STUDIES ON THE INGESTION OF PLASTIC AND LATEX BY SEA TURTLES

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ABSTRACT

Small pieces of latex and plastic sheeting were offered to sea turtles on different occasions and the turtles' feeding behavior was noted, as well as the time taken for the turtles to pass ingested materials. The physiological and clinical status of turtles that had consumed plastic sheeting was also monitored. We observed that green sea and loggerhead turtles actively seek out and consume the offered material. Some color preference was shown, clear plastic having the lowest acceptance rate. The amount consumed was influenced by appetite. At the low feeding levels allowed in these experiments, we detected no effects of plastic ingestion on gut function, metabolic rate, blood chemistry, liver function, or salt balance. However, blood glucose declined for 9 days following ingestion, indicating a possible interference in energy metabolism or gut function. The sojourn of the ingested latex material in the gut ranged from a few days to 4 months. Moreover, some of the turtles passed multiple pieces all bound together, although they had ingested the individual pieces at different times. Since the gut clearance time for food is in the order of days, it appears that some of the latex pieces were being held up in the intestine. Latex pieces that had been retained for the longest time in the gut showed evidence of deterioration.

INTRODUCTION

As man's use of nonbiodegradable products increases, so does the amount of such material dumped into the ocean. Offshore garbage dumping by ships at sea was legal until recently and the ocean is considered by some (e.g., Osterburg 1986) as "nature's trash basket." However, one consequence of this practice is that contact by marine animals with nonbiodegradable refuse such as plastic bags and Styrofoam products also increases. Hopefully, the ratification of the MARPOL V agreement will help

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to alleviate the problem, but recent incidents of entanglement and ingestion in marine mammals and seabirds (Cawthorn 1985) suggest that harmful contact with refuse may occur much more frequently than previously thought.

It is becoming increasingly recognized that the ocean dumping of plastic waste presents a particularly serious hazard for sea turtles. Sea turtles consume a wide variety of debris and, in the man-made category, plastic bags and sheets appear to be the most prevalent material ingested (Balazs 1985). In some instances, the level of contamination can be very high. For example, plastic bags were found in 23% of a sample of green sea turtles in Peruvian waters (Hays de Brown and Brown 1982), and in one analysis 44% of adult nonbreeding leatherbacks were found to have plastic in their stomachs (Mrosovsky 1981). It has been suggested that one cause for ingestion is that turtles mistake the plastic for their natural jellyfish prey (Fritts 1982). More recently concern has been expressed over spent balloon material in the ocean, the result of increasing popularity of massive balloon launches.

Is the ingestion of plastic and latex by sea turtles any cause for concern? Clearly, if sufficient material is swallowed to cause a complete stoppage of the gut, death will result from starvation. However, there are only a few such documented cases (Balazs 1985; Cawthorn 1985), and most of the evidence for turtles swallowing plastic comes from butchered turtles (Balazs 1985). In domestic vertebrates, persistent partial blockage of the intestine can interfere with gut function (Fraser 1986). In the sea turtle, a coating of the gut wall by plastic could cause a reduction in absorption efficiency and also cause mechanical damage to the gut lining. Sublethal ingestion, therefore, where complete intestinal blockage does not occur, may be quite common and could adversely affect behavior, growth, reproduction, and general homeostatic physiological functioning and lead to other potentially lethal situations.

There is, unfortunately, no information on whether the ingestion of such material is accidental or deliberate, or information on the effects of sublethal ingestion of plastics by sea turtles. Given the critical position of most sea turtle populations and the huge magnitude of ocean dumping (van Dolah et al. 1980; Horsman 1982), it is clearly important to determine if the swallowing of such inert material by sea turtles is harmful and to establish the seriousness of any harm.

The purpose of this study was to document the mode of plastic and latex ingestion in sea turtles and to give a first estimate of how serious the resultant harm might be.

MATERIALS AND METHODS

This is the first study of its kind, and since there were no previous data to use as a guide, and as we did not wish to cause any lasting harm to the experimental sea turtles, we were particularly careful and cautious in designing our experimental protocol.

Animals

Green sea and loggerhead turtles were kept in tanks of approximately 3,785 L (1,000 gal) capacity. Each tank was supplied with running, filtered seawater. The turtles were fed a specially formulated feed for sea turtles (Purina sea turtle chow) each day during the experiments unless otherwise noted.

Ingestion

In the initial experiment, green and loggerhead yearlings (ca. 1 kg weight) and juvenile (10 to 18 kg) turtles were allowed to consume a small single piece of plastic sheeting (1 to 10 cm^2) and were observed for about 2 weeks during which time various behavioral (yearling and juvenile) and physiological (juvenile) measurements were taken. The animals were fed turtle chow daily during this experiment. Since a preliminary examination of the data showed no adverse effects, a second set of experiments was undertaken at an increased (but still modest) level of plastic ingestion. Seven loggerheads weighing 13 to 18 kg were used in this section (four experimental, three control) and were fed five to seven small pieces of plastic. They were also fed daily and observed for 2 weeks. In these experiments the initial measurements before feeding plastic served as individual controls. In order to understand the effects of simple food limitation per se, a third set of turtles was starved for 2 weeks and the various physiological parameters were monitored. This set also served as a control for those turtles in the previous experiments that occasionally refused food for a few days.

An additional study on latex was undertaken in order to determine whether the ingestion of balloon material was accidental or deliberate and if the latex material was altered on passage through the gut. Five turtles were isolated in separate tanks and were offered small (ca. 1 cm^2) pieces of colored latex and clear plastic sheeting under different conditions. The turtles' feeding behavior was noted, as well as the time taken for the turtles to pass ingested materials. The passed material was collected for examination.

Gut Function

Food consumption was measured as the number of pellets consumed each day. The pellets weighed on average 0.918 ± 0.085 g. Feces were collected in plastic bags attached to the turtles and stored frozen at -20°C. It was noted that defecation usually started 1 to 2 h after feeding. Samples of food and feces were dried at 67°C for 48 h and their calorific value measured using a Parr 1241 Adiabatic Calorimeter.

The ash content of food and feces was estimated by weighing samples before and after being heated in a muffle furnace at 600°C for 24 h. Ash was used as a digestibility marker (Conover 1966). Although this method has been criticized because of its unproven assumption that ash-forming materials are neither added nor absorbed as food passes through the gut (Bjorndal 1985; Newman et al. 1985), it is used fairly commonly in studies of digestibility in marine organisms, and gives values in reasonable agreement with the acid insoluble method in sea turtles (Vargo et al. 1986). It also has value as a comparative estimate.

Gut passage time was determined from the first appearance in the feces of the plastic sheets and of small plastic markers (Teflon disks, 2-3 mm diameter) that had been included in the food.

Occult blood in the feces was tested for using the benzidine reaction (Henry 1974).

Dive Time

Dive time was recorded on a stopwatch while observing the turtles' diving behavior in the tank. Surface time was not measured since the interval was, almost without exception, less than 3 sec (usually one breath).

Oxygen Consumption

A closed circuit method was used for oxygen consumption measurements. The turtle was placed in a sealed humidified air chamber connected to an Applied Electrochemistry oxygen analyzer. Chamber air was pumped through the analyzer and returned to the chamber. Carbon dioxide and water vapor were removed from the analyzer input line by chemical scrubbers (Ascarite and Dririte). The experiments were run for approximately 1 h, and the minimal chamber partial oxygen pressures (PO_2) were always >100 torr.

Blood Chemistry

Blood was taken from the dorsal cervical sinus as previously described (Bentley and Dunbar-Cooper 1980).

Blood gases (PO_2, PCO_2) and pH were determined immediately on whole blood using a Radiometer BMS Mk 2 blood-gas analyzer set to the experimental temperature (22°C). Plasma bicarbonate was calculated from the pH and PCO_2 data using the temperature- and pH-dependent CO_2 solubility and dissociation constants of Severinghaus (1965).

The blood was then centrifuged and the plasma divided into two parts. One part was deproteinized with 8% chilled perchloric acid and served for plasma lactate and urea measurements using the Sigma kit No. 826-uv for lactate and the Sigma kit No. 640 for urea. The untreated plasma was analyzed for osmotic pressure using a Wescor 6100 osmometer and saved frozen for measurement of ions and metabolites. Plasma chloride was measured by an Aminco chloride titrator and plasma cations by atomic absorption spectrophotometry (Perkin Elmer PE 403). Column chromatography was used to estimate plasma cortisol, and glutamic pyruvate transaminase levels were measured by spectrophotometry using Sigma kit No. 505. The hematocrit and the percentage volume of white blood cells were read after centrifugation.

RESULTS

Feeding and Digestion

Ingestion

During normal feeding, green sea turtles were each offered, on different occasions, five pieces of pink, blue, and yellow latex, and clear plastic (Fig. 1). Each turtle had its own preference: No. 1, blue; No. 2, pink; and No. 3, yellow; Nos. 4 and 5 refused all. Surprisingly, none of the turtles accepted the clear plastic. On offering yellow material to turtles that had been fasted for 3 days, there was a substantial increase in the amount of ingestion. Turtles No. 1, No. 2, and No. 3 consumed all of the material offered, but turtle No. 5 continued to hold itself aloof from this experiment (Fig. 2). In two additional sets of experiments on fasted turtles, turtle No. 1 ingested clear plastic but the others continued to ignore it (Fig. 3).

Gut Passage Time

The ingested material started appearing in the tank water after a few days and then declined over the next few weeks (Fig. 4). This time course corresponded with normal gut passage time as measured by the Teflon markers (11.3 days, range 10 to 13 days, $n = 3$). Quite unexpectedly, latex material continued to appear in the tank for up to as long as 4 months, peaking at about 8 weeks. Some of the turtles passed multiple pieces all bound together, although they had ingested the individual pieces at different times. The latex pieces that had been held for the longest time in the gut showed evidence of deterioration.

Food Consumption

In the loggerheads, daily food consumption did not vary much on an individual basis and when changes occurred they were fairly smooth (Fig. 5A). There was no noticeable pattern after feeding plastic. The average daily rate of consumption (grams of food per kilogram body weight per day) for individual loggerheads was 5.07 ± 1.97, $n = 7$; 5.9 ± 3.08, $n = 7$; 9.2 ± 1.59, $n = 11$; 9.3 ± 2.06, $n = 8$. In the green sea turtles, the average rates were similar, i.e., 6.7 ± 3.8, $n = 8$; 10.9 ± 1.93, $n = 8$; 11.82 ± 2.8, $n = 9$. However, in one of the green sea turtle consumption gradually diminished to zero on day 4 and then recovered (Fig. 5B). The consumption patterns for the other two turtles were similar to those observed in the loggerheads.

Energy Adsorption

The calorific value of the feces showed no consistent change with time in either the green sea turtles or the loggerheads (Fig. 6). Interestingly, the green sea turtle feces had a higher calorific content than the loggerhead (loggerhead feces $3{,}328 \pm 145$ cal/g, $n = 10$; green $4{,}126 \pm 324$ cal/g, $n = 9$). These differences were statistically significant ($P < 0.01$). It can be calculated that an amount of loggerhead food containing 1 g of ash

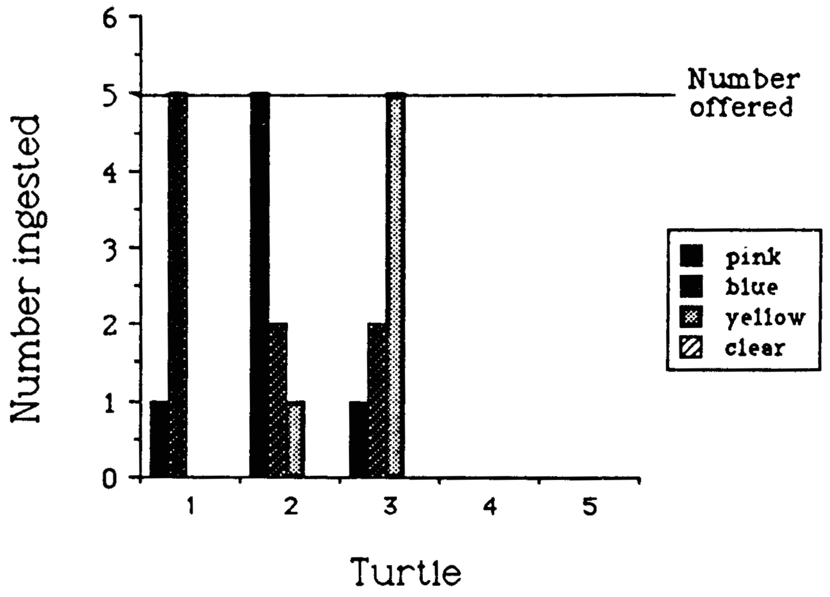

Figure 1.--Voluntary ingestion of latex pieces in green sea turtles.

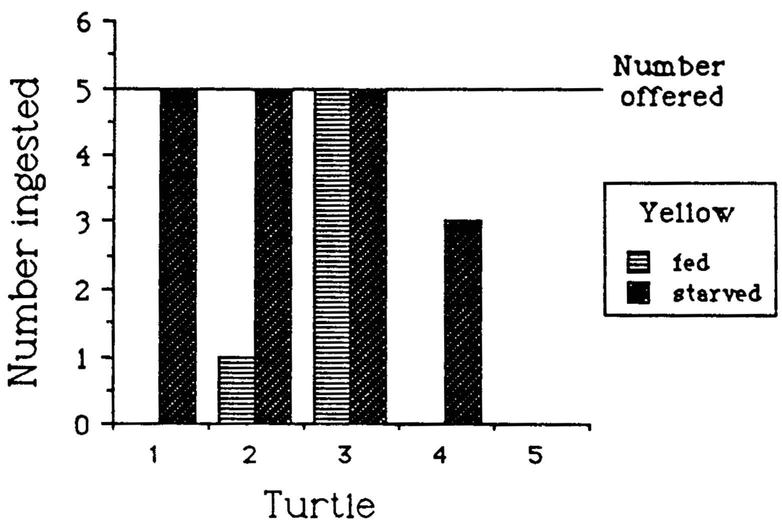

Figure 2.--Effect of 3 days fasting on latex ingestion in green sea turtles.

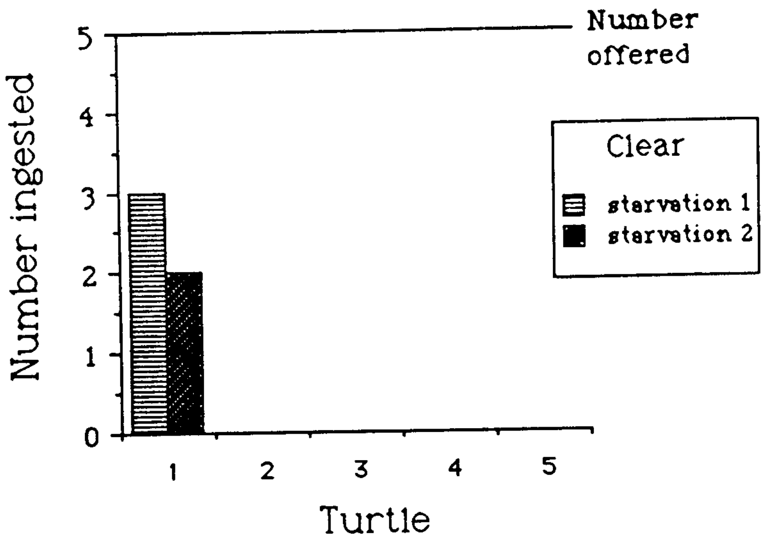

Figure 3.--Effect of 3 days fasting on the ingestion of clear plastic in green sea turtles.

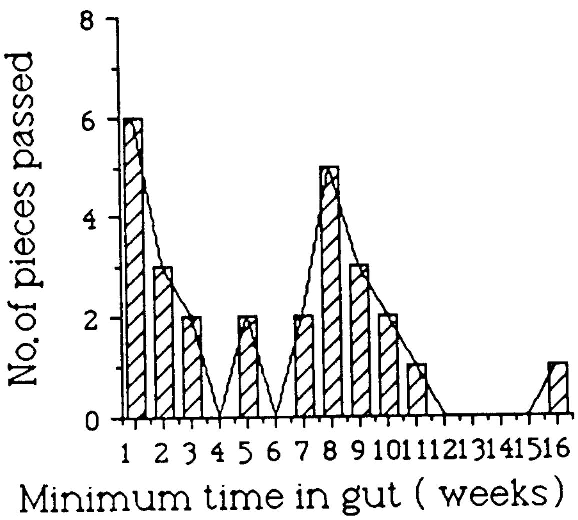

Figure 4.--Gut passage time for ingested pieces of latex in the green sea turtle.

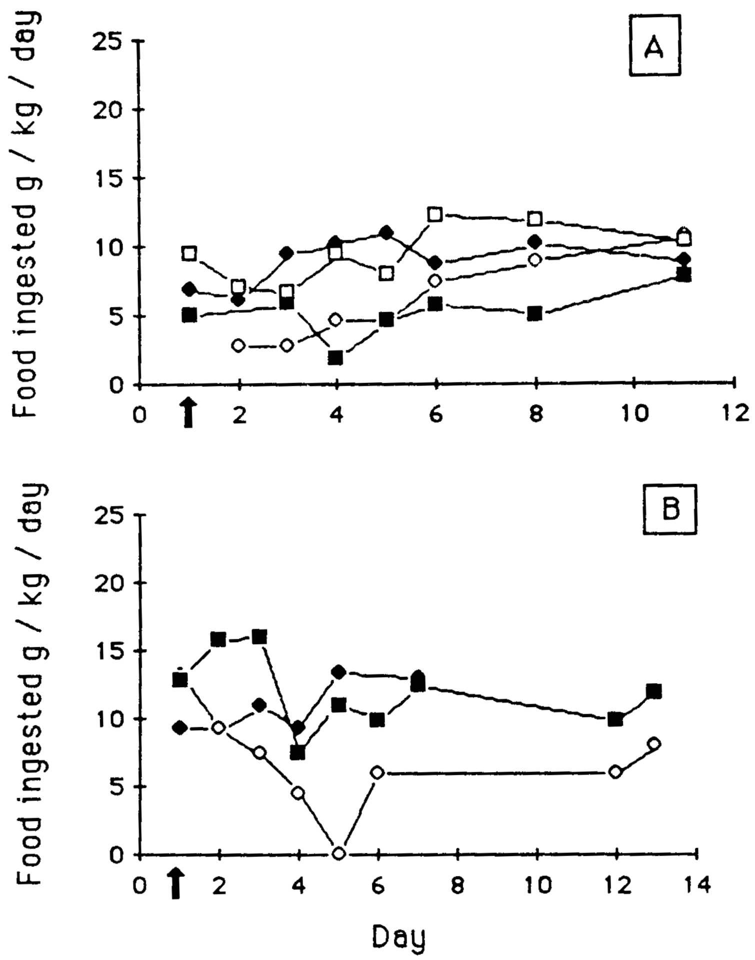

Figure 5.--The effect of plastic ingestion (↑) on food consumption in four individual loggerhead (A) and three green sea turtles (B).

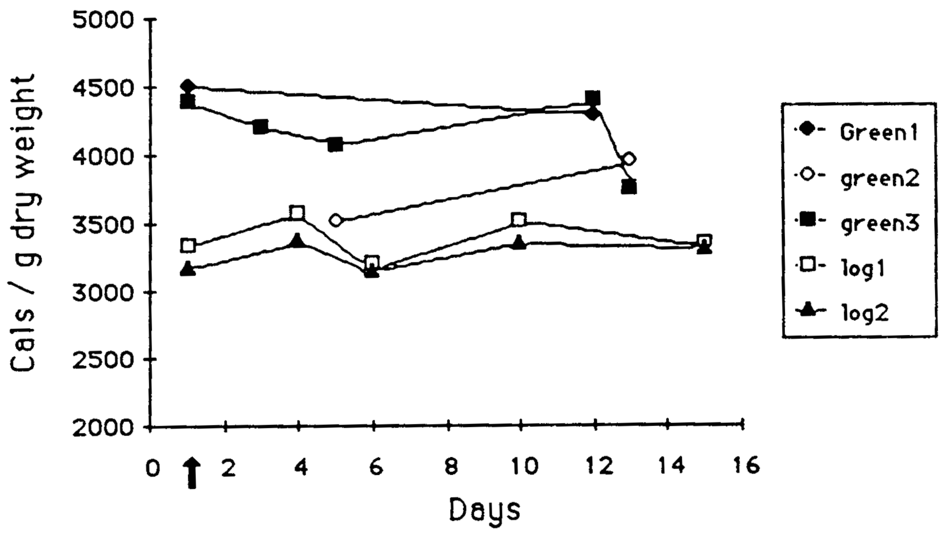

Figure 6.--The calorific value of green and loggerhead turtle feces after being fed plastic (↑).

would have a gross energy content of 51,381 cal, while feces with the same amount of ash would have 12,743 cal. Assuming constancy of ash, this indicates a digestible energy adsorption efficiency of 75.2%.

Stool Culture

In the two control loggerheads, the fecal flora was respectively 99% g positive, 1% g negative; and 98% g positive, 2% g negative. In three turtles that had been fed plastic bags, the fecal floral composition was as follows: 100% g positive; 85% g positive, 15% g negative; 100% g positive. The gut bacterial composition was, therefore, substantially gram positive in nature and this feature was not altered by plastic ingestion.

Occult Blood

No occult blood was observed in any of the fecal samples examined either in the control or the experimental animals. The plastic ingestion, therefore, had not caused intestinal bleeding.

Respiration

Oxygen Consumption

Plastic ingestion had no apparent effect on the oxygen consumption of either the green sea or the loggerhead turtles, and on an individual basis they were remarkably constant over the 2 weeks of monitoring. Metabolic rates for the green sea turtles ranged from 47.9 to 73.8 ml/kg/h, and the loggerhead values showed an almost identical range of from 38.1 to 70.2 ml/kg/h. Similar oxygen consumptions have been obtained for green sea turtles (70.8 ml/kg/h at 25°C, Kraus and Jackson 1985) and loggerheads (62.0 ml/kg/h, Lutz and Bentley 1985) measured in air.

Blood Chemistry and Acid Base Balance

Oxygen

Venous oxygen levels remained relatively constant in both the experimental turtles and in the starved group. There was no significant difference between groups. Since venous oxygen levels are determined by the difference between oxygen supply and tissue use, and since oxygen consumption did not change, it seems likely that the mechanisms for oxygen transport have not been affected by plastic ingestion. The mean venous value for all of the data ($PO_2 = 56.69 \pm 1.59$, $n = 38$) is very similar to that found in an earlier study on the same animals (Lutz and Dunbar-Cooper 1987).

Carbon Dioxide

Venous carbon dioxide remained similarly constant over the course of the experiment, and no statistical difference was found between the control and the experimental groups. The mean value for all of the data is $PO_2v = 24.79 \pm 0.976$, $n = 38$.

Blood pH

For the group of experimental turtles fed plastic, venous blood pH appeared to decline on the first day after feeding plastic ($P \leq 0.5$) and continued to fall in two turtles on day 2 and in one until day 3 (Fig. 7A). No such trend was noted for the starved controls (Fig. 7B). However, the range in pH shifts was very narrow, and for the whole set the average pH was 7.550 ± 0.008, n = 38, close to the predicted normal venous pH for the prevailing body temperature (25°C, pH = 7.442, Lutz et al. 1988).

Bicarbonate

There was no change in venous bicarbonate on the day following plastic ingestion. The overall bicarbonate concentration was 22.6 ± 0.971 mM, n = 38.

Glucose

In the loggerheads fed plastic, blood glucose levels declined for 10 days (Fig. 8), but recovered to initial values by day 14, about the time plastic was expelled from the gut (see below). A least squares linear regression of the relationship between blood glucose (G) and days after plastic ingestion (T) produced the following equation illustrated in Figure 8.

$$G\ (\text{mM}) = 6.683 - 0.445\ T \qquad r = 0.866,\ n = 12$$

The average rate of decline in blood glucose was therefore 0.45 mM/day. Interestingly, starvation by itself caused a marked fall in blood glucose levels (Fig. 9). In both the loggerhead and green sea turtles, blood glucose levels declined sharply on the second day of starvation at much greater rates than the fed loggerheads who had consumed plastic viz., 2.52 mM/day in the green and 2.42 mM/day in the loggerhead.

Glutamic Transaminase

The initial concentration of loggerhead glutamic transaminase plasma (GTP) was 1.67 ± 0.608, n = 7, international units/ml. The GTP values varied somewhat in both the control turtles and the plastic-fed turtles for the first 3 days of the experiment (Fig. 10), but after the fourth day there was a marked decline in values in both groups, possibly related to the fall in plasma glucose.

Cortisol

In all samples tested, the blood cortisol levels were extremely low (≤1.0 μg/dl), indicating that the turtles were not stressed by the experimental protocol. Blood cortisol levels have been seen to increase in stressed loggerheads from similar low initial levels (1-3 μg/dl to as high as 37 μg/dl (D. Owens pers. commun.).

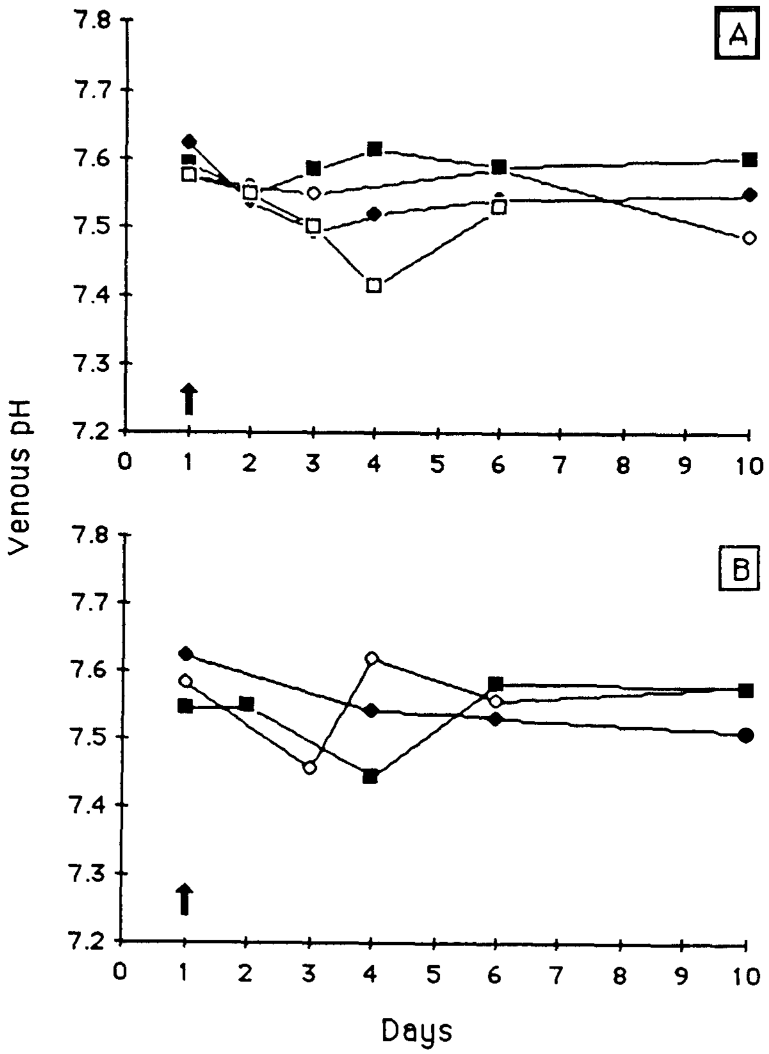

Figure 7.--The effect of plastic ingestion (A, ↑) and starvation (B, ↑) on the loggerhead turtle venous pH.

Hematocrit

The hematocrit values did not change over the course of the experiments in either the loggerhead or the green sea turtle. The loggerhead mean value (28.6%) is less than that found for loggerheads sampled in the wild (35.5%, Lutz and Dunbar-Cooper 1987) and less than that found for the green sea turtle (33.3%); the latter difference is significant ($P < 0.01$).

White Blood Cells

No change was seen in white blood cell volume following plastic ingestion. In the loggerheads, the white blood cells initially made up about 0.2% of the whole blood, and with one exception the values were reasonably constant, ranging between 0.2 and 0.4% for 10 days after plastic ingestion.

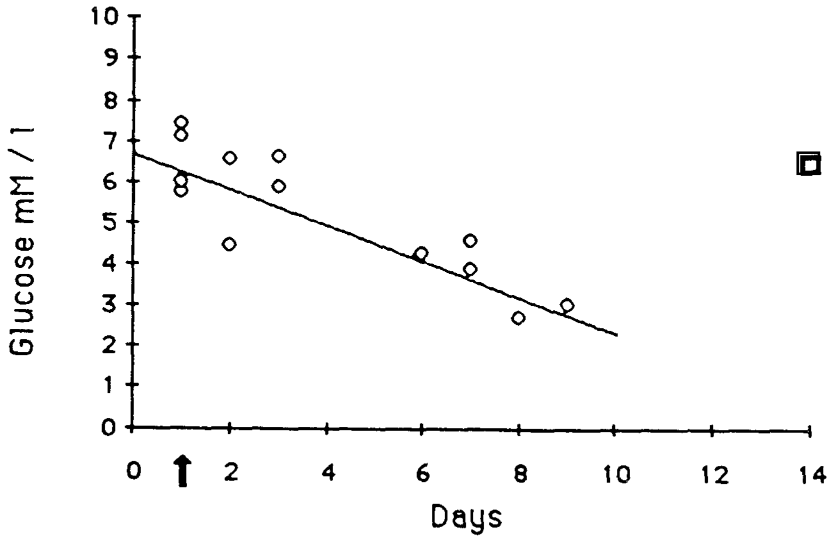

Figure 8.--The effect of plastic ingestion (↑) on blood glucose levels in the loggerhead turtle.

DISCUSSION

We have been able to demonstrate that both green sea and loggerhead turtles do not discriminate against plastic sheeting when they engulf food intermingled with plastic. The experiments with latex ingestion in loggerheads demonstrated that if their appetite is sufficient, they will actively swim towards and ingest latex materials, that all colors are acceptable, and that the amount ingested will depend on their nutritional state. Indeed, it was our impression that hungry sea turtles will swallow almost any material of a suitable size and consistency and will continue to do so until satiation.

No clear evidence of ill effects from plastic ingestion was found in this set of experiments though it should be noted that the turtles were only allowed to consume very small amounts. In fact, the constancy of many of the physiological parameters over the 2 weeks of monitoring is evidence that the experimental setup was not, by itself, a perturbing influence.

Further evidence of a lack of stress is seen in the low blood cortisol levels. The values are similar to those reported for resting blood cortisol levels for vertebrates in general which are around 1-5 μg/100 ml (rainbow trout, 3.8 μg/100 ml, Donaldson 1981; loggerhead, 1-3 μg/100 ml, Owens pers. commun.; dog, 1-5 μg/100 ml, Fraser 1986). For many animals, stress produces a surge in blood corticosteriods, often within hours of the stress, that will persist during the stress and sometimes for days afterwards (Fraser 1986). Compared to resting values, the expected increases in blood cortisol concentrations under stressful conditions can be substantial (16 μg/100 ml in the stressed rainbow trout, Donaldson 1981).

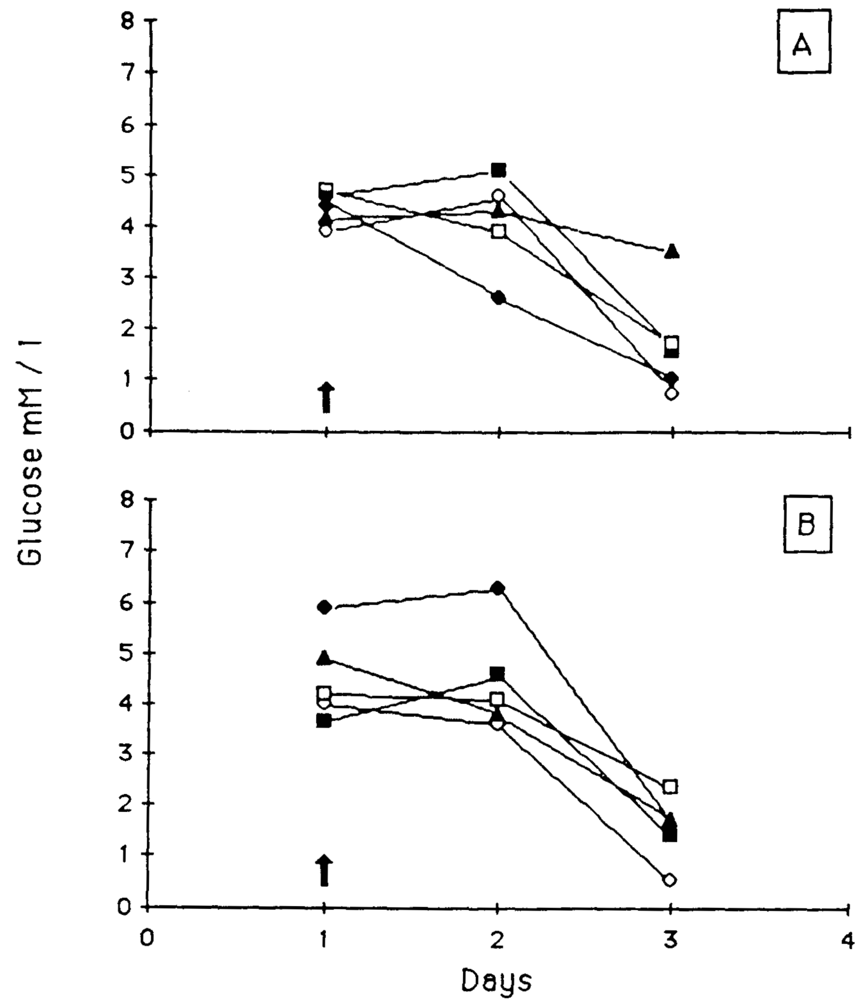

Figure 9.--The effect of starvation (↑) on blood glucose concentrations in the loggerhead (A) and green sea turtles (B).

There was no evidence of plastic ingestion affecting feeding and the handling of food. The rate of food consumption did not change after eating plastic in either the loggerhead or the green sea turtles, and the average daily consumption was similar for both species (9.79 g/kg/day, green; 7.37 g/kg/day, loggerhead). Wood and Wood (1981) found a similar food intake for green sea turtles fed pellets (8 to 12 g/kg/day). The food consumption rates found in this study are equivalent to a calorific intake of 44.2 kcal/kg/day for the green and 33.3 kcal/kg/day for the loggerhead.

The efficiency of food adsorption and the calorific value of the feces were unaltered, and the bacterial composition of the gut was not changed. There was no evidence of blood in the feces, pointing to an absence of mechanical damage as plastic passed through the gut.

No effect of plastic ingestion was detected with respect to any of the measured parameters that are directly associated with respiratory

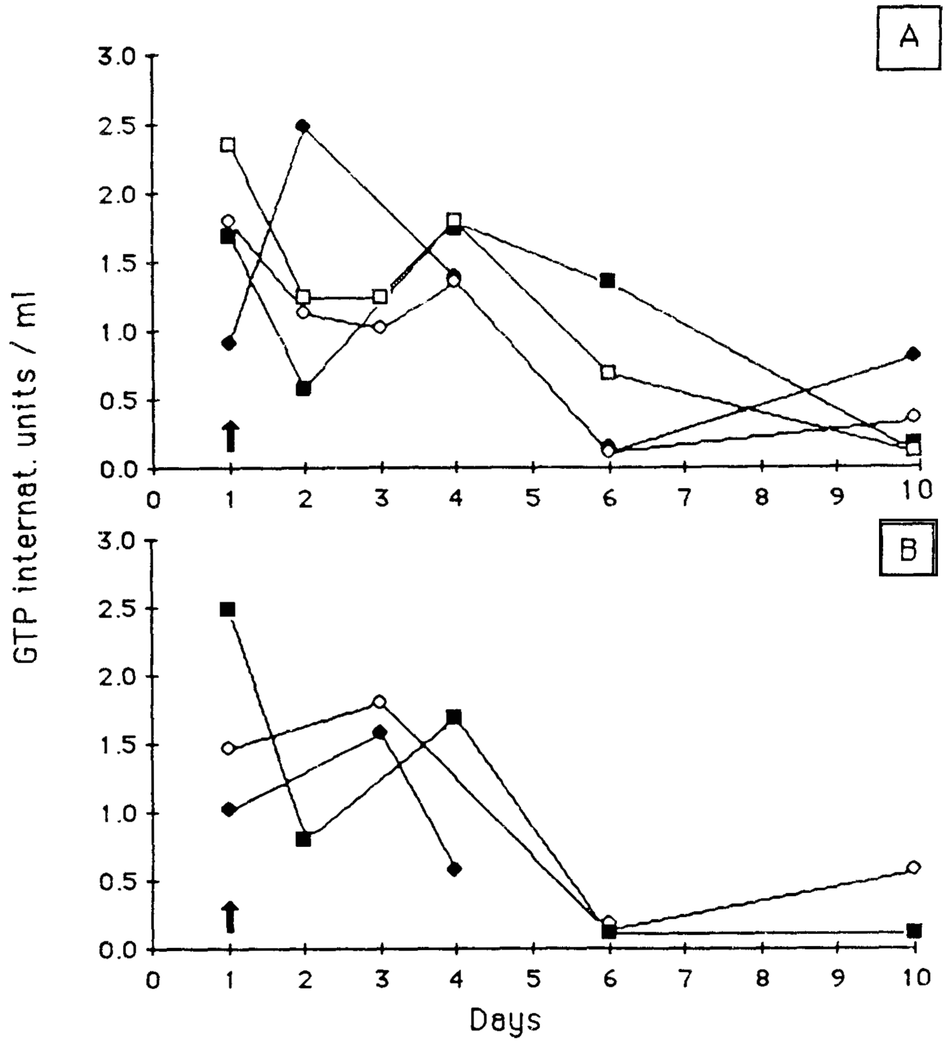

Figure 10.--The effect of plastic ingestion (↑, A) and starvation (↑, B) on glutamic transaminase levels in loggerhead plasma.

physiology, viz., metabolic rate, blood oxygen and carbon dioxide levels, blood acid base status.

The hematocrit was remarkably constant, an indicator of health, and no marked changes were seen in the proportion of white blood cells. A very substantial increase in white blood cell numbers (400%) was one of the most notable features of sea turtles affected by oil pollution (Vargo et al. 1986). No evidence of liver malfunctioning was seen in the lack of increase in plasma glutamic pyruvic transaminase (Fraser 1986).

The rates of change in blood glucose are a possible exception to this pattern. The key observation was that blood glucose declined rapidly in loggerheads that were starved and also fell, although at a lesser rate, in turtles that had been fed plastic sheets. The implication is, therefore, that blood glucose levels in sea turtles are especially sensitive to nutrient uptake from the gut and that this process had been interfered with in those animals that had consumed plastic. Interestingly, the blood glucose concentrations for the control fed loggerheads in this study (5.23 mM ± 1.279 mM, n = 10) were much higher than those recorded in the wild from loggerheads sampled in the Port Canaveral ship channel (ca. 1 mM, Lutz and Dunbar-Cooper 1987) evidence perhaps that the Canaveral turtles had not been feeding. Blood glucose levels, therefore, may serve as a sensitive index of nutritional status for turtles both in the laboratory and in the wild.

The study did point to some interesting differences in the physiology of green sea turtles and loggerheads. On average the green sea turtles had a higher hematocrit than the loggerheads (33.3%, green; 28.6%, loggerhead) and a higher proportion of white blood cells (0.2%, loggerhead; 1.02%, green). In the green sea turtles, the average daily food consumption of the pelleted food was about 32% higher. On the other hand, this was offset somewhat by green sea turtles having a higher feces energy content (24% higher in the green) and, therefore, a lower efficiency in extracting energy from the food.

In summary, when hungry, sea turtles will actively consume plastic and latex material. Except for a possible interference in energy metabolism (declining blood glucose levels), at the levels allowed in this study ingestion produced no measurable changes in the physiological parameters that were measured. However, the observation that pieces of latex can gather up in the gut and remain there for considerable periods of time should be viewed with some concern and certainly needs more detailed investigation.

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