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Determination of formaldehyde residue and histopathological observation in formalin and neutral-formalin treated Korean rockfish(*Sebastes schlegeli*)

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In this study, Residue of formaldehyde and histopathological changes in formalin and neutral-formalin in treated fish(*Sebastes schlegeli*) were observed at two different temperatures(15 and 25℃). Immediately after in treatment, residue of formaldehyde in formalin treated fish was show little bit higher than in neutral-formalin treated fish at 15℃. But, there is no difference at water temperature 25℃. The elimination of formaldehyde was markedly temperature-dependent. The approximated withdrawal time were 72hr and 24hr at water temperature 15 and 25℃. Formalin was more toxic than the neutral-formalin at the same condition. Intensity of tissues damage was increased with increasing concentration of chemical and temperature. Formalin and neutral-formalin treatments caused edema and seperation of epithelium, winding of secondary gill lamella, necrosis in the gill ; congestion and pycnosis, vacuolation in the liver ; hydropic and granulated degeneration, necrosis of epithelial cells in the proximal renal tubule ; increasing mucus cells, cracking, necrosis of epidermis and dermis in the skin tissue.

Key words : Formaldehyde, Neutral-formalin, Formalin, *Sebastes schlegeli*, Histopathology

There are many fish diseases in high density culturing fish husbundary caused by environmental pollutions, bacteria and external parasites(protozoan), and so forth.

In these factor, parasites cause directly harmful effects on fishes and secondary viral infection, especially from spring to late summer. Also, in cultured fish populations, the presence of dense populations of fish kept in particular environmental conditions may favour certain parasite species so that the parasite population increases rapidly to a very high level. It is very serious problem in econmic raised since earlier aquaculture.

Thus, the number of species of fish parasites have been reported by parasitologist and some of chemicals, in order to control, developed in many way. Use of aquaculture chemicals has proven to be an effective way of controlling both noxious weeds and outbreaks of diseases in fish pond, and hatcheries (Tucker and Boyd, 1985). There are many therapeutic and prophylatic agents as well as herbicides available. Two of these com-

monly used chemicals are formalin and copper sulfate (Reardon and Harrell, 1990).

Between the two, formalin have been widely used in fish husbundary for the control of protozoan and such external parasites as *Costia* sp., *Gyrodactylus* sp. and *Trichodina* sp. Formalin(Formaldehyde 40% dilluted solution), produced by contact reaction of the methylalcohol and the air from $CH_2OH+O \rightarrow HCHO+H_2O$, is very strongly stimulative chemical and may can kill almost micrroorganism with a extremely small quantities and exhibit a effect of sterelization. When formalin is handled, however, formaldehyde vapours at a hatchery are often objectionable, because of they are caused burning of the eyes and skin, weeping, irritation of the upper respiratory passages.

It was revealed that an imprudent formalin use, not considering the species-specificity of fishes and other environmental conditions, cause such severe damage as histopathological changes in gill, liver, kidney, skin tissues. Therefor, an important consideration in the use of chemical for treatment is a knowledge of the tolerance level of fish and the effect of the chemical on fish tissues (Cruz and Pitogo, 1989). Histopathological study of formalin toxicity to some species have been conducted and nowaday, health hazards of formaldehyde have stimulated interested in residue of formaldehyde in seafood (Radford and Dalsis, 1982) but basic information of this is very insufficient.

Formalin reacts with many chemicals in the water to produce compounds that might be toxic to fish. These reations are dependent on the acidity or alkalinity of the water temperature, metabolic products from the fish, waste from excess food. And formalin develop a white cake paraformldehyde precipitate polymer under old, cool storage and when react with bicarbonate in the sea water. It was reported that paraformaldehyde cause more toxiside effects on fishes when suspending in the water. Until now, however, there is no report that infulence of paraformldehyde on treated fish and investigation of factor which effect to formaldehyde reaction.

For that reasons, in this study, residue of formaldehyde and histopathological changes in formalin and neutral-formalin, completely removed the paraformaldehyde, in treated fish were compared. And also, infulence of temperature was observed in order to render basic information on successful treatment.

Meterial and Methods

Korean rockfish, *Sebastes schlegeli*, culturing in Korea, were obtained from hatchery in Tol-san, Yochun kun, Chunnam province. Fishes were acclimatized and rared in circular-filteration tank which equiped with raring tank(78cm×76cm×55 cm) and filtering tank(79cm×76cm×59cm) for four month at 15 and 25℃. All tanks were aerated and fishes were starved 24 prior to and during the entire experimental period. All tests were conducted under stastic conditions in black glass-closed system aquaria filled with 20ℓ of clean sea water. Apparently healthy Korean rocktish average body weight 30± 1 g were selected for the test. Each testing tank contained 15 fish per 20ℓ. Each test concentrations used were 0, 100, 300, and 500 ppm formalin and neutral-formalin, with three replicates per concentration. Exposure time was one hour and each of these treatment was tested at two different water temperatures

15 and 25℃. Right after the exposure, fishes were immediately moved into the water tank filled with fresh sea water. Samples of each testing group were rendomly removed at the desired time intervals(hours 0, 24, 48, and 72hr) for determination of formaldehyde residue and histopathological observation. Colorimetric procedures was used for determination of formaldehyde. Samples taken from exposure group were homogenized. Sample homogenate 20 g was distillated with Kjeldahl distillation apparatus. 2 mℓ Nash,s regent and 1 mℓ H_2O were added to 1 mℓ distillated solution, and heated 30 minute in H_2O bath at 37± 1℃ to develop the color. The colored solution transferred to 1cm cell and mesured at 415 nm with spectrophotometer(AOAC, 1990). Statical analysis of formaldehyde residue was calculated using by student,s t test($p<0.05$). Gill, liver, kidney and skin tissues of each sample for histological examination were fixed with Bouin,s solution for at least 24 hr. Dehydration and clearfying in a graded series of alcohol-xylene and embadded in paraffin. Tissue sections were cut at 5~6 μm and stained with haematoxylin and eosin. Stained tissues were examined microscopically.

Results and Discussion

During the experiment, fishes(Sebastes schlegeli) used for test were survived except 60% mortality in 500 ppm formalin treatment at 25℃.

Fig. 1 showed that residual quantity and withdrawal time of formaldehyde in course of time after the one hour exposure at two different temperature 15 and 25℃. In formalin treatment(Fig. A, B), right after the exposure, formaldehyde residue was showed little bit high in proportion to each concentration at 15℃. After 72 hours, however, all of each equipment was showed less than 1.0 ppm. Under water temperature 25℃ condition, formaldehyde residue was showed little low compare to 15℃ and after 24 hours, it was showed less than 1.0 ppm.

In neutral-formalin treatment(Fig. C, D), formaldehyde residue was showed low compare to formalin treatment right after the exposure at 15℃. Also, it was showed lower than 1.0 ppm at all tests after 72 hours. Under 25℃ condition, residue of formaldehyde was not differ from formalin treatment. After 24 hours, it was showed less than 1.0 ppm at all test.

In previous report, Bjorklund *et al.*, (1991) showed that residue of oxolinic acid and oxytetracyclin, administered to salmonid fish mixed in feed for treatment of fish diseases caused by Gram-negative bacteria, were completely eliminated with 10~15 days and 25~30 days. These result are similar to present study. Merely for differentiation of withdrawal period, it was assumed that antibiotics, administered with food and react in the body, were stayed longer than formaldehyde which has strong volatility and react outside the body.

On the other hand, however, formaldehyde develops post-mortem in marine fish and crustaceas, probably from enzymatic reduction of trimetylamine oxide(Radford and Dalsis, 1981).

As a result in this study, excretion of chemicals to outside the body were highly dependent on temperature. The approximated withdrawal time of formaldehyde were 72hr and 24hr at 15 and 25℃. This is in accordance with Bjorklund and Bylund *et al.*, (1990) who described that the half-life of oxytetracycline in rainbow trout serum was 4.8 days at 16℃, 6.1 days at 10℃ and 8.9 days

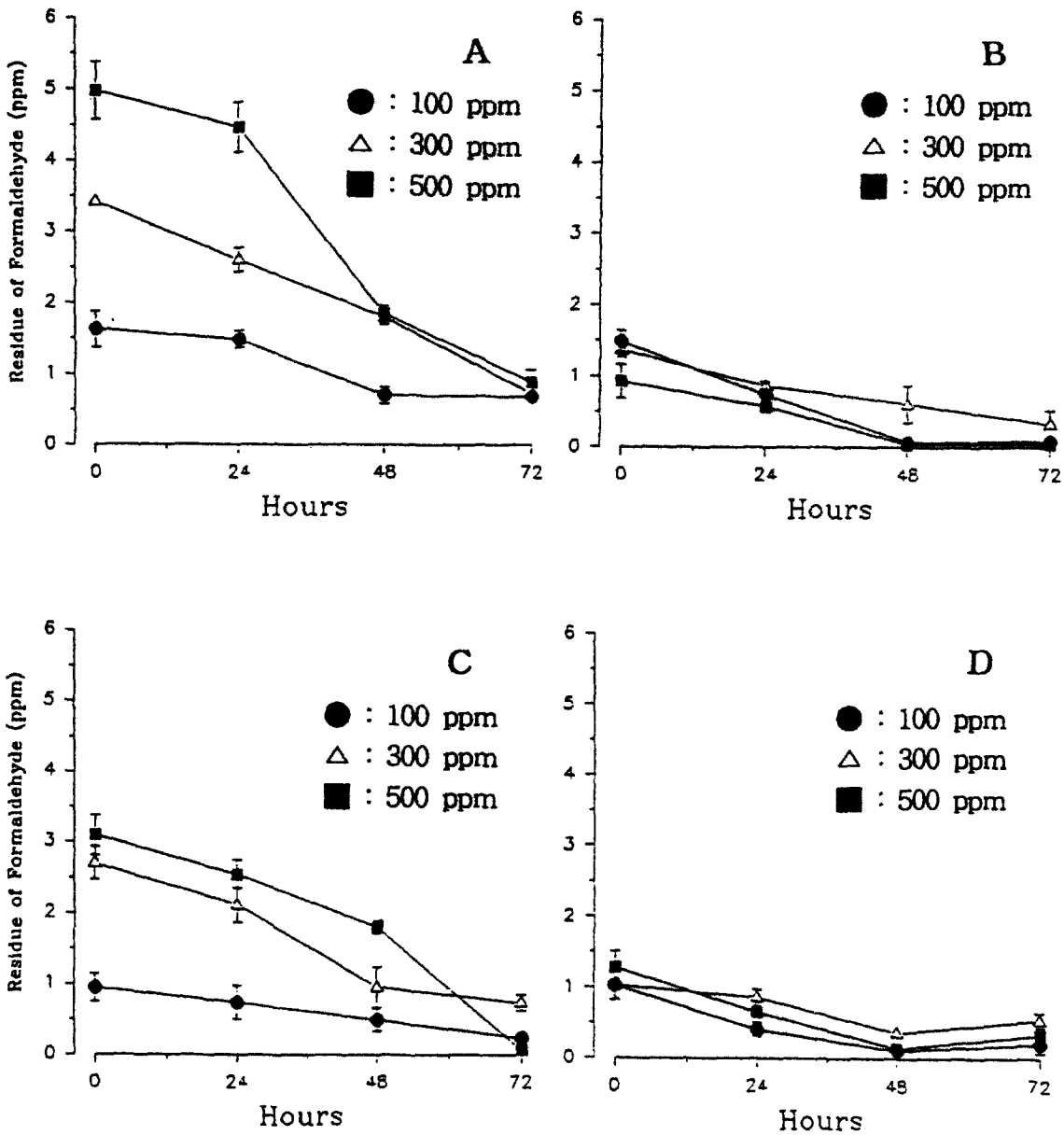

Fig. 1. Residue of formaldehyde in formalin treated korean rockfish at 15℃(A), 25℃(B), and in neutral-formalin treated fish at 15℃(C), 25℃(D).

at 5℃. Especially in this study, residue of formaldehyde was influenced by physiological behavior of fish react to toxic condition. Right after the formalin treatment, formaldehyde residue between at 15 and 25℃ make difference about 3± 0.5 ppm in 500 pmm, 2± 0.5 ppm in 300 ppm. And, stressful unusual behavior and vommiting of fishes were observed in high concentration and temperature.

Moreover, there is no significantly difference of formaldehyde residue in low concentration at two different temperature conditions. It was asummed that absortion of formaldehyde showed little at high temperature by activation of species-dependent behavior. But, in spite of defence reaction, there were high mortality in a short period beyond at tolerance limit condition.

In a result of histopathological observation, it was demonstrated that formalin was more toxic than the neutral-formalin which completely eliminate paraformaldehyde(white precipitate) at the same condition. Similar result was reported by Rucker *et al.*, (1963) indicated that formalin which contained paraformaldehyde more toxic than clean formalin in 3-inch chinook salmon. And, in high temperature was more toxic than in low. This would indicated that intensity of tissues damage were dependent on temperature. Therefore, it should be considering that the relationship between concentration and temperature of treatment in fish husbundary and hatchery.

Histpathological changes in the gill, liver, kidney and skin tissues were observed for all treatment. Lesions of each tissue were similar for all treatment, althought the intensity of cell damage increased with increasing concentration and temperature.

At first, fish gill, most important organism for survive, are effected by external conditions. The normal histology of the gill fillament is shown in (Plate. 1-①). This structure showed that uniformed arrangement of gill lamella and close adhesion of each caillary wall and epithelial cell. Following exposure to chemicals, differences in histological structure were noted. edema with thrombosis of gill lamella was observed in fish treated with 300 ppm formalin at 15℃(Plate. 1-②). seperation of epithelium and winding of secondary gill lamella occurred in 300 ppm at 25℃(Plate. 1-③). The most extreme gill damage was noted in moribund fish exposed to 500 ppm at 25℃(Plate. 1-④). Proliferating undifferentiated cells fill the interlamellar regions resulting in fused secondary lamellae in these fish. These histological changes were similar with lesions caused by aluminum and formalin treatment (Mueller *et al.*, 1991 ; Wedemeyer and Yasutake, 1974). Epithelial seperation can cause asphyxiation, partial or complete loss of secretory or excretory function, impairment of oxygen-carbon dioxide exchange, and loss of plasma electrolytes and protain(Smith and Piper, 1972).

Plate. 2-① shows normal liver tissue from control fish. edema in company with congestion and pycnosis of liver cell were observed in 300 ppm neutral-formalin at 15℃, 24hr after the treatment(Plate. 2-②). Particularly, vacuolation of hepatocytes was observed. in formalin 300 ppm treated fish at 25℃(Plate. 2-③). This lesion may be due to temperatual stress on toxicity responce. Vacuolation phenomenon was reported to occur in winter flounder exposed to copper sulfate and rainbow trout exposed to ammonia(Soderberg, 1985 ; Baker, 1969). And plate. 2-④ was showed severe necrosis of liver tissue in 500 ppm formalin treatment at 25℃.

The kidney tissue was characterized by tubules surrounded by hemopoetic tissue. Normally the tubular cells were well formed with little cellular debris within the tubule lumen(Plate. 3-①). Following the treatment, slight swelling of cytoplasm and nuclear(Plate. 3-②) was showed in 300 ppm neutral-formalin treated fish at 15℃. Hydropic

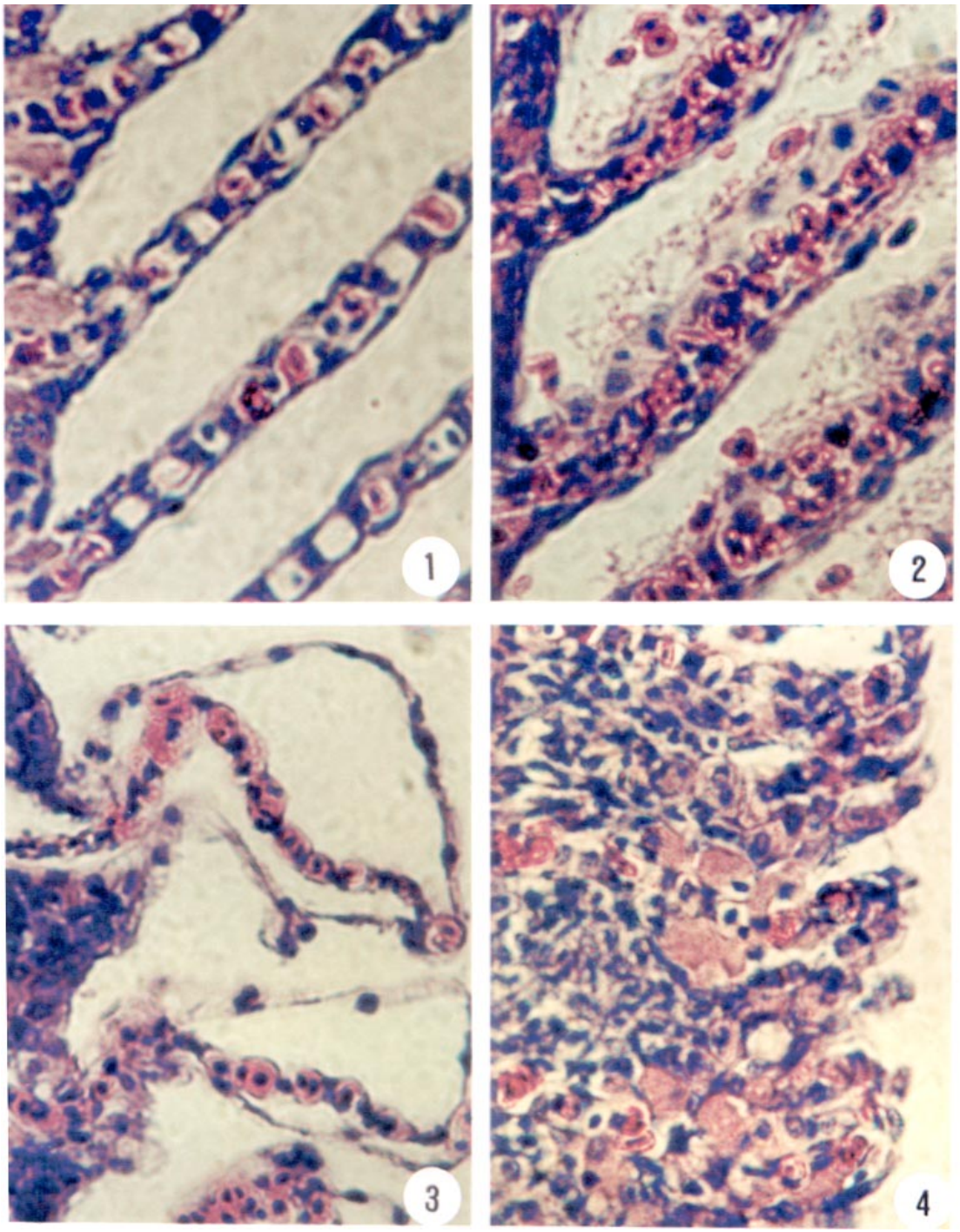

Plate. 1. ① : Normal gill tissue from control. ×450. ② : edema and thrombosis of gill lamellae. ×450. ③ : seperation of epithelium and winding of secondary gill lamellae. ×450. ④ : proliferating undifferentiated cells fill the interlamellar regions resulting in fused secondary lamellae. ×450.

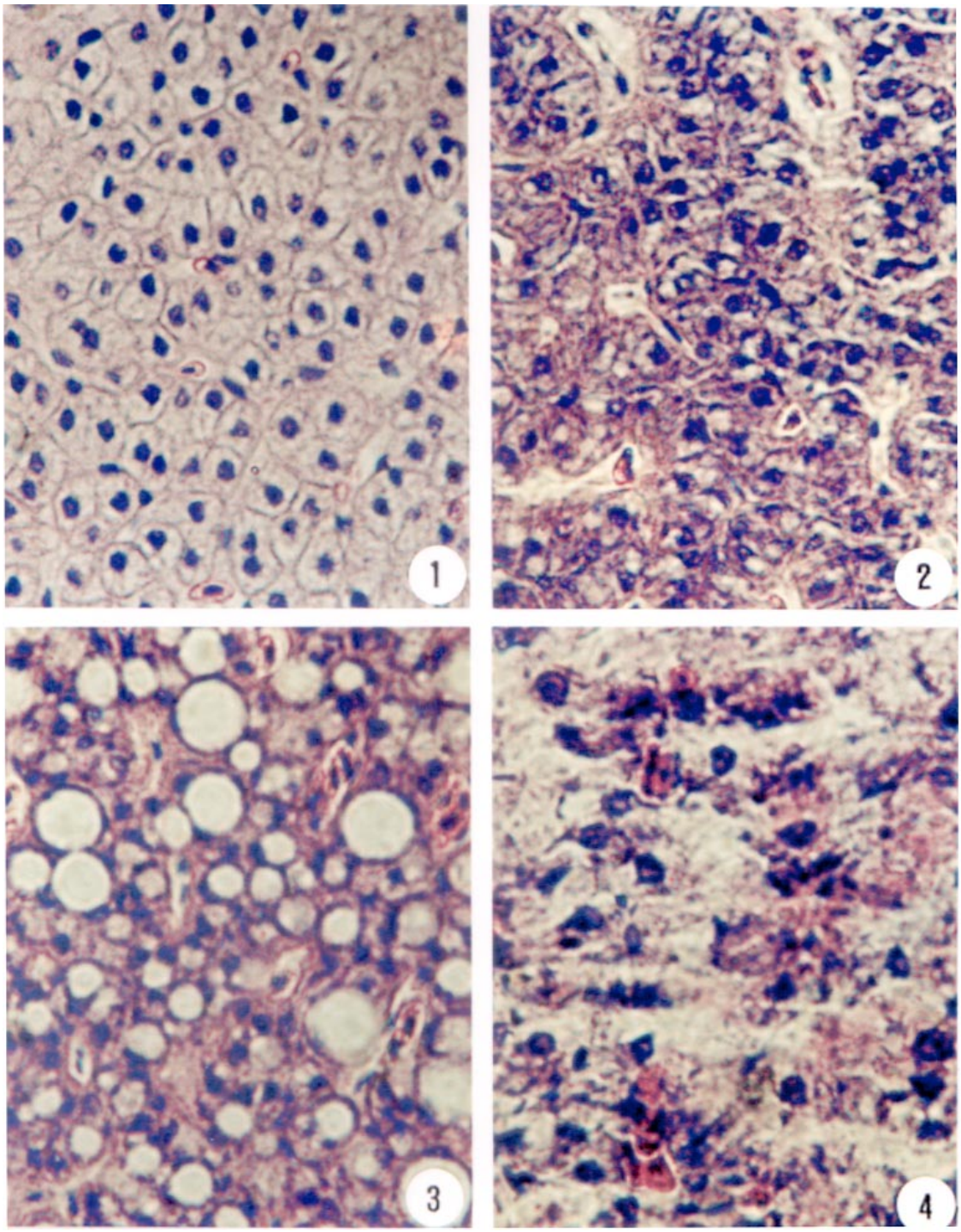

Plate. 2. ① : Normal liver tissue ×450. ② : edema in company with congestion ×450. ③ : vacuolation of hepatocytes ×450. ④ : severe necrosis ×450.

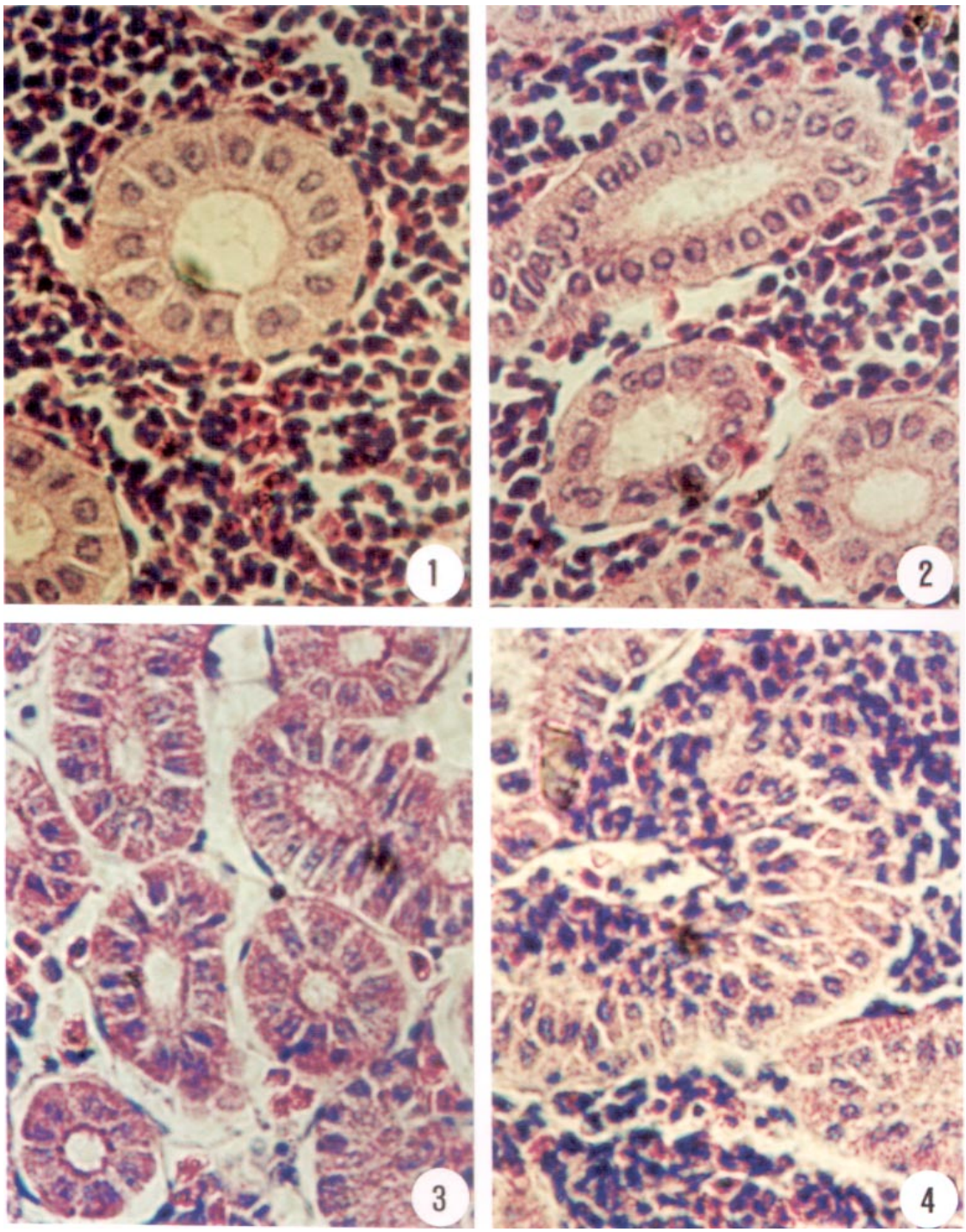

Plate. 3. ① : Normal kidney tissue ×450. ② : slight swelling of cytoplasm and nuclear ×450. ③ : hydropic and granulated degeneration of epithelial cell of renal tubule ×450. ④ : severe necrosis of entire kidney tissue ×450.

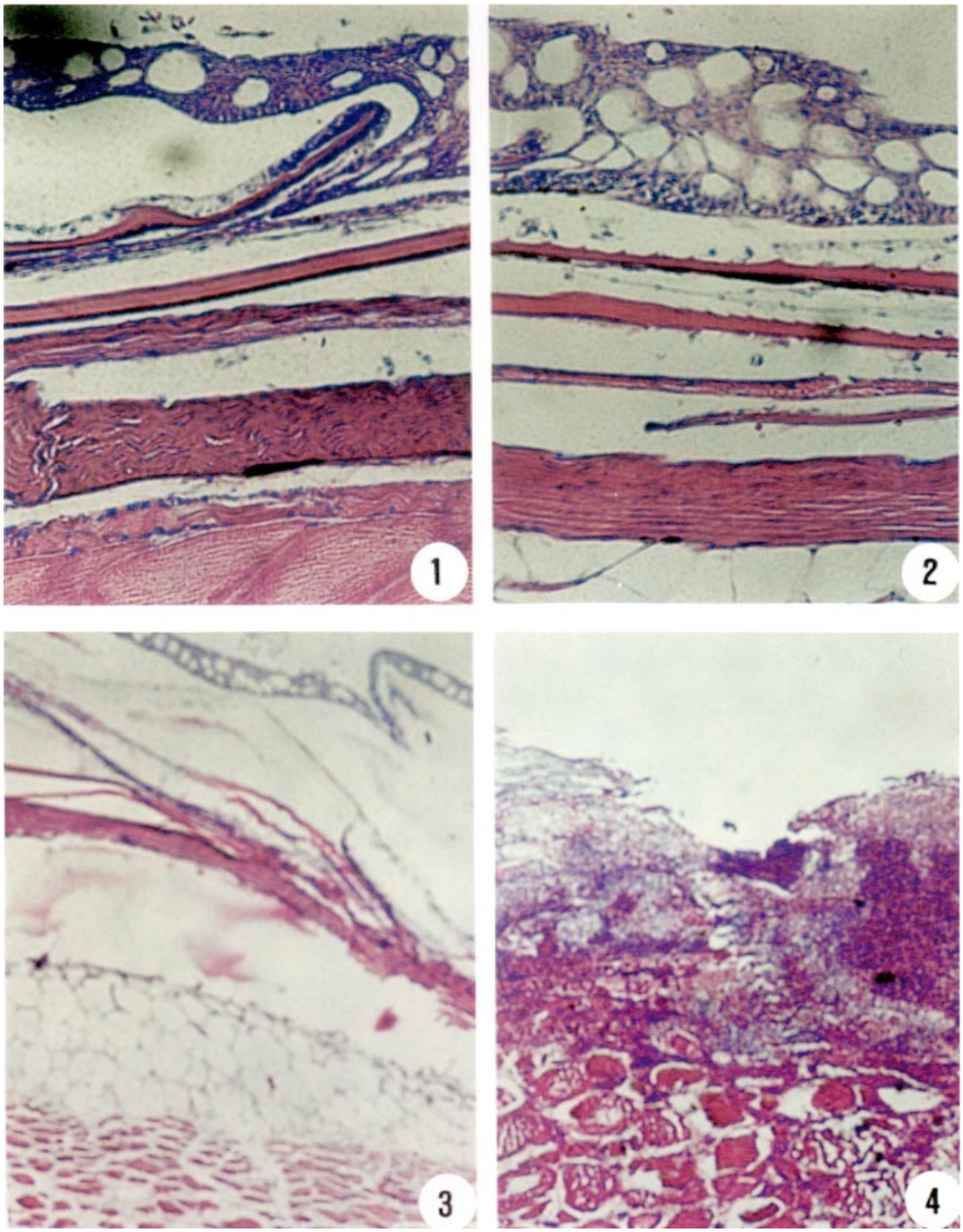

Plate. 4. ① : Normal skin tissue was show that epidermis, dermis, hypoderma and muscle layer were well formed ×450. ② : increasing of mucus cells in epidermis ×450. ③ : cracking of dermis and hypoderma ×150. entirely necrosis and infusion of skin tissue ×450.

and granulated degeneration was noted in fish exposed to 300 ppm formalin and neutral-formalin at 25℃(Plate. 3－③). In 500 ppm at 25℃, severe necrosis of entire kidney tissue was observed (Plate. 3－④). Similar results were occurred in estuarine teleost exposed to cadmium (Gardner and Yevich, 1970).

Normal skin tissue was showed that epidermis, dermis, hypoderma and muscle layer were well formed in control(Plate. 4－①). Skin tissue is directly effected on external conditions. After the treatment, increasing of mucus cells(Plate. 4－②) was shown in neutral-formalin treated fish at 15℃. And cracking of dermis and hypoderma(Plate. 4－③) were observed(formalin 300 ppm at 25℃). The most extreme skin damage, entirely necrosis and infusion, were occurred in moribund fish(500 ppm, at 25℃)(Plate. 4－④).

Process of lesions had no relation to residue of formaldehyde. Especially in 500 ppm treatment, there was no particular recovery to the end of experiment. Cruz and Pitogo, (1989) was described that partial recovery of tissues was observed in fish after 10 days in formalin-free sea water.

These results of formaldehyde determination and histopathological observation, considering the fact that formalin widely use for control of external parasites in fish husbundary, will be a basic information of aquaculture food safety and effective treatment.

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포르말린과 중성포르말린 약욕한 어류의 Formaldehyde 잔존량 측정과 병리 조직학적 관찰

조 재 권 · 양 한 춘

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이 실험에서는 조피볼락을 대상으로 중성 formalin과 formalin으로 약욕하여 잔존량과 잔존기간 및 병리 조직학적 변화를 관찰하고 온도가 미치는 영향에 대해서 알아보았다. formalin 과 중성 formalin의 잔존량은 15℃일 때 약욕 직후 약간의 차이를 제외하고는 별다른 차이를 보이지 않았다. 잔존기간은 수온 15℃와 25℃ 조건에서 각각 72 hr과 24 hr으로 온도의 영향을 많이 받는 것으로 나타났다. 어체에 미치는 독성은 중성 formalin보다 formalin이 강하게 나타나고 수온과 농도가 증가 할수록 조직에 미치는 영향이 심해졌다. formalin약욕으로 인하여 아가미 조직에서는 부종과 상피세포의 박리, 뒤틀림, 괴사증상이 그리고 간에서는 부종과 핵농축, 공포화 현상이 나타나고 신장에서는 수증성 퇴행적 병변과 세뇨관 상피세포의 과립화 증상, 괴사가 일어나고 피부에서는 점액 세포의 증가와 표피의 괴사 및 피하조직과 진피층의 균열이 나타났다.

Key words : Formaldehyde, Neutral-formalin, formalin, *Sebastes schlegeli*, Histopathology