MUCOSAL AND CUTANEOUS ULCERATIVE SYNDROME IN BLACK RHINOCEROS

Linda Munson

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Munson: What I am going to be talking about is this mucosal and cutaneous ulcerative syndrome that we are seeing in black rhinoceros. We are also seeing it in other rhinoceros, but the prevalence is by far much much greater in the black rhinoceros. We see rare cases in the Indian or in the white rhinoceros. Contrary to what Eric [Miller] said last night, I think it is the most prevalent disease. I have no idea how many instances there are, but we have 42 cases to date. This is also a very persistent and serious problem because it is not just irritating little ulcers on these rhinos. Some have died as Raoul [du Toit] has mentioned, and some have died in captivity too.

Originally when we first were working on this disease... I should say this is something I have been harping about *in the background* all the time. We see this in captivity. We see it very frequently in the United States in zoos, it has also been seen in England, and it has been mentioned in some zoos in Europe. Apparently it is being seen in some of these translocated rhinos when they are kept in captivity for a while, but not in the wild ones, as Raoul [du Toit] has repeated many times. Originally when we saw this in the biopsies that had been taken in the past, we had it at this ulcer stage. This is a big and deep ulcer and you want to go right for the diagnosis, so they were taken right out of the middle and they were sent to the pathologist, and of course they are chronic granulation tissues. It is a typical ulcer, no primary etiology at all. These were seen with multiple different disease conditions. The skin often manifests disease conditions when there are other systemic diseases, but it is usually different types of patterns and morphologies of the skin disease when we see it. So what we decided to do was to do this perspectively, because also in talking to the clinical veterinarians who had seen this disease, they said it does not look like this at the beginning. It starts out as a wheal or a welt, then it turns into a vesicle, then that ruptures, then we get this. So what we did was initiate this prospectus study involving a number of you here as well as several other zoos, to try to get good biopsies from the margins of the lesions when they first show up to try to find what the initial lesion was; to try and see if this in fact is an entity or if this is multiple skin diseases that just seem to be something we can see clinically. I want you to notice too that this is a broad spread of geographies and climates involved in the United States, because that is a point I am going to come back to later. So we have a broad spectrum of where this is showing up in the United States.

Some of the data is not on slides, because we just got more information that I just added. The data that I am presenting now is based on this. I can tell you a unified theory of the skin disease, but I am not going to be able to tell you what is causing it. I am hoping that it might come out from some of the expertise we have here. It is not going to quite fit, maybe it will, but I do not know if it is going to fit with the enzyme theory that Don [Paglia] is going to talk about tomorrow; but it will fit with the "novel

metabolic disease." Any rate, we now have 35 cases that are skin and oral ulcers. Then we have seven additional cases with oral ulcers only. So we have a total of 42 cases. Seventeen of these I have examined histologically, as well as had other pathologists look at them. So we know what the histology is on at least seventeen of these. The rest of the cases are clinical records that we have derived the data from. There is no sex discrimination with this disease, and there does not seem to be any age discrimination with this disease also. It is spread all across the age groups. There has been some suggestions from the clinical veterinarians in zoos that there was some seasonality to this. So this is the number of incidence that we have seen spread out over time. Again, there is a variety of geographies involved in here. I have not separated it. How on earth could you separate this, it is this temperature here, there and then, it might be a little bit more in the winter. With 444 there is a lot of mention that when there is a rapid change in temperature, then they break with these ulcers or these vesicles or something. Probably if you did statistics on that it would not fall out to be anything significant at all.

In terms of the clinical pattern of this disease... When I took all of this data from all of these cases and we had these biopsies, this fell into a very distinctive pattern. We do not have just a multitude of different skin diseases here. What this has, and you have been hearing it all along during various talks, is that we have a disease that affects certain areas of the body. It is essentially a symmetrical disease. It affects the tips of the ears usually, it affects the mucocutaneous junction along the lips, there are glossal ulcers along the tongue and usually along the margins of the tongue in contact with the teeth. We also get it down along the coronary bands. You get them on the pressure points. You get them on the tip of the tail, and rarely if they get quite serious, they go along the *ventrum*, along the back, on the shoulders, anywhere. This is "Chifumbi," here. These are all along the inside of the leg. This shows you some of the more serious progressive lesions that have shown up as they get more serious. This was at necropsy, so this is when he had been progressing for quite a while. This is one of the more serious cases, this was a terminal vasiculation that occurred. It is the worst case that I have seen. Has anyone seen anything of this severity? I do not think so. This is a really acute severe form of it. This rhino also had these chronic ulcers, so this was just an acute manifestation terminally. This is the lip, and along the lip margin. This is along the margin of the tongue. I want to emphasize that this is along the dental arcade and it was bilaterally symmetrical. To summarize, these are symmetrical lesions in general. Certainly the pressure points show up most frequently at the tip of the tail, tip of the ears. We have all seen these in the oral cavity. So there is a very typical pattern of lesions on them. If we take that into actual numbers, the skin and oral cavity are both equally affected in the number of cases I had. Occasionally we will see it in the nostrils, but the pressure points are most typically involved. Again, I am going to get back to this distribution when I talk about the disease we see in other species.

We talk about how the lesion develops, what is going on in the skin. I am going to do this diagrammatically because I know you all love histo so much, but I am still going to force you to look at some even though it is this late at night. It starts basically as a papule, a little elevation in the skin. A lot of

you have seen this clinically. It just gets these little elevations in the skin. They then seem to get a little vesicle in them, but the vesicle is as you will see within the epidermis. The swelling is actually within the epidermis. It has nothing to do with any inflammation or reaction in the dermis. You then get a vasiculation within the epidermis. Then what happens is that this becomes devitalized and it ulcerates. So the terminal event is that ulceration.

Bolin: How deep is that vesicle, what stratum in the...?

Munson: It is in the superficial side of the stratum on spinosum. It is right at the junction. It is right at the granular layer. To show you clinically, this is what it looks like in the vasicular form. It is quite a discrete lesion, it will be fluctuant. In this case it looks like there is also hemorrhage in there, but it would just be an acute lesion. If you want to take biopsies, take them from these margins right here, because the activity we are going to see is right at that edge. That is where we want to find what is going on with the cases. To orient you here, this is the dermis, this is the epidermis, this is the stratum corneum. This is what we see with the very very acute lesion. There is virtually minimal to no inflammation in the dermis, very very minimal. So it is really essentially a degeneration of the epidermis that we are seeing. In the upper layer of the stratum spinosum, these epithelial cells undergo hydropic degeneration. You get both intercellular and intracellular edema, and you get some parakeratosis occurring over here. These changes progress. This again is in all of the cases with a few exceptions, like your little calf we saw which did not fit into this, in all these other cases it is fitting in.

As it progresses you are going to get a very linear area with this hydropic degeneration and edema here. You are getting marked hyperplasia of the epithelium. This is your stratum spinosum and right here is your stratum corneum up here. Right at that margin where you normally see your granular layer you are getting all of this linear edema. You are getting parakeratosis here at the surface, hyperplasia and some dyskeratosis. Dyskeratosis is abnormal keritization of the epithelium, so these are degenerative and dystrophic changes that we are seeing in the epithelium. You are getting a little bit more of a reaction here, because what happens is the overlying epithelium starts to slough off and you are getting secondary bacterial invasion there. The inflammation we see is related to surface bacteria coming in in this case. Just another example, a little bit more severe, you get these big vesicles. This is where you start getting vasiculation here. Ulceration is starting to occur. This is where the epithelium has undergone complete degeneration and necrosis, and then you are getting bacteria coming in here. This is where this lifts off. People talk about big sheets of it just coming off the surface. That is these sheets of skin coming off right there. This is where we get more superficial infection coming in. Sometimes we get these big subcorneal pustules occurring here.

I want to mention this directly to Don [Paglia] right now, how this differs from the *acatalasemia* and the ulcers you see with that, is you do not see any of these degenerative changes in the epithelium with that. You get a change that is much more similar to this. You get bacteria and neutrophils coming in from

the surface toward the inside and causing necrosis probably from the neutrophil infiltrate. You do not see all of these other changes in the epithelium that we are seeing that are very characteristic. So that is why... As I said, I did not know if it was related to that and it does not seem to be. These are the dystrophic changes, these are abnormal skin cells, keratinocytes. They are not undergoing normal keritinization. They are all becoming vacuolated. This is a really severe case of dyskeratosis. The other thing that I want you to notice is that normally there are a lot of blue granular cells in here, in the superficial layer of the stratum spinosum, and normally that has all kinds of these very prominent keratohyaline granules there. There were none present in the areas of these lesions. That is a point I want to get back to later.

Later on, when you get the ulceration you get these marked *adreno* granulomas, and that is what a lot of the diagnosis came back originally as. They came back as eosinophilic granulomas or just *granulomita* skin disease. That is what Dick [Montali] had on some of the cases. It is just the end stage of this disease and they are all secondary changes, it is not the primary initiated lesion.

So what I want to emphasize is that all of these cases have very similar clinical characteristics, and they have very similar histological characteristics.

Paglia: Are the granulomas specific... *Fairly* obviously you have stained for critters. Is there keratin in there, are they getting a reaction..

Munson: No, they are this entity that horses also sometimes get, which are called eosinophilic granulomas. There is a large influx of eosinophils in there, and new granulation, you can not find anything in there except for necrotic collagen. And it is possibly the necrotic collagen that is causing the foreign body response. But no, we find no critters in there. You find lots of critters in these lesions once they are ulcerated. But they are typical *Staph. epidermidis* and *Staph. aureus* and things like that.

Paglia: There is nothing in the macrophages *except from the* granuloma.

N. Kock: I think you are probably right and that is probably a reaction in rhino. Because in my 603 skin lesions that are obviously parasitic, I get the same sort of granuloma 604.

Munson: Yes, there were some that I looked at when I was over there. I should say also that this is a very unusual lesion, this is a very distinctive lesion and unusual lesion that is characterized by these many points, which are: 1) acanthosis, which is hyperplasia of the epithelium; 2) parakeratosis, which is retention of the nuclei in the keratin layer, which is again abnormal differentiation of development; 3) spongiosis or swelling in the epithelium, which says something is going wrong in those cells, that they are unhealthy; 4) the hydropic degeneration again; and 5) the loss of granules in that granular layer.

In humans and in dogs there is a rare skin disease that fits these characteristics exactly. But the thing that is unusual, and these are different names for this disease [(superficial necrolytic dermatitis, necrolytic migratory erythema, hepatocutaneous syndrome, diabetic dermatopathy, generic dog food dermatitis)], is the same histological pattern and clinical distribution in the body and this waxing and waning of lesions and the poor chronic healing. All of these things are very characteristic. They have just been

given all of these different names in the publications, so if any of you have heard of any of these, it is the same disease. It is the same histological pattern, it is the same clinical pattern that we are seeing. What does not fit is that in humans this is very very rare, it is a little more common in dogs, but we are seeing this in 50% of the rhinos, maybe more. This is very very common in captive rhino.

Miller: At some point in there life time, yes.

Munson: Yes, in terms of number of instances. I guess I will get back to these points. This has obviously been seen in diabetic dogs, [diabetic dermatopathy]. This is in dogs on poor nutritional status, [generic dog food dermatitis]. This is what is seen in dogs with certain types of hepatic disease, [hepatocutaneous syndrome]. These are the different names that have been given to the human and canine form that are seen under certain conditions of hyperglucagonemia, [superficial necrolytic dermatitis and necrolytic migratory erythema]. Glucagon is one of the main glucose homeostatic hormones in the body. This disease was first described back in the forties with people that had tumors that made too much glucagon. And you say, you are crazy, of course rhinos do not all have tumors that produce glucagon. But if you remember what glucagon does... I should also say in some dogs that have it, they have seen high glucagon levels, but not all dogs that have it. It has also been seen now in some humans that have essential fatty acid deficiencies, celiac disease and malabsorption.

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Lots of different conditions have been seen with people; and pellagra, which is why I was harping on the B vitamins. It is starting to show up more frequently than before.

If you remember what causes glucagon to increase, besides a tumor, is the stress hormones epinephrine and corticosteroids. Liver disease causes elevation of glucagon, because glucagon is taken up and metabolized by the liver. So if the liver is diseased, glucagon can not be taken up and metabolized. Interestingly enough there have been some recent studies in which they have adrenalectomized mice and rats and subjected them to cold stress. Cold alone causes an elevation in glucagon. Now glucagon is usually very transient effect in inducing gluconeogenesis and ketogenesis in the liver. They know from these cases with tumors in people that prolonged exposure to glucagon... That what glucagon does is bind the gluconeogenic mechanism and causes hypoaminoacidemia over a prolonged period of time. They have in fact found in many people with this disease, and in all of the dogs that they have tested, low amino acids in the serum and low serum proteins, which is why I have been harping about the protein business too. I do not think in the rhinos that we should measure total protein because a lot of them with this skin disease have *hypergammaglobulinemia*, so their total protein is going to be relatively normal. Eric [Miller] has sent a survey out to supplement the data we have on this, and thank you all for returning it if you did. That determined that the albumin values are quite low in them. We have the ISIS data saying that 1.9 is normal, which seems low to me for a species. But nonetheless, a lot of these are one or less in terms of their albumin. So we have evidence that they are hyperproteinemic, in this case too.

Harvey: The albumins, depending on your method... We get a lot of albumins on our cats just because of our method. Even though they all say *pharmatusal* green, not all *pharmatusal* greens are the same. So it is possible that rhino albumin does not react quite the same.

Munson: Yes, this is done in 15 million different labs. I have no idea what the significance of...

Miller: One of the things I did not bring with me was Dr. Stockhom's folder. When we get home I will call you. It does not answer your question, but we could produce 20 albumins done in one lab that *used one* technique.

Harvey: That is about our normal for cats too, but everyone else would say it was too low, but with our methods that is the way it comes out.

Munson: But I guess there have been some cases that have had normal amino acid levels and normal glucagon levels that are also showing this exact disease syndrome in humans and dogs. The bottom line is not in. As I said, in the last five years this has just been expanding in terms of our awareness that this is actually something specific rather than just these strange changes.

Miller: It is anecdotal now, we already know of a several cases, but a couple of cases seem to have been very responsive to corticosteroids. Where might that...

Munson: In people that have the hyperglucagonemia syndrome, if you treat them with corticosteroids they get a little better temporarily. How they have rationalized that is that there is all that secondary inflammatory change which exasperates the lesions, not the initial lesion. Remember the initial lesion is an epithelial regeneration that then turns into a massive inflammatory response. That has been their justification for that. Otherwise I agree, there is a discrepancy there.

Miller: I do not know if you agree, but I do not think there is enough cases that we can say absolutely steroids work. Because three or four where they have had marked improvements...

Munson: I would say they are just decreasing inflammatory response in the skin, but I do not know that it is curing anything. Interestingly enough, in some people and dogs with this disease they have tried i.v. infusions of amino acids and have gotten an absolutely remarkable improvement. They have also fed eggs and yolks to them and had a remarkable improvement quite rapidly, but the yolk of course is not just high protein, that gets into this whole essential fatty acid business that we get into. That is why there have been as I said, some human cases just recently reported that are essential fatty acid deficiency. So I think what the bottom line of this is is that there is a lot of evidence that this might be either related to nutrition or stress.

Just so you understand how this relates to the skin, because otherwise there is a quantum leap of faith here... First of all the skin is a rapidly replicating organ, so it is something that constantly needs nutrition to be normal. It is not like your heart that basically does not ever grow or do anything other than need the energy to work. Skin needs everything to develop. I mean these cells, I do not know in the rhino how often they turn over, in the human they are turning over once a month--you get the whole epithelium

regenerated. Now with this trauma it is regenerating more frequently. So first of all nutrition and all of these essential vitamins and essential fatty acids and protein and everything are needed for healthy skin.

This is your whole epithelial surface diagrammatically, these are the cells that replicate and they move up and differentiate. Part of differentiation is forming all of this keratohyalin granules. This is the granular layer that I said was missing in the rhinos. These granules are made up of certain lipids and certain amino acids, there is a lot of histidine and lysine there, very critical to the formation of these granules. And you say, granules, so what, they are like little spots in the skin. What these granules do is connect with the keratohyalin granules and with the tono filaments, the structural parts of the cell, to give the entire skin structural integrity. So if those granules are not formed because there is not enough protein or other essential things in there, they are made up of lipids and proteins and RNA and a few other things, then the structural integrity of the skin is not present and it will be incredibly fragile. This layer is the entire protection from any external trauma or any other damage like that. So if that is not there, the structural integrity of the skin breaks down.

That is why they think it has this distribution. Remember it is at the pressure points, it is at the points that have a lot of movement and contact, tongue and the lips. So that is why they think it has the distribution in the body because of this. They do not know if it is protein depletion of the skin leading to this. This is all still so hypothetical. I know for sure that this is the lesion we have. I have seen it in all but one or two of the rhinos. To me the literature is very confusing as I said. It is still being worked on, but the bottom line is that it seems to point most frequently to glucose and amino acid metabolism, and I think that is the direction we should be going in. Whether the amino acid metabolism or the glucose is affected by stressors, I do not know. That is really just hypothetical. I think what we should try to do is get sera which has been banked back from the time when they had the ulcers. We are going to run amino acid levels on them, we are going to run glucagon levels on them just to be thorough, and then I do not know where to take this beyond that. I am open to suggestion.

Stover: Can you get those same amino acid levels run on free-ranging serum?

Munson: Sure, it would be really possible.

N. Kock: You would have to make sure before you do anything that the serum samples are not too old. Because there are some hormones...

Munson: It actually has to be done on the plasma.

N. Kock: Even so, if it has been stored too long you might lose *activity*...

Stover: Do you?

Munson: Yes.

Stover: We ran these on "Chifumbi," but they meant absolutely nothing to us because we had nothing to compare them to.

Munson: You ran glucagon levels?

Stover: Not glucagon, amino acids.

Munson: The other thing I want to mention... This gets back to why I kept harping on the fact that all of these emaciated starving to death rhinos do not have skin lesions, is that to me it can not just be an energy level thing. It has to be more complicated, like an essential fatty acid or something. Because otherwise those nutritionally depleted animals should have had lesions, right? In my mind that would make more sense.

Sadler: What about zinc status?

Munson: The parakeratosis is typical of a zinc lesion, but the other are not typical of a zinc lesion. We have no idea what the zinc status is. Yes I am sure this is multifactorial, I am sure it is. That is why I said I can tell you what the skin lesion is, but I can not tell you what is causing it. But in my mind it is serious enough so that we need to figure out more of this before more animals are brought in 094 any of that.

Sadler: Are there currently animals in the population that are affected right now with it?

Munson: Many, yes

Miller: This is a syndrome that if you just wait six months, almost invariably there will be another one pop up.

Munson: Even more often, this waxes and wanes, I mean some individuals... There have been a couple of them that have just...

R. Kock: Raoul [du Toit] said he thought had seen this in the boma. Nancy, have you seen this?

N. Kock: 009* I have never seen anything in the rhinos like what she is talking about in any 101. I mean I have seen a lot of ulcers in the animals. The ulcers look like that, but histologically they do not.

M. Kock: That last picture of that rhino with the leg. I mean we saw lesions in one rhino along its flank that healed spontaneously. They look very similar to that.

Munson: These do heal spontaneously, then they come back.

Miller: That has been one of the problems with the milder cases. Early on people say, well I treated them with this and they got better. And you realize that a high percentage of these just got better on their own. We treated ours with Kopertox, I suspect strongly it would have gotten better on its own.

M. Kock: One of the animals that went to Australia had a persistent skin problem, but it also had a ulcerative lesion that refused to heal. Has that healed now Dave [Blyde]?

Blyde: Yes.

M. Kock: But that was a problem for a while.

Munson: Some of them heal right away, some of them persist for a very long time, some of them spread out and coalesce. From this data that we just got from this questionnaire we are going to see if there is any treatment. From what I heard, you could do nothing or multiple things and it makes no difference. Corticosteroids might decrease the inflammation and antibiotics might clear up the superficial infections

that are exasperating it, but I do not think it is going to fix the primary thing. The other thing that I think is important has to do with what we are seeing this in. We are seeing this in many of these with toxic hepatopathy. I also think it is very interesting in that people that have this disease, with hyperglucagonemia, are anemic; but it is normocytic and normochromic. But they are anemic, so anemia is often associated with this in humans. "George" is the only one that did not have some other major disease problem that showed up. So this is evidence of the stress issue in my mind.

Citino: The two we had were both normochromic and normocytic anemias.

Munson: Of the dogs that they did, 50% or something had anemia at the same time they had these lesions, from multiple other causes. Some were diabetic and some they did not know what it was. That comes back to something else I discussed with Nancy [Kock] when I was over in Zimbabwe. This is an adrenal gland from a rhinoceros, if you cut that, the cortex of most of the ones we see in captivity just absolutely bulge out. I asked Nancy, do you think this is normal. Do you?

N. Kock: No.

Munson: In my opinion this is not normal. People say, that is just a normal rhino adrenal, they are all like that. Well, they are all like that in captivity. I do not think this is normal. To me this is also more evidence of chronic stress, it is something we see fairly commonly. So that also gives some evidence that chronic stress might be a factor in this. I do not know what we can do about it under that circumstance, other than try to reduce stresses in their life and look at their diet. I think those are the two avenues we should go to try to 133 this.

Paglia: Is that all cortex? It looks like that is cortex going through the center of it. It looks like normal cortex in the center.

Munson: Yes this is cortex and this is cortex. I appreciate this is a terrible picture. In general, their adrenals are very very large, with enormous bulging cortexes.

Paglia: Is it bilaterally symmetrical?

Munson: Bilaterally symmetrical, yes. It just looks like adrenocorticohyperplasia, like as if it was a pituitary tumor or something. It is the same type of lesion.

N. Kock: I have seen larger adrenals in some of the animals that have been held captive or translocated for a long time. But nothing as big as the ones you are describing.

Paglia: What is the weight difference between the ones you are seeing with this hyperplasia and the normal rhino? Is it twice as big, ten times as big?

Munson: I do not know. The trouble is, I have seen the captive ones and Nancy [Kock] has seen the wild ones.

N. Kock: And none of us are that sure either.

Munson: Actually, *Marilyn* 143 probably has this stuff measured. I am sure she probably weighs this stuff, so I can probably get that from San Diego Zoo's files. But you do not have the wild ones when you are out in the field. But any rate, this leads...

Paglia: That is fascinating.

Bolin: What is the mechanism of hyperglucagonemia diabetes?

Munson: Oh my, you want me to talk about...

Bolin: We can discuss it over a beer!

Munson: Please!

END