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HEPATOPATHY AND HYPERCHOLESTEROLEMIA IN RED PANDAS ASSOCIATED WITH A DIET CHANGE

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Bamboo is a staple for the red panda, *Ailurus fulgens*, in the wild and has been used successfully as a mainstay in their diets while in captivity (Glatston, 1980; Roberts, 1982). Due to the fact that bamboo is not plentiful in many geographic regions, it remains a limiting factor in the propagation of red pandas in captivity. A feeding trial was conducted in the autumn of 1986 at the National Zoological Park (NZP) in Washington, D.C., to search for a suitable fiber replacement. The purpose of this paper is to describe a condition characterized by liver dysfunction and elevated serum cholesterol that developed during these feeding trials in five red pandas. The liver problem (hepatopathy) was discovered inadvertently in one of the red pandas (no.3, Dusty) that had undergone a pre-shipment health examination early in December of 1986. Although not ill at the time, the red panda had developed a markedly elevated alanine aminotransferase (ALT), a serum enzyme used to monitor liver function in domestic carnivores. As a result of these findings the four other red pandas on the feeding trial were tested eight days later and were also found to have similar serum chemical abnormalities.

Materials and Methods

The feeding trials were carried out in five red pandas (4 males, 1 female) born and mother-raised at the NZP's Conservation and Resource Center in Front Royal, Virginia. Details of these trials are described by Warnell *et al.*, (this volume). Diets of all five animals were returned to the original gruel and bamboo in December, 1986, immediately after discovery of the liver abnormalities. Complete blood count (CBC) including hemograms and serum chemical profiles were performed serially on each animal from December 1986 through July 1987. These included ALT, alkaline phosphatase (AP), Bilirubin, total protein and other serum chemical parameters that were measured in the NZP clinical pathology laboratory. Values for these tests were also available for some of these red pandas prior to the feeding trials performed in December 1986. Serum cholesterol tests were performed in the NZP laboratory from January through July 1987. Serum triglycerides and thyroid functions test (T3, T4) were undertaken at the Veterans Administration Hospital in Washington, D.C., and lipid profiles including high density lipoprotein (HDL), low density

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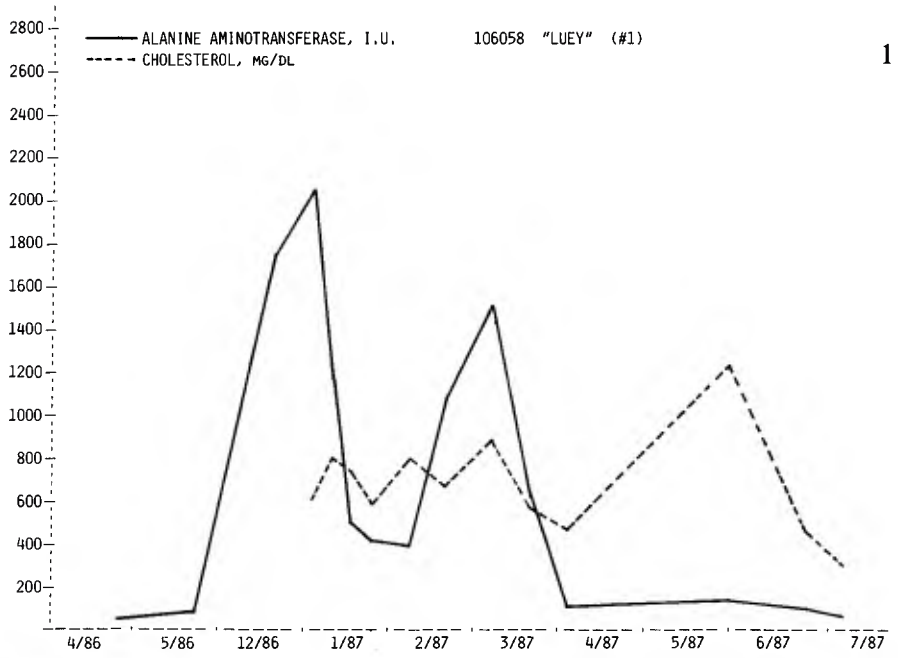
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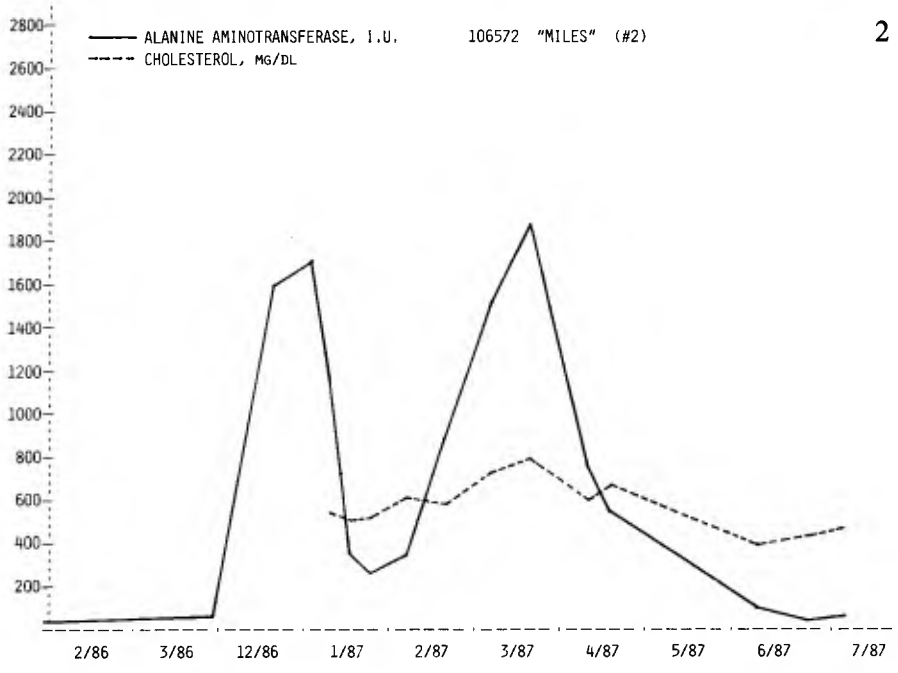
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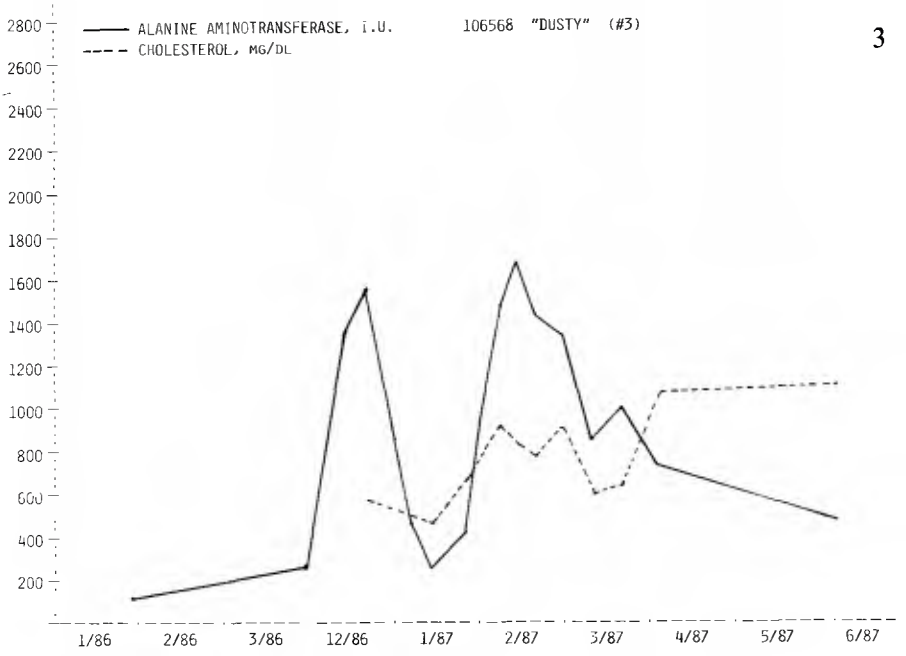
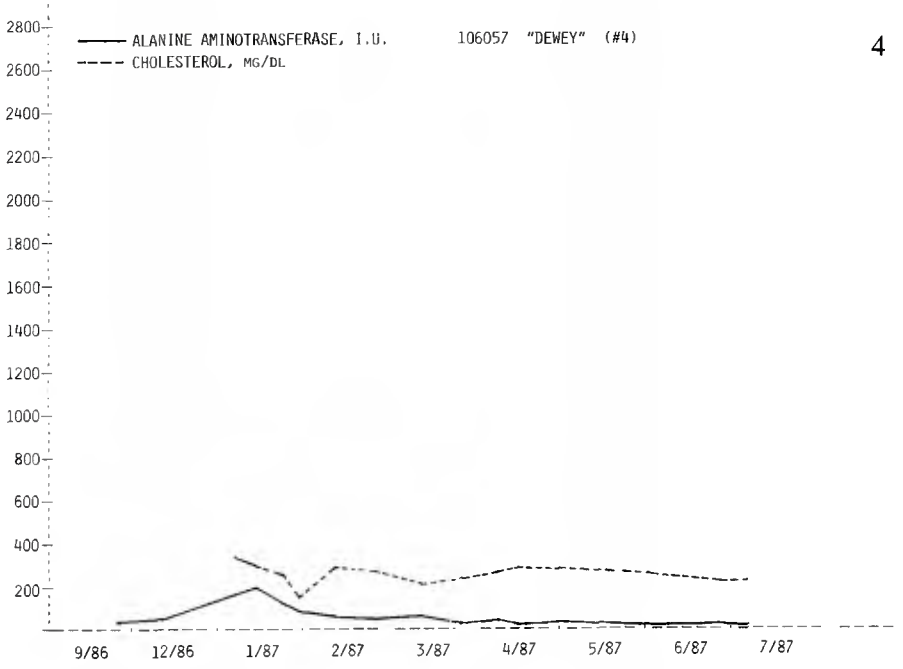


Figs. 1–3. Alanine aminotransferase (ALT) and cholesterol values in 3 high-effect red pandas with hepatopathy.

lipoprotein (LDL), and very low density lipoprotein (VLDL) were all analyzed by Metpath, Kensington, Maryland. Each of the five pandas underwent two liver biopsies at two to three month intervals, between January 2nd and April 10th, 1987. The red pandas were anesthetized using a combination of ketamine and halothane and closed liver biopsies were performed with the aid of a laparoscope (Bush *et al.*, 1980). Liver biopsy specimens were fixed in Trump-McDowell combined fixative and processed routinely for light and electron microscopy. Ultrastructural and special histochemical studies were conducted at the Department of Hepatic Pathology, Armed Forces Institute of Pathology (AFIP) which included oil-Red-o stains and polarized light microscopy on frozen fixed liver tissue. Specimens of biopsied liver were also routinely cultured for bacteria and fungi. As the dehydrated alfalfa meal used in the feeding trials was the only newly introduced substance, samples of this were analyzed by the Michigan State University (MSU) Toxicology laboratory for mycotoxins, abnormal mineral concentrations and toxic xenobiotic compounds by gas chromatography/mass spectrometry methods. (via Dr. D.E. Ullrey, MSU).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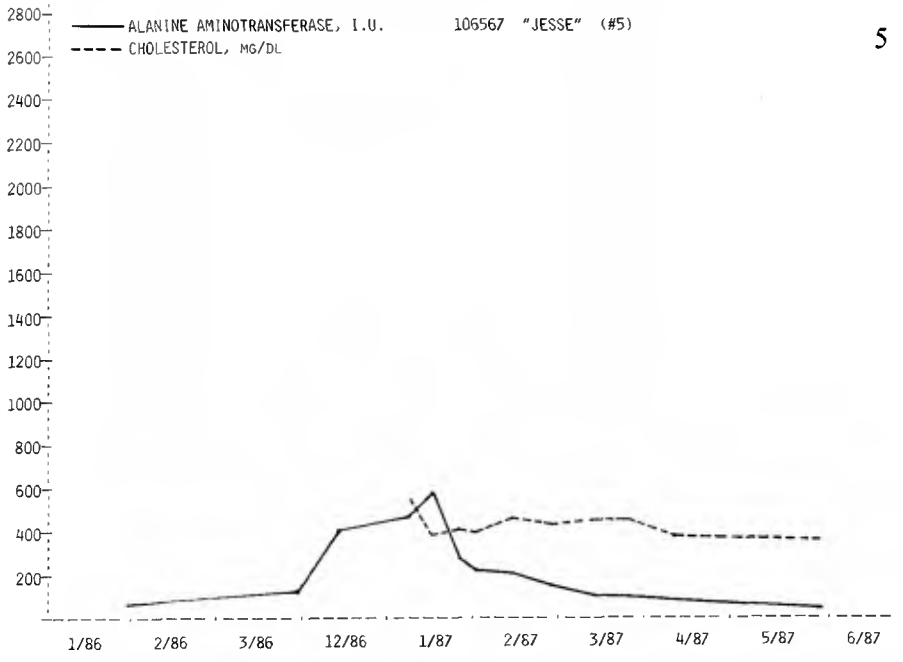
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Table 1. Results of thyroid function test in three high-effect red pandas with hepatopathy.

Red panda	Date	T3 (NG/DL)	T4 (UG/DL)
#1 (Luey)	4/23/86	80	2.4
	1/16/87	203	6.0
	3/19/87	103	2.5
#2 (Miles)	1/09/87	117	4.2
	3/19/87	112	2.4
#3 (Dusty)	2/28/87	82	3.5
	3/19/87	68	1.6

Results

Results of the feeding trials with bamboo, gruel and alfalfa meal applicable to the palatability, digestibility, and effects on stool consistency are discussed by Warnell *et al.* (1989).

There were marked elevations (high-effect) of the ALT and cholesterol values in three of the five red pandas involved in the feeding trials (Figs. 1, 2 & 3) and milder elevations (low-effect) of these components in the two other animals (Figs. 4 & 5) that persisted for up to 7 months after the original observations. In each of the high effect animals the ALT showed distinct biphasic curves. Liver enzyme levels returned to normal or near normal over the seven month period. Lipid profiles showed a high ratio of LDL cholesterol to HDL cholesterol, up to 4:1 in the high-effect red pandas, and a ratio in the low-effect animals that approximated 1:1. Analysis of the dehydrated alfalfa meal was negative for mycotoxins, mineraltoxins and toxic xenobiotics. Thyroid function tests in the three high-effect red pandas were within the normal range for domestic carnivores (Table 1). Liver biopsies showed varying degrees of fatty change with hepatocyte degeneration and evidence of cholesterol accumulation both in the degenerated hepatocytes and within Kupffer cells and the giant cells reacting to these changes (Fig. 6). The hepatic lipidosis was confirmed with the oil-Red-o stain; the cholesterol clefts were birefringent with polarized light indicative of cholesterol ester deposits. The cholesterol was also evident in electron micrographs of liver cells (Fig. 7) and there were small amorphous nonmembrane bound granular inclusion material in the cytoplasm of some of the hepatocytes. The significance of this latter change could not be determined. Liver biopsies showed more intense histologic changes in the three

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Fig. 4,5. Alanine aminotransferase (ALT) and cholesterol values in 2 low-effect red pandas with hepatopathy.

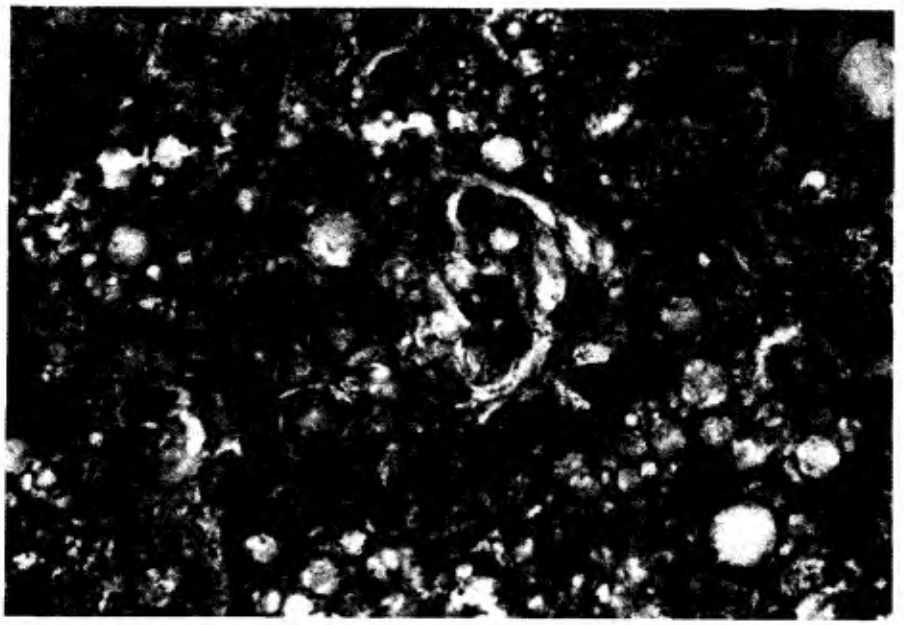

Fig. 6. Photomicrograph of liver from red panda no. 1 with hepatopathy shows lipidosis (clear vacuoles) with heptocyte degeneration (arrow) and a cluster of reactive macrophages (center). H&E, ×256.

high-effect red pandas but they were not any further advanced in the follow-up biopsies taken 2-3 months later.

Discussion

Although the five pandas were clinically normal from the onset and remained so throughout the study, liver enzyme studies and biopsies were indicative of a progressive insult to the liver that might have resulted in liver failure if allowed to continue. NZP range for ALT, considered the most liver-specific enzyme in carnivores, was 16-113 I.U. in normal red pandas, (mean = 49 I.U., n = 34). Alanine aminotransferase values exceeded 2000 I.U. in some of the high-effect animals. Specific causes of the liver cell injury could not be determined but infectious conditions were ruled out by the negative cultural findings and the lack of pathological changes consistent with an infection. As alfalfa meal was the only newly introduced item in the feeding trials, it was

Fig. 7. Electrophotomicrograph of liver from red panda no. 2 with hepatopathy shows degenerated hepatocyte containing cytoplasmic lipid material (clear vacuoles) and spindle-shaped profiles indicative of cholesterol accumulation. ×11, 570.

highly suspect, hence scrutinized for toxic substances. Mycotoxins have been associated with hepatic injury in domestic dogs via food contamination (Wilson *et al.*, 1967), but the alfalfa product used in the red panda diets was found to be free of these toxins. Alfalfa also contains endogenous substances, including phytoestrogens, saponins, and soluble proteins; the latter lead to bloat factors in cattle. Alfalfa sprouts and other legumes contain analogues of arginine called conavanine and indospicine which inhibit the incorporation of arginine into the liver. Conavanine from alfalfa sprouts has been associated with lupus erythematosus in humans and simian primates, and indospicine has caused liver cirrhosis in rats fed tropical legume seed containing this substance.

Natural exposure to indospicine has also been associated with liver disease in cattle and sheep (Cheeke and Shull, 1985; Christie *et al.*, 1975). It is currently unknown whether high concentrations of legumes would have any untoward effects on an animal such as the red panda which is herbivorous but has a digestive tract more typical of a carnivore (Flower, 1870). However, given the quantities of alfalfa and leguminous products fed to other species, any toxic effects due to the substances mentioned above must be both uncommon and sporadic.

A possible role of cholesterol in the pathogenesis of the hepatopathy was also considered. True normal serum cholesterol values are unknown in red pandas as, for the sake of accuracy, they would need to be obtained from animals in the wild. NZP values for cholesterol in captive red pandas ranged from 172/588 mg/dl, (mean = 330 mg/dl, n=31) as compared with 95-210 for domestic carnivores (Duncan and Prasse, 1986). When all diet components were re-examined it was clear that the original gruel was quite high in cholesterol (93 mg/100 ml of gruel) and that there may have been an absolute increase in the cholesterol intake since the red pandas appeared to ingest more gruel during the dietary manipulation. A larger percentage of the cholesterol in the high-effect red pandas was of the LDL type but the significance of this in red pandas is unknown. Although the liver changes resemble some of the familial cholesterol ester storage diseases of humans which can lead to liver cell injury (Scarpelli, 1985), ALT levels returned to normal or near normal in the face of rising cholesterol levels (up to 1200 mg/dl) in the three high-effect red pandas (Figs. 1-3). This suggested the hypercholesterolemia may have been an effect rather than a cause of the hepatopathy observed, since the liver is the primary site of cholesterol catabolism and excretion via the biliary system (Linder, 1985).

In humans and other mammals, serum cholesterol rises abnormally during hypothyroid states since thyroxin is important for the integrity of the LDL receptors which, in effect, are important in maintaining low serum cholesterol levels (Linder, 1985). Although clinical hypothyroidism has been diagnosed (and treated) in a red panda at the NZP (Alvarado *et al.*, in prep.), thyroid function was found to be normal in the three high-effect red pandas.

When the first observation of elevated liver enzymes was made in the index case (red panda no.3), all the pandas were returned to their original diet of gruel and freely available bamboo, and panda no.3 was placed on corticosteroids. The steroids had little effect on the course of the hepatopathy and ALT values in all red pandas eventually reverted to normal or near normal, by the end of the 7 months. Throughout this follow-up period, there was considerable fluctuation of the ALT values and the significance of the biphasic peaks noted in the three high-effect animals was not determined.

Summary and Conclusions

Five young adult red pandas developed a liver disease that was characterized by biochemical and histological changes but no clinical signs and appeared to be associated with the removal of bamboo and the incorporation of dehydrated alfalfa meal into their diet. Despite a change back to the original diet right after the condition was discovered, seven months elapsed before the serum chemical values reverted to normal or near normal. The specific cause of this condition was not determined but it had the pathological hallmarks of a toxic hepatopathy. Although the clinicopathologic studies suggested that elevated serum cholesterol and hepatic cholesterol ester accumulations were secondary to the hepatopathy, new low-cholesterol gruels have been formulated (2 mg/100 mg and 44 mg/100 mg of gruel) and are currently being evaluated. Efforts to further elucidate the hepatopathy will be carried out in another feeding trial with different red pandas, to test low and high cholesterol combinations with alfalfa and bamboo. These animals will be followed periodically with liver biopsies and serum chemical evaluations as before.

This paper also points out the importance of periodic health screening with serum chemical panels for early detection of abnormalities before clinical signs develop.

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