GRFs will be more potent than GH, thus reducing production costs, but other costs will remain relatively constant and the benefit should be similar to that seen with GH itself. The paradox with the grading/pricing system must still be addressed. The largest advantage may come while addressing the challenges of delivering the peptide. The molecule is smaller than GH (44 vs. 192 amino acids) and more potent analogs have been identified with testing in the rat (Lance et al., 1984). Increasing the potency of GRFs would allow formulation of smaller amounts of material for delivery, and perhaps would allow some of the problems to be overcome.

Summary

Much more basic information is needed to conclusively demonstrate the potential for GH or GRFs in growing livestock. The potential for GH per se seems limited due to delivery challenges, economics and the current grading/pricing system, all of which inhibits active research and development by the animal health industries. The prospects for GRFs are somewhat brighter, but overcoming the research and development challenges will require enthusiastic acceptance of the challenge and innovative solutions.

Acknowledgements

The author gratefully acknowledges the contributions of his coworkers for some of the work and ideas reported herein, with special acknowledgement to W.M. Moseley, A.R. Friedman, L.F. Krabill, R.A. Lepley, J.P. Peters, M.D. Charlton and R.G. Zimbelman. Conversations with R.A. Merkel and D.E. Bauman were particularly helpful.

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Discussion — Growth Biology

R.G. Kauffman: You implied that the genes could not be transferred if they are directly incorporated into the zygote, why would you not suspect that they could be transferred on?

Smith: I do know that it has been demonstrated in the number systems that it is successfully transferred; where it might not be, is if it's just in some of the cells of the tissue but not incorporated into the chromosomes. The gene is being expressed, but it may not be incorporated in such a manner that it would be transferred. That may not be a clear answer, but I really can't give you a specific one.

G.R. Schmidt: What makes you believe that you can engineer an animal better than one that you can get through selection from a genetic pool?

Smith: Because the genetic pool may not carry the potential of the gene we wish to introduce. It may not be able to produce enough of what we want, or because of natural selection a long time ago, it may no longer have the genes required. A good example is that we know animals produce a great deal of saturated fats, why aren't they producing more unsaturated fats? The gene product, the enzymes that would synthesize that are not present, which would strongly indicate that the genes which code for those enzymes are not present. What I'm talking about is really modifying the animal to do something it does not have the genetic background to do. So the genetic potential, right now, does not exist in animals. This will also speed up genetic selection for other traits. But I'm really talking about changing animals to do something that they could not do before.

R.G. Cassens: Steve, it seems rather "dead-end" if the animal can't transfer it. I think some people are working on the concept of chopping up the embryo or the fertilized egg after they've incorporated the material into 50 animals instead of just one. Do you know anything about that? And secondly, does the fluorescent dye have any adverse effect on the pronuclei?

Smith: Let me answer the second question first. I do know

that is a major concern they have. Will the dye be tetragenic? They won't know that until we start bringing animals to term. But it is a major concern, it's one that Dr. Cramer is addressing right up front. As to the other question, about chopping up the zygotes so that they produce the intended effect which is cloning, rather than to have more zygotes to implant. I can see major potential with this technique, especially with the difficulty to micro-inject DNA; also, in case they don't transfer the product to the next generation.

D.B. Anderson: Cathy, could you comment on the improvement in carcass yield through use of repartitioning agents?

Ricks: No, I actually cannot.

J.M. Regenstein: Would you comment on the question of residuals in the meat after slaughter; and, what is the FDA status of beta agonists at this time?

Ricks: That is clearly a concern any company watches very carefully. There is no question that we cannot have residuals of these kinds of compounds in meat because of the potential they can have in increasing heart rate. Basically, these are beta-2 agonists and there are always some crossover effects which can stimulate heart rate, which would be unacceptable. We need no residuals in meat tissue and that's obviously something we're striving for by testing other analogs. I can't speak for the FDA, but essentially what they said was about an 100X safety factor over the no-effect level on heart rate.

D.H. Kropf: I wonder if you have any observations on behavioral aspects, such as nervousness, of animals that are administered these repartitioning agents?

Ricks: That's a very good question. The one thing that was noticeable in steers, and in no other animal, is that in the first 10 days or so of drug treatment we saw muscle tremors. We did not see that in any other species. We have not observed hyperactivity, but then we have not observed test animals on a 24-hr basis.

W.R. Jones: In respect to quality, you discussed marbling. Did you notice any differences that would cause changes in color, texture or palatability?

Ricks: We were concerned about color, but we did not observe any differences for swine or in one study with steers. We have not conducted any tests on taste.

G.W. Davis: What are Cyanamid's plans at this time for commercialization of product?

Ricks: The product is in what we call "development for poultry," unfortunately. The goal we're striving for, and you never know in a situation like this because you have to go through very stringent testing procedures, is the 1990's for other species. Whether we'll make it or not, I don't know.

N.G. Marriott: If I interpret your data correctly, there were no adverse effects related to the product acceptability as well as performance. However, when we have observed muscle hypertrophy, we have had problems related to reproduction and product acceptability. Would you speculate as to why you did not experience these problems in your experiments and to what might happen with additional replications?

Ricks: Since we have not conducted any reproduction experiments, we don't know. In terms of the PSE lean quality, we look very carefully to see if we increased PSE, but there was no difference.

Davis: Rick, do you think the regulatory review process would be simple or complex?

R.F. Olsen: At this point, we seem to be in good shape as far as the regulatory review process is concerned because growth hormone, being a natural peptide, doesn't raise many concerns. The fact that it's the protein which reduces basic amino acids once it gets in the gastro-intestinal tract minimizes it. We and others are looking at various analogs of growth hormone and growth hormone-releasing factors and they become non-natural. How FDA is going to respond to that is an entirely different question.

Kauffman: Rick, I would like to ask you to comment on the results now being released by the Cornell people concerning the effects of growth hormone on lactation as compared to some of these other things. I know you weren't asked to address this, but I'm sure you're well aware of this research. They're working strictly with purified growth hormone for this purpose.

Olsen: I'm not sure, Bob, exactly what comments you're looking for. I find the data to be very, very encouraging. One of the things I've always asked myself is what sort of parallels can we draw between the dairy animal and the meat animal. I think one must keep in mind that the milking animal has a tremendous sink of nutrients and energy flow away from the animal. What implication that might have as opposed to the meat animal, which is essentially going to sequester all that energy and nutrients in house, I think is very, very questionable. But I think it's encouraging. It certainly demonstrates the biological concepts. Whether or not we can apply them to meat animals remains to be seen.

W.G. Moody: My question concerns the increased muscle mass you reported, resulting from lambs fed some repartitioning agents. Has Don Beerman or anyone else looked at the muscle itself to identify the organelles? Also, is this a uniform increase in muscle mass throughout the body, or is it just in the leg and the shoulder?

Ricks: Yes, Don is beginning to look at some of these parameters. It appears that the biggest muscle increase is in the leg muscle.

D.H. Beerman: Bill, in general we don't see any physical property differences in the treated animals but what Catherine was saying is true, although we haven't looked at individual muscle weights in different anatomical sections of the body posterior toward anterior. But at least in the preliminary data, we're getting the biggest response for individual muscle weight increases in the posterior limb muscles. On wholesale cut weight data, it looks as if there is very little increase in wholesale shoulder weight, but a very significant increase in rack, loin and leg weight exists. As far as cellular parameters, we're just getting into that work at this point and we have no comments at this time.

Regenstein: Is there any evidence in Cathy's work that you're changing the composition of the meat animal fats?

Ricks: We haven't looked at that at all, but it is something we need to consider in future studies.

Lindy Miller: Dr. Ricks, with clenbuterol there's going to be a necessary withdrawal time for these animals. Have you conducted studies showing any carcass compositional changes that would occur during these withdrawal times?

Ricks: In the poultry, there was a three-day drug withdrawal and yet we maintained the treatment effect. There is some loss of effect with withdrawal and it's primarily in the fat, the

muscles do not seem to lose the effect so rapidly. In swine, we are thinking in terms of a five- to seven-day withdrawal for clenbuterol, but this drug will never be registered.

R.L. Henrickson: Since fat is deposited in the connective tissue, what effect is it having on the connective tissue? Does it suppress the action or the differentiation or do we get an increase or decrease in amount of connective tissue?

Ricks: I really don't know, we haven't addressed that question in our studies.

R.C. Benedict: Are there any beta adrenergic receptors in the hypothalamic area that could affect the releasing factors?

Ricks: Yes, there are known to be affects of beta adrenergic agents in the hypothalamic area. In fact though, the literature doesn't make sense on this, because the beta agonists supposedly decreased growth hormone released from the anterior pituitary. Clearly, it may be that we're having these effects mediated through hormones in some way. We've done two studies, one in sheep, in which we appear to elevate base line growth hormone levels with these agents. We tried another study in swine, and observed no difference so we really don't know at this point.

R.E. Allen: First a comment and then a question for Rick concerning the growth hormone work. It strikes me that a lot of this work tends to be focused on the growing and finishing parts of development. Now, a lot of us who tend to work in this area and deal with growth hormone and somatomedons, think of growth hormone's action through somatomedons working primarily on issues of cell proliferation. Has there been an interest in focusing on the cellularity aspect of this growth problem, which would be a neonatal, early-development area, as opposed to strictly metabolic issues of protein turnover, protein synthesis, degradation, etc.

Olsen: That's a very good question, Ron. I think the honest answer is that there's been a lot of interest but not much activity. The muscle system, as you know, is very difficult to work with, which has delayed progress in this area. You really come down to culture systems at this point, as the systems that you can work with. How you skip from that to the whole animal is such a huge transition. It's difficult to translate what you're finding in your muscle cultures and those we're finding in our animals. I think that, over the years, we need to build up that transition point.

Jerry Lipsey: What about removal or masking of genetic defect genes that either act in a dominant or additive manner? Do you see that in the future? Physical removing or masking of genes that are now a problem in production of livestock?

Smith: That is certainly in the future, but it is much more difficult than the work I've described. We need to learn at this point, with the studies that I've described, more about what regulates gene expression as far as operator-type sequences that are naturally occurring but controlling the actual expressions. If we can actually go in and talk about these, I doubt if we'd be able to move the gene sequences. What we may be able to do is modulate its activity by inserting an operator-type sequence, something that can regulate this activity. But it is quite a bit more difficult than the less-structured approach we're using right now.

Steve Bartle: With the increase in muscle mass that each of you observed, did you notice any differences in water-to-protein ratios?

Ricks: Our data shows the normal ratio that you would expect for normal muscle tissue. That was something we were very clearly concerned about. We weren't just adding a sum of water to these animals, but they are maintained at the normal muscle protein/water ratio.

Olsen: The same is true for growth hormone. What little data available is that the ratio seems to hold.