TOLERANCE AND RESPONSES OF NORMAL AND DISEASED LOGGERHEAD TURTLES (Caretta caretta) TO SOME CHEMOTHERAPEUTICS

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ABSTRACT

No external injury was observed in early hatchlings of the loggerhead turtle (Caretta caretta) when they were bathed for 10 days in seawater containing either of the following chemicals: Potassium permanganate (0.1 to 0.4 ppm), Formalin (25 to 400 ppm), and malachite green (0.1 to 0.4 ppm). The following concentrations produced toxic or irritating effects: Potassium permanganate, 0.4 and 0.8 ppm; Formalin, 800 ppm; and malachite green, 0.8 ppm and 1.6 ppm.

Thirty-five-day-old loggerhead hatchlings survived subcutaneous injections (10 days) of penicillin G (2,500 to 20,000 units/100 g body wt/day) or gentamicin (0.5 to 0.4 mg/100 g/day). Gentamicin appeared toxic at dosages greater than 0.05 mg/100 g.

Coupled with 7-day Formalin bath treatments, subcutaneous injections of either ampicillin or chloramphenicol for 3 weeks produced survival rates of 87.5% and 62.5% in loggerhead hatchlings afflicted with papillary eruption disease and emaciation. Only 20% of the control (untreated) animals survived, and they were in very poor condition at the conclusion of the experiment. Although injection with chloramphenicol plus bath treatments with methylene blue resulted in a 70% survival of medicated turtles, the overall appearance of the animals was poor. The results indicate that among the 3 drug combinations tested, the ampicillin-Formalin combination should be the preferred treatment of papillary eruption disease, chloramphenicol-Formalin a second choice, and chloramphenicol-methylene blue the last alternative.

1Contribution Number 80-24G, Southeast Fisheries Center, National Marine Fisheries Service, NOAA, Galveston, TX 77550.
INTRODUCTION

Hatchlings of the loggerhead turtle (Caretta caretta) develop many diseases when reared in captivity. The application of chemotherapy against these diseases was considered. Therefore, a number of drugs and chemicals were tested to determine the turtles' tolerance level and to evaluate the therapeutic effectiveness of these compounds for certain pathological conditions.

Drug selection was primarily based on one or more of the following considerations: success in general use against fish diseases; results of in vitro sensitivity tests on microorganisms isolated from sick turtles; broad-spectrum antimicrobial activity; relative safety; and immediate availability. In attempts to develop treatment methods against papillary eruption disease (PED) in loggerhead hatchlings, three combinations of drugs—ampicillin-formalin, chloramphenicol-formalin, and chloramphenicol-methylene blue—were chosen for further testing out of a host of chemicals which were screened in preliminary experiments. In this paper we describe the efficiency of three combinations of drugs.

In human medicine, it is common to administer a drug several times in a 24-hour period to maintain a sufficiently high blood serum level of the drug. This is impractical in the mass culture of sea turtles because of the limited manpower available to deal with a large number of sick animals. We tried to approach actual production conditions in our experiments, and therefore tested injectable drugs in only single inoculations each day to assess their suitability for use in turtle disease treatment. Unless otherwise specified, all injections mentioned in this paper were done once a day. It is possible that the single-dose-per-day approach may lead to the abandoning of some chemicals which otherwise might be potentially effective chemotherapeutics if applied through a multiple-dose-per-day regimen.

Sometimes we tested a relatively low daily dosage of a specific chemical, e.g., penicillin G, to avoid inducing unnecessary mortality with untested chemicals in the loggerhead turtle, which has been listed in Appendix I to the Convent on International Trade in Endangered Species of Wild Fauna and Flora (U.S. Department of the Interior 1978a). Once the lower range of drug tolerance is determined, higher dosages can be tested in the future when needed.

MATERIALS AND METHODS

TOLERANCE TO POTASSIUM PERMANGANATE

Aliquots of appropriate volumes of a stock solution (2,000 ppm) of potassium permanganate (K MnO₄) were added to seawater in gallon-sized glass jars. The final level of K MnO₄ in these jars was adjusted to one of the following concentrations: 0.1, 0.2, and 0.4 ppm. Two jars were assigned to each concentration, and two additional jars which did not receive K MnO₄ were used as controls in the experiment.

After the K MnO₄ was thoroughly mixed with the seawater, one 2-day-old loggerhead turtle, weighing about 15 g, was placed in each jar. The experiment lasted 3 days. During this period, both medicated and non-medicated waters were changed daily. The water temperature was maintained at 24 ± 1°C.

TOLERANCE TO PENICILLIN AND GENTAMICIN

Sixty 34-day-old loggerheads were assayed for their tolerance to penicillin G and gentamicin sulfate. Test animals were individually held in gallon-sized glass jars and divided into 10 groups of 6 turtles per group. Each of the first 9 groups of turtles received daily subcutaneous injections of one of the following 9 preparations: penicillin G 2,500, 5,000, 10,000 and 20,000 units/100 g body wt/day; gentamicin sulfate: 0.05, 0.1, 0.2 and 0.4 g/100 g body wt/day; sodium chloride, bacteriostatic (Abbott Laboratories: containing NaCl, 0.9%; and benzyl alcohol, 0.9%) 2 a diluent for the antibiotics: 0.1 ml/100 g body wt/day. No injection was given to the 10th group (control). Medication was continued daily for 10 days. The aquarium water was changed every day, and the temperature was maintained at 26 ± 1°C. The concentration of dissolved oxygen in the water was monitored as described later. The animals were held for observation and monitored for possible mortality for 2 weeks after stopping the medication.

TOLERANCE TO FORMALIN AND MALACHITE GREEN

Seventy-two 2-month-old loggerheads were tested for their tolerance to Formalin and malachite green bath treatments. Test animals were individually held in gallon-sized glass jars with water temperature maintained at 26 ± 1°C, and divided into 12 groups of 6 animals per group. Eleven groups were bathed in seawater containing either one of 6 concentrations of Formalin (25, 50, 100, 200, 400, and 800 ppm) or one of 9 concentrations of malachite green oxalate (0.1, 0.2, 0.4, 0.6, and 1.6 ppm). The 12th group was maintained in untreated seawater to serve as a control. Treatments lasted 10 days with daily replacement of medicated or non-medicated waters at about 24-hour intervals. Oxygen levels in the water were monitored as described later. All turtles were held in seawater for post-treatment observations.

PAPILLARY ERUPTION DISEASE

Papillary eruption disease was widespread among the loggerhead hatchlings in the Galveston Laboratory in 1977. The illness was characterized by small, papilla-like, light tan-colored eruptions on the skin. The eruptions usually occurred in clusters on the eyelids, around the cloacal opening and on the inner wall of the esophagus.

The etiology of PED is unknown, although bacterial infection is suspected. The disease was often accompanied by emaciation of the turtle body, but it has not been determined whether or not the papillary and emaciation syndromes share a common etiology. It has also been noted
that emaciation could occur without papillary eruption and vice versa.

Papillary eruption by itself did not seem to be fatal, but when emaciation happened, the affected animal would become progressively weakened and usually would die. There is no known cure for emaciation.

CHLORAMPHENICOL AND AMPICILLIN INJECTIONS PLUS MEDICATED BATH vs PAPILLARY EREPTION DISEASE

Sixty-eight 12-week-old loggerheads with papillary eruption disease were used as test animals. Individually isolated in glass jars, they were divided into 4 groups, each having either 16 or 20 turtles. Treatment regimens included: 1) subcutaneous injection of chloramphenicol (Chl) (5 mg/100 g body wt/day) plus 7-day long, daily-replaced methylene blue bath (1 ml of 1% methylene blue per gallon of seawater); 2) subcutaneous injection of Chl (dosage as above) plus 7-day long, daily-replaced Formalin bath (50 ppm); 3) subcutaneous injection of ampicillin (Amp) (0.5 mg ampicillin sodium/100 g body wt/day) plus 7-day long, daily-replaced Formalin bath (50 ppm); 4) no medication (control). Chl and Amp injections were given once daily for a total of 19 injections with two 2-day interruptions on days 10-11 and 15-16.

MEASUREMENT OF OXYGEN IN AQUARIUM WATER

Oxygen levels in aquarium water medicated with either KNO₃ or Formalin or malachite green were monitored with an oxygen meter, YSI Model No. 57 (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). Measurements were made in the high, medium and low drug concentrations and in the control (non-medicated). For KNO₃, oxygen measurements were taken at the beginning in freshly prepared medicated bath water, and at the end of the 3-day experiment. For Formalin and malachite green, 2 duplicated sets of oxygen measurement were taken for each specific drug concentration. Each set consisted of one measurement in freshly medicated bath water and one after 24 hours just before replacement of the water.

RESULTS AND DISCUSSION

OXGEN IN AQUARIUM WATER

Oxygen concentrations in the experimental aquarium water are summarized in Table 1. Dissolved oxygen (DO) was not reduced to less than 60% of saturation after either 24 hours for Formalin and malachite green or 72 hours for KNO₃. The results have conformed with the DO levels recommended by the U.S. Environmental Protection Agency for toxicity tests with aquatic organisms (Committee on Toxicity Tests for Aquatic Organisms 1975).

Oxygen concentration dropped in either 24- or 72-hour-old bath water, but there was no significant difference in percent decrease between control and medicated water for each specific type of drug. These results indicate that the depletion of oxygen was not caused by the added chemicals but was perhaps primarily due to biological demands in the system, such as demands by organic wastes and microorganisms, which exist in both the control and medicated baths.

In the Formalin and malachite green test series, the 24-hour-old baths had become relatively turbid and odorous, indicating a need for replacement soon if not immediately. The general quality of the bath water seemed to correlate inversely with the DO level.

Table 1. Level of Dissolved Oxygen in Medicated Aquarium Seawater Either Freshly Prepared or After 24 or 72 Hours

<table>
<thead>
<tr>
<th>Chemical added</th>
<th>No. of</th>
<th>Fresh (ppm)</th>
<th>24-hr (ppm)</th>
<th>72-hr (ppm)</th>
<th>Difference&lt;sub&gt;a&lt;/sub&gt; (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>replicates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KNO₃</td>
<td></td>
<td>0 ppm (Control)</td>
<td>2</td>
<td>5.5</td>
<td>81.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 ppm</td>
<td>2</td>
<td>5.6</td>
<td>81.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2 ppm</td>
<td>2</td>
<td>5.3</td>
<td>76.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4 ppm</td>
<td>2</td>
<td>5.4</td>
<td>78.3</td>
</tr>
<tr>
<td>Formalin</td>
<td></td>
<td>0 ppm (Control)</td>
<td>4</td>
<td>7.0</td>
<td>101.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 ppm</td>
<td>4</td>
<td>7.1</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 ppm</td>
<td>4</td>
<td>7.1</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400 ppm</td>
<td>4</td>
<td>7.0</td>
<td>101.5</td>
</tr>
<tr>
<td>Malachite Green</td>
<td></td>
<td>0 ppm (Control)</td>
<td>4</td>
<td>6.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 ppm</td>
<td>4</td>
<td>7.0</td>
<td>101.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4 ppm</td>
<td>4</td>
<td>7.0</td>
<td>101.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6 ppm</td>
<td>4</td>
<td>7.0</td>
<td>101.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Percent of saturation, with 100% calculated as being equal to 6.9 ppm dissolved oxygen at 28 ppt and 28° C (Green and Carrick 1967).
<sup>b</sup>Between aquarium seawater either freshly prepared or after 24 or 72 hours.

The 72-hour DO levels in KNO₃ baths were either similar to or higher than those in 24-hour Formalin or malachite green baths. It was probably because the turtles in the KNO₃ baths were only 2 to 5 days old, while those in the Formalin and malachite green experiment were 2 months old. The very young hatchlings, unlike the older ones, excreted no or very little feces, and therefore created less oxygen demand through organic wastes as well as microorganisms, which would utilize the wastes as growth substrates.

Although sea turtles do not respire via water, our experience has indicated that they need clean water for maximal survival. Oxygen levels are good indicators as to how fouled the water has become. Our data and observations have indicated that under current experimental conditions, daily replacement of bath water should be adequate for maintaining reasonably good water quality.

DRUG TOLERANCE EXPERIMENTS

One-day-old loggerhead turtles suffered no mortality when given 3-day baths containing from 0.1 to 0.8 ppm KNO₃ (Table 2). However, at the 0.4 and 0.8 ppm levels, the animals became quiescent after the 2nd day of medication, showed very little movement while floating on the water surface, and refused to eat. They resumed normal behavior after the 3-day medicated bath treatment was terminated. In view of the unfavorable responses to prolonged applications of KNO₃, newborn sea turtles should not be treated with more than 0.2 ppm until the significance of the lethargy can be determined.
Table 2. Mortality and Behavior of One-Day-Old Loggerhead Turtles Receiving Potassium Permanganate Bath Treatment

<table>
<thead>
<tr>
<th>KMnO₄ in bath (ppm)</th>
<th>Replicates</th>
<th>Duration (days)</th>
<th>Mortality (%)</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>Turtles active and ate well</td>
</tr>
<tr>
<td>0.2</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>Turtles active and ate well</td>
</tr>
<tr>
<td>0.4</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>Turtles showed signs of irritation, later became lethargic and ate very little; resumed normal activity and feeding after treatment was stopped</td>
</tr>
<tr>
<td>0.8</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>Same as 0.4 ppm</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>Turtles active and ate well</td>
</tr>
</tbody>
</table>

*One turtle per replicate.*

Unfortunately, 0.2 ppm KMnO₄ may not be an effective dosage and the utility value of KMnO₄ for combatting disease in newborn turtles may be limited. Commonly recommended dosages of KMnO₄ for prolonged use on freshwater fishes ranged from 2 to 8 ppm, depending on environmental conditions (Davis 1953; Meyer 1966; Quick 1977; Schick et al. 1979; Snieszko, unpublished). Nevertheless, in our later experiments, it was found that 3-month-old Atlantic ridley turtles (Lepidochelys kempi) could be bathed daily for 8 hours in 5 ppm KMnO₄ in seawater without being killed, although they also became highly immobile and weak and refused to eat soon after treatment had begun (J. K. Leong, unpublished data). Thus, it appears that KMnO₄ may still have a place for short-term uses on older turtles for the control of external parasites.

Thirty-five-day-old loggerhead turtles tolerated penicillin G subcutaneous injections (Table 3). The data, after adjustment against those of the control (non-treated) group, showed an insignificant loss in average body weight at the 10,000 units/100 g and 20,000 units/100 g injection series at the end of a 10-day treatment regimen, but no turtles died or exhibited any abnormal signs. There was an increase (adjusted) in average body weight in the 2,500- and 5,000-unit injection series, and again, no animals died or appeared abnormal. The results indicate that for therapeutic use, penicillin G at up to 20,000 units/100 g/day may be administered subcutaneously in single doses to young turtles. Further tests should be conducted if higher dosages are required for disease control.

Adjustment data from the gentamicin sulfate experiment showed a slight (2.8%) decrease in average body weight in the 0.4 mg/100 g injection series (Table 3). In the 0.1 mg/100 g series, one turtle (16.7%) became emaciated and died; the average body weight lost was 7.8%. (Because of the emaciation, and because no turtle died at higher gentamicin dosages, it is questionable if the mortality was drug related.) The lower dosage of gentamicin (0.05 mg/100 g/day) produced an adjusted gain of 2.9% in average body weight, and no animal died. Apparently, this lower dosage is a safe level for subcutaneous injection of the drug in loggerhead hatchlings. Gentamicin is well known for its neurotoxic and nephrotoxic effects on humans. There are unconfirmed reports that reptiles generally are also highly sensitive to gentamicin.

Table 3. Mortality and Body Weight Changes of 35-Day-Old Loggerhead Turtles Injected with Antibiotics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of turtles</th>
<th>% Avg G/C/L in body wt Unadjusted</th>
<th>% Mortality during treatment Unadjusted</th>
<th>Deaths post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin(\text{G})</td>
<td>2,500 units</td>
<td>6</td>
<td>20.5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>5,000 units</td>
<td>6</td>
<td>18.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>10,000 units</td>
<td>6</td>
<td>15.7</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>20,000 units</td>
<td>6</td>
<td>16.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Gentamicin(\text{S})</td>
<td>0.005 mg</td>
<td>6</td>
<td>21.0</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>0.01 mg</td>
<td>6</td>
<td>10.3</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td>0.02 mg</td>
<td>6</td>
<td>17.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.04 mg</td>
<td>6</td>
<td>15.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Saline(\text{S})</td>
<td>6</td>
<td>17.8</td>
<td>-0.3</td>
<td>0</td>
</tr>
<tr>
<td>None (Control)</td>
<td>6</td>
<td>18.1</td>
<td>0</td>
<td>16.7</td>
</tr>
</tbody>
</table>

*One turtle per aquarium jar. \(\text{G}\) = gram or liter.\nPercent adjusted G/C/L = percent unadjusted G/C (treatment) - percent unadjusted G/C (control).\n*Days consecutive days.\n*Corrected mortality = \(\%\text{ death (treatment)} - \%\text{ death (control)}\) \times 100.\n*Two weeks.\nUnits/100 g body wt/day, subcutaneous injection.\n*The death turtle was highly emaciated; cause of death might not have been drug-related.\n* Sodium chloride inj. bacteriostatic (Abbott’s Lab.), containing 0.9% NaCl and 0.9% benzyl alcohol; used in experiment as diluent for penicillin and gentamicin.

The usual dosage of gentamicin sulfate recommended for human use is 3 mg/kg/day I.M. (Huff 1977), 6 times higher than the proposed safe dosage (0.05 mg/100 g) for loggerhead hatchlings. The 0.05 mg/100 g dosage may not be effective for disease treatment, unless further evidence shows that the turtles retain gentamicin far better than man. Therefore, despite the advantage of being a broad-spectrum antibiotic, gentamicin sulfate does not appear to be potentially useful as a chemotherapeutic for use on loggerhead hatchlings.

In the Formalin bath experiment, 2-month-old loggerheads tolerated up to 400 ppm in static baths, which were replaced daily for a period of 10 days (Table 4). Grossly, there was no discernible adverse effect, and no animals died. However, at 800 ppm the animals appeared irritated and scratched their eyes with their front flippers. To prevent unwanted damage to the turtles, the 800 ppm test series was ended ahead of schedule.

The above results indicate that the loggerhead turtles have a very high tolerance level to Formalin, probably higher than that of fish. According to a list provided by Hoffman and Meyer (1974), the LC₅₀ of Formalin for striped bass (Morone saxatilis) is 12 ppm when exposed for.
In actual practice, we have developed a treatment region in which in the loggershead up to a drug concentration of 12 ppm, the test results were observed. In the malachite green experiment, no visible injury was noted. A 5 ppm formalin bath, replaced daily for 7 days, was also effective against an external tumorous growth in both loggershead and ridley hatchlings. In the malachite green and the white suckers, umbilical eggs, subcutaneous injection of 5 mg chloramphenicol sodium succinate/100 g body wt/day in subcutaneous injection, plus 7-day methylene blue bath (2.7 ppm), replaced daily.

### Table 5. Mortality and Body Weight Changes in 2-Month-Old Loggerhead Turtles Infected with Papillary Eruption Disease and Treatment with Ampicillin or Chloramphenicol plus Medicated Bath

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of turtles</th>
<th>Avg G/L</th>
<th>% Mortality</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
<td>During treatment</td>
</tr>
<tr>
<td>Chlm + M.B.</td>
<td>16</td>
<td>-2.1</td>
<td>+12.4</td>
<td>18.8</td>
</tr>
<tr>
<td>Chlm + Fm</td>
<td>16</td>
<td>-6.6</td>
<td>+7.9</td>
<td>18.8</td>
</tr>
<tr>
<td>Amp + Fm</td>
<td>16</td>
<td>-1.9</td>
<td>+12.6</td>
<td>12.5</td>
</tr>
<tr>
<td>None (control)</td>
<td>20</td>
<td>-14.5</td>
<td>0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

*aOne turtle per aquarium jar.  
bGain or loss.  
cUnadjusted.  
dAdjusted.  
e% Adjusted G/L = % unadjusted G/L (treatment) - % unadjusted G/L (control).  
fUncorrected.  
gCorrected.  
h% Corrected mortality = % death (treatment) - % death (control) x 100.  
i5 mg chloramphenicol sodium succinate/100 g body wt/day in subcutaneous injection, plus 7-day methylene blue bath (2.7 ppm), replaced daily.  
jChlm dosage as in (i), plus 7-day Formalin bath (50 ppm), replaced daily.  
k0.5 mg ampicillin sodium/100 g body wt/day in subcutaneous injection, plus 7-day Formalin bath (50 ppm), replaced daily.  

96 hours at 21° C, and the LC50 of the same chemical for exposure at an unspecified temperature (pH 7.0) is 167 ppm for 48 hours exposure. The channel catfish suffered no mortality when exposed to either 5 ppm formalin for one hour or 50 ppm for 96 hours, both at 25° C. It must be noted, however, that in the initial experiment, the exposure time to formalin was continuous in the same aquarium, while in the loggershead test, the medicated solution was changed daily.
CHLORAMPHENICOL AND AMPICILLIN INJECTIONS PLUS
MEDICATED BATH VS PAPILLARY ERUPTION DISEASE

Nineteen days following the onset of medication with 3 types of
drug combinations—ampicillin-formalin, chloramphenicol-formalin,
and chloramphenicol-methylene blue—treated sick loggerheads showed marked
improvement in health. Average group data (adjusted against those of
the control group) revealed that medicated animals gained weight (7.9%
to 12.6%) and had 0% to 4.5% mortality compared to 25-80% in the un-
treated groups (Table 5). Although the unadjusted data showed a reduc-
tion in average body weight of 1.9% to 6.6% in the medicated turtles,
those figures are relatively low when compared to the 14.5% loss in the
control (non-medicating) group. The benefit of chemotherapy is readily
apparent. Our experience has been that turtles undergoing medicated
baths, especially of Formalin, may lose some body weight during treat-
ment, but the loss is soon made up upon recovery from illness and termination
of medication.

Ampicillin-Formalin treatment produced the best overall results in
adjusted weight gain (12.6%) and in survival rate (87.5%). In direct
observation, most turtles treated with Amp-Formalin and Chl-Formalin
were much better in physical appearance than those treated with Chlm-
methylene blue and the untreated (control). They also were more active,
and had more healed lesions. Many turtles in the control groups developed
a high degree of emaciation and died.

Extended observations revealed that 10 days after a 21-day treat-
ment regimen, only 20% of the untreated turtles survived. The survival
of medicated turtles ranged from 87.5% in the Amp-Formalin group to
62.5% in the Chl-Formalin group (Table 5). Although the 75% survival
in the Chlm-methylene blue group is higher than the 62.5% in the Chlm-
Formalin group, the overall health appearance of the animals was worse
in the former group. It appeared that methylene blue is unsuitable for
prolonged use against PED in loggerhead turtles, despite the fact that a
similar concentration (2.7 ppm) is recommended for the control of external
parasites of fish in long duration baths (Amacher 1970).

In the order of effectiveness against PED, Amp-Formalin was best,
Chl-Formalin was second, and Chlm-methylene blue was the least desir-
able. Later, actual application of Amp-Formalin successfully cured nu-
umerous cases of PED, especially when the sick animals were not accompa-
nied by the emaciation syndrome. For undetermined reasons, neither Am-
or Formalin applied alone was as effective as the combination.

In conclusion, our study has shown that loggerhead hatchlings are
highly tolerant to several chemicals and drugs traditionally used for
treatment of either fish or other vertebrate diseases. Notable excep-
tions were observed in the case of gentamicin and perhaps KMnO4. These
findings are important because captive hatchlings of sea turtles are
highly vulnerable to many diseases and require chemical treatments if
they are to survive. Since the loggerhead turtle has become a rare spe-
cies, it is highly desirable that every turtle be cured when sick and that
no accidental deaths occur due to toxic effect as a result of the
use of chemotherapeutics.

In addition to evaluating drug tolerance in loggerhead turtles, we
were able to demonstrate a practical example of successful chemotherapy
of PED, a serious turtle malady, under production conditions. Our pres-
ent work represents only a start in the search for information regarding
chemical treatment of turtle diseases. Since there are many other kinds
of disease found not only in the loggerhead turtle but also in other
species of sea turtles, such as the Atlantic ridley and the green sea
turtle (Chelonia mydas), more research is needed to provide technical
knowledge to enhance confident and successful treatment and control of
turtle diseases.

ACKNOWLEDGMENTS

We thank the Florida State Department of Natural Resources for a
supply of newborn loggerhead turtles. We also express our appreciation
to Dr. F. P. Meyer, National Fishery Research Laboratory, Department of
the Interior, LaCrosse, Wisconsin, who read the manuscript and offered
valuable suggestions.

LITERATURE CITED

Amacher, E. 1970. Textbook of Fish Diseases (English translation by
D. A. Conroy and R. L. Herman). T.F.H. Publications, Neptune City,

Committee on Toxicity Tests for Aquatic Organisms. 1975. Methods for
acute toxicity tests with fish, macroinvertebrates, and amphibians.
U.S. Environmental Protection Agency, Corvallis, Oregon. EPA-660/
3-75-009.

Davis, H. S. 1951. Culture and Diseases of Game Fishes. University of

Green, E. J., and D. E. Carritt. 1967. New tables for oxygen satu-

Hoffman, G. L., and F. P. Meyer. 1972. Parasites of Freshwater Fishes:
A Review of Their Control and Treatment. T.F.H. Publications,
Neptune City, N.J. 224 pp.

Huff, B. B. 1977. Physicians' Desk Reference, 31st ed. Medical Eco-
nomics Co., Oradell, N.J.

6. Parasites of catfishes. Fish Disease Leaflet 5, U.S. Fish and

in C. J. Sprendler (ed.), Disease Diagnosis and Control in North
American Marine Aquaculture. Elsevier Scientific Publishing Co.,

registered for fishery uses. Fisheries 4:18-19.

Snieszko, S. F. Chemicals used most frequently for control of infec-
tious diseases of fishes. Unpublished lithographic copy, Extension
Wildlife and Fisheries, Mississippi State University.

U.S. Department of the Interior, Fish and Wildlife Service, Fact Sheet,
1977. A guide to endangered species regulations—the Endangered
Species Act (with attachment: 50 CFR17, revised 8/77), pp. 1-16.
