SERUM AND TISSUE IRON IN BLACK RHINOCEROS

Joe Smith

TAPE 8A 160

Smith: ...you may not know it is the second most abundant metal and the fourth most abundant element, so there is some kind of dichotomy there. The reason is that so much of it is in a form that can not be used. A lot of iron oxides... If you live where they put a lot of salt on the roads, then your car gets these kind of lesions on them. One of the problems with iron is that free iron is not good in the body. All species need iron, from bacteria on up. But on the other hand, free iron can lead to free radical formation. Yesterday there were a couple of slides that had all that peroxide and radicals. Free iron is what metabolizes that, so in the body you can not afford to have free iron around, because it can cause all those oxidase dangers that people talked about before. In mammals there are these number of iron binding proteins. We will talk about some of them and some of them we will not.

Transferrin is in the plasma, iron is bound to it. Ferritin is stored in the hemosiderin. The reason why they talk about hemosiderin is when you fix tissue, the ferritin falls out. The ferritin is water soluble and it tends to fall out. So what you are left with is hemosiderin. Hemosiderin is really just a by-product of ferritin. In the lysosomes there seems to be some changes that occur to make it hemosiderin, and it tends to stay around so they can stain with Prussian blue; but it is made as ferritin. Lactoferrin is another compound around that has bacterial studies.

If we look at the iron compartments, where is iron in the body in the rhino for example? Most of it is in the form of hemoglobin. In various species it ranges from 50% to 75% under normal conditions. That is the main place for it, but not the only place. It also occurs in myoglobin, what makes the muscles red. We do have a storage compartment where we store our iron in case we need it. In the storage compartment we also have ferritin and hemosiderin. That is where most of it is stored. Ferritin is kind of an interesting compound. It is made up of 24 of these individual units in the form of kind of a hollow sphere. This is kind of like a cross section so that we have the iron on the inside, then we have these *doors* where the iron goes in and out. So it is kind of like a tennis ball. The protein is on the outside and the iron is in the inside. That way we keep iron away from causing all the damage it does. We do have some transport compartments. The main one in this case is transferrin that occurs in plasma. Ferritin we will talk about a little bit. Lactoferrin we are not going to mention this time, I guess mainly because I do not work on lactoferrin. We do have tissue iron. Tissue iron is important iron that occurs in... All of you remember the Krebs Cycle, and you have all those cytochromes, catalase for example, *kona*tase. There is a whole series of enzymes that require or have iron. And those are really important. There is some indication that in iron deficiency in some species, that iron is preferentially taken to hemoglobin. So you can have human patients that are phebotomized real severely that have polycythemia, can show symptoms of iron deficiency, and have pack

cell volumes in the 45, 50, 55 range. This is a very small part of the iron, but you can not minimize it under iron deficiencies. It occurs in heme-*comp*, as heme as in hemoglobin, so non-heme, some as cofactors, some of them we do not know.

We measure non-heme iron. If you take a piece of rhino liver, digest it, and do atomic absorption, however much hemoglobin you have in that sample, you are going to get that iron too. The technique that we use only measures non-heme iron. So basically this just confirms what people have seen in slices. Black rhinos have a tremendous amount relative to other species in particular, and more than the white rhinos [(back rhinos *2529±725* mg/g, white rhinos *650±100* mg/g)]. This is statistically significant. There is a marked variation in this. As you can see, this is a standard error here. There is a lot of variation in that. So we plotted this versus age. We do not have age data for all the samples we have, some of them are captive, some of them are not. If you kind of look at this, you get some impression that there is an increase here over time. There is a significant correlation there. The line really goes off like this. You can see that the young ones do not have very much iron, and then as time goes on it can go up. There are some kind of notable exceptions in this data.

Harvey: Is the horse that way too in age?

Smith: We have never done the ages in horses.

Harvey: I think the 239 *single* iron in the horse marrow they claimed was a lot higher in older animals. But I do not know about ferritin.

Smith: When we did non-heme iron and looked at the spleen, we did it from a slaughter house and we did not do ages.

Dierenfeld: What would the normal levels for a horse be for comparison on that slide?

Smith: We will run into them in a minute. They are much much lower than that. A dog for example runs about 100µg. You can get them to go up. We will talk a little bit about that.

Stover: Is that dry weight?

Smith: No, it is wet weight.

N. Kock: Are these animals that have had the ulcers?

Smith: We do not know. Well, some of them come from animals that have died during hemolysis, some of them do not.

N. Kock: Are they all captive animals?

Smith: They are all captive animals. I will have to take that back. We have some of those that died that came in [to the United States in] 1992. We have some of those livers, but we do not have... I can not remember if they are mixed in this or not. But the iron in those are real low relative to the others. You can see here with the whites it is kind of a different story, there does not seem to be any effect of age at all. If you did a statistical analysis, there is not any difference.

Well, it is kind of nice I suppose to look at it and see a lot of hemosiderin, or do the assay when the animal is dead; but that is kind of a little late in the game. So we also have been looking at other serum analytes. Obviously in man where there is a lot of interest in iron, they do not do liver biopsies. So some of the serum analytes you can have are iron and total iron binding capacity (TIBC). Total iron binding capacity is really a measure of transferrin, which is the thing that is circulating around that gets bound to; but it is expressed as iron content. It is kind of easier to explain from my stand point if you look at it like this. The whole line is the total iron binding capacity. That is, if you put iron into this sample, you could get this much before you see the binding capacity. There are various ways to measure that. The way we do is we have a commercial system that does it. We have a little stand alone iron machine that we [use to] measure for serum irons. We can measure the iron itself in 25µl, it takes 100µl to do total iron binding capacity. Basically there is a resin of iron on it that equilibrates with the transferrin, so if their binding capacity will fill it up, then we just measure the iron. On the other hand, if the iron exceeds the total iron binding capacity, the resin is not saturated so 281 there. We have two or three rhinos that we have done that there was more iron in the sample than there was binding capacity. Normally about a third of the binding capacity is saturated. [The rest] is called the unsaturated iron binding capacity. You can get that by subtraction. What we actually measure is the amount of iron and then the amount of total iron binding capacity. Another thing you can do is make a percent saturation by dividing this number [(total iron)] into that number [(TIBC)]. If you get any contamination, it may exceed this. If you get anemia, at least in the dog, the iron tends to go up, and the TIBC tends to go up. In iron deficiency, the iron will go down in severe cases. At least in the dog, it is shown by John Harvey and we have seen this a lot, the TIBC does not go up. In man the TIBC goes up in iron deficiency and in pigs it is astronomical. In pigs it would be 900µl over here in a really severe case. The infection tends to go down in an acute phase reaction. With corticosteriods what happens depends on the species.

Munson: Is that the stress response people talk about? They say there is increased iron deposition with stress. Is that the glucocorticoid response?

Smith: Probably, but that would depend on the species.

Munson: In calves they mentioned it.

Smith: In calves the corticosteriods go down, the iron goes down. In dogs..

Munson: We are talking serum, right?

Smith: We are talking serum.

Munson: Does tissue then go up?

Smith: We never have measured it. I think under the conditions you have that would not change. I do not know about the absorption of iron under those conditions.

Jessup: Is the reason that iron goes down with infection is because it is scavenged by bacteria and used?

Smith: There is a lot of mystery about that. But, kind of the simplistic view point is that it is captured in macrophages. What you are trying to do is starve the bacteria down. The whole thing is that bacteria have to have iron to grow. So if the body can get that iron away... So there is a whole series of reactions that occur. Basically the serum iron will go down in infections or any other acute phase reaction pretty dramatically. We did some experiments where we took a lot of heat. The classical way to produce and look at an acute phase reaction is to give turpentine. Turpentine is kind of like creosote, it is a mixture of compounds, so you can not pick out a name that does not say turpentine and say we injected it. We did that in a horse. Within 24 hours the iron had dropped from 70 or 80 μd down to 12 μd then went to 8 μd and then it came back up. If the infection does continue, they do not come back up.

Here we measured the serum iron in blacks and whites. You can see that black rhinos have a tremendous amount of serum iron relative to other species. In other species about 200 μd is probably nearly an excess number, [normal seems to be] 100 to 120 μd. You can see that the total iron binding capacity has gone up to compensate for that. As I say, we have a few animals that virtually exceeded it. The other thing is that percent saturation has gone up. Percent saturation is used a lot in iron overload in man with hemochromatosis, which is an iron overload disease. That is a fairly common disorder. It is basically a disease of middle aged men; because women tend to lose iron during menstrual cycle, so they tend not to do this. Men over a long time frame accumulate iron until it starts causing a problem. One of the keys is to look at the percent saturation to decide whether you have it or not. And they phlebotomize those people.

Keitt: What was your *background* 344?

Smith: We are in the rhino book now, so... Most of these are animals that have been caught and bled for some other purpose. That creates some problem, because some of them are not cases where the animal is healthy. When we get to the next analysis you can see it really... That is one of the problems with this. I think we started this in 1987. We have just slowly been accumulating samples. These are all captive animals.

Keitt: What are the symptoms of the disorder in humans?

Smith: With hemochromatosis it is liver serosis, diabetes, and skin discoloration. It can be a lethal disease, but it can be treated relatively easy by phlebotomy. When they find this in somebody, we are talking about taking lots of blood over a short time, and then maintain that over years. I have a friend that every three months I think he gives a unit of blood. The blood banks will not use that blood for anything else, because the blood banking industry is very conservative to begin with and they are afraid there is something else that might have caused it. Even before HIV they would not use that blood. There was a big push by an organization called Iron Overload Group that this is a waste of resources to pour that blood down the drain. In *Manhattan*, Kansas which has a population of 35,000 there are about four hemochromatotic patients that are phlebotomized on a regular basis. It is a good source of blood for researchers.

This is serum iron. You could fantasize and say it looks like it is going up, but in real life there is no significant correlation there. And these values do not necessarily correspond to the other values because sometimes we may just get the liver when an animal dies. Sometimes we just get serum, because the animal is still alive.

The other analyte we have been working on it seems like for a life time in different species is ferritin. This is the same ferritin that is a storage molecule in liver and spleen, *however* it does occur in the serum and it can be measured immunologically. The problem from an analytical standpoint is that it is an immunologically driven assay, and most species do not cross react. So if you take a human kit and try to do a horse, nothing happens; or a horse assay and you try to do the cat, it does not work. Cats are particularly bad. This is some work we did a long time ago with pigs. It was the first species we got into. We got into the iron business because of baby pigs. These happen to be three month old pigs, and you can see there was a correlation with the non-heme iron. This is not in the same units as the others, so you can not pay attention to that. I think these were total irons in the liver and spleen, or something like that. Anyway, you get the same kind of correlation. This is a group of horses from the slaughter house. There is a slaughter place in Nebraska where they ship horse meat over to Europe. We went up there and took this. Here you can see, this is in $\mu g/g$, this is combined liver and spleen we just put them in there together. There is a significant correlation here in the horse. We have done this in dogs. This is to show you that if you looked at iron or total iron binding capacity, your ferritin is the best one... This is not a striking 409 type correlation, but we are talking about I think it is 99 dogs with varied backgrounds. These are dogs coming from the Humane Society to be euthanized. So they have some backgrounds that might create a little problem. The other species I have not shown you is cats. We spent about ten years working on a cat assay off and on, but we finally got it. All of this work started in humans. In humans it is one of the best 418 storage of iron is serum ferritin.

We had done different species, and we tried to react one with the other and never had any luck. About a month ago, I decided that maybe we should try the rhinos in the horse assay. Inspiration I guess. This is an anti-horse ferritin. This is horse ferritinate, isolated horse ferritin. This is isolated ferritin from a rhino. So you can see they cross react here, there is no line of identity. So I would like to tell you that is the way we did it. In real life it is easier to do it in the assay and see what happens. But once it did work in the assay, then we had to go back and make sure that we were not just measuring something else in there. So this is from a black rhino. Sometime this week we will do it from a white rhino to make sure it is still the same thing. This is data we have gotten from that. You see some enormous numbers here. If you take all the samples together you get this and it is not significant. This number here is huge relative to that one. That is caused by one animal that had a ferritin of 750,000. This is just unreal. You can take 750,000 and divide by 27 and you get a big part of that mean that is pretty average. And there is one white that is real high. This standard of error here is real close to that number there. So I threw the two highest ones out. When you do that, now you get something that is really different from each other.

Then I did one other for some reasons that may become apparent I guess. I took what I call the living animals. I defined that as we did not have a liver or spleen to go with it. Now we do this one versus age. Again you see that the younger animals are down here and that there is a significant correlation, not real striking. There is this sample that is up there somewhere. I thought if I put that one in it might make the correlation better, but it did not, it made it worse. It was kind of surprising, because the line is coming off through here somewhere and that point is up there. But there is a significant correlation. There are some things that are very interesting about this. One is that we have these real high values, but we have this value right here that represents a black rhino that is over forty years old. If you believe all this stuff I have told you so far, it should not be accumulating any iron. There are couple of problems with that. If you do a non-heme iron to serum ferritin in rhinos, you will not get a significant correlation. But it is a small sample size and there is a lot of noise in that whole business. We can not do it like we do in horses, we can not go to a slaughter plant and pick out a whole bunch, or dogs in the pound or cats in the pound, or pigs that are going to slaughter or something like that. So I do not know if we will ever be able to lay out a number of animals and show this wonderful correlation.

Harvey: You might mention what inflammation does to that. That is probably part of the problem.

Smith: Yes, I think that is a big part of the problem. This warrants some of what is going on there, at least in my mind. This is white rhinos, in this case I was able to plot that. This is the one 489 one. And you can see all the white rhinos are way down here like this, so they do not seem to be accumulating it. If you look at serum ferritin values, they are low in iron deficiency. If you really want to know whether you are iron deficient or not, this is the way to do it.

We can talk about iron overload. In the dog in hemolytic anemia, ferritin will go way up. We do not know about horses, but in dogs it will go way up. The other thing is that serum ferritin is what is called an acute phase reaction. An acute phase it what happens when you get an infection. You get a temperature and feel bad, there are a number of things that happen. One is that serum iron goes down, we talked about that before that that is an acute phase reaction. The one that most veterinarians are around is fibrinogen. They measure fibrinogen, it goes up. That is an acute phase reaction. This is some data from horses where we had two groups of horses. At this point we created an acute phase reaction by injecting bovine recumbent alpha interferon. We had a nice little temperature spike, it went up and came back down. It peaked at 12 hours and was back to normal by 24. The iron went down, it was back by 28. Here you see the ferritin went up and then slowly came down. Along in here we injected this other group, we did not get quite as good of a response. So ferritin is an acute phase reaction, it will go up when you have some kind of inflammatory process going on in the body, just like fibrinogen for example.

One of the other acute phase reactions is ceruloplasmins and copper containing. Under normal conditions ceruloplasmin, at least in our hands, correlates with serum copper pretty well. Now if you get

into 528 situations, that is not necessarily true. You can see the ceruloplasmin when we injected these animals went up, and it never quite got back down to where it belongs. This data is pretty fresh. We were still running assays on Tuesday. What we have to do next week is go back and run ceruloplasmins on these animals to see... Like that one that has 750,000 probably has a big acute phase response. That animal *is* dead, but to see whether they have a good acute phase response or not. We have about got the situation now with all of our ferritins that we have run, some other acute phase reaction. Because *the serum fibrinogens are* out... The other opportunity is *hetra*globin. It kind of depends on how we feel on which one we use I guess. Real 546 you can do across species and 547 to be really honest, I think you would need to use hemoglobin for the same species. So we tend to run 549.

I have a few comments I would like to make about the iron overload, or *ferrion* overload. Basically, iron is a one-way street in an animals. You absorb it and it is so hard to get that the body holds on to it so tenaciously that it will not let it go. The only way you lose it is in the little intestine as it sloughs off its normal process, a little in the skin, a little in the sweat, but really not much. Once you get it in there, it stays. There are one or two things that can happen that will disrupt that, one of which is that if you have a severe anemia, then the absorption will increase. At least in the dog, the absorption goes up dramatically. In dogs that have chronic anemia, like pyruvate kinase deficiency, and possible fructokinase deficiency that John [Harvey] described, we have done one or two of those animals. We did one PK deficient dog, and we did another beagle we had for a long time that ran a retic count around 10% for years and years and years. Those dogs will load up on iron. There is some experimental data on radioactive absorption to say that if you have a problem over here where you do not have enough, the body's responding will turn on this iron absorption. So that is one complicated factor in trying to figure out what is going on with the rhinos.

There are different kind of dietary absorptions. But, basically the non-heme iron, that is if you are not a carnivorous animal, is absorbed differently than iron. One of the things that I think is striking is that it is inhibited by tannins here, so you can load the iron up. Just because you take iron does not necessarily mean it is going to be absorbed, a very small amount of it is absorbed. On the other hand, this system can be overloaded. In general, you only take in iron when you need iron. If the body iron goes down, then you tend to take some up, otherwise you do not tend to take it in. But that system is not a fool proof system, because if you give the animal, this is human data, but if you give them more and more iron, they absorb more and more iron. That is the reason why there is a certain number of kids poisoned every year from 606 iron tablets. They take it in and boom, in it goes. If you present the animal with a lot of iron that is not inhibited, than it is likely to be absorbed. So in the case of rhino, the way that I interpret it, is either they have to have a continuing hemolytic scenario going on, which is increasing their iron absorption; or there is something in the captive diet that is causing the iron absorption to go up. You are presenting the animal with more iron in a form that is different. This animal is a browser and he is used to having a lot of tannins in there, and suddenly if you are giving them inorganic iron, maybe they are just taking it in. Those to me are the two possibilities in this particular scenario.

Dierenfeld: Ascorbic acid increases the absorption of iron. Is it a pH affect or is that unique to ascorbic acid?

Smith: I do not know the answer to that.

Kettt: I think it is a 627 phenomenon.

Smith: I have tried to stay out of the absorption arena. People have been working on that for 50 years. There is a new hypothesis by Conrad now that has to do with *musins* things. They try to indicate ferritin in it, apoferritin and all that story. It turns out that all that ferritin that you get intestinal, that is a way to keep it out. If you make a lot of apoferritin, and the ferritin gets in there when the mucosal cells slough off, then that iron goes out the back.

Dierenfeld: So the dietary fiber with *musins* that would increase sloughing would help take that iron out.

Smith: One of the reasons of course I am interested in those samples from the captive, is to look at the ferritin and see what the ferritin is in those animals.

Dierenfeld: The free-ranging?

Smith: The ones we were talking about that they did the lepto work on. We want to look at the serum ferritin in those and see what the serum ferritin is like in those animals that are basically in the wild. If they are low, the indications are that the ones in the wild do not seem to accumulate iron. It seems to be a captive phenomenon.

Sadler: Ellen, do you remember your numbers from yesterday, you had iron didn't you in your browse?

Dierenfeld: The iron levels in the browses eaten by these guys really are variable, which is typical of most browses. It went something like around 4 ppm to over 200 ppm on a dry basis. Horse iron requirements are about 80 ppm. So certainly, depending on the tannins.

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It is condensed tannins rather than hydrolyzable that do that binding. There are a lot of studies with tea drinking in Britain for example with much lower iron availability in people that drink a lot of condensed tannins. So this might in fact be one of the good benefits of condensed tannins, if indeed that is an issue. But it is certainly something we should pay attention to when we are formulating diets, is that not to dump too much in.

Smith: That I guess is the message when I asked you about the iron. If I were formulating that, I would take the iron out.

Sadler: That is what I was writing down.

Smith: It is only a third of your iron, but...

Stover: Is it inhibited by *phytanes*?

Smith: Yes. So it could be when you are formulating a diet in captivity, versus what they are seeing... The availability of iron may be totally different. These animals may be adapted so that when they get iron, they take it. This is a precious commodity and we want to get a hold of it. Now you are making it where it is too available.

Blumer: In some of these massively hemolyzed animals, questions come up. What is the value of trying to chelate out much of that iron that is being dumped out into the system all of a sudden?

Smith: I am not sure if the amount of hemolysis and the iron you get in that is bad. It is just how much you have got accumulated over whatever time frame. That is a different story. I do not know whether it is worth trying to chelate that out or not.

Blumer: Would there be anyway to tell? There are some products now that are experimental, but in the current situation we are willing to try it.

Smith: I guess my feeling is that if this is an animal that looks like it has iron overload, it would probably be advantageous to try and take it out. The evidence is that it does not occur in the wild and having that much iron around is certainly, from my standpoint, not going to be healthy over a long time frame.

Blumer: But it is a long-term issue and not an acute issue?

Smith: Yes.

Keitt: It is bound iron. It is really the free iron that is the problem with acute toxicity.

Blumer: If it is coming out in hemoglobin, it is bound and not an issue?

Keitt: I would not think so.

Smith: The hemoglobin goes out in the urine, it is gone. It takes that iron out with it. The hemoglobin that stays there gets taken up by macrophages, etc. That iron is protected and so we are only dealing in quantities, how much hemolysis, what is the amount of iron in there, that kind of stuff. I would not be too worried about that. I think of the longer haul of loading these animals up with lots of iron... There are lots of interesting stories about iron overload. There are two stories I have heard about people with hemochromatosis tripping off those things at the airport! That is a lot of iron! The other one was at the first International Symposium on Equine Hematology there was a horse that was presented in Pennsylvania and stayed for a while. The owner admitted giving this horse so much iron, and if you calculate that out it is two and a half pounds of iron over a two year time frame. It is a handicap as far as I was concerned.

Jessup: Some of these slides of liver disease we were looking at yesterday show a lot of iron in different cells. Is that type of picture typical of animals that absorb more iron? They have more iron in the diet than they use, and absorb it?

Smith: I think it kind of depends on the species. The dogs that John [Harvey] has seen is both in the parenchymal and the Kupffer cells. The ones that were shown yesterday in the rhino, most of them

were in Kupffer cells and RE system. Now he showed one yesterday that had a lot in the parenchymal, so I want to find out whether we...

Harvey: In intravascular hemolysis, the 047 iron is taken up by the hepatocytes and macrophages.

Jessup: But aside from hemolysis, if an animal has evolved with and adapted to a relatively low iron diet and you feed it lots of dietary iron, is that the kind if picture you will see?

Smith: I do not think we know the answer to that.

Munson: Not in rhino. They know in humans that that is the case. It is first picked up by Kupffer's cells, then the parenchymal cells pick up the overload. That is how they distinguish a primary hemochromatosis from hemolysis, the relative amount of iron in the parenchymal cells versus the Kupffer's cells.

Jessup: The livers from mynas that have iron storage disease look anything like that?

Munson: They have so much at the point that we see it, that it is hard to say what is more, whether it is Kupffer's cells or parenchymal cells. We see iron in a lot of zoo species. Cheetahs have tons of it, I am sure you have seen it in a lot of stuff you have seen too.

Smith: It kind of depends on what the effects are. There must be a lot of species that effects this. Back in the 1940's they tried to produce hemochromatosis in dogs by injecting iron. They just gave them tons of iron. They made them sick eventually, but they did not get the liver serosis, they did not get the diabetes, and they did not get the kind of things that you would expect with that. You will get serosis in like thalassemia where it is a chronic hemolytic, but you are also pumping units of blood in there. If you transfuse somebody somewhere around 100 units of blood, you will start having trouble with iron overload, and there will be some 063.

Sadler: We went through our study yesterday. What would you be measuring in the blood in the sense of iron areas that would perhaps give us a better picture of what we are trying to do?

Smith: I think those three serums should handle it: iron, TIBC and ferritin. I have to be kind of careful I guess the way I do that. The serum iron and TIBC are not a problem, those are not species specific, you can get them done anywhere. The ferritin, there are only two or three places I know that are doing that. We run it sporadically. I think North Carolina was doing it for a while. But it can be set up. The horse one is all done with commercially available stuff. You know when the EM people use ferritin for labeling? That is horse ferritin. So you can buy ferritin and anti-ferritin antibody. We tried to do pig and could not make it work, so we 074 horse. Once we got the horse in we said, well now what do we do? We published the horse assay, then *we went* looking for iron deficiency in horses.

Sadler: So basically where we are now, you measure the ferritin iron...

Smith: And the serum iron and TIBC. And probably ceruloplasmins so you know where you are.

Blumer: This may be a simplistic question, but in terms of *cows*, if we have an individual animal that has survived a hemolytic event, or a chronic hemolyzer, do we give them iron?

Smith: No, do not give them iron. Based on what we are seeing they have enough iron. The response of the red cell, at least in man, is related to the amount of iron that you have available, but you have got enough iron there to go forever.

Dierenfeld: That is why it is so hard to get low in any manufactured diet, because it is just ubiquitous in any of your mineral supplements and a lot of feed stuffs. It is really difficult to put together a diet that has a physiological normal level.

Sadler: We have gone to the extent to make low iron diets and literally calculating... People have said it came from the machinery and the dyes, etc. When we calculated how much iron we would have to be losing, we would have to build a new plant ever ten years or something. So it is not that. It is back to what Ellen [Dierenfeld] is saying, it is ubiquitous. And it goes up, it varies.

Dierenfeld: But it needs to be paid attention to, you get a lot of problems with it.

Smith: They have had trouble in primates, particularly with small primates, where they put them on experiments and they take a lot of samples. When you take a blood sample, you are effectively taking iron out. You can also get it in blood donors, in dogs and cats that are bled. Ohio State got in trouble because they were bleeding their dogs real frequently and not giving them any iron. So you can produce it, but it is kind of hard to do.

Munson: I am confused because you were saying that if they have a bleed and that iron is sequestered in the macrophages that you do not need to supplement iron. And yet yesterday you were saying that that iron is there forever and it is 099.

Smith: Iron is in the body. There is no mechanism to take it out. When they have a bleed, you have the hemoglobin, so that has got to be processed down to the iron that can be reused. So it will be recirculated.

Munson: During the processing?

Smith: Yes, during the processing.

Munson: But at the point for instance like where Nancy [Kock] is seeing it, where it is in those macrophages, that is not assessable?

Smith: No, it is assessable. It turns over.

Munson: Yes, I thought it was. So what you were saying yesterday about that is there forever, is not necessarily true.

Smith: It is in the body forever, that is what I meant. There is no way to get rid of it unless you bleed them out.

Munson: If the iron was to 107 the iron you are seeing in the tissues for instance, would eventually go away.

Stover: No, it is available in the bone marrow.

Smith: It is available in the bone marrow. If you were to take one of these animals that have that and you started bleeding them, you could eventually get that iron out by taking the hemoglobin out. That is the way to do it in hemochromatosis. You just bleed, bleed, bleed. In thalassemia where the patient is anemic to begin with, you can not do that, so then you have *dextriferroxamine* that is used, because that is the only thing they have got to get rid of the iron, but it is not a neat... They have been working on these iron chelators probably longer than 30 years. I think this *ferroxamine* came from bacteria. The other side of this story is that when you have an infection and the iron goes down, now the bacteria have to get a strategy to get iron, so they put out these siderophores. Sometime they trap transferrin. They have transferrin receptors and they just take it off the transferrin. So there is this battle going on over iron in the acute phase response.

R. Kock: Have you looked at any other browsers at all?

Smith: No.

R. Kock: That would be interesting.

Smith: We have an Indian and some other rhinos, but only a couple of animals, so it is little hard to tell. But I think it fits this story of the ones that are browsers have a lot more iron and then ones that are not. But we have not looked at other species. *Evan* [*Blumer*], you were talking about in browsers, but it is a different situation then a neonatal problem.

Sadler: Just for the group's fun, I went ahead and calculated that extra 150 ppm on a 20 kg intake. It represents one kilo of iron excess per year. I do not know what the absorption is, so you have to multiple it by an absorption factor, but even if it is 10% and then take it times ten years, you are still back to your kilogram of excess iron in an animal.

END