Metabolic Epidermal Necrosis as a result of a Glucagonoma in a Mixed Breed Dog

Introduction:

A nine-year-old neutered male Labrador mixed-breed dog initially presented with crusted serum between the metacarpal and digital pads of the left forepaw. This gradually progressed to more widespread skin lesions, anorexia, and weight loss. Histopathology of the skin lesions, ultrasonography, and an amino acid profile were consistent with metabolic epidermal necrosis. Eight months later, a necropsy of the dog including histopathology of liver sections with biochemical staining revealed the underlying cause of the skin lesions was a metastatic glucagonoma.

This report describes the clinical course of metabolic epidermal necrosis in a dog caused by a glucagonoma, the diagnostics performed to determine the underlying cause, and the attempts at therapy to alleviate the symptoms of a disease that carries a grave prognosis.

History of the disease

Metabolic epidermal necrosis (MEN), also known as superficial necrolytic dermatitis, necrolytic migratory erythema, and hepatocutaneous syndrome, is a manifestation of internal disease presented on the skin. It was first recognized in 1986 and was called diabetic dermatopathy because the first four cases were reported in diabetic dogs.[1] Since then it has been seen in conjunction with metabolic diseases of the liver, pancreas, and gastrointestinal tract. Examples are pancreatitis, pancreatic atrophy and fibrosis,[2] and gastrointestinal disease such as protein-losing enteropathy.[3,4] It has been reported with mycotoxin ingestion,[5] phenobarbital or primidone administration,[4,6] copper-associated

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hepatopathy,[3] and idiopathic hepatopathy.[3] It has also been seen with glucagonomas, hyperglucagonemia, and glucagon-secreting liver metastasis (no primary tumour identified).[3] In general, hepatopathies have been the most common underlying cause of MEN in dogs, whereas glucagonomas are more often associated with human MEN.[3] Because of the role glucagon plays in cases of people with MEN, it has been extensively studied. Glucagon is a 29 amino acid polypeptide secreted by the alpha cells of the islets of Langerhans in the pancreas.[7] Normally it is secreted in response to hypoglycemia to stimulate glycogen breakdown, promote gluconeogenesis in the liver, and block glycolysis.[8] The net result is to markedly increased the release of glucose by the liver.

To date canine glucagonoma has only been reported in the literature in eight other dogs.[9] Cats have also been documented to get MEN. Although rare, reported cases include a cat with pancreatic carcinoma, a cat with thymic amyloidosis, and a cat with chronic hepatopathy.[3] There have been no reports of glucagonoma in cats.[10]

Pathophysiology

The role of amino acids

The exact pathogenesis of MEN is unknown; however, there are several hypotheses.
The first theory is that cutaneous lesions in dogs with MEN arise as a result of increased catabolism of amino acids by the liver, resulting in depletion of plasma amino acids.[4] This finding differs from dogs with hepatitis where compromised liver metabolism results in increased amino acids. (Dogs with hepatitis have increased amino acid levels due to decreased uptake of amino acids by the liver and lysis of necrotic liver with release

of amino acids into the blood).[4] The low amino acid levels seen with MEN are proposed to be responsible for the characteristic skin lesions as their depletion makes them unavailable for the health and repair of normal skin (see Figure 1). Amino acids were reduced by 32% of mean normal values in a case of canine glucagonoma with characteristic skin lesions of MEN.[11] An increase of 102% occurred in 22 of the 24 amino acids measured 30 days after the tumour was removed.[11] Further support for the role of amino acids in MEN comes from the favourable response seen when dogs are given intravenous administration of amino acids. The intravenous route bypasses portal circulation and removes the role the liver plays in amino acid catabolism. As a result, amino acids are delivered directly to the peripheral tissues.[4]

Table 1 summarizes all the cases of canine glucagonoma reported in the literature to date. Three out of eight cases actually measured amino acid concentrations in the plasma of affected dogs. In all three cases, the majority of amino acids were reduced and the reduction was highly significant.

The question that still remains is what stimulates the liver to begin breaking down amino acids?
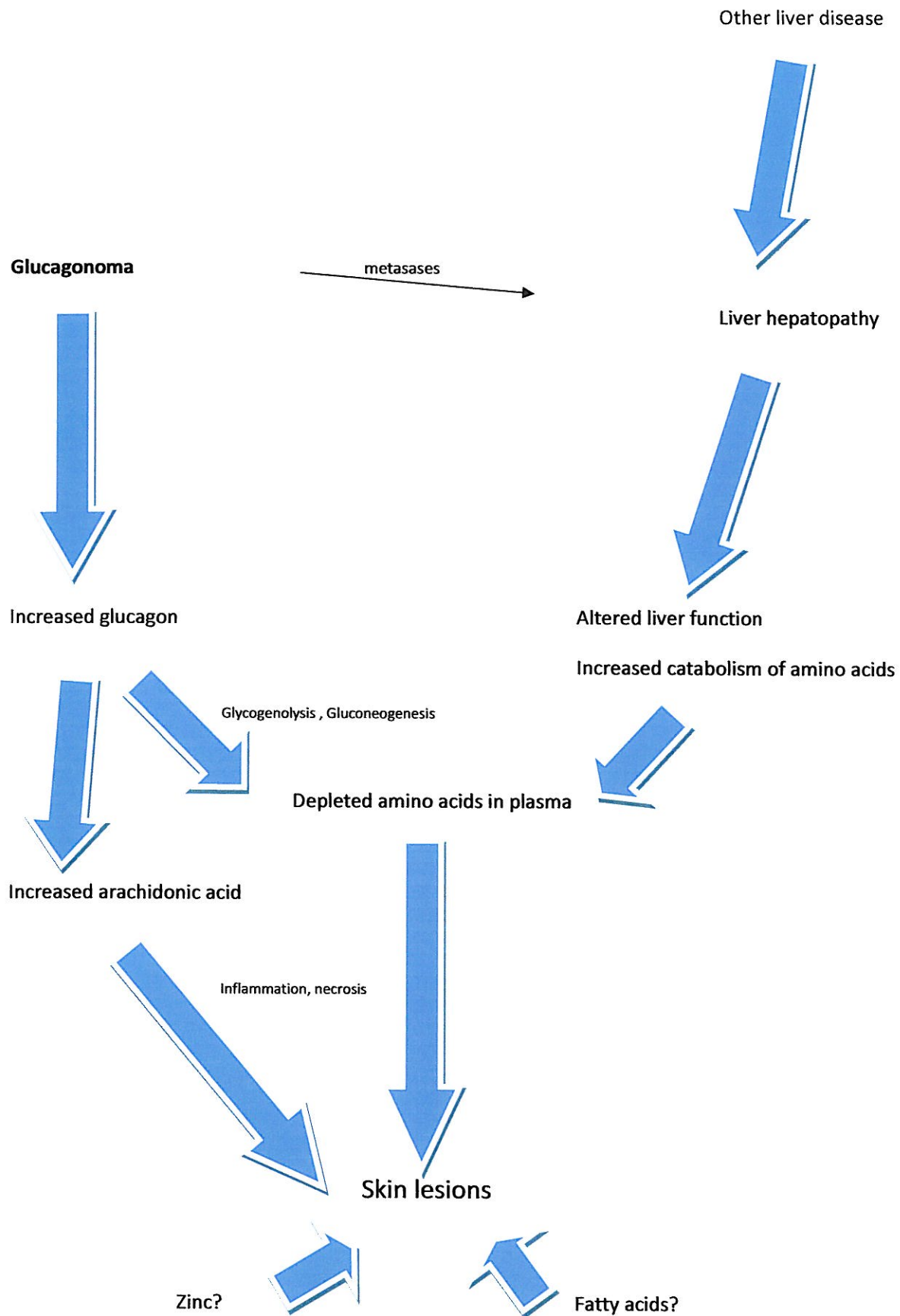
Other liver disease

Glucagonoma → metasases →

Liver hepatopathy

Increased glucagon

Altered liver function

Increased catabolism of amino acids

Glycogenolysis , Gluconeogenesis

Increased arachidonic acid

Depleted amino acids in plasma

Inflammation, necrosis

Skin lesions

Zinc?   Fatty acids?

Figure 1: Proposed Pathogenesis of Metabolic Epidermal Necrosis

Ref	Authors, year	signalment	skin lesions	glucagon level (reference range)	amino acid levels	imaging	primary tumor	metastasis	surgery	outcome	histo pancreas	histo liver
12 1990	Gross, O'Brien, Davies, Long, 1990	Fox terrier, 11y, M	yes	na	na	u/s (possible panc mass)	right pancreatic limb	na	yes	died 3 d post-surgery (pancreatitis)	carcinoma amyloidosis vacuolation	diffuse vacuolation
12 1990	Gross, O'Brien, Davies, Long, 1990	Lab retriever 9y, F	yes	na	na	u/s (WNL)	pancreas	na	yes	euth 3 d post surgery (pancreatitis)	carcinoma	diffuse vacuolation
13	Miller, Anderson, McCann, 1991	Mixed Breed, 5y, FS	yes	528 (130 pg/ml)	na	u/s (small liver)	not found	to liver	no	euth 6 w post presentation	necrotising pancreatitis	mild vacuolar degen, nests of islet cell tumors
14	Bond, McNeil, Evans, 1995	Boxer, 8y, MN	yes	830 (45 pg/ml)	13 of 18 reduced	u/s hyperechoic area in liver	pancreas	to liver and hepatic lymph node	yes	euth 5 d post surgery adhesions, pancreatitis	tumor, hyperplasia, tumor foci, no vacuolation	low grade nodular
11 O'Brien, 1987	Torres, Caywood, Poodle, 11y, MN		yes	700 (25-250 pg/ml)	20 of 24 reduced	u/s WNL	pancreas	to mes. lymph nodes	yes	alive at time of publication; euth 9 m post sx (ref 5)	tumor	na
15 Hardy, 1997	Torres, Johnson, McKeever, Springer spaniel, 13y, M		yes	397 (25-250 pg/ml)	na	u/s (WNL)	pancreas	to liver	no	euth 2 w post presentation	eosinophilic matrix, amyloid	endocrine tumor
16	Allenspach, 2000	Bernese mountain dog 9y, FS	yes	375 (<50 pmol/L)	4 of 5 reduced	u/s (multiple liver nodules)	not found	to liver	yes	euth 4 w post presentation	hyperplasia	endocrine carcinoma
9	Langer, Jergens, Miles, 2003	Lab retriever 6y, MN	yes	820 (<200 pmol/L)	na	u/s: multiple liver lesions, CT:mass in pancreas	pancreas	to liver	yes	alive 4 m post surgery	endocrine neoplasm	endocrine tumor

Table 1: Dogs with glucagonoma reported in the literature

na= test not available

The role of glucagon

The second theory is an embellishment of the first but is unclear due to conflicting reports. It is thought that glucagon may be the driving force in the pathogenesis of MEN. Increased production of glucagon by a glucagon-producing tumour (or its lack of degradation) causes excessive gluconeogenesis. Gluconeogensis, which occurs primarily in the liver, involves the catabolism of amino acids to produce glucose, resulting in decreased plasma amino acid levels. Hypoaminoacidemia causes the skin to become susceptible to lesions because of the requirements for histidine and lysine-rich keratohyalin granules in the stratum granulosum.[17,18] Furthermore, increased glucagon may stimulate arachidonic acid synthesis in keratinocytes and lead to arachidonic acid build up and subsequent inflammation and necrosis of the skin.[8,19]

The theory that glucagon is responsible for cutaneous lesions is supported by documentation of decreased intensity of skin lesions following surgical debulking of a glucagon-secreting tumour in a human and one dog.[3,11] For dogs with reported glucagonomas, all six of the eight cases that measured glucagon had elevated levels compared to the reference range (see Table 1).[9,11, 13-16]

Discrepancy exists as to whether glucagon is actually responsible for the clinical signs of MEN.[3] It has been argued that elevated glucagon may be detected not only in glucagonoma patients, but also in patients with diabetes mellitus, pancreatitis, or chronic liver disease, irrespective of skin lesions.[18,20] However, glucagon has also been reported to be in the normal range when typical skin lesions of MEN are documented. Glucagon

was normal in 5 dogs with skin lesions characteristic of MEN caused by a hepatopathy.[19] In addition, human patients were reported to have signs of MEN, regardless of the underlying cause, with apparently normal glucagon levels.[21] A review of 11 dogs with hepatocellular degeneration and nodular regeneration consistent with MEN (without verification of a glucagonoma), showed elevations in glucagon in all five dogs that were tested.[22] Four of these five dogs, however, were also diabetic, and the glucagon levels were higher in these dogs than the levels traditionally reported in diabetic dogs.[22] Since the fifth dog with hepatopathy was not diabetic, the observations indicate more than just diabetes might be influencing the glucagon levels.[23] It has been speculated that there may be undetected primary tumours in other areas of the gastrointestinal tract that hypersecrete glucagon.[13]

Another problem with glucagon measurement is accuracy and validity. The blood for the glucagon assay has to be handled carefully. It must be collected in a chilled glass potassium EDTA tube with a special reagent, held on ice, centrifuged, then separated into its plasma components and frozen at $-20^{\circ}C$.[14] It is not known whether the assay is accurate enough to differentiate neoplastic from non-neoplastic conditions in dogs.[13] Furthermore, different values have been reported for the same sample at different laboratories because labs may perform different assays, which may lack sensitivity or specificity.[23] In addition to these inconsistencies; the peripheral concentrations of glucagon do not always correlate with the pancreatic levels being secreted. Glucagon secretion by the pancreas can triple without significantly affecting the peripheral glucagon concentration due to significant (up to 50%) first-pass extraction of glucagon by

the liver.[2] In other words, increased glucagon in portal blood could result in increased gluconeogenesis in the liver with a concurrent depletion of amino acids, but without detectable elevated glucagon in the peripheral plasma after hepatic extraction.[19]

The role of fatty acids and zinc

Lastly, it has been proposed that essential fatty acid and zinc deficiency may contribute to the pathogenesis of MEN because of the similar histological appearance of the skin lesions in cases with these deficiencies.[3] However, neither supplementation with zinc nor fatty acids has been proven to ameliorate the cutaneous signs of MEN in most dogs .[25] In humans, there have been reports of zinc and fatty acid supplementation, in combination with adequate protein, improving clinical signs of MEN.[3] For this reason, it has been speculated in the human literature that an altered metabolic pathway from a deficiency of one or all three of these elements is the cause of the skin lesions.[3]

The fact that MEN lesions are seen in a variety of metabolic disturbances (namely hepatopathy and glucagonoma) is perplexing because the underlying link to the dermatological manifestations still remains unknown.

Presentation

Dogs suffering from MEN are usually middle-aged to older (4 to 16 years of age),[7] and males are more commonly presented than females.[4] The most common clinical signs of MEN are the distinctive skin lesion. These lesions include erythema, alopecia, and crusting erosions. Animals may present with ulcers and scaling around the nose, muzzle,

eyes, perineum, ventrum and extremities, especially the footpads.[7] Pressure points and mucocutaneous junctions may be affected due to friction or trauma.[7] An animal may be presented for sore feet or their reluctance to walk due to the hyperkeratosis, fissuring, and crusting of the footpads. The skin lesions may become secondarily infected with bacteria, dermatophytes, and yeast.[4] Affected animals may be lethargic, anorexic, depressed, and have lost weight.[7] Depending on the underlying pathology, dogs may also be polyuric and polydipsic, especially if they have diabetes mellitus. If dogs become diabetic, it is usually late in the course of the disease and the diabetic state is subsequent to persistent hyperglycemia.[9] Elevated blood glucose is a result of glucagon triggering glycogenolysis and gluconeogenesis. Insulin production may be normal or impaired but the secreted levels cannot keep up with glucagon production, leading to diabetes mellitus.[9]

Laboratory Findings

Blood work may be variable depending on the underlying cause of MEN. There may be a nonregenerative (to mildly regenerative), normochromic, normocytic anemia due to glucagon-induced protein catabolism.[16,9] Abnormalities in red cell morphology include polychromasia, anisocytosis, poikilocytosis, and target cells.[3] Some dogs may show serum biochemical abnormalities, but this is not a consistent finding. Elevated serum aminotransferase (ALT), serum aspartate aminotransferase (AST) and alkaline phosphatase (ALKP) are commonly elevated.[16,9] Bile acids may be normal or severely abnormal in dogs with MEN caused by a hepatopathy.[3] Dogs with glucagon-secreting tumours may have no biochemistry abnormalities, or they may have elevated liver

enzymes with normal bile acids.[9] Blood glucose levels may be considerably high or may fluctuate;[3] again, this is not a consistent finding, even with a glucagon-producing tumour. Albumin may be low, and may be reflective of the hypoaminoacidemia.[3] Other possible biochemistry abnormalities are hyperinsulinemia, concurrent diabetes mellitus and/or hyperadrenocorticism.[11]

Imaging

Ultrasound examination of the abdomen may reveal a completely normal abdomen, even in cases of pancreatic neoplasia. Alternatively, a pancreatic mass, liver pathology and/or metastatic disease in the liver may be found.[3]

A pancreatic mass was found in only one of the eight cases that underwent an abdominal ultrasound (Table 1).[12] Five of those cases had a primary tumour in the pancreas that was not detected by ultrasound, but was later confirmed at surgery or necropsy.[9,11,12,14,15]

The ultrasonographic appearance of the liver of a dog with MEN and underlying hepatopathy may have a 'honeycomb' or 'swiss cheese' pattern that has been reported to be pathognomonic for the disease.[2] The pattern is caused by variably sized hypoechoic regions measuring 0.5 to 1.5 cm in diameter, which correspond to regenerative nodules, surrounded by highly hyperechoic borders.[2] The echogenic borders reflect degenerating, fat-laden, vacuolated hepatocytes, bile ductules, and a network of reticulin and collagen fibers of collapsed hepatic lobules.[2] The first two dogs reported with glucagonoma in the literature in 1990 were not reported to have the characteristic honey-comb appearance of

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the lesions on ultrasound.[2,12] However, it was reported in 2003 that of the eight cases of glucagonomas causing MEN, half of them did have ultrasonographically visible liver abnormalities, including multiple hypoechogenic foci throughout the liver.[9]

The use of computed tomography (CT) has been used to locate a glucagonoma in a dog for the first time.[9] The advantages of CT imaging are the relative non-invasiveness of the procedure, the ability to locate small masses, and the ability to determine the extent of metastases from the primary site of neoplasia. Most importantly, in the study mentioned, the CT scan was able to locate a pancreatic mass that was not detected by ultrasound.[9]

Skin Histopathology

MEN can be reliably diagnosed by the unique histopathology of the skin. Classic lesions show diffuse parakeratotic hyperkeratosis, intercellular and intracellular epidermal edema of the outer epidermal cells, and basal cell hyperplasia. When stained with hematoxylin and eosin, a red, white, and blue pattern is seen. The superficial layer stains red, the middle layer of vacuolated pale keratinocytes stains white, and the deep layer of the basal cells stains blue.[26,27] The parakeratotic hyperkeratosis layer may extend into the hair follicles. Clefts or subcorneal vesicles may also be present. Bacteria, yeast, or dermatophytes may be seen in the stratum corneum as secondary invaders.[28] Chronic lesions demonstrate the epidermal edema and have marked parakeratotic hyperkeratosis, epidermal hyperplasia, and surface crusting.[28] Very chronic lesions may not show the characteristic high-level epidermal edema.[23] It is important that multiple skin biopsies are

examined because the characteristic red, white, and blue pattern may not be uniformly present in all tissues biopsied.

Liver Histopathology

Histopathology of the liver is useful for confirming the characteristic appearance of the hepatopathy as the underlying cause of MEN, and for detecting metastases in cases of glucagonoma. Wedge biopsies (versus fine needle aspirates or even tru cut biopsies) are superior for displaying collapsed liver parenchyma with vacuolated hepatocytes.[3] Increased fat deposition within vacuolated hepatocytes may be confused with cirrhosis if only vacuolar hepatopathy is reported; however, the extensive fibrosis and small size usually associated with cirrhosis is not seen with MEN.[2] In addition, Masson's trichrome stains have been negative for increased collagen deposition, which is a feature of cirrhotic livers.[19] The liver pathology for MEN is unique in that it is secondary to chronic hepatocellular degeneration with severe intracellular zonal fat accumulation within the hepatocytes. Lobular collapse with minimal collagen formation and nodular regeneration are evidence of ongoing hepatocellular regeneration and necrosis with resultant parenchymal loss.[2]

Unfortunately specific histopathology of the liver from dogs with glucagonoma is less helpful. Two papers (3 cases) mention mild diffuse hepatocellular vacuolation.[12,13] In the case of the Boxer with a primary pancreatic tumour and metastasis to the liver, there was no vacuolar degeneration observed in the liver; instead, periacinar congestion with nodular hyperplasia was seen (Table 1).[14] The remaining published cases of MEN caused

by a glucagonoma do not describe the histopathology of the liver itself, aside from the identification of endocrine tumour cells within the parenchyma. They appear as multifocal, well-demarcated masses composed of discrete to coalescent nests, small sheets, or cords of basophilic cells.[9,15,16]

Postmortem /Exploratory

The liver of dogs with MEN caused by hepatopathy may appear enlarged and nodular with round edges. The nodules may be red, brown or yellow and are interspersed within more normal parenchyma.[19] In these dogs, the pancreas is grossly normal, with the exceptions of small nodular foci and interstitial fibrosis.[14]

In dogs with MEN caused by glucagonoma, the liver may contain tumour foci-forming nodules throughout the lobes, as already described.[14] The pancreas may be atrophied, inflamed or fibrotic, sometimes with adhesions, and typically a mass is found. In eight reported cases, four dogs had metastatic lesions in the liver;[9,13,15,16] one dog had metastases to the regional lymph nodes;[11] and one dog had metastases to both the liver and regional lymph nodes[14] (Table 1). In two of the eight cases documented, a primary tumour could not be identified, despite finding metastatic glucagon-producing nodules in the liver. [13,16]

Differential diagnoses

The differential diagnoses for MEN are skin diseases such as pemphigus foliaceous (PF), systemic lupus erythematosus, erythema multiform, generic dog food dermatosis, and

zinc-responsive dermatosis, which present with similar scaling and crusting lesions. MEN can be differentiated from all of these disorders simply by the lack of inflammatory cells seen in the vesicular fluid. For example, a direct smear of the pustules from a dog with PF would contain non-degenerate neutrophils, eosinophils and acantholytic keratinocytes;[9] none of these cells would be seen in vesicular fluid from a dog with MEN. Furthermore, zinc-responsive dermatosis would respond to replacement therapy.

Other differentials for MEN are vitamin A responsive dermatosis, niacin deficiency, contact hypersensitivity, toxic epidermal necrolysis, and pyoderma. Hyperkeratosis is the distinguishing feature of vitamin A responsive dermatosis, while niacin deficiency causes ulcerated mucous membranes.[28] Histologically, neither has the classic red, white, and blue pattern observed in MEN cases. Both respond favourably to replacement therapy. Contact hypersensitivity (otherwise known as allergic contact dermatitis or plastic dish syndrome) may have a similar clinical appearance to MEN, but a good history of food and dish type would help rule this out as well as histopathology. Superficial perivascular dermatitis, with neutrophils as the predominant inflammatory cell, is observed with contact hypersensitivity.[28] Toxic epidermal necrolysis, secondary to drug therapy, *Staphylococcus sp.* infection, or of idiopathic origin, which may resemble MEN clinically, would also differ histologically.[7, 28] Furthermore, a distinguishing feature of toxic epidermal necrolysis is a positive Nikolsky sign. When pressure is applied to vesicles or edges of ulcers, the outer layer of the skin easily rubs off, demonstrating poor cellular cohesion.[28] Lastly, bacterial pyoderma could also be a differential for MEN due to its crusting and sometimes erosive nature. Again, this could also be ruled out by

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histopathology, which would show epidermal hyperplasia with superficial pustulation and crusting, and not the pathognomonic red, white and blue pattern that is observed with MEN.[7,28]

If liver pathology is suspected as the underlying cause of skin lesions, copper storage disease, mycotoxicosis and phenobarbital administration should be ruled out.[6,18]

Treatment:

Treatment of MEN has been aimed at removing the insult to the liver if the cause is identified (i.e. toxin exposure or phenobarbital drug administration). Some cases have not resolved with discontinuation of phenobarbital.[29]

If a glucagonoma is present, complete removal of the tumour is the treatment of choice. At the time of the journal article publication, an eleven-year-old standard poodle was considered cured after partial resection of a glucagon-secreting pancreatic islet tumour.[11] However, the dog was euthanized nine months after surgery due to return of clinical signs.[9]

Unfortunately in most cases when the decision is made to go to surgery, the tumour has already metastasized. Despite this reality, resection of the primary tumour may still be of some palliative value. A six-year old Labrador retriever with MEN was reported to be maintaining weight and have mildly improved skin lesions after removal of a pancreatic

mass. This dog had widespread metastatic liver tumours noted at the time of surgery that were positive for glucagon secretion.[9]

The most common complication of surgical resection of the pancreatic tumour is severe necrotizing pancreatitis, which resulted in the death or euthanasia of several dogs.[12,13,14,15,16]

Medical therapy for the skin lesions consists of octreotide, a somatostatin analog that has been used in humans with glucagonomas and has been shown to achieve remission of the MEN skin lesions.[9,28] Octreotide antagonizes glucagon and decreases the concentrations of insulin, gastrin, secretin and motilin.[9] However, the use of this drug has not been as successful in dogs for the treatment of glucagonomas as it has been in humans. In a study conducted to determine the effect of octreotide on glucagon and other hormones, both healthy dogs and dogs with insulinomas were administered octreotide. It was found that octreotide had no affect on glucagon in either group.[30] In another case, octreotide was used in a dog with metastatic glucagonoma to ameliorate the dermatologic manifestations of MEN; however the dog was euthanized due to worsening of the systemic signs.[3] The cost of octreotide, the lack of evidence of efficacy, and unestablished dosages for dogs makes this drug prohibitive.[9]

Antibiotics are recommended if there are secondary bacterial infections with *Staphylococcus sp*. If there are fungal hyphae and spores in the stratum corneum then antifungal shampoos or oral antifungals may be considered. Analgesics such as opioids

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or non-steroidal anti-inflammatories may be used if the dog is uncomfortable. Antihistamines have been used symptomatically for pruritis.[3,28]

In cases where the underlying etiology is unknown, nutritional supplements aimed at replacing the depleted amino acids can be given. Egg yolks (1 yolk/4.5 kg q 24 h) may be beneficial. An amino acid solution (Aminosyn 10% Crystalline Amino Acid Solution made in Illinois or Travasol, made in Canada) can be given intravenously at a dose of 25 ml/kg or 500 ml/dog over an eight to twelve-hour period via the jugular vein and repeated weekly until clinical signs resolve. Prolonged remission has been noted after only one infusion. If minimal to no response is observed then the infusion is repeated every 7 to 10 days for 4 treatments. Since there is a risk of hepatic encephalopathy with this treatment, ammonia levels should be measured before the infusion.[31] In addition to the intravenous amino acids, a highly digestible diet with good quality protein and a powdered protein supplement should be given orally.[31]

Zinc supplementation and fatty acids may be beneficial. The response has been variable to disappointing with improvement seen in only a few cases. Suggested treatments include omega-3 fatty acids (80 mg/kg PO q 24 h), zinc sulphate (10 mg/kg PO divided q 12 h), or zinc methionine (2 mg/kg PO q 24 h). Zinc levels measured before and after surgery in a dog with a glucagonoma did not change significantly after the tumour was removed, indicating the benefits of zinc are questionable. In addition, the zinc level measured for both assays was in the normal reference range.[11]

Glucocorticoids may be beneficial to some dogs (unless they are diabetic or polyuric/polydipsic) but the effect is usually transient. S-adenosylmethionine has also been used empirically to increase liver concentrations of glutathione, an important antioxidant that helps maintain and protect liver function.[9]

Prognosis:

The prognosis for this disease is poor.[31] Mean survival time was reported to be 5.3 months from the onset of skin lesions and 1.6 months from a diagnosis.[2,7,23] Another study cites an average survival time of 6 months, and all dogs that survived longer than 12 months were receiving oral nutritional supplementation and intravenous amino acid infusions.[4]

<u>Clinical Report</u>:

A nine-year-old neutered mixed breed male dog was presented for evaluation of a sore left front paw (Day 1). The dog was current on vaccinations against distemper, adenovirus, parvovirus, parainfluenza virus, and rabies. There was no history of travel outside the province of British Columbia, Canada. The dog was fed a commercial maintenance dry dog kibble.[a] There was no history of polyuria or polydispsia; conversely, the owner stated that the dog was eating and drinking a little less than normal. There was no history of vomiting and the stool was normal frequency and consistency. The owners had not noted any coughing or unusual sneezing, but they did report hearing some abnormal breathing sounds, especially when excited or on walks.

The owner perceived the dog to be painful on the front paw for the last two days, and seemed less eager to walk the entire distance of the regular route.

On physical exam, the dog was alert and anxious. The paw appeared crusted and hyperemic in the interdigital area between the third and fourth digits with accompanying purulent exudate. The rectal temperature was 38.6 °C, the heart rate was 120 bpm, and the respiratory rate was approximately 40 breaths/min, with an audible inspiratory stridor heard over the proximal trachea. The oral mucous membranes were pink with a normal capillary refill time. The sclera were white. There was no organomegaly or pain detected on abdominal palpation. The rest of the dog's skin appeared normal. The dog weighed 37.7 kg and was slightly overweight with a body condition score of 5/9. The initial clinician who examined the dog suspected it had unilateral laryngeal paralysis and a localized pyoderma of the paw. The tentative diagnosis of laryngeal paralysis was based on the history of decreased exercise intolerance and an ascultated mild inspiratory stridor. Pyoderma of the left front paw was suspected based on the crusting and purulent exudate of the interdigital skin. The dog was treated with amoxicillin/clavulanic acid[b] (16.5 mg/kg) q 12 h PO for 5 days and the foot was bandaged over an application of furacin[c] ointment.

On Day 12, the dog was presented to a second veterinarian for bleeding from the plantar aspect of the left forepaw. Necrotic tissue was noted between the digital pads as well as ulceration in that area. An ulcerated, depigmented area on the nasal philtrum and some hyperkeratosis on the nasal planum was also documented at this visit (Figure 2).

Figure 2:   Ulceration of the philtrum on Day 12

Hyperkeratosis and fissuring of the nasal planum

The assessment was inflammation of the paw and nasal philtrum with secondary pyoderma, likely due to an underlying auto-immune disorder. The treatment administered was an injection of penicillin[d] (20,000 Units/kg IM), methylprednisolone acetate[e] (1 mg/kg IM) once, and cephalexin[f] (13.3 mg/kg PO q 8 h) for 10 days. Some comments on why the treatments given on Day 1 and Day 12 did not significantly improve the affected skin can be found in the discussion.

On Day 33, the dog was admitted for a progressively worsening condition of his left front foot, and was examined by the author of this paper. The plantar aspect of the left forepaw was erythematous, fissured, and thickened between the metacarpal pad and digital footpads. There was minimal hyperkeratosis of the pads themselves. There were no obvious lesions on the right front footpad, the hind footpads, or the interdigital areas of these paws. The owner reported mild improvement on the amoxicillin/clavulanic acid therapy. At this time the owner reported some "soreness" in the hind end when taking him for walks. A neurologic and orthopedic exam was performed and the only abnormality observed was a slightly delayed placing reaction in the hind legs. It was thought that this might be neurological in origin or secondary to weakness. The owner also reported a decrease in appetite. Cephalexin[f] was prescribed at 26 mg/kg PO q 12 h for 20 days with plans of biopsying the affected area prior to finishing the antibiotics.

A geriatric panel, including a complete blood count and chemistry profile was performed at an external laboratory (Table 2 and 3). There were a few abnormalities in the geriatric panel but these were minor. A slight increase in platelet numbers was noted and

CBC with differential	value	ref range
WBC (10^9/L)	12.6	4.0-15.0
neutrophils (10^9/L)	7.89	2.80-10.56
lyphocytes (10^9/L)	2.59	0.96-4.80
monocytes (10^9/L)	1	0.0-0.980
basophils (10^9/L)	0.4	0.0-0.10
rbc (10^{12}/L)	7.06	5.5-8.0
Hb (g/L)	163	138-199
Hct (L/L)	0.446	0.39-0.56
MCV (fl)	63	63-77
MCH (pg)	23.1	22.0-27.4
MCHC (g/L)	365	326-374
RDW	12.5	10.0-190
PLT (10^9/L)	476	170-400
mean plt vol (fl)	16	7-14

Comments:
clumped platelets (absolute numbers are
increased), slight echinocytosis;
poikilocytosis is present

Table 2: Complete Blood Count Results on Day 33
Reported by external veterinary laboratory
Reference values supplied by external veterinary laboratory

Chemistry	value	ref range
Glucose (mmol/L)	**2.3**	3.0-6.6
Gray Glucose (mmol/L)	4.1	3.0-6.1
BUN (mmol/L)	**1.9**	2.5-9.2
Creat (umol/L)	69	68-141
Bun/Cr ratio	7	
Sodium (mmol/L)	147	140-151
Potassium (mmol/L)	4.6	4.0-5.4
Na/K ratio	32	
Calcium (mmol/L)	2.62	2.24-2.83
Phosphorus (mmol/L)	0.79	0.72-2.08
Total Protein (g/L)	71	54-71
Albumin (g/L)	41	31-42
Globulin (g/L)	30	20-40 g/l
albumin/globulin ratio	1.4	0.8-2.3
Bili total (umol/L)	hem	0-7
AlkP (iu/L)	38	0-113
ALT (iu/L)	60	0-113
GGT (iu/L)	8	2-20
Chloride (mmol/L)	115	108-118
Carbon dioxide (mmol/L)	**14**	15-25
osmolality (mmol/kg)	286.2	278-306
anion gap	**23**	10-22
CPK (iu/L)	**507**	0-314
amylase (units)	1021	206-1250
lipase (units)	**666**	40-560
cholesterol (mmol/L)	4.65	3.48-9.3
T4 (nmol/L)	18	13-44

Comments:
no significant findings

Table 3: Serum Chemistry Results on Day 33
Values reported by external veterinary laboratory
Reference values supplied by external veterinary laboratory

was attributed to stress (epinephrine release and splenic contraction). Slight monocytosis was most likely due to mild chronic inflammation. A mild elevation in mean platelet volume (MPV) was present and is speculated to be caused by the blood sample being held in EDTA at 4°C for greater than 4 hours.[32] Albumin and total protein were high normal, but blood urea nitrogen (BUN) was low. The low BUN was attributed to anorexia. Alternatively, low BUN could indicate hepatic insufficiency but was less likely with normal albumin and normal liver enzymes. Hemolysis of the sample prevented acquiring a value for bilirubin. Carbon dioxide (or bicarbonate) was slightly decreased, which corresponds to a slightly increased anion gap. Most likely this was due to sampling or sample transport. Creatinine phosphokinase (CPK) was slightly elevated due to difficulty sampling from a somewhat uncooperative patient. Lipase was not elevated enough to be significant, and clinical symptoms did not fit with pancreatitis. The value for glucose was normal, as seen from the gray top sample.

The dog was presented again on Day 52, after nineteen days on cephalexin. The dog weighed 35.0 kg, a loss of 2.7 kg of body weight. The left forepaw now appeared crusted with hardened serum on the plantar surface of the interdigital areas. The plan was to continue on the cephalexin at 26 mg/kg q 12 h until biopsies could be done. Since the owners reported some coughing and gagging, the laryngeal function would also be assessed prior to intubating the dog. In addition, radiographs were to be taken of the pelvis and lumbar area to document any bony changes.

On day 60, after 27 days of consecutive antibiotics, the dog, weighing 33.6 kg (a loss of 4 kg since first presented), was pre-medicated intramuscularly with a mixture of hydromorphone[g] (0.08 mg/kg) and acepromazine[h] (0.025 mg/kg). The dog was induced with 4 mg/kg propofol[i] IV, and maintained on isofluorane inhalational anesthestic.[j] The larynx was assessed just prior to intubation and, at this time, the right arytenoid cartilage did not appear to abduct adequately on inspiration, and the mucosa appeared thickened and erythematous. This observation was judged consistent with unilateral laryngeal paralysis, but the clinical signs associated with the laryngeal dysfunction were not thought to be severe enough at this time to warrant surgical intervention. Biopsies were taken of the haired skin area between the metacarpal and digital foot pads, and also of the philtrum using a 3.5 mm biopsy punch.[k] The biopsies were fixed in neutral buffered 10% formalin. They were closed with 3-0 polydioxanone[l] (nasal philtrum) and 2-0 nylon suture[m] (foot). The biopsies were sent to a diagnostic laboratory to be examined by a board certified pathologist. The paw was bandaged after applying 0.25% methylprednisone and 0.25% neomycin cream.[n] The pelvic radiographs showed very mild arthritic changes in the hips, and the lumbar spine appeared normal. The subtle degenerative changes observed on the radiographs and the previous orthopedic exam were not consistent with the "soreness" perceived by the owners on walks. Radiographic findings do not always correlate with the degree of clinical lameness in a patient; however, further questioning of the owners revealed the dog was "stumbling." The clumsiness was observed ambulating over curbs, suggesting more likely a neurological disorder. At this stage the owners were finding it very difficult to administer oral

25

medications to their dog. They reported the dog was uncooperative, and they could no longer hide the pills in food because the appetite was even more reduced.

The pathology report, received on Day 67, suggested a diagnosis of metabolic epidermal necrosis based on the foot and philtrum biopsies. Sections of ulcerated/eroded skin had been processed according to standard techniques, sectioned and stained with hematoxylin and eosin. The diagnosis was based on the characteristic "red-white-blue" pattern observed in the sections (Figure 3). The red represents the outer parakeratosis with pyoderma (mixed inflammatory cells), the white in the centre is the outer epidermal pallor/necrosis and sloughing, and the blue represents the inner, but intact epidermis with basophilic staining and acanthosis. The dog was started on a liver diet[o] supplemented with 3 to 6 egg yolks per day to provide increased amino acids. The owner was told that the skin on the foot was prone to secondary infections from bacteria and yeast and to try to bathe/soak the foot in warm water baths containing diluted chlorhexidene.[p]

On Day 81, the sutures were removed from the foot and the nasal philtrum. The owners were supplementing the liver diet with a recuperative canned diet[q] and egg yolks. The owners declined giving the intravenous amino acid therapy, Aminosyn, as they felt the dog would be too stressed by the weekly hospitalization required for this procedure. The owners consented to a bile acids panel, but made it clear at this time that invasive tests such a liver biopsy or an abdominal exploratory would not be permitted. Their decision was based on the information given that the prognosis for this disease is poor.
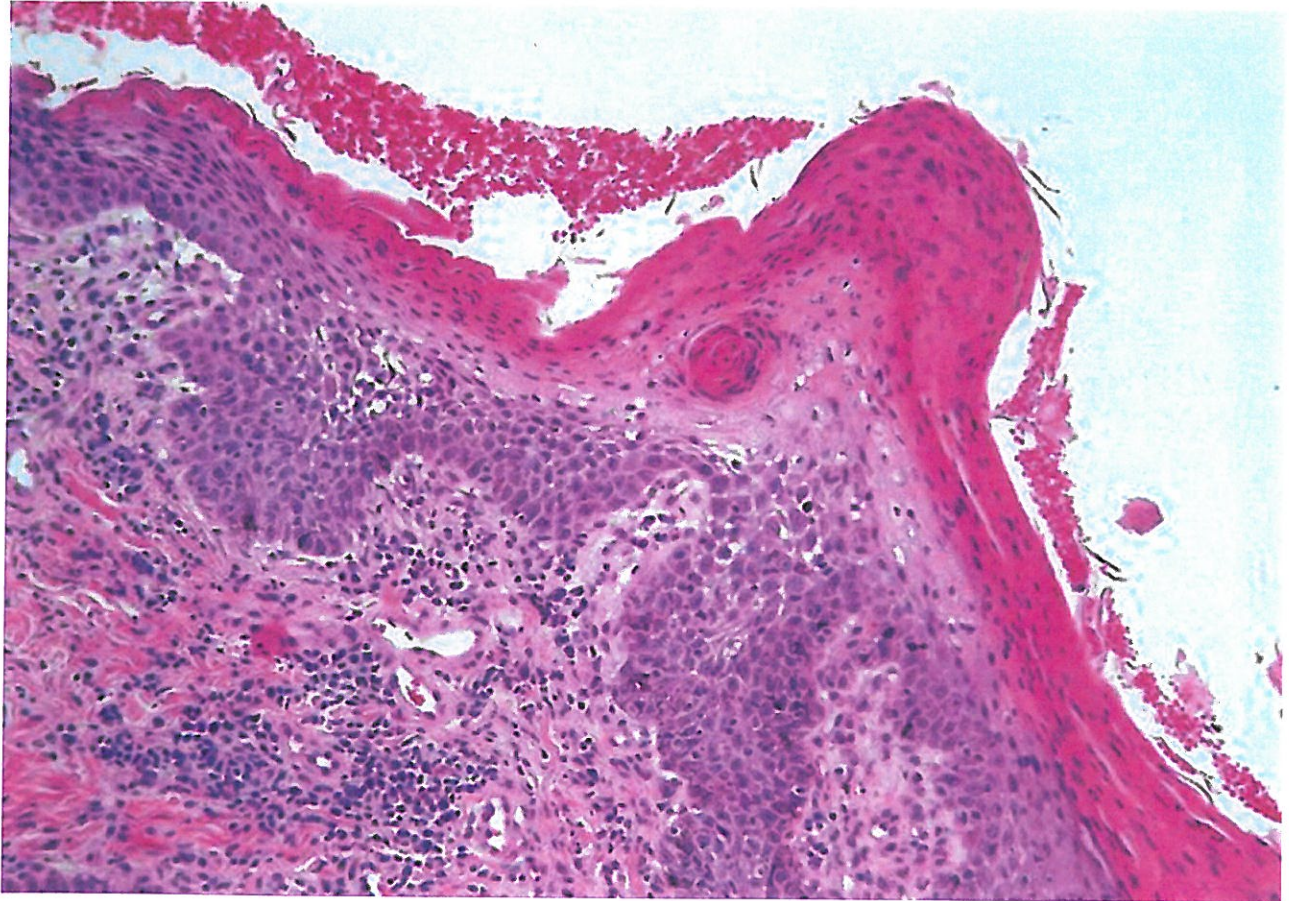
Figure 3:   H&E stained biopsy of footpad on Day 60

(x180)

Red - outer parakeratosis

White - epidermal pallor/necrosis

Blue - intact epidermis with basophilic staining

Meanwhile, biopsies of tissue taken from the foot and philtrum on Day 60 were sectioned and viewed a second time, in order to confirm the diagnosis. The concern regarding the diagnosis was due to the fact that the skin lesions seemed to be focal (on one foot) rather than diffuse, as was expected for MEN. The other differential at this time was contact hypersensitivity; however, this was not considered likely because a plastic contact dermatitis does not have the classic red-white-blue pattern that is pathognomonic for MEN. Histopathology for contact hypersensitivity is more consistent with superficial perivascular dermatitis and secondary bacterial pyoderma[28] and this was not observed by the pathologist. The results from further sectioning were still most consistent with MEN.

The fasted and fed blood samples for the bile acids panel, taken on Day 88, was reported by an external laboratory to be normal (See Table 4).

Three months after initial presentation, on Day 120, the owner brought the dog in for a weight check. The dog weighed only 33 kg. He had an ulcer on the lip margin near the upper right canine tooth. The haired area around the ulcerated lip was swollen and crusted. It appeared that skin and mucous membranes susceptible to trauma were at risk for showing exaggerated signs of the disease. Hydrotherapy was used to remove some of the crusts from the dog's footpad. The owner was told to concentrate on increasing his daily caloric intake. They started grilling the dog steaks for dinner, as his appetite was becoming more and more finicky, and they felt steak was a good source of protein.

	value	ref range
fasting base-line bile acids (umol/L)	0.1	0-15
post-prandial bile acids (umol/L)	1.0	0-22

Table 4: Bile acid panel result on Day 88
Values reported by external laboratory
Reference values supplied by external laboratory

On day 130, the dog's weight had gone up to 35.3 kg but it was noted that the furred skin adjacent to the commissure of the lips appeared ulcerated, crusted and swollen.

On Day 135, the dog's weight was 37.7 kg and blood for an amino acid panel was collected after a 12 hour fast. On that day, an abdominal ultrasound was performed, which showed hypoechoic regions surrounded by echogenic areas in the liver, giving rise to a reticular pattern (Figure 4). No other abnormalities in the abdomen were found. Permission for a liver biopsy was declined. The owners declined any further work up at this time, including an exploratory laparotomy.

The amino acid panel, which was analyzed at the University of California at Davis, showed low and "below detectable limits" in 24/26 amino acids tested (See Table 5). Over 60% of these measured amino acids were less than 30% of the normal values and 77% were less than 50% of the normal values. Two amino acids were higher than the mean. These were tryptophan, and *3*-methyl-*L*-histidine. The rest of the amino acids were significantly lower. *L*-Glutamic acid and *L*-aspargine were below detectable limits. Other amino acids that were very low included *L*-Proline, *L*-Glutamine, *L*-Arginine and *L*-serine, which were less than 5% of the normal value.
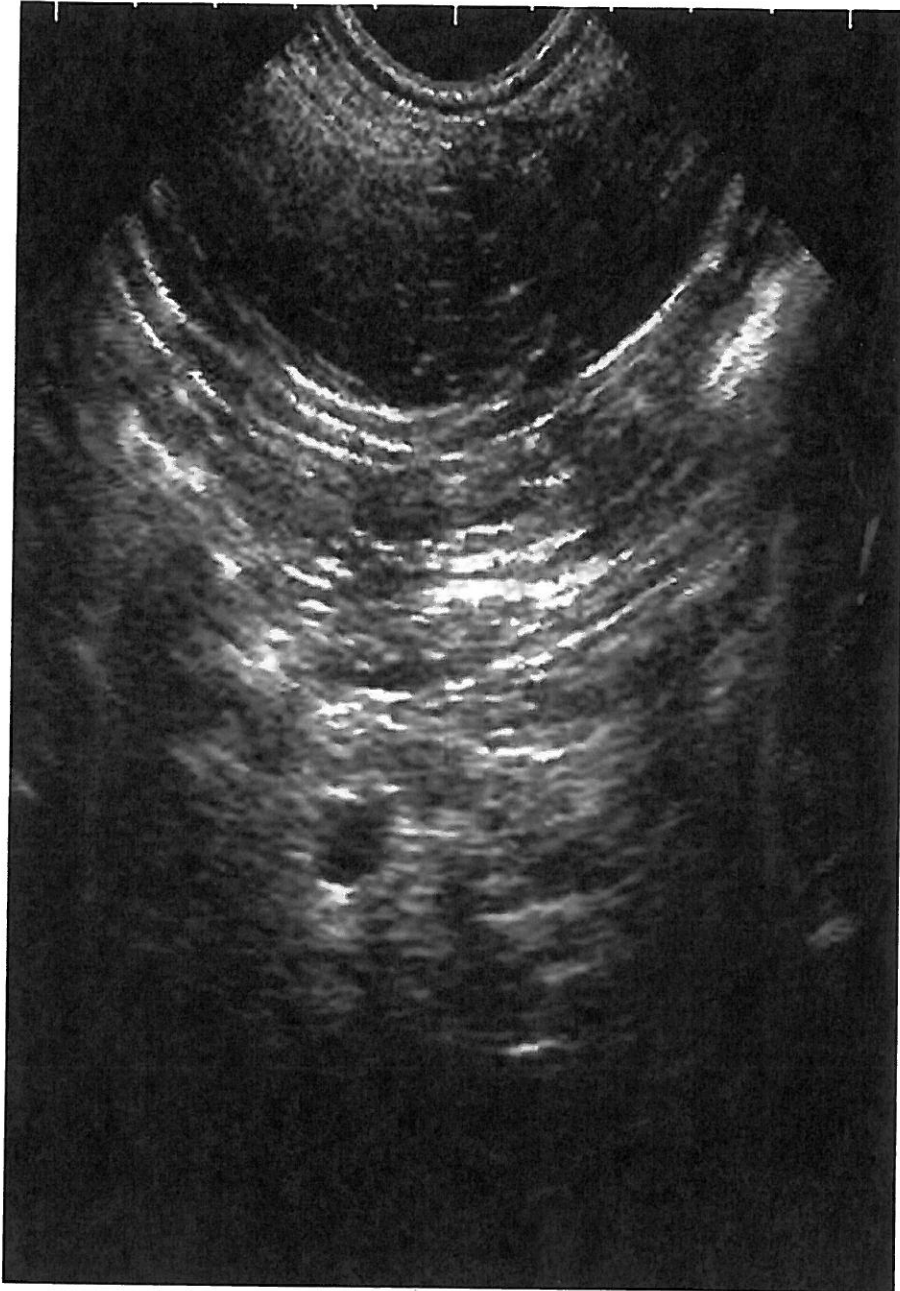
Figure 4:   Ultrasound of Liver on Day 135

                     Transverse view near mid-line

                     Falciform ligament at top of photo

				% NORMAL		
amino acid	value (nmol/ml)	ref range (nmol/l)	% normal	Bond et al, 1995 [14]	Torres et al, 1997 [11]	Allenspach et al, 2000 [16]
L-aspargine	**BDL**	40 + 1	<1			
L-glutamic acid	**BDL**	23 + 1	<1	100	102	
L-proline	**5**	246 + 6	2	14	12	
L-glutamine	**16**	495 + 9	3	20		
L-arginine	**3**	102 + 3	3	31	6	4
L-serine	**4**	107 + 3	4	23	42	
L-isoleucine	**3**	51 + 1	6	55	30	
Haf-L-cystine	**4**	46 + 1	9			
L-methionine	**5**	57 + 2	9	60	30	
L-alanine	**39**	388 + 9	10	22	37	
L-leucine	**12**	120 + 3	10	59	41	
L-threonine	**19**	178 + 5	11	24	22	
Glycine	**54**	268 + 8	20	26	39	
L-valine	**35**	157 + 4	22	61	38	
L-ornithine	**9**	35 + 2	26		9	
L-phenylalanine	**12**	45 + 1	27	100	81	91
L-citrulline	**14**	41 + 2	34		16	
L-tyrosine	**14**	39 + 1	36	50	44	
L-aspartic acid	**3**	7 + 0.2	43		18	
L-lysine	**64**	132 + 5	48	36	24	37
L-histidine	**49**	71 + 2	69	49	67	87
taurine	**59**	77 + 2	77	34	15	
L-amino-n-butyric acid	**5**	6 + 2	83		26	
Cystathionine	4	3 + 1	100		317	
tryptophan	70	60 + 2	117	83	97	
3-methyl-L-histidine	20	6 + 1	333		207	165

Table 5: Amino Acid Analysis of Fasted Plasma on Day 135
Values reported by external laboratory
Reference values given by external
laboratory
BDL- below detectable limits

Approximately four months after initial presentation, on Day 128, the dog was presented for scratching the left ear. Purulent exudate was observed in the vertical canal and a diagnosis of otitis externa was made. This development was not surprising, as all locations of skin could theoretically be susceptible to secondary skin invaders such as yeast and bacteria. The dog was treated with a combination of miconazole nitrate, polymyxin B and prednisolone acetate.[r] Ten to fifteen drops were given in the left ear q 12 h for 10 days and this seemed to resolve the infection.

On Day 176 the dog presented for periodic shivering and shaking of the torso. Now all four paws appeared affected; the left front markedly worse. The footpads were thickened, fissured and crusted (Figure 5). The owner was placing nylon booties on the feet to keep them protected. The dog seemed to walk with careful placement of the feet and a slight high-stepping gait (even without the booties on). The dog weighed 31.8 kg (a loss of 7.9 kg since Day 1). The feet were soaked in chlorhexidene soap and warm water and some of the necrotic tissue and crusts were manually removed.

Almost seven months after initial presentation, on Day 206, the dog's weight had decreased to 31.0 kg. The hair coat appeared unthrifty. It had not grown back since it was last groomed 3 months ago, and was thin and coarse-looking. The owners reported the dog was only eating steak and eggs at this point, refusing all dog food including the canned recuperative formula. All four paws had a foul odour and appeared severely hyperkeratotic and fissured.

Figure 5:   Hyperkeratotic Footpads observed on Day 176

Injectable enrofloxacin[s] was dispensed at a dose of 6.5 mg/kg q 24 h SQ to treat the secondary infection, as the owners could no longer give any oral medication.

Two weeks later, on Day 220, the dog's weight dropped to 30.6 kg. The skin over the entire body was scaly and alopecic in many areas. The feet were deeply fissured and had a very foul odour. It appeared that the response to enrofloxacin was poor. The muzzle and chin were crusted and cracked with purulent discharge (Figure 6). The skin around the anus was also ulcerated adjacent to the rectal mucosa. The owners generously offered their dog's body for necropsy when the time came for euthanasia.

The dog was euthanized approximately 8 1/2 months after initial presentation on Day 258 with sodium pentobarbital[t] injectable solution, and a postmortem followed. On the day of euthanasia it was noted that the periocular skin was ulcerated and crusted. The dog had lost approximately 8.7 kg during these 8 1/2 months. The body condition score was now 3/9.

The necropsy was surprisingly unremarkable. Several photographs were taken of the outside of the body, which showed the most obvious lesions. As mentioned previously, the ulcerated and crusted skin around the eyes was one of the last skin changes to be noted prior to euthanasia (Figure 7). The dog had severe crusting around the lips and ventral muzzle. All four footpads were hyperkeratotic with a crusting dermatitis between the pads. The nose was hyperkeratotic with fissures and ulcerations on the philtrum and nostrils (Figure 8). There was black pigment around the prepuce, thickened

Figure 6:   Ventral muzzle showing ulcerated, crusting dermatitis on Day 220

Figure 7:   Periocular ulcerative dermatitis at Postmortem on Day 258

Figure 8:   Fissured, ulcerated philtrum

Hyperkeratotic nasal planum

Crusting around lips at Postmortem on Day 258

hyperpigmented skin on the points of the elbows (Figure 9) and well-demarcated black areas on the inner surface of the pinnae. The anus was ulcerated and moist with serous secretions at the mucocutaneous junction (Figure 10). Histopathology results of these tissues were consistent with the footpad results taken on Day 60.

Grossly the chest cavity looked normal. The abdominal cavity also looked surprisingly normal. The liver had a smooth serosal surface, was firm and homogenous in texture, had a uniform red-brown colour, with relatively normal to slightly thickened edges. Mesenteric lymph nodes and other abdominal lymph nodes appeared normal. The kidneys were both a normal size and shape. The left and the right arms of the pancreas appeared as two long, thin bands of light pale pink tissue that was normal in appearance. The lobules were distinctly visible and arranged in a normal fashion. There were no apparent masses in this organ. The intestines appeared normal.

Sections of the liver and pancreas were sent for histopathology. The liver was found to have numerous large clusters of endocrine cells scattered randomly throughout the parenchyma (Figure 11). These clusters of cells were obvious even on low power of the microscope. The endocrine cells had basophilic cytoplasm and were arranged in a lobular pattern that is typical of an endocrine tumour (Figure 12). The histopathology of the remainder of the liver was normal. There was no evidence of vacuolar degeneration. Immunohistochemistry was performed at an external laboratory to determine if the clusters of cells were producing glucagon. Paraffin-embedded tissues were cut to 5 micron sections and mounted on 0.1% poly-D-lysine-coated glass slides.

Figure 9:   Hyperpigmented and parakeratotic elbows on Day 258

Figure 10:   Crusted anus and mucocutaneous junction at post-mortem on Day 258
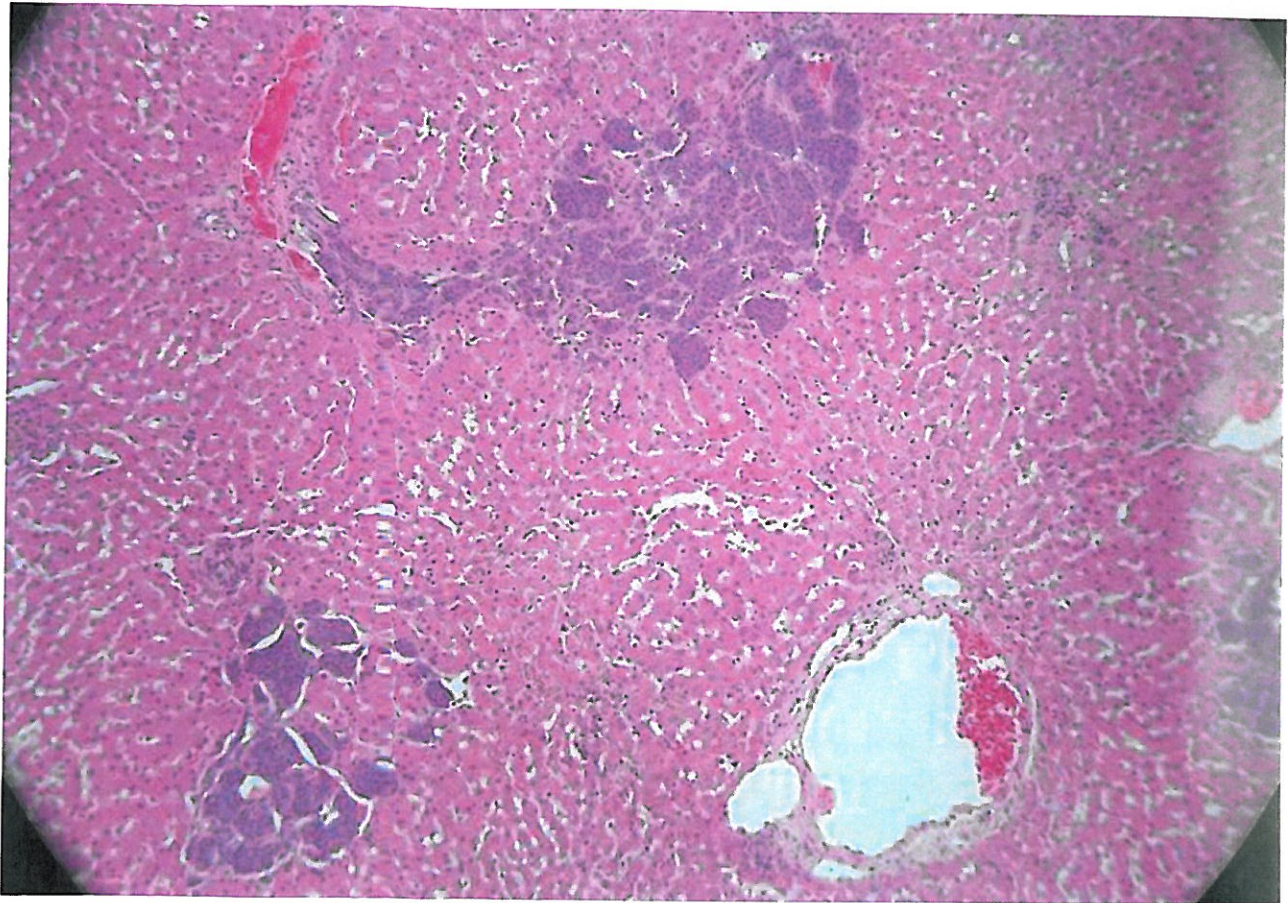
Figure 11:   H&E stained liver cells on Day 258 showing darkly stained clusters of endocrine cells (x100)
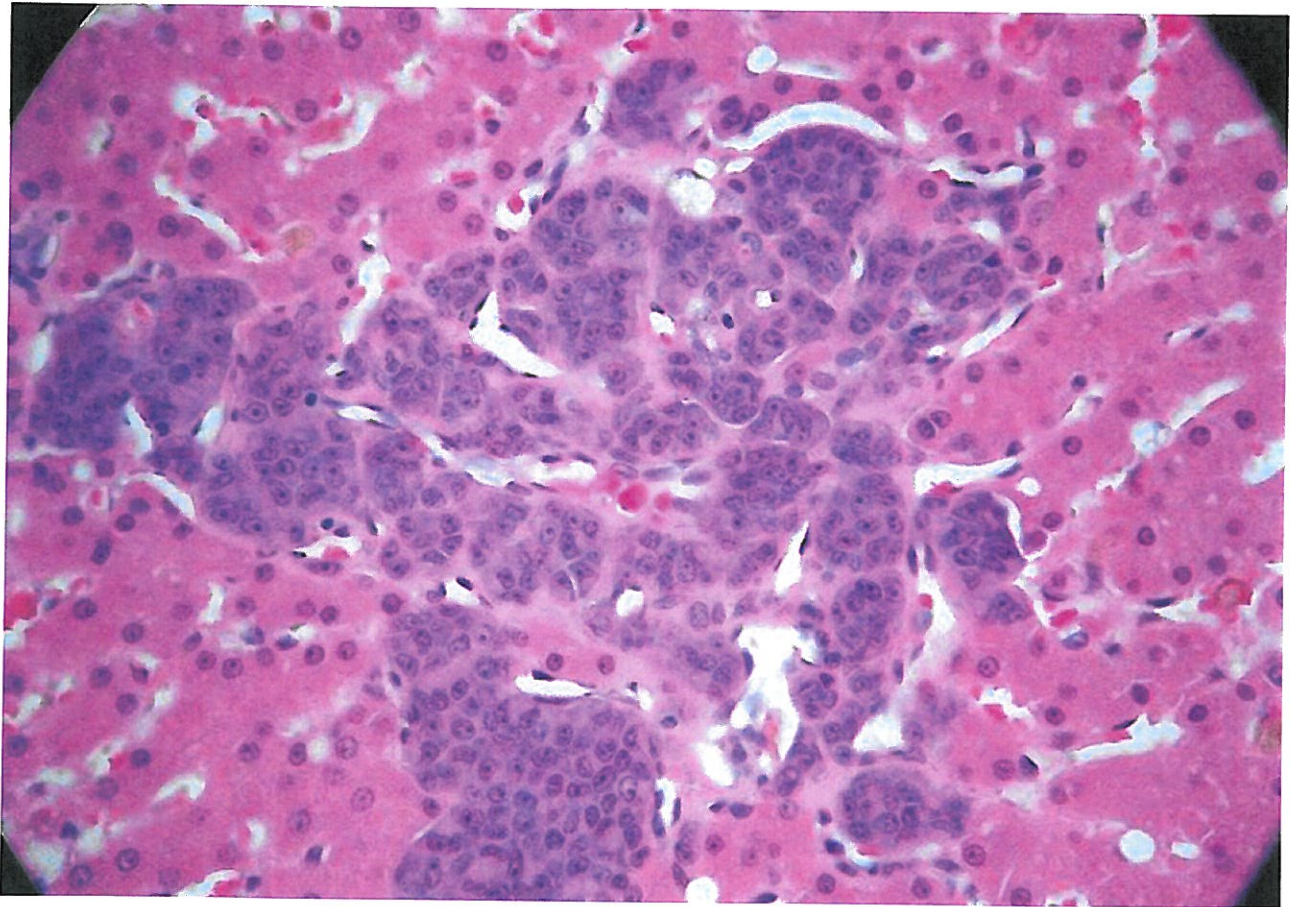
Figure 12:   Multiple coalescing clusters of H&E stained liver cells magnified on Day 258

(x400)

Following protease XIV digestion of the tissue for 20 minutes, sections were immunohistochemically stained using a 1:500 dilution of rabbit anti-porcine glucagon antibody. The secondary antibody, goat biotinylated anti-rabbit IgG was used at a 1:400 dilution, and binding was visualized using the avidin-biotin complex immunoperoxidase method. The large clusters of tumour cells in the liver were highly positive for glucagon production (Figure 13). A few cells of most islets in the pancreas stained positive for glucagon, and this served as a positive control, but large areas of clustering tumour cells in the pancreas were absent.

Figure 13:   Section of liver demonstrating immunohistochemical staining of glucagon-secreting cells

Stained with glucagon anti-sera

(X 180), Day 258

Discussion:

This report describes the diagnosis and clinical management of a nine-year-old mixed breed dog with MEN caused by a glucagonoma of unknown origin. The primary tumour was not found in the pancreas but numerous nests of glucagon-producing cells were randomly scattered in sections of the liver.

There were several puzzling or unexpected results during the work up of this case. Initially, although the histopathology of the skin was consistent with MEN, it was not typical for only one foot to be affected for five months before the other feet became affected. In previous reports, the disease is more diffuse in nature. This case showed a definite progression in the lesions observed, beginning with one foot, then the philtrum, followed by the muzzle, the remainder of the feet, haircoat, anus, and finally the periocular area, as observed immediately prior to euthanasia. The literature reports that dogs may present for reluctance to walk because of painful feet as a result of their pathology, and perhaps this was why the dog had difficulty walking over curbs.

Conversely, only the left front foot was affected when the owners reported this observation, and the "clumsiness" they saw seemed to be more in the hind end. The dog may have been beginning to develop signs of a generalized neurological disorder that included laryngeal paralysis. Most clinicians agree that hypothyroidism does not cause laryngeal paralysis; however, it is worthwhile ruling it out as an additional problem. In this case hypothyroidism was ruled out in the initial chemistry panel, and was not suspected to be a potential cause of a polyneuropathy. Furthermore, blood work did not

support the possibility of a diabetic neuropathy. An alternative explanation is that the dog was becoming weak from amino acid catabolism. Weight loss and deteriorating body condition was documented during the progression of the disease, most likely due to catabolic effects of glucagon on fat and protein metabolism. Finally, a paraneoplastic neuropathy may also explain the clinical signs seen in the hind end. Interestingly, there are no reports of neurological symptoms in cases of MEN noted in the literature.

The treatment given by the first two veterinarians who initially saw the dog for dermatitis of the left forepaw was not successful. The reasons for lack of resolution are discussed below. On Day 1, the dog was treated with a satisfactory dose of amoxicillin/clavulanic acid, but the duration, 5 days, was inadequate. On Day 12 when the dog was treated by a second veterinarian with different antibiotics (penicillin and cephalexin) and a steroid, the improvement seen was minimal. Penicillin is not generally a suitable choice for pyoderma of the skin, as it is not *B*-lactamase resistant. The cephalexin that followed the penicillin was acceptable for skin pathogens such as *Staphylococcus intermedius* (the most important pathogen involved in canine pyoderma), but it was prescribed at the lower end of the dose range and for too short of a duration. Typically, the treatment length for superficial pyoderma should extend past a clinical cure plus another 7 to 10 days, or up to 21 days longer if it is deep pyoderma.[34] The second veterinarian used methylprednisolone acetate at an anti-inflammatory dose, most likely to provide some relief for the painful-looking lesions of the left forepaw. Ideally an accurate diagnosis with skin biopsies is preferable to empirical treatment with this drug since it is in a repositol form and cannot be withdraw if side effects occur.

The initial blood work was unremarkable and not that helpful, except to exclude other diagnoses. There was no elevated glucose, as one would expect to see if the disease process involved a glucagonoma causing glycogenolysis and gluconeogenesis. All given liver values were within normal limits, which suggested minimal hepatocellular injury. One can speculate that most of the liver parenchyma was preserved in this case, as the magnitude of increase in ALT roughly parallels the number of hepatocytes or liver mass affected.[32] There are exceptions to this general rule in cases of chronic liver disease. Over time, as the disease progresses, fewer hepatocytes are undergoing necrosis at any one time, and the value for ALT measures lower than would be expected.[32] Another explanation is that the blood sample may have been taken before metastasis occurred, or before significant tumour cell crowding caused enzyme leakage. The ALT in this case did not even begin to approach the high normal reference level. This, and the fact that albumin was high normal supports the deduction that the liver was, for the most part, unaffected. (Approximately 80% of the liver functional mass has to be lost before albumin levels decline).[32] High normal albumin levels are not typical for a disease with a pathogenesis that involves hypoaminoacidemia. Hence, dogs with glucagonoma usually are reported to have a hypoalbuminemia.[7,9] It may have been interesting to see if albumin decreased over time due to the catabolism of amino acids as the disease progressed, and if the liver enzymes increased over time. Decreased BUN is a consistent finding in dogs with MEN.[9,16] Urea is produced from the breakdown of amino acids into ammonia and carbon dioxide. As the amino acids become less available, ammonia levels, and consequently urea, may decrease accordingly.

Without the histopathology report of the skin, liver involvement would not have been suspected with this clinical presentation, normal liver enzymes, normal albumin, and the normal bile acid result. A normal bile acid result is typical of MEN when it is caused by a glucagonoma. Normal liver enzymes and bile acid findings in a dog with MEN diagnosed from skin biopsies should raise the suspicion of a glucagon-producing neoplasm, as early recognition and intervention are essential before metastases occur.

A urinalysis probably would not have been helpful, but should have been performed for completeness when the geriatric blood panel was collected.

The dramatic decrease in amino acids that was found in this case (over 60% of the amino acids were less than 30% of their normal value) is supported by other cases of glucagonoma causing MEN that reported performing an amino acid panel (Table 5). Only three out of 26 amino acids in this case were normal or increased. Tryptophan and methyl-histidine were 117% and 333%, respectively, their normal values. Interestingly, in a study of the regulation of amino acid metabolism, tryptophan catabolism is not influenced by glucagon, while the catabolism of almost all the other amino acids is increased by glucagon.[33] In the case reported by Allenspach and colleagues, 4/5 amino acids were decreased from the reference range.[16] Tryptophan was the only amino acid that wasn't reduced. Furthermore, tryptophan was only reduced by 3% in the study that measured 24 amino acids.[11] Arginine was the most consistently low amino acid in this case plus three other cases that report MEN caused by a glucagonoma.[11,14,16]

Reduced amino acid levels observed in cases of MEN caused both by a glucagonoma and a hepatopathy can be used to support a diagnosis of MEN. However, the pattern of reduction in specific amino acids cannot be used as a tool to determine the underlying cause of MEN until there are further published studies of amino acid levels in dogs with a glucagonoma.

The exact pathogenesis of the skin lesions in this disease remains unknown but is most likely linked to the deficient amino acid levels, as a result of increased catabolism by the liver. The role of glucagon is less clear. As stated previously, in all cases of MEN caused by a glucagonoma where the glucagon was measured, values were found to be higher than normal, while cases of MEN caused by hepatopathy have reported either elevated or normal values for glucagon. This incongruity makes glucagon measurements less helpful in making a diagnosis of MEN or determining the underlying cause. Further studies need to be carried out to determine why glucagon is elevated when a glucagonoma is not the underlying cause of MEN.

Unfortunately a glucagon assay was not conducted in this case. It would have been interesting to see if it was elevated and if so, how high. The limitations with this assay have already been discussed; however, an elevated glucagon level may have suggested that a glucagonoma existed, but would not rule out a hepatopathy as the underlying cause.

The best diagnostic indicator of MEN in this case was the skin biopsies that were taken of the forepaw, repeated, and then repeated again with different tissues samples taken at the

time of necropsy. The classic red-white-blue pattern was indicative of MEN and ruled out immune-mediated diseases and other contact (allergic) or generic dog food dermatoses. Immune-mediated skin disease could have been initially ruled out by microscopic exam of smears from intact pustules for neutrophils, eosinophils and acantholytic keratinocytes.

Radiographs of the abdomen are also seldom helpful and were not done in this case. For completeness, radiographs of the thorax may have been helpful to rule out metastatic disease; however, glucagonomas rarely spread to the lungs in humans.[3]

Ultrasound imaging of the abdomen is useful, but not diagnostic, and very seldom can a pancreatic tumour be identified by this means. Furthermore, the honeycomb appearance of the liver corresponding to the regenerative nodules surrounded by the fat-laden, vacuolated hepatocytes that is observed on ultrasound examination is supportive if the skin histopathology has been performed and MEN already assumed. When Nyland and colleagues called the honeycomb appearance 'pathognomonic' they were referring to cases of MEN caused by a hepatopathy. Although there was a vacuolated appearance of the liver in this case, it was not pathognomonic. This was because there was absolutely no hepatocellular vacuolar degeneration (as described above) observed in the histopathology. Aside from the nests of metastatic endocrine cells, the liver was completely normal. As stated previously, 3 out of the 8 cases of dogs with a glucagonoma reported a mild diffuse hepatocellular vacuolation on histopathology, but no corresponding characteristic vacuolar pattern was reportedly observed on ultrasound

examination. The remainder of the cases did not report any other significant findings in the liver, other than the endocrine cells. There are other liver conditions that may result in a vacuolar hepatopathy pattern that may resemble the appearance of the liver in this case and these are steroid hepatopathy, nodular hyperplasia, chronic hepatitis, diffuse neoplasia, and macronodular cirrhosis. Nyland and colleagues felt the honeycomb pattern in their 5 cases of MEN was distinguishable by the unique network of linear echogenicities,[2] which had a 'lacey-patterned' appearance, rather than a randomly distributed pattern than was seen in this case. Their deduction that the ultrasound appearance is pathognomonic applies only to MEN caused by a hepatopathy.

The necropsy submissions should have included samples of the lymph nodes, small intestine, and stomach, and even kidney to look for further metastasis and also determine other possible locations of the primary tumour. In hindsight, had an exploratory been carried out, the primary and secondary tumours could not have been confirmed at the time of surgery without on-site cytology/histopathology because the organs all looked grossly normal. Biopsy of the liver would potentially have been helpful in confirming the tentative diagnosis of MEN. However, even with the discovery of endocrine cells in a liver biopsy, the primary tumour location would likely have been undiscovered at surgery.

Disappointingly, submission of the entire pancreas, which appeared to be grossly normal, showed no evidence of a mass of any kind and did not reveal the primary tumour. The pathologist reported that a small primary tumour could have been missed during the

processing as only five-micron sections were used to make up the slides. Other possible sites of a glucagon-producing tumour could have been anywhere along the gastrointestinal tract, particularly the stomach, the salivary glands, the bowels and the kidneys.[13] Since two of the eight published cases of glucagonomas reported the primary tumour was not found, it is conceivable that other sites for primary glucagon-producing tumours, besides the pancreas, do exist.

There was not enough overwhelming evidence to support the administration of octreotide in this case, as the efficacy in dogs does not appear to be good. The oral amino acid supplementation with eggs, recuperative diet, and steak, barely seemed to maintain this dog's body weight. Fortunately, this dog lived for 6 and ½ months from the date of diagnosis by skin histopathology to the date of euthanasia. This is the reported expected survival time when dogs are not receiving intravenous amino acid supplementation.[4] If the owners had agreed to the amino acid infusions, perhaps the dog may have lived longer, but this consideration was outweighed in the owner's minds by what they viewed as a diminished quality of life and the hours the dog would spend in the clinic receiving the intravenous drug.

As more diagnostic tools become readily available, it appears that CT scanning would be superior to other forms of imaging such as radiography and ultrasonography to determine if there is a mass in the pancreas and the extent of the metastasis prior to taking the animal to surgery. For the most part, surgical removal of glucagonomas does not seem to

have a good survival rate due to complications such as pancreatitis and also because the tumour is likely to metastasize early in the course of the disease.

Summary:

This is the ninth report of a dog showing symptoms of MEN caused by a glucagonoma. Clinical signs and histopathology of the skin, ultrasound of the liver, and depleted amino acids all support the diagnosis of MEN. It was not until the dog succumbed to the disease and a necropsy was performed, that a metastatic glucagonoma was determined to be the cause of the skin lesions. Unfortunately, the primary tumour was never found. The pathogenesis of MEN is unknown, but depleted amino acids are thought to play a role. Sadly, the prognosis for MEN is poor. Even if the primary tumour can be found and removed, in most, if not all cases, the tumour has already metastasized. Medical therapy for glucagonomas is palliative at best.

Endnotes

a. Kirkland Signature Maintenance Dog Food Costco Wholesale, Ottawa K2E 1C5

b. Amoxicillin/clavulanic acid 500/125 mg, Apotex Inc., Toronto M9L 1T9

c. Furacin ointment, Vetoquinol, Quebec J5T 3S5

d. Depocillin, Intervet, Canada, Ltd. Whitby, Ontario L1N 9TS

e. Depomedrol, Pharmacia Animal Health Orangeville, Ontario L9W 3T3

f. Novolexin, Novopharm, Toronto, Ontario M1B 2K9

g. Hydromorphone. Sandoz Canada Inc., Quebec J4B 7K8

h. Acepromazine (Atravet) Ayerst Veterinary Labs, Guelph, Ontario N1K 1E4

i. Rapinovet, Schering-Plough Animal Health, Pointe Claire, Quebec H9R 1B4

j. Isoflo Abbott Laboratories Ltd. St. Laurent, Quebec H4S 1Z1

k. Acu punch (3.5 mm) Acuderm Inc. Ft. Lauderdale, Florida 33309

l. PDS, Ethicon, Somerville, New Jersey, 008876-0151

m. Ethilon Ethicon Somerville New Jersey 008876-0151

n. Neomedrol cream Glovers Medicine Centre Pharmacy, Kamloops V2C 1T8

o. L/d, Hill's Pet Nutrition, Topeka, Kansas, 66601

p. Hibitane soap. Wyeth, Guelph, Ontario N1K 1E4

q. A/d, Hill's Pet Nutrition, Topeka, Kansas, 66601

r. Surolan Janssen Animal Health, Merial, Quebec, H9X 4B6

s. Baytril, Bayer, Inc., Toronto, M9W 1G6

t. Euthanyl – Biomeda MTC Cambridge, ON N3C 2W4

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2. Nyland TG, Barthez PY, Ortega TM, Davis CR. **Hepatic ultrasonographic and pathologic findings in dogs with canine superficial necrolytic dermatitis.** *Vet Radiol* 1996; 37: 200-205.

3. Taboada J, Merchant S. **Superficial necrolytic dermatitis and the liver.** In: Proc *Intl Vet Emerg Crit Care Symp 2003.*

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