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STEREOLOGICAL ANALYSIS OF ARSENIC-INDUCED CYTOLOGICAL STRUCTURES

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ABSTRACT

Necrotic and fibrous bodies have been reported to occur with greater frequency in hepatocytes of green sunfish accumulating higher levels of arsenic during environmental exposures. These structures are believed to represent nonspecific degenerative changes rather than changes specific for arsenic poisoning. The accepted pattern of hepatocyte degeneration and necrosis begins with the ballooning, then shriveling, of dying cells followed by extrusion of the markedly shriveled, pyknotic hepatocyte from the hepatic cord into the sinusoid. Dying hepatocytes are also engulfed by neighboring parenchymal liver cells by heterophagy. Three pieces of evidence suggest that the necrotic body is the end product of unicellular death. Necrotic bodies occasionally contain what appears to be disintegrating organelles similar to those observed in hepatocytes. These bodies, in addition, are shriveled and highly pyknotic; moreover, they occur in a variety of locations within the liver of the green sunfish (i.e. within hepatocytes, between hepatocytes within the hepatic cord, in the space of Disse, and within the sinusoid). Fibrous bodies, although occasionally pyknotic, generally occur within or between hepatocytes. Stereological methods were, therefore, employed to mathematically describe the necrotic and fibrous bodies and to determine whether statistically significant changes in the volume and surface of various components occur to indicate that the necrotic bodies "age" on the basis of definite trends in compartmentalization.

INTRODUCTION

Environmental exposure of *Lepomis cyanellus* (green sunfish) to arsenical wastes has been reported to result in the appearance of two structures in the liver—the Both structures were present in fish livers which had accumulated from 6 to 64 ppm arsenic, on a dry weight basis, and which also showed submassive necrosis, irregular lobular architecture, abnormally-shaped hepatic parenchynal cells, and numerous nucleolar abnormalities (Sorensen *et al.*, 1980a). Necrotic and fibrous bodies, as well as other abnormalities, were reported to occupy a greater volume of the hepatocyte in livers containing higher levels of arsenic (Sorensen *et al.*, 1980a).

Nonspecific degenerative changes, such as hepatic necrosis (Gilderhus, 1966; and increased numbers of autophagic vacuoles (Brown *et al.*, 1976; Yeh *et al.*, 1974), have been reported following arsenic poisoning. Human exposure to arsine, a toxic arsenical gas, has been reported to result in the appearance of the Councilman, or acidophilic, inclusion body which is recognized as the remnant of unicellular death of hepatic parenchymal cells (Neuwirtova *et al.*, 1961). An entire hepatocyte is transformed into a single-membrane delimited, Councilman or acidophilic structure which is eventually engulfed during parenchymal hepatocyte heterophagy (Hug. 1973). Councilman bodies and necrotic bodies share certain characteristics: both are located within hepatocytes, within liver plates between hepatocytes, in the space of Disse, and in the sinusoidal lumen. Both structures contain nuclei and remnants of organelles at some stage of disintegration, are surrounded by a single membrane, are spherical, and lack the Golgi apparatus, as well as fibrillar arrays of material. The necrotic body, like the Councilman body, is therefore assumed to be the product of unicellular degradation of entire hepatocytes.

Fibrous bodies also appear to represent nonspecific degenerative changes, morphologically akin to Mallory's hyalin body which has been observed in the livers of

According to Tanikawa's studies, dying hepatic parenchymal cells balloon, then shrivel, becoming increasingly pyknotic prior to extrusion into the sinusoid from the hepatic cord (Tanikawa, 1968). Hug's studies show that single dying hepatocytes can be engulfed by neighboring hepatic parenchymal cells by heterophagic mechanisms (Hug, 1973). It was, therefore, assumed that the variable pyknosity observed in necrotic bodies of green sunfish was due to a unicellular aging process whereby single dying cells are reduced in size for eventual extrusion or heterophagy. This hypothesis was tested using well-established stereological procedures for volume and surface density estimation of subcellular components.

MATERIALS AND METHODS

Fish determined to be less than three years of age (from scale annuli counts) were collected from Municipal Lake in Bryan, Texas, three years following the first report of elevated arsenic levels in the water; therefore, fish were assumed to have been exposed for an entire lifetime. Arsenical wastes (including calcium arsenate, arsenic acid, and arsenic trioxide) leached from earthen waste ponds into Municipal Lake and were presumably biomethylated to form inorganic arsenicals, as has been reported in other studies (McBride and Wolfe, 1971; Wood, 1974). The green sunfish collected for this study were, therefore, probably exposed to a variety of chemical forms of both organic and inorganic arsenic.

Livers of fish were cut into about ½ mm cubes for fixation in phosphate-buffered 2.5% gluteraldehyde, post-fixed in buffered 1.0 or 1.3% osmium tetroxide, washed in buffer and water, *en bloc* stained in 1% aqueous uranyl acetate, and dehydrated in ethanol and propylene oxide prior to embedding in Epon 812. Thin (pale gold) sections were cut with a diamond knife on a Sorvall MT-1 ultramicrotome, placed on 200 mesh copper grids, stained with uranyl acetate and lead citrate, and photographed at 3000 × by an unbiased sampling procedure on a Hitachi HV 11-E electron microscope operating at 50 kV.

For stereological analyses, a 100 point multipurpose test system having 50 equal lines ($z = 6$ mm) was cast at random over prints of necrotic bodies or fibrous bodies. Necrotic bodies were placed in categories based upon exact location within various regions of the liver. Based on well-established morphometric procedures, three-dimensional information can be obtained from two-dimensional data provided adequate numbers of micrographs are measured (Weibel and Bolander, 1973). In this study about forty necrotic bodies and about thirty fibrous bodies were measured, providing 3704 and 886 test points, respectively, fro a confidence level of 96 to 98% for the necrotic body and 86 to 97% for the fibrous body. Volume density of vesticles (V_{ves}), cisternal space V_{cis}), electron dense deposits (V_{edd}), and bundles of fibrous material (V_{fib}) were measured as the fraction of points falling over these structures compared to those falling over the entire body. Surface density of density (S_{ves}) and cisternae (S_{cis}) of the necrotic body was determined by using the following formula:

$$S = 2I_l/L_T$$

Where S is the surface density of vesicles or cisternae, L_l is the total number of intersections wit hthe specified membrane, and L_T is the total line length (Weibel and Bolander, 1973).

RESULTS AND DISCUSSION

Volume density estimates of the subcomponents of all necrotic bodies, regardless of location, show that about 29% of the entire structure is represented by cisternal (i.e. 12%) and vesicular (i.e. 17%) volume. Only 4% of the volume of the average necrotic body is composed of electron dense deposits. Theoretically, one might expect to see necrotic bodies of increased pyknosity and reduced cisternal and vesicle volume in the direction: space of Disse > parenchymal hepatocyte > hepatic cord—assuming that necrotic bodies represent single dead or dying hepatic parenchymal cells which have been engulfed from the hepatic cord by heterophagy and gradually processed by hepatic cells prior to extrusion from these cells into the space of Disse and finally into the sinusoid. Theoretically, the entire process might represent digestion of dying cells to minimize nutrient loss from the body.

TABLE I. *Volume and surface density estimates for necrotic bodies located in different regions of the liver. Values are expressed as mean fractional volume or surface ± one standard error of the mean; the number (n) of necrotic bodies in each group is indicated.*

Location	n	S_{ves}[a]	S_{cis}[b]	V_{ves}	V_{cis}	V_{edd}[e]
Parenchymal cell	12	0.09 ±0.02	0.27 ±0.09	0.18 ±0.04	0.09 ±0.03	0.04 ±0.02
Hepatic cord	25	0.05 ±0.01	0.28 ±0.06	0.11 ±0.03	0.14 ±0.03	0.07 ±0.02
Space of Disse	22	0.11[f] ±0.02	0.24 ±0.06	0.21[f] ±0.04	0.12 ±0.02	0.01[f] ±0.01
Mean		0.08 ±0.02	0.26 ±0.01	0.17 ±0.03	0.12 ±0.01	0.04 ±0.01

[a] Surface density of vesicles (ves) in mm^{-1}.

[b] Surface density of cisternae (cis) in mm^{-1}.

[c] Volume density of vesicles (ves) in cm^{o}.

[d] Volume density of cisternae (cis) in cm^{o}.

[e] Volume density of electron dense deposits (edd) in cm^{o}.

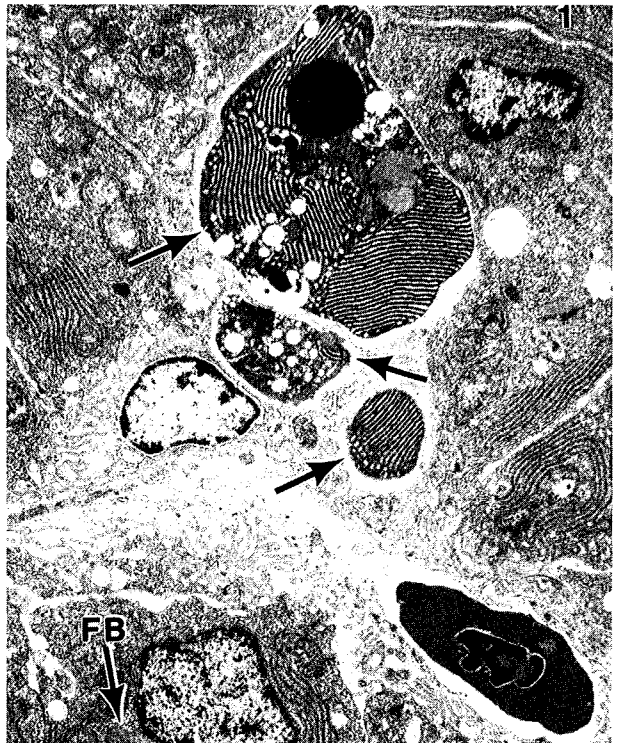
[f] Differs from necrotic bodies in the hepatic cord, $p < 0.05$.

The groups into which individual hepatocytes were placed is indicated in Table I. Since insufficient numbers of necrotic bodies were observed in the sinusoid, this group was excluded from consideration. Generally the surface area of cisternae of the average necrotic body was three times that of vesicles when necrotic bodies were considered without respect to location within the liver. Vesicels and cisternae show repeating, but alternate, patterns for surface and volume measurements with regard to location. Surface and volume measurements for vesicles were significantly higher ($p < 0.05$) for those necrotic bodies located in the space of Disse compared with the hepatic cord, with intermediate values for necrotic bodies in parenchymal cells; whereas, measurements for cisternae were slightly higher for those necrotic bodies in the hepatic cord. The volume density of electron dense deposits was significantly higher ($p < 0.05$) for necrotic bodies in the hepatic cord than for those in the space of Disse. Electron dense deposits in parenchymal hepatocytes were intermediate in value between those of the hepatic cord and the space of Disse. Electron dense deposits and vesicles were estimated with 78 and 95% confidence, respectively, based on Weibel's (1963) computation of total test points (P_T) required per sample as follows:

$$P_T = \frac{0.453\ (1\text{-}V_v)}{V_v \cdot E^2\,(V_v)}$$

Where VV_v is the volume density of the component measured and $E^2\,(V_v)$ is the error one is willing to accept.

These statistically significant patterns are opposite to those anticipated. Instead of becoming more pyknotic (and, therefore, less vesiculated) in the order: space of Disse > hepatic parenchymal cell > hepatic cord, the necrotic body becomes less pyknotic (i.e. significantly reduced V_{edd} estimates) and more vesicutated (Fig. 1, 2). Since necrotic bodies in parenchymal cells show intermediate V_{edd}, S_{ves}, and V_{ves} values, the majority of necrotic bodies are assumed to move from a position in the hepatic cord, to the interior of parenchymal hepatocytes (by heterophagy) to the space of Disse, and finally into the sinusoid—rather than directly from the hepatic cord to the space of Disse. The process of necrotic body "aging", therefore, appears to be measurable in terms of morphological correlates, namely vesicle volume and surface, as well as electron dense deposit volume (i.e. pyknosity). Presumably, necrotic bodies are fully "aged" at the time of extrusion into the sinusoid.



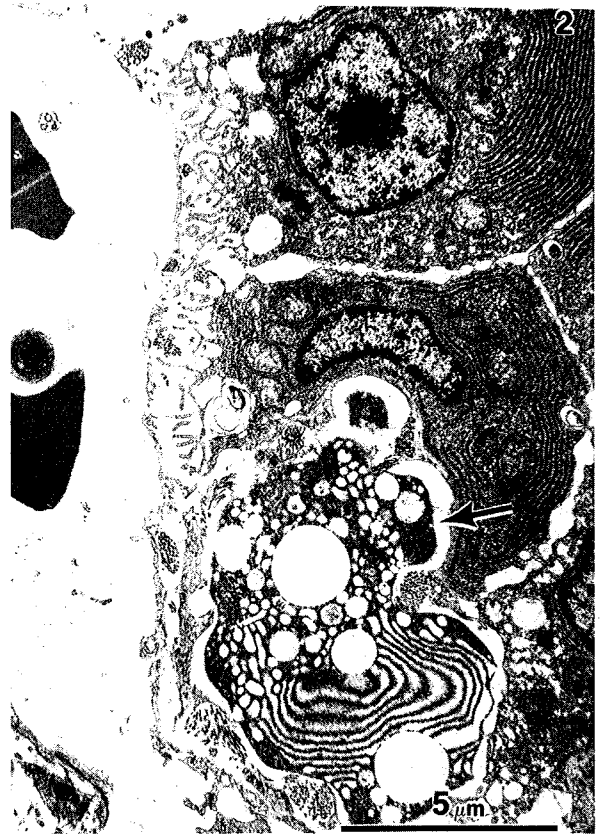


FIG. 2. *Liver cells of a green sunfish which accumulated 64.2 ppm arsenic in the liver. A necrotic body (arrow) is present in the space of Disse; extremely large vesicles occur within this necrotic body. Uranyl acetate and lead citrate.*

Volume density estimates of the subcomponents of the fibrous body were computed based on measurements for about thirty fibrous bodies of an average diameter of 2.05 μm ($\pm$0.11 standard error of the mean, S.E.M.). These measurements show that approximately 53% ($\pm$2%, S.E.M.) of the fibrous body volume is composed of bundles of fibrous material. An average of 19% ($\pm$2%, S.E.M.) of the volume consists of electron dense material and 14% ($\pm$2%, S.E.M.) is composed of material of an intermediate electron density. The volume density of the compartments of the fibrous body was estimated with 86, 89, and 97% confidence for material of intermediate electron density, electron dense areas, and bundles of fibrous material, respectively (Weibel, 1963). Morphologically no differences appear to occur in volume density of compartments of

out an increase in the total number of these structures, or *vice versa,* the maximum and minimum diameters of 600 fibrous bodies were measured from negatives using an illuminator box and a 7 × telecentric lupe with a graticule of 20 mm total scale length, subdivided in 0.1 mm units. Livers concentrating 6.1, 11.4, 15.0, 30.5, and 64.2 ppm arsenic contained fibrous bodies with maximum diameters of 0.88, 1.20, 0.44, 0.78, and 0.90 μm, respectively. Fibrous body size was not significantly different for different concentrations of arsenic in the liver except that fibrous bodies in livers concentrating 15 ppm arsenic were significantly smaller ($p < 0.05$) than those concentrating 64.2 ppm arsenic. The increased fibrous body volume in livers concentrating more arsenic was, therefore, presumed due to increasing numbers of fibrous bodies rather than increased size of individual fibrous bodies.

These data, therefore, provide a mathematical description of the necrotic body and the fibrous body, as well as indicate that significant differences in volume and/or surface density occur in subcompartments of the necrotic body dependent upon location within the liver. Apparently, as the necrotic body passes from the hepatic cord (between hepatocytes), to the hepatocyte, to the space of Disse, and finally to the sinusoid, it became less pyknotic and more vesiculated—indicative of increased necrosis of the structure.

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ZINC AND CADMIUM RESIDUES IN STRIPED BASS FROM CHEROKEE, NORRIS, AND WATTS BAR RESERVOIRS

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ABSTRACT

Zinc and cadmium concentrations in muscle, liver, and kidney were measured in striped bass (*Morone saxatilis*) from Cherokee, Norris, and Watts Bar reservoirs in East Tennessee to determine if these metals had contributed to fish kills observed in Cherokee during the 1970's. The range of mean concentrations of zinc from collections of Cherokee striped bass (muscle 11-14, liver 98-106, kidney 88-105 mg Zn/kg dry weight) were comparable to ranges in fish from Norris and Watts Bar (muscle 12-13, liver 83-132, kidney 96-108 mg/kg dry weight.) With the exception of concentrations in the kidneys of one collection, cadmium residues from Cherokee striped bass (muscle 0.02-0.09, liver 0.3-0.7, kidney 0.2-4.0 mg Cd/kg dry weight) were also similar to residues from Norris and Watts Bar fish (muscle 0.05-0.13, liver 0.3-2.1, kidney 0.3-0.5 mg Cd/kg dry weight). There were significant differences in tissue residues among seasons (summer 1979, spring 1980, summer 1980) in Cherokee Reservoir, as well as significant differences among the three reservoirs (Cherokee, Norris, Watts Bar) during the same season (spring 1980). All concentrations, however, were well below those reported for fish exposed to the maximum non-harmful concentrations of zinc and the lowest

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potentially harmful concentration of cadmium and moreover, were within the range typically reported for fish tissues. It is, therefore, believed that in at least the last two years, zinc and cadmium in the tissues of striped bass from Cherokee Reservoir have not been harmful to the fish.

Introduction

Striped bass (*Morone saxatilis*), an anadromous fish native to the Atlantic Ocean and Gulf of Mexico, have been stocked in East Tennessee reservoirs since the mid-1960's. Annual introductions of juveniles have been successful in establishing landlocked fisheries for this large pelagic species in Cherokee, Norris, and Watts Bar reservoirs. Beginning in the late summer of 1971 die-offs of adult striped bass occurred in areas of Cherokee Reservoir. At the time, stricken fish were sent to Auburn University for disease analysis, but nothing was found that would explain the mortalities. Fish were also analyzed for mercury by Oak Ridge National Laboratory because of known mercury contamination in the upper Holston River, however, the concentrations found were not sufficient to have been the cause of mortalities (Coutant 1978). Mortalities of striped bass continued to occur, usually but not always in the summer months, in Cherokee throughout the early and mid-1970's. During this time, no problem with striped bass were reported from Norris or Watts Bar. On the basis of biotelemetry studies, accompanied by dissolved oxygen and temperature data, Coutant (1978) hypothesized that summer stratification in Cherokee Reservoir that resulted in warm temperatures (> 27C) in the epilimnion and low dissolved oxygen (< 3 mg/1) in the hypolimnion forced striped bass to seek cool, well-oxygenated waters (refuge areas) near the inflows of creeks and springs. In these refuge areas, the fish were thought to be exposed to elevated levels of zinc and cadmium, known to occur in ores in the watershed, contributing to multiple stress in the striped bass and ultimately their death (Coutant 1978). The objective of our study was to determine if striped bass in Cherokee Reservoir contained higher levels of zinc and cadmium in their bodies than striped bass from Norris and Watts Bar.

Materials and Methods

Adult striped bass of both sexes ranging from 57-97 cm in total length, in weight from 2-10 kg, and in age from 3-7 years were collected by electrofishing. Fish were collected during the spring of 1980 on their spawning run from Cherokee (one mile below the John Sevier Dam, $N = 10$), Norris (the 25-E bridge on the Clinch River, $N = 9$), and Watts Bar (five miles below the Melton Hill Dam, $N = 9$) reservoirs. Striped bass were also collected in Cherokee Reservoir during the summers of 1979 ($N = 16$) and 1980 ($N = 16$) from Mossy Creek Cove. Immediately upon capture the livers and head kidneys were removed using a stainless steel knife, placed in individual polyethylene bags, and frozen on dry ice. The remainder of each fish was placed on wet ice and brought back to the laboratory where a whole fillet was taken from the left side of each fish and then stored along with the livers and kidneys at -10 C prior to analysis. A 100 ml water sample also taken at each collection site, placed in a polypropylene bottle, and acidified with 2 ml of nitric acid (1:1) for later analyses of zine and cadmium concentrations. The Norris Reservoir water sample was not taken at the time of the spring collection, but in the fall of that same year.

Muscle was prepared for analyses by taking a sample of tissue from the anterior, middle, and posterior portions from each fillet, mincing them, and combining the portions. Two samples from each fish were then dried at 110 ± 5 C until there was a consistent weight; they were then dry ashed at 550 C and reweighed. Ash was dissolved in 6 N HCl and brought to a final dilution with double distilled, deionized water. Livers were prepared for analyses by taking two samples from two different portions from the large lobe of the liver, mincing them and combining the portions. Kidney samples were so small that the samples were simply split into two portions for analyses. Liver and kidney samples were dried, ashed, and dissolved in the same way as the muscle samples. Each tissue (muscle, liver, and kidney), for each fish, was analyzed in duplicate by atomic absorption spectrophotometry. Zinc analyses were conducted at the University of Tennessee using an Instrumentation Laboratory aa/ae Spectrophotometer Model 551. Cadmium analyses were run at Oak Ridge National Laboratory using a Perkin-Elmer Model 603 absorption spectrophotometer with a HGA-1200 graphite furnace. Muscle, liver, and kidney residues for each collection period were subjected to a nested analysis of variance with an F-test of significance ($P = 0.05$) and subsequent analysis with a Duncan's multiple range test (on all significant F-values) with Kramer's adjustment (Kramer 1956) for unequal cell frequencies.

Results

Zinc residue analyses indicated greatest concentrations in livers and kidneys with levels an order of magnitude lower in muscle tissue (Figure 1), metal concentrations are reported on the basis of dry weight tissue. There were statistically significant differences in muscle tissue concentrations between seasons from Cherokee Reservoir. The significant differences, however, depended on low variability than larger differences in means. Comparing the three sample periods (summer 1979, spring 1980, summer 1980) from Cherokee Reservoir, the mean concentrations of zinc in the muscle from fish collected during the summer of 1979 and spring 1980 were both statistically higher than those from fish taken during the summer of 1980; yet the difference between the highest and lowest mean concentrations was only 2.7 mg Zn/kg dry weight. Looking at the three reservoirs (Cherokee, Norris, Watts Bar) sampled in the same season (spring 1980), the mean concentrations of zinc in the muscle tissue from fish from Watts Bar were not significantly different from Cherokee and Norris fish. The concentration of zinc in the muscle from Cherokee fish were statistically higher than those levels found in Norris fish; the difference between the two means, however, was only 1.87 mg Zn/kg dry weight.

In contrast to muscle, when comparing the three sample periods (summer 1979, spring 1980, summer 1980) from Cherokee Reservoir, the mean concentrations of zinc in the livers were not statistically different. The concentration of zinc in the kidneys of striped bass collected during the spring, however, were significantly higher than the levels in striped bass collected during the summer of 1979 with spring fish containing 19% more zinc than summer 1979 fish. Also, the relative proportions of zinc from one tissue to another were inconsistent. Looking at Cherokee, Norris, and Watts Bar reservoirs during the same season (spring 1980) the inconsistency was evident again. Striped bass collected from Cherokee Reservoir contained the highest

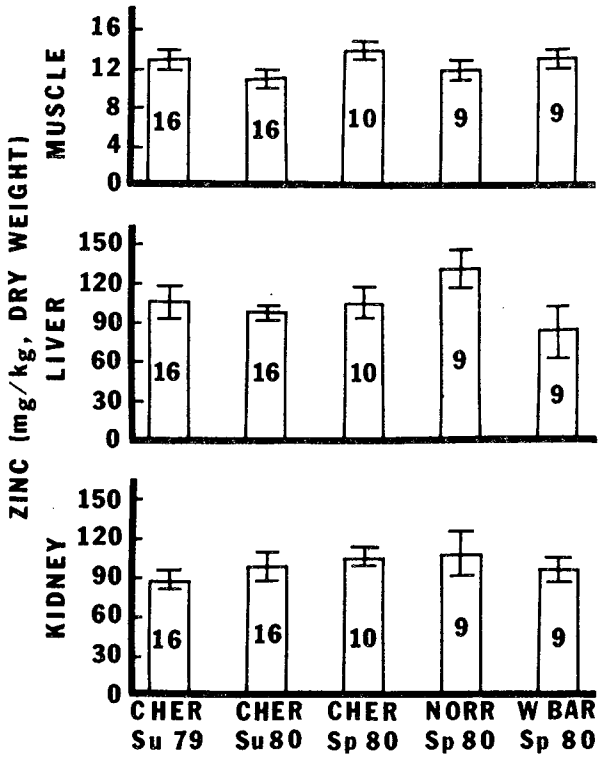

FIGURE 1. *Mean concentrations ±95% confidence intervals for zinc residues (mg/kg) on a dry weight basis in striped bass muscle, liver, and kidney tissues. Sample size indicated inside bars (Cher = Cherokee; Norr = Norris; W Bar = Watts Bar; Su = Summer; Sp = Spring; 79 = 1979; 80 = 1980).*

mean levels of zinc in the muscle tissue, however, liver and kidney did not contain the highest levels of zinc. Instead, fish taken from Norris which had the second lowest mean concentration of zinc in the muscle tissue, had the highest levels in both liver and kidney tissues (Figure 1). The mean concentration of zinc in the liver of striped bass collected from Norris were significantly higher than the concentrations in both Watts Bar and Cherokee striped bass livers; however, there were no significant differences in the concentrations of zinc in the kidneys among the three reservoirs.

Comparisons among the three sample periods (summer 1979, spring 1980, summer 1980) from Cherokee Reservoir indicated that the mean concentrations of cadmium in the muscle tissue were not statistically different between summers (Figure 2). The levels of cadmium in the muscle tissue from the spring, however, were significantly higher than those from the summer of 1980. The three sample periods from Cherokee Reservoir did not contain statistically significant differences in the mean concentrations of cadmium in the livers. The levels of cadmium in the kidneys of Cherokee fish from the summer of 1980, however, were as much as 24 times higher than the mean concentrations found in summer 1979 and spring 1980 fish (Figure 2). Comparisons of the three reservoirs (Cherokee, Norris, Watts Bar) during the same season (spring 1980) reveal inconsistencies in the relative proportions of cadmium in the tissues. Striped bass from Cherokee Reservoir had the second highest mean concentration of cadmium in the muscle but had the lowest concentration of cadmium in the kidneys during the spring. Fish from Watts Bar which contained the highest mean concentrations of cadmium in the muscle tissue and contained 3 times as much cadmium in the muscle as Norris fish, did not have the highest levels of cadmium in the livers or kidneys. Norris fish contained 6 times the amount of cadmium in the livers than the levels found in Cherokee and Watts Bar fish (Figure 2). The concentrations of zinc and cadmium in the water collected at the various sampling times was at the limit of detection for the procedure used (0.0001 mg Zn/liter and 0.00002 mg Cd/liter respectively).

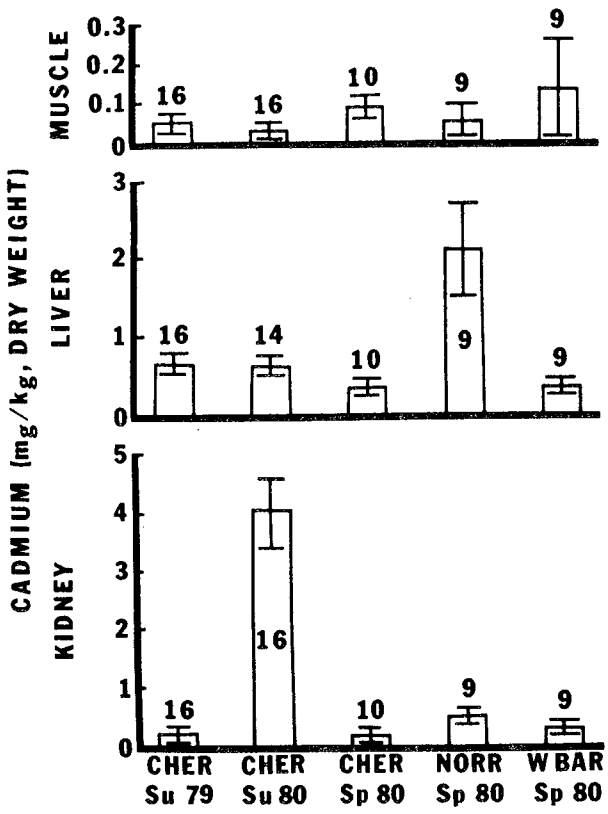

FIGURE 2. *Mean concentrations ± 95% confidence intervals for cadmium residues (mg/kg) on a dry weight basis in striped bass muscle, liver, and kidney tissues. Sample size indicated inside bars (Cher = Cherokee; Norr = Norris; W Bar = Watts Bar; Su = Summer; Sp = Spring; 79 = 1979; 80 = 1980).*

DISCUSSION

Laboratory studies of zinc and cadmium have established the maximum concentrations of each metal that can be carried in the tissues without apparent harm to the fish. Tissues concentrations for wild fish populations can be used to determine if there has been expos-

ure to harmful concentrations of zinc and cadmium. Holcombe et al. (1979) found 300 mg Zn/kg dry weight in the livers and 200 mg Zn/kg dry weight in the kidneys of brook trout exposed for 24 weeks to the highest concentration of zinc (0.534 mg Zn per liter that did not result in any harmful effects (unexposed fish contained half those concentrations). Levels in the muscle tissue did not differ from the unexposed brook trout (approximately 14-20 mg Zn/kg wet weight). Joyner (1961) also found that zinc did not accumulate in any substantial amounts in muscle tissue of fish. Striped bass collected in Cherokee, Norris, and Watts Bar reservoirs, had about ⅓ as much zinc in the liver and ½ as much zinc in the kidneys (average mean concentrations for the three reservoirs) as the brook trout exposed to the highest, non-harmful concentration of zinc. Striped bass from all three reservoirs contained similar concentrations of zinc in muscle tissue as those found by Holcombe et al. (1979) in exposed and control brook trout. The mean concentrations of zinc in the tissues of striped bass from this study were in the same approximately range as concentrations reported for apparently healthy saltwater striped bass and for other species of fresh and saltwater fishes collected throughout North America (e.g. Uthe and Bligh 1971, Kohlhorst 1973, Windon et al. 1973). Although there were significant differences in tissue zinc concentrations between samples from Cherokee Reservoir, as well as some significant differences among the reservoirs, no consistent pattern could be established for the slightly, though significantly different concentrations.

Benoit et al. (1976) determined that the maximum acceptable toxicant concentration (MATC) for brook trout exposed to cadmium was between 0.0017 and 0.0034 mg Cd/liter and that an equilibrium was reached in tissue concentrations after 20 weeks of exposure. Brook trout exposed for 70 weeks to a concentration of 0.0034 mg Cd/liter contained 65 mg Cd/kg dry weight in the kidneys and 9 mg Cd/kg dry weight in livers. These values were approximately 50 and 20 times higher respectively, than those concentrations found in unexposed fish. Muscle tissue levels were not significantly different from those in the unexposed fish (approximately 0.05 mg Cd/kg dry weight). Mount and Stephen (1967) also found that cadmium did not accumulate in any substantial amounts in muscle tissue of fish. Brook trout that were exposed to the lowest potentially harmful concentration of cadmium (Benoit et al. 1976) contained 4.5 times more cadmium in the livers and 16 times more in the kidneys than the highest concentrations in similar tissues of striped bass from Cherokee, Norris, and Watts Bar reservoirs. Like zinc, the mean concentrations of cadmium in the tissues of striped bass in this investigation were in the same approximate range as concentrations reported for apparently healthy striped bass and other fresh and saltwater fishes (e.g. Uthe and Bligh 1971, Kohlhorst 1973, Windon et al. 1973). Since the concentrations of zinc and cadmium in the liver and kidney tissues were well below those tissue concentrations reported for fish exposed to marginally harmful concentrations of the metals and since the concentrations in the muscle, liver, and kidney tissues were in the same approximate range reported for saltwater striped bass as well as other species of fresh and saltwater fishes, it appears that striped bass from the three study reservoirs do not contain concentrations of zinc or cadmium sufficient to harm them.

Even though the concentrations of cadmium found in the striped bass from the three reservoirs are not believed to be harmful, there were substantially higher concentrations of cadmium in the livers of Norris striped bass and in the kidneys of striped bass collected during the summer of 1980 from Cherokee Reservoir. These concentrations seem inconsistent because the levels were not reflected in any of the other tissues, however, other investigators have reported similar inconsistencies. Rowe and Massaro (1974) and Benoit et al. (1976) found that the kidneys of fish exposed to cadmium had substantially higher concentrations than the livers, while Mount and Stephan (1967) and Eaton (1974) found substantially higher levels of cadmium in the liver. Since only one collection was made on Norris Reservoir, there is no way of knowing if those concentrations of cadmium in the liver accumulated over a short period of time or over a period of years. In Cherokee Reservoir, however, the striped bass collected during the summer of 1980 contained 24 times more cadmium in the kidneys than those collected 6 months previously in the same reservoir. Water samples from the summer collection did not reveal high concentrations of cadmium where fish were collected, possibly an episodic exposure or the sampling of a different subpopulation of striped bass caused the apparent increase.

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