Z. vergl. Physiologie 71, 129—141 (1971)
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Dolphin Thyroid: Some Anatomical and Physiological Findings

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Received October 23, 1970

Summary. 1. Plasma thyroxine (T_4), protein bound iodine (PBI), inorganic iodine, total iodine, thyroxine binding globulin (TGB), unsaturated TBG (resin T_3 uptake test) and free thyroxine were measured in a series of tests on two species of porpoise (or dolphins) and two species of small whales. This was the first such work done on cetaceans. The values presented for the bottlenose dolphin, *Tursiops truncatus*, were derived from 86 plasma specimens from 31 different animals (Table 2) and should be clinically useful for the future diagnosis of thyroid disease in that species.

2. A total of twenty-four plasma specimens from the Pacific white-striped dolphin, *Lagenorhynchus obliquidens* (Fig. 3), the killer whale *Orcinus orca* (Fig. 4) and the pilot whale *Globicephala scammoni* (Fig. 5) gave values that must be regarded as preliminary until a larger number of animals can be sampled.

3. All of these marine cetaceans have larger thyroids and smaller body surface areas than terresterial mammals of comparable weight.

4. In bottlenose dolphins and Pacific white-striped dolphins the total thyroid weight is about 2.25 times the total adrenal gland weight. The adrenals and the thyroid are both about one-third larger (on a gland weight to body weight basis) in Pacific white-striped dolphins.

5. Differences in water temperature of 6° C to 9° C did not appear to affect the plasma thyroid indices of bottlenose dolphins or killer whales (Fig. 4). A 72-hour fast caused an increase in plasma T_4 levels of each of 3 bottlenose dolphins with an average rise of 1.2 µg/100 ml.

6. The high level of plasma T_4 and PBI appear to correlate well with the relatively high metabolic rate observed in *T. truncatus*. However, the species that appears to have the highest metabolic rate, the Pacific white-striped dolphin, *Lagenorhynchus obliquidens* (Fig. 3), had the lowest plasma level of T_4, PBI, total iodine, TGB capacity and cholesterol (Table 2). This data points out the need for a comprehensive study of thyroid function in wild and captive white-striped dolphins and other species that are difficult to maintain in captivity.

Introduction

Ondontocetes (toothed whales including dolphins and porpoises) are considered to have a high metabolic rate (Crile and Quiring, 1940; Irving *et al.*, 1941; Kanwisher and Sundness, 1966). They live in a relatively cold environment that would appear to require large heat production for homeostasis as well as special mechanisms for heat conservation (Kanwisher and Sundness, 1966; Scholander and Schevill, 1955; Bel'kovich, 1965).

The thyroid gland in most toothed whales is relatively large. Crile and Quiring (1940) compared the thyroid weights of the Beluga *Delphinapterus leucas* with a thoroughbred horse and found the cetacean to have about three times the thyroid tissue per kg of body weight as the equine. Bottlenose dolphins *Tursiops truncatus* have about 400 mg of thyroid tissue/kg of body weight (Ridgway, 1968), whereas the dog has about 85 mg of thyroid/kg (Ganong, 1967). The size range for adult humans in North American is 15–25 g (about 250 mg/kg)—just over half that of bottlenose dolphins (Williams, 1962). The largest thyroid known to us (on a ratio to body weight basis) is that of the Dall porpoise *Phocoenoides dalli*: 650 to 900 mg/kg. Harrison (1969) gives values of 480–900 mg/kg for the harbor porpoise *Phocaena phocaena*. Our Pacific white-striped dolphins *Lagenorhynchus obliquidens* (both wild and captive animals) have thyroids of about 600 mg/kg which is in close agreement with data given by Harrison. One juvenile pilot whale *Globicephala scammoni* had a thyroid weight of 200 mg/kg (Harrison, 1969). Thyroids of these odontocetes are about 2.25 times as large as the adrenals. This ratio was similar among the species that we studied. Fig. 1 is a drawing of the thyroid gland and its regional anatomy on a bottlenose porpoise. The gland is quite lobulated and has a rich blood supply. Harrison (1969) has recently reported on the histology of the thyroid of several species of cetaceans in detail. Therefore we will not discuss the histology of the gland.

The protein bound iodine (PBI) determination has been employed as a routine clinical diagnostic test in our porpoise health program (Ridgway, 1965). We noticed that the PBI, determined by the modified alkaline ash method of Barker *et al.* (1951), is unusually high in our primary research species, the bottlenose porpoise *T. truncatus*. These values were so high that by human clinical standards virtually all of the animals would be judged hyperthyroid. In contrast the Pacific white-striped porpoise *L. obliquidens* exhibited a relatively low PBI even though this species is more active, has a higher metabolic rate, lives in colder waters (Horvath *et al.*, 1968; Ridgway and Johnston, 1966), and as mentioned previously, has a large thyroid gland in proportion to body weight. The differences in PBI were quite sizable and we wanted to try to determine what they were due to.

First it was necessary to determine whether or not specimens collected from *T. truncatus* were in some way being affected by exogenous organic iodine of which we were not aware. The methods of Murphy and Pattee (1964) and Pileggi *et al.* (1961), were employed to measure thyroxine

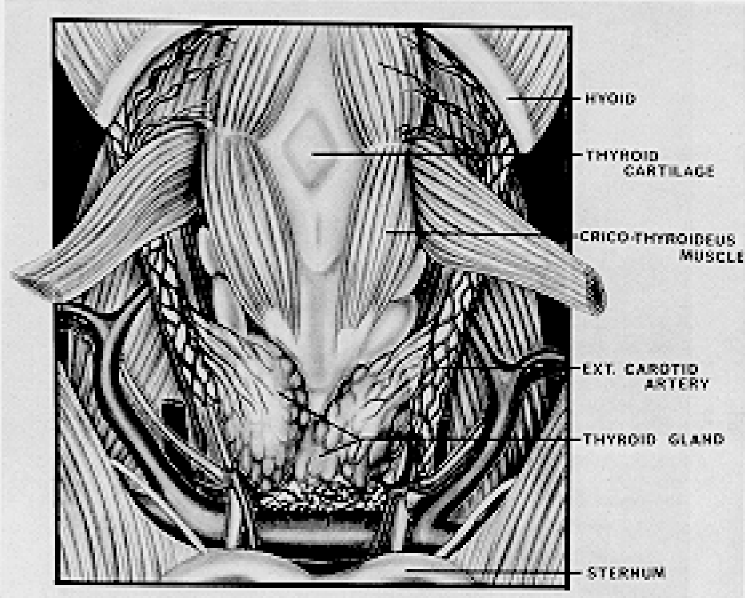

Fig. 1. A drawing of the ventral neck region of a bottlenose dolphin *Tursiops truncatus* showing the relationship of the thyroid to the other major anatomical landmarks of the region. In this species the gland has a lobulated appearance

Table 1. *Comparison of T_4 by column, PBI, total iodine, inorganic iodine, and T_4 by Murphy-Pattee in three* T. truncatus *and an* L. obliquidens. *All of these determinations were done at the Bio-Science Laboratories, Van Nuys, California*

	T_4 (µg/100 ml) (Column Chromatography)	PBI (µg/100 ml)	Total I (µg/100 ml)	Inorganic I (µg/100 ml)	T_4 (µg/100 ml) (Murphy-Pattee)
T. truncatus "Delphi" ♂	7.8	10.3	12.1	1.8	8.0
T. truncatus "Rounder" ♂	7.7	11.0	11.7	0.7	8.6
T. truncatus "Peg" ♀	6.4	9.2	13.7	4.5	7.1
L. obliquidens "Peanuts" ♂	2.4	5.8	6.8	1.0	2.7

(T_4) in three *T. truncatus* and on *L. obliquidens*. The former method is not affected by any iodine or mercury contamination (iodine gives a falsely high, and mercury a falsely low, PBI), and the latter technique

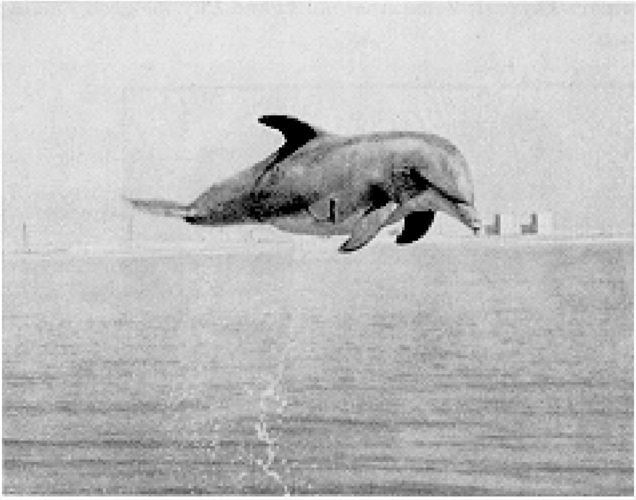

Fig. 2

Fig. 3

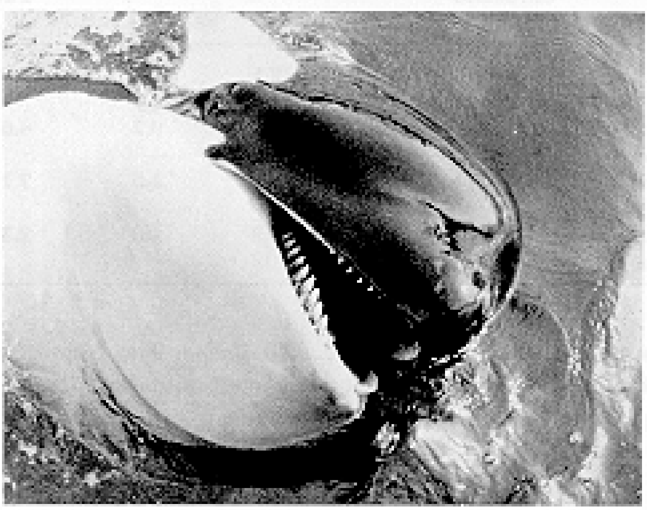

Fig. 4

is accurate in the presence of inorganic iodine or mercury contamination. The results, as shown in Table 1, indicated that no significant amount of contamination occurred and that the observed species difference was real.

To our knowledge no detailed tests of thyroid hormones have previously been done in any cetacean species. We decided to investigate plasma thyroid hormone levels in the four cetacean species available to us.

Methods

The animals were kept in several types of enclosures and were randomly moved from one to another. These enclosures include lagoon pens, concrete tanks, a wooden tank, and floating pens in the open ocean. There was no filtration in the concrete tanks; water quality was maintained by continuous water flow. The wooden tank has a sand and gravel filtration system, and copper sulfate ($CuSO_4$) was added. No chemical treatment was used in the other holding facilities and water osmolarity ranged from 852 to 918 mOsm. Air temperature normally varied from 14° C to 22° C while water temperature ranged from 11° C to 22° C.

Blood samples for these studies were drawn from the central veins or arteries of the tail fluke (Ridgway, 1965) or rarely from the flipper (Medway and Geraci, 1964). In the killer whales, dorsal fin (Fig. 6) or tail flukes (Fig. 7) could be utilized for blood sample collection. Only tail fluke samples were utilized in this study, however. Specimens were collected as animals were bled on a routine clinical basis over an 18 month period. Some research animals are bled on a regular schedule while others are examined and bled when their holding tanks are cleaned or when they are moved from or to an ocean pen.

We had opportunities to sample wild porpoises freshly caught in the open sea. Thirteen *T. truncatus* (Fig. 2) had been recently captured, but most of the samples from *T. truncatus* were from animals that have lived at our facility for two to six years. Several of these porpoises are normally maintained in ocean pens in the Pacific Ocean off the Marine Bioscience Facility, and frequently participate in experiments that require them to be free and untethered in the open sea. One *L. obliquidens* female (Fig. 3) was with us for five years and another male for over two years, while the others were recently captured. Two killer whales *Orcinus orca* were sampled about ten days after their capture and again six and ten weeks later. These two young males were apparently friendly from the outset and adapted more readily to captivity and human association than any other marine mammal with which we have been associated. Two pilot whales *Globicephala scammoni* were sampled soon after capture and then periodically during the next six months. In most cases, blood was drawn when the animals had been fasted overnight, but this was not practical in all cases and a few of the *T. truncatus* samples were taken from animals not fasted.

Fig. 2. Picture of *Tursiops truncatus*, the Atlantic bottlenose dolphin (or porpoise) (U.S. Navy photograph)

Fig. 3. Picture of *Lagenorhynchus obliquidens*, the Pacific white-stripped dolphin (or porpoise) (U.S. Navy photograph)

Fig. 4. Picture of *Orcinus orca*, the killer whale (U.S. Navy Photograph)

Fig. 5

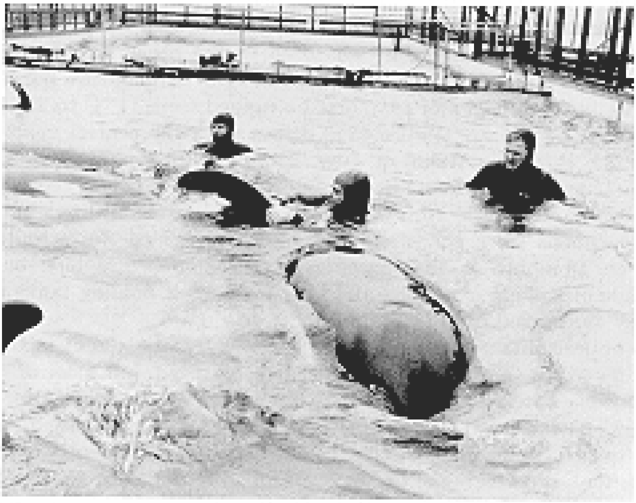

Fig. 6

Fig. 7

The heparinized samples were centrifuged at low speeds and the plasma removed and frozen for later analysis, with the exception of the Murphy-Pattee tests which were run immediatley. In addition to methods mentioned above (Barker *et al.*, 1951; Murphy and Pattee, 1964; Pileggi *et al.*, 1961), the method of Sterling and Brenner (1966) as modified by Lee (1969) was used in the determination of free thyroxine (f_{T_4}). Thyroxine binding globulin (TBG capacity) and unsaturated TBG (resin T_3 uptake test) were estimated by methods summarized by Henry (1967) and Sisson (1965).

Results

The results of the various groups of tests for circulating thyroid hormone are presented in Table 2. Values for the *T. truncatus* and *L. obliquidens* are in the same general range as the preliminary values in Table 1 and the previous clinical data on PBI's done in our laboratory.

The *O. orca* and *T. truncatus* specimens were similar as were the values for *G. scammoni* and *L. obliquidens*. Animals of the latter two species had low TBG, higher T_3 test, with low T_4 and PBI. Of possible significance is the fact that the pilot whales showed a high f_{T_4} while in the *L. obliquidens* it was lower. The really striking finding is the lower value in every index for the *L. obliquidens* save T_3 uptake value which was slightly increased in line with the lower TBG capacity.

Discussion

Sisson (1965) has reviewed the various thyroid function tests and their interpretation. The reader is referred to his discussion for the details of thyroid evaluation.

Crile and Quiring (1940) pointed out that particularly large thyroids occur mainly in aquatic mammals and in terrestrial mammals that dwell in the Arctic. Thus, we sought to find some relationship between circulating thyroid hormone and water temperature.

Three of our *T. truncatus* blood samples were taken from animals being maintained in Hawaii where water temperature was about 6° C warmer, and thirteen specimens were taken from animals upon arrival from southern Florida where water temperature was also about 6° C

Fig. 5. *Globicephala scammoni*, the California pilot whale in a large oceanarium tank with several dolphins in the background. Photograph courtesy of Mr. John Prescott, Marineland of the Pacific

Fig. 6. Small blood samples may be taken from the killer whale's dorsal fin. These whales adapt very readily to captivity and to human association

Fig. 7. Blood samples for these studies were taken from the central vessels of the tail flukes. The larger killer whale is being sampled here

Table 2. *Values (± standard deviation) for various thyroid tests performed on porpoises and whales. N = the number of test series while the number of animals used is written on the table. The analyses were performed at the Bio-Science Laboratories, Van Nuys, California*

N	T_4 (μg/100 ml)	PBI (μg/100 ml)	Inorganic I (μg/100 ml)	Total I (μg/100 ml)	T_3 uptake (%)	TBG (μg/100 ml)	f_{T_4} (μg/100 ml)	f_{T_4} (%)
				Fifteen ♂ *T. truncatus* (70–205 kg)				
15	8.18 ± 1.46	14.54 ± 4.43	1.28 ± 0.81	15.65 ± 4.80	9.85 ± 1.26	29.33 ± 3.24	3.48 ± 0.85	0.042
				Sixteen ♀ *T. truncatus* (61–189 kg)				
71	7.38 ± 1.72	11.79 ± 5.70	3.55 ± 2.34	15.34 ± 5.42	11.02 ± 1.36	23.65 ± 3.81	3.58 ± 0.90	0.048
				Two ♂ *L. obliquidens* (70–75 kg)				
4	2.58 ± 1.06	4.03 ± 1.25	1.07 ± 0.7	5.10 ± 1.47	12.0 ± 2.6	14.66 ± 5.51	1.73 ± 0.45	0.067
				Five ♀ *L. obliquidens* (55–94 kg)				
6	3.72 ± 0.95	4.67 ± 0.71	0.65 ± 0.67	5.32 ± 1.08	11.74 ± 0.82	14.40 ± 2.07	2.30 ± 0.51	0.060
				Two ♂ *G. scammoni* (390–520 kg)				
8	4.26 ± 1.57	6.67 ± 1.98	2.30 ± 1.30	8.96 ± 1.88	12.77 ± 1.57	14.21 ± 3.41	3.99 ± 1.58	0.093
				Two ♂ *Orcinus orca* (1730–2240 kg)				
6	6.05 ± 1.70	13.28 ± 4.00	2.70 ± 1.40	17.47 ± 4.55	9.10 ± 0.91	25.75 ± 3.50	2.78 ± 0.71	0.046
				Ranges for *Homo sapiens*				
	2.9–6.4	4.0–8.0	—	—	10–14.6	10.0–26.0	1.0–2.9	0.040–0.050

warmer than at Point Mugu. Two of these animals were sampled before, during and after 19 days in a heated pool (8° C warmer than their normal environment). The two *O. orca* were first sampled on their arrival from Puget Sound where the water temperature was 9° C colder than at Point Mugu. In none of these cases did there appear to be significant differences in the levels of the measured thyroid indices that could be related to these differences in water temperature.

Even waters of 25° C, which was the highest water temperature that we tested, represent a marked mammalian thermoregulatory problem because water has 20 or more times the cooling effect of air. Because of the apparently well regulated anatomical and physiological mechanisms for heat conservation (Scholander and Schevill, 1955; Bel'kovich, 1965), variations of 9° C or less in water temperature might be too small to produce metabolic changes.

A *T. truncatus* placed in a 4° C ice water bath showed a rapid 1° C rise in core temperature and some shivering after one hour. The critical temperature for *T. truncatus* must lie somewhere between 4° C and 11° C which is the lowest water temperature that we normally have at Point Mugu.

Three bottlenose porpoises (two female and one male) that were fasted for 72 hours had an average increase of 1.2 μg in plasma T_4 concentration as compared with prefast levels.

According to Williamson (1962) the thyroid of man produced sufficient hormone to maintain an average resting metabolic rate of 37 calories per hour per square meter (kcal/m²/h) of body surface area. An *L. obliquidens* of 80 kg had a body surface area of 1.5 square meters. To maintain body weight this animal required a food intake of 7700 kcal/day. This indicates a metabolic rate of about 200 kcal/m²/h. Oxygen consumption of a 128 kg, 228 cm, *T. truncatus* female was measured in a closed chamber partially filled with 17° C water for periods ranging from two to six hours (Fig. 8)[1]. The animal had been with us since 1963 and had previously been accustomed to staying in this tank for long periods of time. She was not stranded but had sufficient water depth to support her whole body. Previously this animal had been trained to hold her breath on command while submerged and exhale into an inverted water filled funnel and to press a paddle *manipulandum* on signal while in this same container. Although these measurements may not represent a basal rate completely comparable with such measurements in man and other terrestrial mammals, the porpoise was quiet, she was not actively swimming and in a state, as nearly as we could determine to a dolphin at rest. Average oxygen consumption was 900 ml/min which corresponds to about 6220 kcal/day for a measured respiratory quotient (RQ) of 0.77. The

1 A much larger chamber has been described by Pierce (1970).

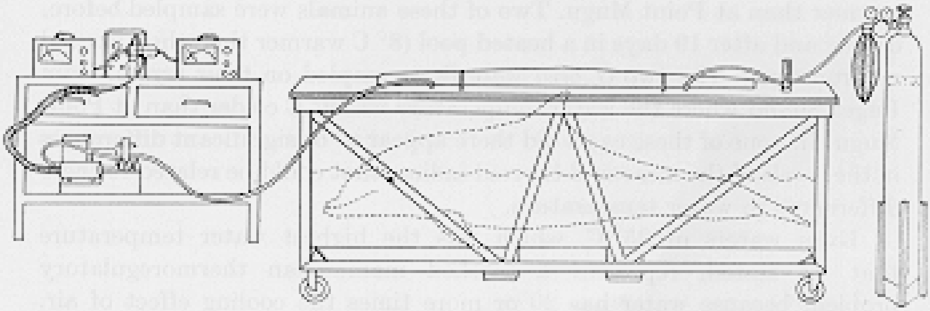

Fig. 8. Set-up for oxygen consumption measurements on a bottlenose dolphin

body surface area of this *T. truncatus* was calculated as approximately 2 meters for a metabolic rate of 138.5 kcal/m²/h. This is over three times the resting rate for man on a surface area basis and if we use the accepted formula of $W^{0.75}$ our *T. truncatus* had over twice the BMR of man. A man of 228 cm and 120 kg would have a considerably greater body surface area. A pig of the same surface area would be heavier, and one of identical weight would have a BMR of about 2150 kcal/day. Measurements of oxygen consumption made by Irving *et al.*, also showed bottlenose porpoises to have a high metabolic rate. The metabolic rate data on the *T. truncatus* appear to correlate well with the observed levels of thyroid hormone. The *L. obliquidens* values do not seem to correlate at all.

In most mammals the plasma serum cholesterol varies somewhat inversely with the PBI or T_4 (Ganong, 1967; Henry, 1967). Hyperthyroid individuals or those with high metabolic rates have a low cholesterol. The cholesterol is high in hypothyroidism. The cholesterol level of *L. obliquidens* is comparatively low (male 152 ± 43 mg/100 ml and female 155 ± 21 mg/100 ml) *T. truncatus* is higher (male 223 ± 27 mg/100 ml and female 219 ± 32 mg/100 ml). The *G. scammoni* (281 ± 63 ml/100 ml), and *O. orca* have the highest cholesterol (335 ± 61 mg/100 ml). These values for the killer whales are particularly interesting in view of the fact that both whales are sub-adult, have a diet (fish) low in saturated fats, and appear to have a relatively high metabolic rate, PBI, and T_4. The cholesterol value appears to be somewhat proportional to the average body weight of the various species. The cholesterol level of the *L. obliquidens* also indicates that these animals have the highest metabolic rates of the group if we assume that plasma cholesterol level has an inverse relationship to metabolic rate.

Our *L. obliquidens* subsist on the same diet as do the *T. truncatus* although their diets in the wild may vary somewhat. Thus, the iodine consumption of the two groups of animals should be similar. It is difficult to imagine how an animal living in an environment of ocean water which contains a relatively large amount of iodine could develop a dietary deficiency of this element. The low plasma values for total iodine and inorganic iodine seem to indicate either an impairment in absorption, a dietary deficiency or a more rapid utilization and thus faster turnover rate.

Sterling and Brenner (1966) showed significant increases in serum free thyroxine level in euthyroid "sick" individuals (mean 4.76 mμg/100 ml as opposed to 2.76 mμg/100 ml in normal adults). In man the absolute free thyroxine concentration is better correlated with thyroid function than the PBI. Euthyroid individuals with low thyroxine-binding alpha-globulin (TBG) exhibit an abnormally high percentage of free thyroxine. It is possible that our pilot whales fall into the category of euthyroid sick individuals as described by Sterling and Brenner (1966). During the course of these studies, they both appear to be in good health although one of them has since developed a chronically poor appetite.

Chronic stress is known to depress thyroid function (Williams, 1962). Stress may also be a factor in our *L. obliquidens*. This species does not adapt as readily to captivity as does the bottlenose dolphin. One male and one female that we sampled were trained to work free and untethered in the open ocean (Fig. 3). These animals were being trained for deep diving experiments similar to those reported with the bottlenose dolphin (Ridgway *et al.*, 1969). At the time of sampling they appeared to be in excellent health. However, the male died of a severe acute pancreatitis and the female later swam away from her trainer when a large wild male of the same species showed up at the diving site about two miles off Point Mugu. They apparently joined a large herd of *L. obliquidens* that was seen swimming through the area about one mile seaward.

The difference in total blood volume between *L. obliquidens* and *T. truncatus* may be of some importance in assessing thyroid hormone levels. The *L. obliquidens* have a total blood volume of about 11% of body weight whereas the *T. truncatus* have only 7% to 8% blood volume (Ridgway and Johnston, 1966). If the hormone in the blood can be viewed as a total intravascular pool, then the total amount of f_{T_4} of an *L. obliquidens* would be closer to, but not equal to that of *T. truncatus*.

In its evolution in a completely aquatic life, the bottlenose dolphin (*T. truncatus*) has adjusted to the increased metabolic requirements of such an existence by an increase in thyroid size and plasma thyroxine level. In the white-striped dolphin (*L. obliquidens*) the increase in thyroid size is still more pronounced, but plasma thyroxine level is much lower and it seems to us that the thyroid must be providing for metabolic requirements in some additional way. A more comprehensive study of thyroid function in species such as *L. obliquidens* and *P. dalli* that have a very large thyroid-to-body-weight-ratio seems clearly indicated.

We thank Dr. John Simpson and William Gilmartin for valuable technical assistance. Dr. N. D. Lee of Bio-Science Laboratories reviewed the procedures and made many helpful suggestions. Dr. William Medway, Dr. Richard Harrison, and F. G. Wood reviewed the manuscript.

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