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## PATHOLOGY IN CAPTIVE PLATYPUS (*ORNITHORHYNCHUS ANATINUS*) IN VICTORIA, AUSTRALIA

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**ABSTRACT:** Between 1978 and 1981, 20 platypus (*Ornithorhynchus anatinus*) which had been held in exhibits for varying periods, were submitted for necropsy. The most common gross and histologic lesions were adrenal enlargement, pulmonary pathology consistent with shock or aspiration pneumonia, intestinal coccidiosis, the presence of trypanosomes, myocarditis and nephritis. Other conditions encountered included infestation with ticks (*Ixodes ornithorhynchi*), a mild infection of intestinal trematodes (*Mehlisia ornithorhynchi*), myocardial toxoplasmosis, and focal hepatic necrosis. Adrenal weights, both absolute and relative to body weight, were determined in 12 specimens, and used as parameters of each animal's response to the stress associated with captivity. The results showed that, in platypus held in captivity from about 1 day up to 6 mo, both parameters were higher than in animals which were examined within a few hr of capture. In view of the general lack of conclusive necropsy findings, it was considered that these results indicated that stress may have been a significant underlying factor in the death of these animals in captivity.

### INTRODUCTION

The Ornithorhynchidae (platypus) and the Tachyglossidae (echidna) are the only surviving families of the class of egg-laying mammals known as the Monotremata. The platypus (*Ornithorhynchus anatinus*) is found in fresh-water streams and lakes of eastern Australia, and although it is apparently quite common in the wild (Frith, 1979) there are still large gaps in our knowledge of its biology and diseases. Apart from a brief record of a few parasites (Mackerras, 1958), this appears to be the first description of pathologic findings in the platypus.

### MATERIALS AND METHODS

From May 1978 until March 1981, 20 platypus were submitted for necropsy. Seven of these originated from the well-established platypus exhibit at the Sir Colin MacKenzie Fauna Park, Healesville, Victoria (FP), and the remaining 13 were submitted by the Royal Melbourne Zoo, Melbourne, Victoria (RMZ) where a new exhibit was opened in 1980.

On arrival each animal was weighed and examined at necropsy. Parasites were removed and preserved in 70% alcohol pending identification, and, in some cases, direct microscopic examination of wet smears from various levels of the alimentary tract was undertaken. In 12 of the platypus, the adrenal glands were removed, cleaned of adhering tissue, and weighed. Tissue samples were fixed in 10% formal saline, paraffin embedded, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin. Examination of

the brain, bone marrow, and bone structure was rarely possible at necropsy as the intact skeleton of each specimen was sought for teaching purposes. Bacteriologic examinations were made when considered necessary, and in these cases samples were cultured on horse blood and McConkey's agar, and inoculated into tetrathionate broth.

### RESULTS AND DISCUSSION

Details of the age class, sex, duration of captivity, body weight, and adrenal measurements of each animal are given in Table 1. The age of each platypus was estimated on the basis of body weight and the general condition of the animal. The time that each animal was held in captivity before it died varied from 2 hr to 4 yr, with 13 (65%) dying within 3 wk of capture. An assessment of the behavior of these 13 indicated that each had adapted very poorly to captivity.

The major necropsy findings encountered are summarized in Table 2. The most consistent were adrenal enlargement, pulmonary changes associated with shock and aspiration, endoparasites, and the presence of mild inflammatory lesions in the heart and kidneys. Descriptions of the gross and histopathologic findings in various organ systems are summarized below.

#### Endocrine system

Gross pathology of the adrenal glands was observed in only one of 16 platypus (6%), an immature male which had been held for 3 mo at the FP. The adrenals were markedly enlarged, each one being about one-third the size of its adjacent kidney.

The ratio of total adrenal weight (mg) to body

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TABLE 1. Age class, sex, duration of captivity, body weight and adrenal parameters of 20 captive platypus.

Age class	Sex	Duration of captivity	Body weight (kg)	Total adrenal weight (mg)	Adrenal wt./body wt. (mg/kg)
Mature	Male	1 day	1.54	300	195
		1 wk	1.25	840	672
		2 wk	1.42	—	—
		4 yr	1.05	—	—
			(1.31) <sup>a</sup>	(570)	—
	Female	1 day	—	—	—
		1 day	0.94	—	—
		1 wk	1.06	—	—
		1 wk	1.04	440	422
		1 wk	1.10	640	582
		2 wk	0.93	—	—
		3 wk	0.79	570	722
		12 wk	1.27	510	402
		12 wk	0.87	540	619
		24 wk	0.71	510	718
			(0.97)	(535)	—
Immature	Male	1 wk	0.43	640	1,488
		2 wk	0.27	340	1,259
		6 wk	0.85	—	—
	Female		(0.52)	(490)	—
		1 day	0.34	170	500
		2 day	0.50	—	—
		6 wk	0.31	260	839
			(0.38)	(215)	—
Weiss <sup>b</sup>		1 day	1.510 <sup>c</sup>	385 <sup>c</sup>	257 ± 41 <sup>d</sup>

<sup>a</sup> Figures in parentheses are mean values.<sup>b</sup> From Weiss (1973). Three mature male platypus examined soon after capture.<sup>c</sup> Mean value.<sup>d</sup> Mean ± standard deviation.

weight (kg) (ad.wt:bd.wt ratio, mg/kg) was determined in 12 specimens and the results are shown in Table 1 with the results of Weiss (1973) who examined three mature male platypus at necropsy within hours of catching them in the wild. This table shows that the ad.wt:bd.wt ratio of all animals held in captivity beyond 1 day was much greater than for the three wild animals which were examined soon after capture. Comparison of Weiss' results with the mean values of body weight and total adrenal weight for each age class and sex suggests that the main reason for the higher ratio in the captive platypus was the absolute increase in total adrenal mass, although a slightly lower average body weight may also have contributed to this increase.

Adrenal weights, both absolute and relative to body weight, are parameters indicating an animal's response to stress (Barnett, 1973). This study suggests that platypus held in captivity for at least a day have been subjected to more stress than wild animals since both parameters are higher in captive animals.

The ad.wt:bd.wt ratio for the immature platypus with the grossly enlarged adrenals was the highest (1,488) in the survey, and the total adrenal mass for this animal was greater than for many of the mature platypus. This may reflect the management of this animal which included a daily weighing. Three other immature animals in the survey also had high ratios, but none of these had a high total adrenal mass. However, this may be normal since relative adrenal mass is elevated in young animals of a number of species (Hartman and Brownell, 1949; Chester Jones, 1957; Weiss and Ford, 1977).

The characteristic architecture of the adrenal of the platypus (McDonald, 1978) was evident microscopically, and hypertrophy and hyperplasia of the steroidogenic cells was present in animals with high relative adrenal mass.

#### Hemopoietic system

*Blood and bone marrow:* One platypus had many slender, flagellated trypanosomes in blood smears and tissue sections stained with Giemsa.

TABLE 2. Prevalence of major necropsy findings in captive platypus.

Age class	Mature	Immature
Adrenal enlargement	7/8*	4/4
Pulmonary pathology		
Congestion	10/14	2/6
Aspiration	6/14	3/6
Intestinal coccidiosis	8/12	2/6
Trypanosomes	7/14	0/6
Myocarditis	4/11	1/6

\* No. positive/no. examined.

Similar organisms were found in the lung, liver or heart in seven of 20 (35%) platypus. All host animals had been collected from the wild and held in captivity at the RMZ for 1–3 wk before death. Although two of these animals were also infested with *Ixodes ornithorhynchi*, a possible vector, serial sections of two ticks failed to reveal crithidial stages. Trypanosomes have been observed previously in platypus (Mackerras, 1958), but their pathologic significance is unknown. (Specimens have been deposited in the Queensland Museum, Brisbane, Australia as nos. GL 2005 and GL 2006.)

The bone marrow was examined histologically in one mature animal only and appeared normal. Although very few megakaryocytes were present, numerous plasma cells were observed.

**Lymphoreticular tissues:** No gross lesions were in the 19 spleens examined, but histologically all cases had few to many megakaryocytes in the red pulp. Possibly their presence reflected the normal hemopoietic role of the spleen in many of the lower vertebrates and marsupials (Bryant, 1977). Frequently they were aggregated around the periphery of the white pulp, almost forming syncytial sheets in some cases, but there was no apparent relationship between the number of megakaryocytes and either the estimated age of the animal or the presence of trypanosomes. Plasma cells and immature lymphocytes were also present in the red pulp of most animals, but were unrelated to any other gross or histologic finding.

There was a wide range of histologic development of the white pulp in the spleens. Most specimens with a high ad.wt:bd.wt ratio had poorly developed lymphoid tissue suggesting involution associated with the elaboration of steroids from the adrenal and consequent

depression of the immune system (Claman, 1972). The normal histology of the monotreme spleen appears to be different from most eutherian mammals, but an adequate description is lacking. In this series of platypus the white pulp consisted of nodules of lymphoid cells, each nodule being centered on an arteriole. Germinal centers were observed in some nodules, and at the periphery of all nodules was a highly vascularized region. Peripheral aggregation of plasma cells was occasionally observed at one pole of some nodules. Progressive involution of the white pulp was characterized by poor development of the peripheral vascular zone and eventually by loss of definition of the nodule due to reduced concentration of lymphoid cells. Occasional platypus had high ad.wt:bd.wt ratios associated with apparently normal lymphoid tissue.

There was no obvious relationship between the estimated age of a platypus and the presence or absence of a thymus. Of the 20 animals examined, two which appeared to be mature were found to have a thymus, while five which were considered to be juvenile had no thymus. Other lymphoid organs were histologically well-developed in four of the latter and poorly developed in one which also had a very high ad.wt:bd.wt ratio.

#### Circulatory system

No gross lesions were observed in the heart or blood vessels of any specimen. Histologic examination revealed a chronic myocarditis or endocarditis in five of 17 (29%) platypus. These lesions were characteristically mild with focal infiltrations of lymphocytes and plasma cells, but no etiologic agent was observed.

A number of *Toxoplasma*-like cysts (Hartley, pers. comm.) were observed in the myocardium of a mature platypus which had been held in captivity at the FP for 4 yr. The cysts were thin-walled, contained numerous, small zoites, and measured  $18.5 \times 31.4 \mu\text{m}$  ( $14.8\text{--}22.2 \times 25.9\text{--}37.0$ ). Although the cysts were not directly associated with any inflammatory reaction, there were foci of chronic myocarditis in the heart of this animal. The cysts probably developed following ingestion of sporulated oocysts from the straw bedding used in the platypus nests. Fecal contamination of feed and straw by domestic and feral cats was a problem at the FP until recent years. The infection could also have

been acquired from a transport host such as annelid worms which comprise a substantial part of the diet of captive platypus.

#### Respiratory system

In 16 of 20 (80%) platypus gross pulmonary changes were noted. The lungs showed patchy or uniform reddening, they were often firm and failed to collapse when the thorax was opened, and frequently there was a copious frothy fluid in the airways including the trachea.

Histologic examination suggested two main pathologic processes. In 12 of 20 (60%) platypus there was marked congestion of the alveolar walls and occasionally some leakage of proteinaceous fluid into the alveoli. This was possibly a manifestation of shock.

Evidence of aspiration pneumonia was found in nine of 20 (45%) platypus, and was the probable cause of death in three cases (15%). Histologic examination revealed severe congestion, frothy, proteinaceous fluid, foreign, aspirated material and numerous bacteria in alveoli and airways, and patchy emphysema. A heavy mixed growth of bacteria including coliforms, *Aeromonas*, *Alkaligenes*, *Proteus*, staphylococci, and streptococci was cultured from each case.

An additional pathologic process was observed in three of 20 (15%) platypus. Grossly the lungs were uniformly reddened and consolidated, with pale foci up to 1 mm in diameter spread diffusely through the entire lung of one specimen. Histologic examination revealed marked perivascular infiltration of lymphocytes and plasma cells, some peribronchial lymphoid hyperplasia, and mild smooth muscle hypertrophy. The pale foci noted grossly in one lung were due to accumulations of foamy macrophages in the alveoli. No parasites were observed and isolation of viruses and mycoplasmas was not attempted.

#### Alimentary system

The only lesions associated with the alimentary tract were the presence of coccidia in 10 of 18 animals (56%), and a small number of trematodes in the duodenum of one of 20 animals (5%).

No gross lesions were associated with the coccidia which were detected during examination of fecal samples, mucosal smears, and routine histologic sections of the small intestine. Tissue

sections revealed no protozoa in the large bowel or the stomach, but various stages resembling schizonts and gametocytes were observed in epithelial cells lining the convoluted crypts of the small intestine, and in cells in the lamina propria. Even when an antemortem fecal examination suggested a heavy burden, histologic lesions at necropsy were only mild. Apart from mild infiltration of eosinophils into the lamina propria, there was no significant damage to the intestinal mucosa in the 10 animals.

Large numbers of unsporulated oocysts were collected from an antemortem fecal sample of one platypus. These were suspended in 2.5% potassium dichromate solution where they sporulated after 3 days at room temperature. The sporulated oocysts, identified as *Eimeria* sp., measured  $17.3 \times 22.2 \mu\text{m}$  ( $15.5\text{--}18.5 \times 21.8\text{--}22.6$ ) and contained four sporocysts. The warm, humid environment of the platypus burrow would appear to be an ideal site for the promotion of coccidial infections, and as these conditions are reproduced in captive displays, the extra stress on the host that is associated with captivity may be all that is required to allow the coccidia to reach pathologic levels.

Ten red dorsoventrally flattened trematodes, identified as *Mehlisia ornithorhynchi* (Pearson, pers. comm.; specimen deposited in the Queensland Museum, Brisbane, Australia as no. GL 1570) were tightly attached to the duodenal mucosa in one animal. There was no significant histologic damage to the gut.

#### Liver and biliary system

Gross liver lesions were restricted to occasional cases of hepatic congestion.

Microscopically trypanosomes were evident in the blood vessels and sinusoids of two of 18 platypus (11%). Small foci of necrosis and abscessation were also found in one of these livers, while the other showed a periportal lymphoid infiltration comprising lymphocytes and plasma cells. Numerous granulomas and minute foci of necrosis were found in the liver of a third animal with no evidence of the nature of the etiologic agent.

#### Urinary system

Although no gross lesions were noted in the urinary tract of any specimen, five of 20 (25%) showed mild chronic interstitial nephritis his-

tologically. No significant organisms were cultured, and a causative agent was not identified.

#### Skin and appendages

From six to 10 ticks, identified as *Ixodes ornithorhynchi* (Adolph, pers. comm.; specimens deposited in the Queensland Museum, Brisbane, Australia, as no. S 1264), were scattered over the bodies of two platypus. Large, engorged females were present in one case, while the other showed only the smaller males or nymphal stages.

Discrete abrasions were present on the dorsum and lateral margins of the muzzle in two animals which had been anorectic. Similar abrasions were noted on the skin of the hind feet in two cases and on the skin of the ventral tail in a further specimen. As these abrasions were not present at capture, they were probably associated with the continuous activity of the newly-captured animals in an unfamiliar environment. Lesions on the muzzle could be quite painful to the platypus due to the dense supply of sensory nerves in this region (Griffiths, 1978). Consequently the platypus might be discouraged from using its muzzle in the search for food. Both platypus with muzzle abrasions had been anorectic, and necropsy revealed scanty contents in the stomach and small intestine of each animal.

#### Miscellaneous

No gross lesions were observed in any other organ system, and no histologic lesions were noted in the four testes or one brain examined.

In view of the general lack of conclusive necropsy findings, it was considered that stress, manifested by increased ad.wt:bd.wt ratios, was a significant underlying factor in the death of these animals in captivity. However, further work is required on the endocrine and histologic responses to stress in platypus. The possible significance of trypanosomes and coccidia in platypus also warrants further investigation.

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