PATHOLOGICAL CONDITIONS IN CAPTIVE BLACK RHINOCEROS

Richard Montali

TAPE 4A 680

Montali: What I would like to do is to start with sort of a rapid review of pathologic findings in captive black rhinos. This is a collaborative effort. Scott Citino actually helped in reviewing this and worked very closely with me; and of course I could not have done much of the work with out Janet [Stover], Eric [Miller], Evan [Blumer] and Linda Munson who have really done a lot of work together trying to sort some of these things out and put them in sort of context and relationship with the very nice overview that Nancy [Kock] gave on the findings in free-ranging rhinos.

I think the experience of the black rhino disease problem in captivity is really very sporadic. There are not a lot of diseases, they are not rampant epizootic things that we have had to deal with. So the ones that I will review basically are one or two sporadic cases, for example the very few bile diseases that I am aware of. Scott [Citino] mentioned EMC which has been now seen in at least two black rhinos in captivity. One is a neonate that EMC virus was recovered from. But certainly it is something that is a disease which is rat borne. It is something that has to be considered when black rhinos are being held in sort of *ex situ* situations. Tox is another disease that I have never seen, but it is reported in some German zoos and I think it is fairly uncommon. In fact...

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...diseases I think are something that we have to deal with. Of course the leptospirosis has been very well covered by Eric's [Miller] paper last night and I think will be covered in detail by Carol [Bolin] this afternoon. Certainly I think it is one of the single infectious diseases that have to be addressed in the rhino. And there are these others, *Salmonella*. Certainly perrisodactyls seem to be very susceptible to *Salmonella* and some of the Clostridial diseases which again have been reported in the literature as sporadic occurrences. Concerns for rhinos in captive settings are that should one use Bacterns? I think it will depend on the experience of those facilities. *Colebacillosis* is based on a very few number of cases. Eric [Miller] had a young calf that had meningitis probably due to an *E. coli* organism that I will illustrate. This is basically the brain, showing a mantle of acute inflammatory cells, the result of the systemic spread of septic *nasal* illness to the meningies in a young black rhino calf.

Some of the other infectious diseases that we deal with in captivity... Mycobacterials I think is another very important disease. I had experience with this in our black rhino collection in the early eighties. We had suddenly, one of the hottest days in August, our male black rhino just keeled over and died. It turned out to be a disseminated case of tuberculosis associated with *Mycobacterium bovis*. Of course his mate was then very closely worked up and found to be also tuberculis and eventually had to be euthanized. I will illustrate to you slides of that. I think mycotic disease syndrome is allegedly associated with

corticosteriod therapy in some of the cases. I think this has been well covered by Eric [Miller] and is a known entity that I think we need to continue to think about and I will illustrate that as well.

The tuberculins do seem to work and using tuberculins in the tail-fold. This is in fact a very large tuberculin reaction in the female that we subsequently tested.

Bolin: Is that regular strength BTB or was it...?

Montali: No, that in those days, we were using OT. Since then we have...

Miller: We were talking on the way in on the bus yesterday. Detroit has what now appears to be classic 037 culture for maybe not *Mycobacterium tuberculosis*, but had a positive 039 and that one did respond to just bovine BTB.

Montali: Yes, it would not surprise me. They are not like horses in the sense of developing nonspecific reactions, I do not think. Tapirs are also one of them, of course they have I think a higher incidence of TB problems. In fact the pathologic picture of TB is caseocalcarious. This is actually a slab of the lung. You can see it is cavitating, sort of typical caseous and calcific lesions that are unlike the more sarcomatoid lesions that we see in horses with tuberculosis, it is much more less specific. So it is more like the bovine type pathologically. So again, it is something that in *ex situ* programs that one must be vigilant about. I do not think it has been a problem in free-ranging. Have you ever seen any TB anybody?

Morkel: Kruger.

Montali: Yeah, I know about Kruger. In black rhino?

M. Kock: Yeah.

Montali: Because they have a problem in *bovis* in the buffalo.

Munson: Is that confirmed? Because *Nick Creek* was just talking and said it was in the buffalo herd, but he had not seen it in other species. I think the answer is no.

Montali: I do not know that it has spilled over into... They have had some that have reacted, but I do not know if it ever eventuated into finding out if they were tuberculis. It would not surprise me if down the road... And since some of the wart hogs and buffalo herds are rampant with it in the southern part of the park. This is just a very nice illustration of the mycotic pneumonia, you can see the nice *fungal* 061 with a background of *inflammatory* cells. I do not think there is much more I can say about that.

Parasites, I think Nancy [Kock] pretty much reviewed the ones that we see. We still see anoplocephalids in the black rhino. This is really a parasite that I have seen in Asian rhinos. I am not sure that it is one that we have seen in the blacks. Of course the ticks being the concern of coming into the country. I think there were several blacks that came in... Gastric bots, as Nancy [Kock] mentioned, are I think considered incidental findings, but could be responsible for some of these things. I agree that that is probably a good bet on the esophageal lesions. They look like bland ulcers, oval that could certainly be related to the bots; I think *Gyrostigma*, the genus and genera of the bots. They are not the same as the equine, but they look pretty much the same.

N. Kock: Yeah, they are just bigger.

Montali: And then *Hematozoa*, I have had really no experience. I know they are claiming to have seen them in some of the animals in the bomas, but we have not been able to attribute any of the hemolysis or any of those hemolytic hemosiderosis syndromes with *Hematazoa* in any of them. This is actually the opening of the intestine to show you some of the segments of the anoplocephalids. They are very sort of dramatic when you compare these segments to a camel tapeworm segments. You can see that they have learned to adapt well, and they are very large...

Tolt: Before we leave that bot issue...We were talking *to a man* 086 in south Africa, being concerned about treatment of rhinos for intestinal parasites by giving them various things. He felt that what would happen is that some of these bot trusses could dislodge in *a massive leave, then the site* could ulcerate; because he had seen this ulceration, including in animals off treatment with 090 intestinal parasites.

Montali: What were they using to treat it with?

Tolt: They used Ivermectin in a couple of cases. I do not know what else they used.

Montali: Anybody else have any experiences with treatments?

R. Kock: I think Ivermectin is very effective.

Montali: Is it something you do routinely, that you are concerned about?

M. Kock: No, but we do treat with Ivermectin *for export.*

Blumer: With the animals that died in the most recent shipment, there were no bots in any of the animals I looked at. They had all been treated with Ivermectin a couple of times.

Montali: What about the bot fly itself and the preventative methods for the eggs. Where do they lay the eggs? In the horses of course you can see them, they are very obvious, they glue them to the hair. Rhinos do not have much hair, so I am wondering what...

Morkel: *I have got some data* from Nairobi, I think *it is the neck.*

Montali: The necks, under the neck...

Morkel: 101

Montali: I just wanted to contrast some of the differences between these... As I mentioned, Nancy [Kock] I think has very nicely given the overview on the hemosiderosis and that combined with Eric's [Miller] earlier detailed coverage of the hemolytic anemia syndrome, warrants my not spending a lot time on it. But I would like to maybe show some of the differences in the pathology, and particularly to show some of the differences that help to distinguish this hepatopathy syndrome which as Nancy [Kock] mentioned and in my experience does include some component of hemolysis. But there is clearly a difference in that the hemolytic anemia syndrome and hepatopathy syndrome which has me confused. In fact, when I looked back on the cases that I looked upon earlier, there was a bit of confusion on my part. I will not really say much about the encephalomalacia, I think it has been covered very nicely.

This is a liver from an animal with the hemolytic anemia syndrome. You can see the liver is sort of dark, sort of reddish black and it is clearly different from the nice picture that Nancy [Kock] showed of the green discolored liver from the hepatopathy syndrome. So just right there, grossly I think there is every good reason to believe that one should be able to tell those syndromes at gross; at least tell the difference between bile obstruction and just straight hemolysis. Because green livers can also occur for reasons other than this hepatopathy. This is an example of the picture you do see with the hemolytic syndrome. What you see is increased amounts of hemosiderin mainly within Kupffer cells, often with a centrilobular location, and often associated with degenerative or necrotic changes of the hepatocytes around the centrilobular area, basically in a *scemic* change. If you do a Prussian-blue stain, you can see again that this is a Prussian-blue positive pigment. When you look at this under a lower power, it has a centrilobular pattern.

Munson: Do you know if they heal with fibrosis when they get centrilobular scarring?

Montali: I think in some of the more prolonged epizootic cases of hemolysis.

Munson: Have you seen them?

Montali: I have one case that has some questionable fibrosis, or are you saying serosis?

Munson: Yes, do you see serosis in the recurrent cases?

Montali: Of the hemolytic syndrome?

Munson: Yes.

Montali: I do not think so, no.

N. Kock: I have not either.

Montali: But I have seen in the one case of one of the hepatopathy syndromes, the Kansas City case that Richard [Cambry] and David [*Jessup?*] *posted*. This is hemoglobinuric necrosis. This is a classic lesion that one sees with acute hemolytic crisis. And you can see you have both degenerative changes in the tubules and you have these very large pigmented *tasks* and hence the reason for the hemoglobinuria. As I mentioned, it is important I think to determine whether it is hemoglobinuria. There is a test you can tell hemoglobin from myoglobin. But again, this is the picture of the classic hemolytic anemia syndrome. And also then siderophagocytosis throughout many of the organs. This is the lung, you can see these macrophages full of hemosiderin. Then depending on... As Eric [Miller] mentioned, the hemolytic syndrome can be a one time thing where you have a severe acute hemolytic crisis with death within 24 to 48 hours, or there have been all sorts of ranges with reexasterbations in animals that sort of survived the first crisis. So the bone marrows for that matter are going to reflect exactly the stage of the recovery. This bone marrow is from a hemolytic animal that does show regenerative changes, so this animal is actually in some phase of regeneration at the terminal event, to suggest an earlier crisis; where as this animal is from the National Zoo, whose hemolytic events were allegedly associated with the *I&H*. This animal shows

considerable hypoplastic bone marrow. You can see quite a bit of hemocyterin in the bone marrow itself. So that is really a stage that varies.

This is really a severe case of hemosiderosis in which you see hemosiderin not only in the Kupffer cells around the *foremen* areas, but also with in the hepatocytes themselves. It shows you how much iron loading the liver can take. This is sort of a complicated case, because this is the case that I think is one of the prolonged extended cases of hepatopathy that then has gone on to serosis. If it is pure hemolysis, then Linda [Munson], this could be the case you are asking about. I mean, as you can see, it is just loaded with iron.

There are some other things that have been alluded to. I would like to also mention my experience that is with the hypovitaminosis E type related lesions. These are based on histologic findings in just a few cases. 183 myopathy and steatitis are pretty well known lesions associated with hypovitaminosis E. The leiomyometaplasia or lipofuscinosis is something that has been also associated with this condition in pigs and in dogs, and I have observed similar changes. This is a young rhino from when Scott [Citino] was at the Metro Miami Zoo, that died and had evidence of steatitis. This is actually coelomic cavity fat with the sort of classic picture that is a problem attributed to Vitamin E. And this is the picture that I mentioned of the leiomyometaplasia or the lipofuscinosis. This is actually found in the smooth muscle coats of the intestinal tract, and also in the heart. This is actually PAS stain, because the granules, when you look at them just by H&E, they are sort of refractile and they do not stain very well; but they are PAS and also acid fast positive. This is one animal in fact I think that really had some cooberative Vitamin E level values that suggested that it was quite low in the range that I think Ellen [Dierenfeld] has seen in captive animals. This is a myopathy that came from a translocated animal that suggests the possibility of Vitamin E related myopathy.

I will go on to just some miscellaneous conditions. I think it is well known that the rhino is sort of like the horse, it is subjected to intestinal obstructions and impactions. I have seen a few of those in captivity. I will go into that a little bit more and to supplement Nancy's [Kock] coverage and anybody else who wants to chime in. This is this *colistatic hepatopathy* it is sort of a mouthful of garbled 212. It really means that as pathologists we need to have job security by creating... But, basically for those who are not medically oriented, it really means bile retention in the liver that is otherwise sick from some other reason. I would like to probably just refer to it as something like green liver disease, but I do not really want to get that into the literature either! It is very interesting and very enigmatic syndrome that has... It is one of those things that one day you think you have it pretty well figured out and then the next day some other piece of information pretty much makes that sort of less likely or compounds it. These are based on 11 study cases that I have reviewed, which includes two cases from the literature, the two cases from Schmidt, that was at least my first exposure to this in the literature. I might add that this syndrome has really a lot of good information for the good of the black rhino in terms of maybe extending ourselves a little bit to get values and to get more baseline data on these animals. The course, at least in my experience, is from one to seven

months, and it is characterized by jaundice, edema, anemia, ulcers, weight loss and hemorrhage. These all do not necessarily happen at one particular time. For example, the anemia and ulcers and hemorrhage usually take place towards the end of the course of the disease.

Munson: Are those GI ulcers or skin or both?

Montali: They are skin ulcers, oral [mucosal] ulcers and GI ulcers. Clinical laboratory findings include persistent hyperbilirubinemia, usually in two figures, that is 10 to 12, up to 15 maybe even higher; with about 50% of it being conjugated. So it is not clearly just hemolytic. Purely hemolytic would be mostly unconjugated. One of the enigmatic things I find is that in spite of the amount of necrosis... At least in spite of what appears to me to be fairly significant liver damage, the liver enzyme values are not that high, but they are elevated to some degree. Of course the decrease in hematocrit, which I think it probably hemolytic; and decreasing phosphorus levels that was brought out by Nancy [Kock] and also I think Evan [Blumer], is good evidence for hypophosphotemia. At some level, I think it is again during the end of being a significant finding.

These are the 11 cases that I have looked at some detail and it includes as I mentioned, these two cases which are two cases reported by Schmidt, who in his article, this is an article in the Journal of Zoo and Wildlife Medicine 1981, who attributed this to a possible exposure to the creosote preservatives. Then as you see here there are a number of cases from... This is the Bentsen Ranch animal from 1984, it was an early case. This is "Roscoe" from the Dallas Zoo. I am going to speak a little bit more about "Roscoe", because that to me I think has some very compelling but circumstantial possibilities. This is the case from Cambry that died at Kansas City; and one of the Bass Ranch animals from one of the earlier Zimbabwe shipments; and the rest are ones that both Evan [Blumer] and Janet [Stover] had overseen at Fossil Rim and here at White Oak; and then the two study cases that I have had the opportunity to look at. I have looked at the livers histologically. It is one of these things where I have a mass of information in all various nooks and crannies and things, and there is still a lot of getting together to conceive to make sense out of these cases.

Gross findings, again I said dark, I mean look at the liver, if you look at it in certain ways it is very concentrated green coloration. The most striking thing is that when you subject liver to aqueous fixatives, it just leaches out this green color and it a beautiful green. Again, I am not sure how to express the green, but it is a very striking green when it is not so concentrated. Jaundice, ulcers, edema, hemorrhage, these are all things that are seen pathologically at the gross postmortem event. This is actually the liver from "Chifumbi", the male that was euthanized after about five months of this syndrome. You can see how it contrasts... You remember the liver of hemolytic anemia? You can see the ulcers at the margins, around the sort of coronary bands. And these are... I am not exactly sure when they began, I have the impression it was somewhere near the middle or the end of the course.

Stover: It was progressive.

Montali: Right, they are progressive and they become infected and purulent and very difficult to deal with. And along the margins of the tongue, you can see these very dramatic ulcerations. Then the suffuse of hemorrhages, sometimes actual almost *hematomatis* hemorrhage. Again, I think part of terminal event of liver failure with lack of the clotting factors.

Histologic findings I think are very characteristic that can not be confused with hemolytic anemia. I do not know of anything else that really looks like this in another species. I do not think it really looks like the coal-tar derivative of hepatic lesions in pigs, I think those look a little bit different. It is characterized by, this is actually now "Chifumbi," it is one of the most well preserved livers that we have because he was euthanized and got and fixed very quickly. So some of the early ones that I was impressed with this individualization or dissociation of liver cells which I think that some of that is just artifact, because you can see that the plates are pretty well intact. But what you do see are mainly the pigmentary findings. There is swelling. There are individual clusters of cells that just do not look very healthy. The liver cells are now are sort of *polygynal*, they are different sizes, some are without nuclei. It is not dramatic necrosis, it is not centrilobular or even patchy. It is sort of individual cells here and there at various stages. Again, this is a very very sick rhino that was euthanized, so we are coming toward the end stage.

Bolin: Dick, do you think there is enough necrosis there that would account for the lack of clotting factors? Because you say the liver enzymes...

Montali: I do not think you need necrosis really. I think there is probably enough metabolic damage in these liver cells to probably knock out clotting factors.

Bolin: But yet not enough to leak very many enzymes?

Montali: Yes, that is one of the things I find a little bit confusing, but my experience is that it is really unusual to be able to correlate histologic findings with clinical values with the liver.

Munson: To me these are very degenerate cells and probably dysfunctional, but not so much necrotic, which is what you need for... The kind of lysis necrosis you need to elevate your enzymes.

Montali: Yeah, they are not quite spilling.

Munson: That is where I think the discrepancy is coming.

N. Kock: Not as much membrane damage perhaps to get them to leak. Because the animals that we had also had mildly elevated liver enzymes.

Montali: Sure, they are up in the hundreds, they are not in the thousands.

N. Kock: Both conjugated and unconjugated.

Montali: So this is a pretty sick liver cell, but they are still functioning, they are ticking along. And this you can see that they are really sort of constipated with this thick... Now this is a *Hall's* bile stain, it clearly distinguishes bile from other pigments. It is not *hematoid*, it is not hemosiderin. These are basically iron negative. You can see the accumulations. The bile is also not necessarily within the canaliculi, you

have some bile *lakes*, but they are still within the liver cell. So to me it is a cell that is not able to transport this bile into the outflow tract.

N. Kock: I have put a lot of different stains on my livers. When I put a bile stain on I did not get nearly the same extent of staining, as when I put one on that was for pure bilirubin. I do not know really 372 test, using a specific method for staining for bilirubin per say, as opposed to bile. I mean I do not know what the difference is between the two.

Montali: Well, bilirubin is the exact product of the red cells. Bile has a lot of other things, all the conjugated things, the salts that are being spilled into the biliary tract. The other thing is that these stains are very erratic, they are not always easy to use, and they do not really tell you a lot of specific stuff. That is my experience.

N. Kock: When I used the one stain, it was much more *clear*.

Montali: This is an iron stain, you can see that clearly here. All these cells still have this pigment, but it is not staining iron. The iron is mainly within the Kupffer cells. And this varies. Again, I have seen cases where there are others that have much more iron. But still the unifying theme is the amount of bile.

Jessup: With this stain, the gold is not iron, the blue is iron?

Montali: The blue is iron, it is like a Prussian-blue or Perl's. I can not remember what it is, but it is a differential stain. I showed you the other one that shows that it is staining for bile, so it is not iron. This is another stain that stains the canaliculi. I can not prove it by this one small field, but it shows that the bile is not particularly being excreted into the canaliculi. This outlines the canaliculus, it is a very nice stain. But the question is, is it energy related? I think that will bring up a lot of questions, a lot of possibilities that fit with Don Paglia's lack of ATP in catalase, and certainly I could make up a good story about that.

I just wanted to quickly remind you... Now this is the hemolytic syndrome, and you can see the difference. The liver cells themselves are pretty much OK. This is a little bit of degeneration, and this is a little bit of autolysis in the liver. But you can see that the pigment is clearly in the Kupffer cells. This is the iron stain, and as I say if that pigment was iron, it would all stain like this. So this is the hemolytic, it is easy to tell the difference, you can not confuse it. We did some EM, this is really one of the canaliculi. What it shows, I do not have a normal one, but it shows there is apparently some toxic damage to the canaliculus. It is probably an effect rather than a cause. But clearly there are changes in the canaliculus that are indicated toxic of 418.

I think it is clear to me that we are dealing with at least some element of a toxicosis. And I think that one thing that we have been looking at is, what is the origin of this? There has been nothing that we have been able to totally home in on and say this is clearly the cause. I think that we are still in the stage where we are looking for common exposures. I am just impressing on all the players in this that we still need to look at feed, we need to look at drugs, we need to look at anything, insulins, antibiotic, anything that can get into these animals and cause this condition. At some point I sent out a questionnaire, and I

have received some... Out of the few that I have gotten back, there has been nothing to further elucidate this problem. We mentioned the "C" word already--creosote. I think it is something that we need to continue to look at with the intention of determining whether it is really a factor. I believe it is one of the things that is at the top of my list. I eluded to Schmidt's report, in fact that is not the original report. There are illusions to some more anecdotal observations of creosote and some sort of effect on rhinos in the 1960's. I think there is a book of captive management and there is an illusion to it too. Of the eleven that I have seen, there are nine potential exposures to creosote that could of taken place.

I want to talk about "Roscoe" quickly, then some of the toxicology work that we are doing with *Azel* 457 at Michigan State University, who is trying to work with us and see if there is any evidence that it is related to creosote. It turns out that we have some preliminary, it is being refined. Everything seems to be preliminary these days... It does suggest at least as a marker, that perhaps... Let me say that Naphthalene is an organic compound that is found in very very few things. It is found in creosote, it is found in mothballs, and it is also found in these crystalline things that they put in men's urinals in the toilets.

Kenny: It is also found in crude oil.

Montali: Right, crude oil. It is definitely some sort of coal-tar derivative, including crude oil. So that limits it. The testing that we have done suggests that there are elevated levels of Naphthalene in the lipid levels of some of the animals. Now these are controls. In controls there is a background, because in extracting the lipid levels they do use solvents that have a trace of Naphthalene, so these are considered background levels. In the three black rhinos, "Toto," "Betsy," and "Jerry"... Does everybody know who there are?

Miller: They are all from St. Louis.

Montali: I did not know them personally. These are the normals.

Blyde: Are these dead animals?

Montali: They are alive and well. And these are the suspects from Fossil Rim and here [at White Oak]. If you look at the data the first thing you see that, at least in these suspects, there were four out of six that had levels significantly above the background normals. But of the ones who died of course, we see that two has levels and one has probably a normal level. Of the three that are well, two of them have the highest levels. I do not know what that means. You can interpret it and look at it in many different ways. I think that it suggests... One thing that I do not know is when the serum was taken in relationship at least to the disease of these animals.

Blumer: The serum came from the arrival in Houston. *"Tula"* died a month later, *"Cheti"* died a month and a half later and "Chifumbi" died about four months later.

Montali: I am not making any conclusions at this point, but it suggests to me that the Naphthalene is a possible marker indicating that these animals did have some exposure to creosote, but that is about as far as I can go at his point.

Munson: Dick, did the Zimbabwe creosote have Naphthalenes in it?

Montali: Yes, creosote has Naphthalene, no doubt about it. It is a fact, they also analyzed it. Now, I want to tell you about "Roscoe," because "Roscoe" to me provides what I think is compelling circumstantial evidence that there could be a relationship.

Blumer: For what it is worth, we felt that serum, even a lipid fraction of serum was really far from the best place to look for any of these polyaromatic hydrocarbons. So we now have from a number of these animal's liver samples, and also for a couple of them fat. So it is going to take a while, he is finding this to be one of the more difficult things he has ever had to try to find. When we were trying to track people down, the more experience we found somebody had with these things, the less willing they were to help you, because they knew what a bear it was going to be.

Montali: This is all by GCMS, which is *mass atomic* spectrometry. He is using now also HPLC, which is much more sensitive. There is a lot of work to do yet on the liver and fat, but at least it is preliminary and it is suggestive of 535. Let me tell you about "Roscoe." "Roscoe" is a 30 year old male who was wild caught and he was at the Dallas Zoo for 20 years. In the spring of 1986, they decided to build a sun porch or do something. Now, "Roscoe" lived with "Marsha," so "Marsha" was also in this situation. They introduced this treated telephone pole. One month later "Roscoe" developed this syndrome characterized by jaundice, mild anemia. They took the pole out thinking maybe there was a relationship. Then a five month course of weight loss and persistent hyperbilirubinemia, a clear ringer for this syndrome, ensued and he died. Again, this is all retrospective. I just got this case a month ago. It shows exactly what I saw here histologically. There is no doubt in my mind that it is the same syndrome.

We have at best very compelling circumstantial evidence based on a number of different scenarios, including Nancy's [Kock], that suggest that we have to really look at creosote as a possibility while still ruling out other things. I intend to pursue the other possible exposures. Because I think there are too many enigmas that might just express in others.

Here we have "Marsha" now from that little scene that I just told you about. She did well, she never came down with anything. And there are at least three other similar situations where there was a surviving mate, one died and the other one lived and is still there and well. That is the case here at White Oak with the female. I think you have one at Fossil Rim?

Blumer: The two were always separate. They had not even been together after being shipped.

Montali: What I am saying is that raises the question of what? As Nancy [Kock] said, dose relationship, idiosyncratic, the animal's individual response to it, or maybe they do not rub it, or all sorts of things. If we are talking about true creosote toxicity. The interval of their being exposed and then suddenly

they are several months later and come down with this syndrome. Again, the dose relationship, the mechanisms of toxicosis--it is just not a straight forward toxicity that we know that creosote can cause in other species. So I have to say that now knowing that the rhino is... What did you say Eric?

Miller: Metabolically a novel.

Montali: Right, I like that. I think that if it does not have anything to do with pouring water in their ears, it has got to be related to something like the relationship of their ATP to their catalase deficiency. These are things that we need to continue to explore. I felt it really important... I know the problems of trying to find the smoking gun, but I still think we need to show as much data, and I wanted to be sure that you all understood where we were with the creosote story. I think the jury is totally not in. To me I think we all might agree that perhaps, and it has already taken effect, that creosote and rhinos probably do not mix. I think I will stop there.

Worley: Histologically, the Sumatran rhino that died at Cincinnati, did you see that liver, was that similar?

Montali: I did see that liver, and that I sorted out finally; in the early stages, there was some confusion. That is basically obstructive jaundice based on a large pancreatic cyst that obstructed the bile duct. In that case, the difference is that the bile is within the canaliculi. It is not just sort of sequestered in the liver cells.

Jessup: You are convinced these are not a part of the same syndrome, the hemolytic anemia and this *prosthetic* hepatopathy? They are not ends of the same syndrome?

Montali: No, they are not. Within the hepatopathy there is hemolysis. That I think is a terminal event, it is probably part of an oxygen stresser effect or whatever. I think it is definitely a component. Because you see the hemosiderin within the Kupffer cells. By the time these animals die or are near death, they have pretty low hematocrits. They are not the same, they are mutually exclusive as far as their pathogenesis goes.

Munson: Is it hemolysis though Dick, or is that hemorrhage, because these were hemorrhaging extensively. Could not that hemosiderin just be from the hemorrhaging, subcutaneous hemorrhages?

Montali: I think some of that is true too, but I have seen enough hemosiderin in the liver in the Kupffer cells to suggest that hemolysis is probably taking place.

Miller: I think it is both because there have been some cases. In the "Roscoe" animal there was a hemorrhage and there was a precipitous drop in hemoglobinuria. So I think that there maybe both factor involved.

Montali: You see the other thing is that it is milder because they are regenerating. They have *hyperplastic* bone marrows, as Nancy observed, and I also have seen too. So I think there is a component of hemolysis, but it is not critical.

Miller: Would you agree it does not appear that the hemolysis is up front, it is sort of the agonal affect of the actual syndrome that is where...

Munson: As the skin lesion comes.

Blyde: In the rhinos that we saw, certainly got jaundiced and their PCV was still 44-45%.

Montali: Yes, early on.

Blyde: And they were not mineralizing it. I just wonder whether it is hemolysis or not just that the red cells are just running out of "puff" and all just dying. There has to be a life span of red blood cells in rhinos. Now whether that is a sign of 655 or whether it is 130 days. I mean do they just get anemic because the bone marrow is not producing any red cells. Certainly the bone marrow can wind a little down 658 and *hypoplastic*.

Montali: Yes, I have seen that. I think that the pattern of the lower hematocrit is sort of later on in the course and I think it really is secondary. I think the jaundice is not necessarily related to the hemolysis, that is just the stasis that occurs in the liver.

N. Kock: I agree with you completely. And I think the amount of hemorrhaging could never account for the drop in hematocrit. I mean you see a bit of hemorrhage, they will not be anemic.

Montali: I never have seen the ones where the legs were quite extensive.

N. Kock: There was quite a lot of hemorrhage, but it is still not... I mean you have to lose a lot of blood...

Montali: The other thing is that there is histologic evidence that there is hemolysis. I do not think there is any doubt about it.

N. Kock: I agree with you.

Harvey: I would recommend doing Prussian-blue stains on the kidneys on all these things, because if the proximal tubules have iron in them, it would suggest it even on anything that dies. The liver could just be phagocitosis, but it you could find iron in the proximal tubules it suggests *intravascular* hemolysis.

N. Kock: I have done that and it is there.

Montali: There is some, but you do not see the hemoglobinuric necrosis that you see in the out right hemolytic syndrome.

Smith: In that case you had where you had iron in the hepatocytes and in the Kupffer cells, was there anything different about that case?

Montali: That was the case from Denver. That turned out to be one that had one of the longest courses. There was more iron than bile in that case, there was also serosis. It did not fit. If it is hepatopathy it is more of a sub-Q chronic form. That animal survived and maybe overcame, but still the hemolysis I think then sort of took over. It is a little bit different.

Jessup: Is somebody gathering a medical history as far as other compounds theses animals are treated with at various times within...

Montali: Let me just say that "Roscoe" was never sedated, he was never anesthetized or sedated.

Jessup: Was he routinely wormed, was there routine bleeding?

Montali: That is the type of information I 708. I am trying to get a feel for, if there is any common source, you know therapeutics, or anything like that.

END