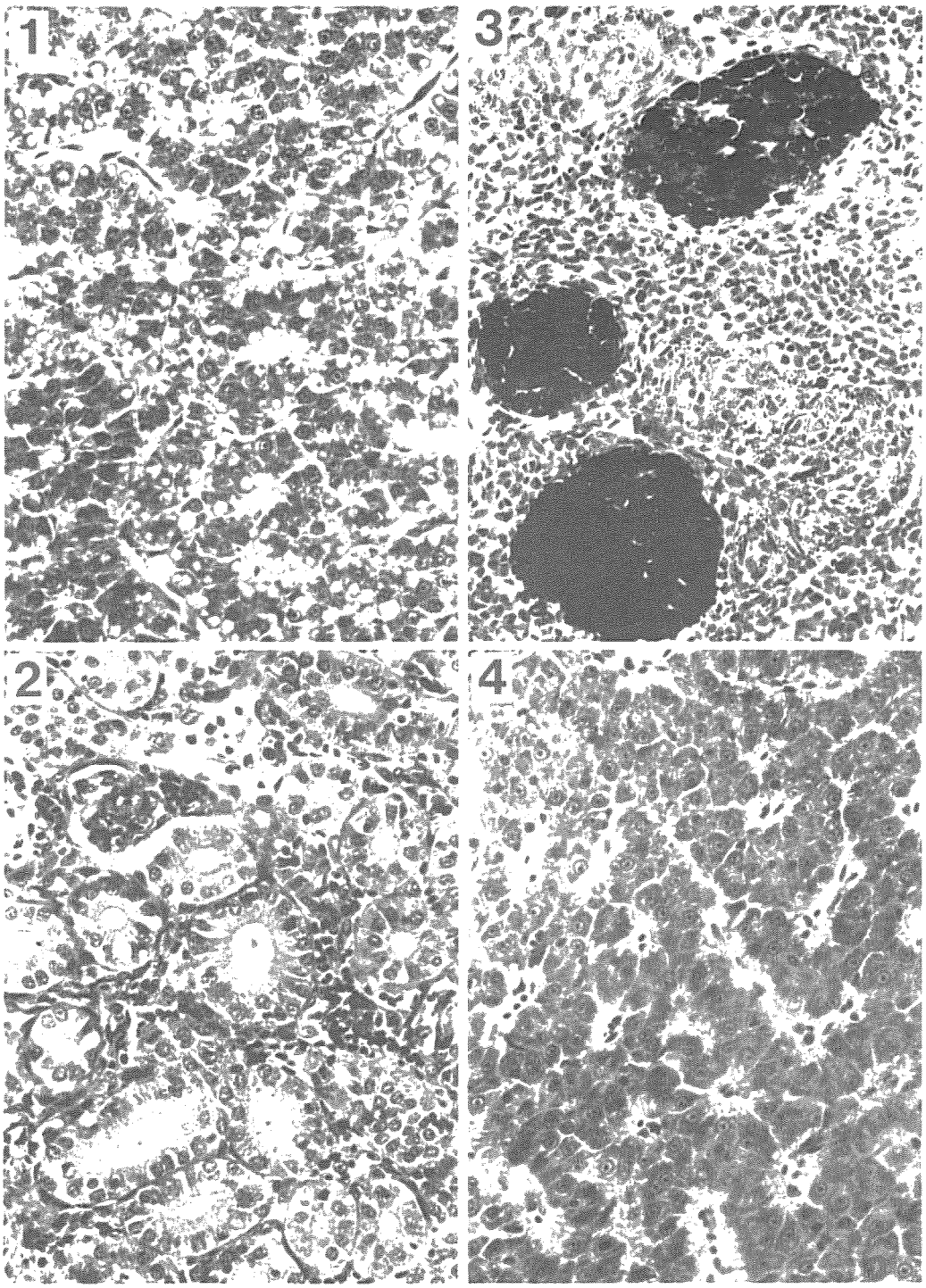

Plate III