17. Souba WW, Pan M, Stevens BR. Kinetics of the Na^+-dependent glutamine transporter in human intestinal cell confluent monolayers. Biochem Biophys Res Comm 1992; 188:746–753.
18. Windmueller HG. Glutamine utilization by the small intestine. Adv Enzymol 1982; 53:202–231.
19. Ziegler TR, Young LS, Benfell K, et al. Glutamine-supplemented parenteral nutrition improves nitrogen retention and reduces hospital mortality versus standard parenteral nutrition following bone marrow transplantation: a randomized, double-blind trial. Ann Intern Med 1992; 116:821–828.
20. Yoshida S, Leskiw MJ, Schulter MD, et al. Effect of total parenteral nutrition, systemic sepsis, and glutamine on gut mucosa in rats. Am J Physiol 1992; 26:E368–E373.
21. Klimberg VS, Salloum RM, Kasper M, et al. Oral glutamine accelerates healing of the small intestine and improves outcome after whole abdominal radiation. Arch Surg 1990; 125:1040–1045.

Discussion

DR. JOHN P. GRANT (Durham, North Carolina): It is a pleasure to discuss this paper and to have the opportunity to review the manuscript before this discussion. There has certainly been a lot of research published lately in the experimental animal model concerning the alterations in gut histology and function during intravenous nutrition. This paper reporting for the first time a generalized decrease in amino-acid transport function in the human is of particular interest in that it does deal with the human model. I have several questions I would like the authors to address. First, do the authors feel that this altered amino-acid transport function is due to bowel atrophy or is it due to some altered metabolism with the intravenous nutrition amino-acid profile? To this end, what would happen, or have they evaluated, the administration of a nonabsorbable bulk agent to these patients who are on intravenous nutrition? Is it simply a matter of bulk stimulation of the mucosa or is it due to a nutrient abnormality? Second question, the authors report on altered amino-acid transport from the terminal ileum in this experiment. This is perhaps the least active area of the intestine with respect to amino-acid absorption and may be sensitive to alterations in dietary intake or intravenous feeding. I wonder if they have looked more proximally in the bowel, say in the proximal two-thirds or, in particular, in the midgut to see if it is as sensitive. Perhaps this is just simply a matter of location of study in the small bowel. Third, this was a short-term study. Most of the intravenous feeding done in the hospitalized patient occurs for 2 or 3 weeks on the average and perhaps this 1-week study is a transient phenomenon. Have they evaluated any other patients who have gone to surgery who have perhaps been on long-term TPN for similar findings? One of the authors in this study, Dr. Inoue, spent 2 years in my laboratory evaluating intravenous nutrition and its impact in an experimental animal model. He, like others, identified significant mucosal atrophy occurring during the intravenous feeding; however, this atrophy was completely reversed when 1 to 2% of the amino-acid content of the intravenous solution was substituted with glutamine. He subsequently did an experiment whereby he injected animals with intraperitoneal *E. coli* and found that those animals given intravenous feeding without glutamine had about a 40% survival, whereas those with supplemented glutamine had about a 95% survival. The question therefore becomes, is glutamine capable of completely reversing their findings and is this simply a matter of its absence in the intravenous nutrition solution? Have the authors studied any patients supplementing either with oral or intravenous glutamine for similar findings of the transport proteins?

DR. JOSEF E. FISCHER (Cincinnati, Ohio): This is another in a series of really excellently done studies by Dr. Souba and Dr. Copeland, the quality of which we have become accustomed. What it shows is that the number of carriers in small bowel of man for the first time is decreased, although the confirmation of the carriers as suggested by KM remains the same. Now the questions is, the data is fairly complex and I suppose I am having a little difficulty in understanding some of the consistencies in the data. One of the ways in which one might explain some of these findings is that one of the effects of TPN on the short term is to increase gut blood flow. And one of the things that happens to the bowel in TPN is it serves as a principal area for transamination. If you have a presentation of a lot of substrate such as amino acids to the gut on the blood side, or the basolateral membrane side, then you might expect an increase in alanine, for example, delivered to the cell from the blood flow side and not from the lumen. It is interesting that of all the amino acids that decreased that were studied, alanine and methyl AIB, which measures the system A for alanine, are the most decreased. I have a few questions. The first really is methodologic. As I review your technique and look at the reference of technique, the scraping and the subsequent homogenization to obtain the membranes is similar except for a couple of changes in the way one goes about with the reagents of obtaining isolated enterocytes. It is true the reagents are a little different and the technique is a little different, but how can you be certain that all of the vesicles that you are obtaining are really from the brush border and some of them are not from another part of the cell, namely, the basolateral membrane. I ask that question because if you assume that glutamine supply is the same via the gut and that there is the appropriate amount of glutamine from the blood that you may be getting maintenance of the glutamine transport if some of your membrane vesicles are really not brush border, but they really are basolateral membrane. There is a discrepancy in system B between glutamine and alanine; whereas the transport of system B alanine is decreased, system B for glutamine remains intact. Presumably they are the same carrier and how do you explain that discrepancy? The other problem I have with the relationship between the systems is that in most other systems, system L for leucine and system Y+ for arginine, usually are linked, and if system L is down, arginine and other dibasic amino acids should be up. One of the ways in which one could explain all of these discrepancies is if the model really does not dissect out brush border alone but has a mixed bag of vesicles and I don't know how one would go about it. In the manuscript you spoke about an x18 enrichment, which I believe, but I am not sure that rules out different vesicles. And finally, I would raise the issue of whether glutamine has other uses other than fuel. Taking off in our laboratory, Per- Olof Hasselgren has pursued some of the data that you raised, in last year's presentation, about the discrepancy between increased use of glutamine and a decrease in glutamine synthase in sepsis. It does appear that there are alter-

nate pathways and other uses for glutamine, and I wonder whether this is not what your data is showing.

DR. J. WESLEY ALEXANDER (Cincinnati, Ohio): I am sure all of the members of the Southern Surgical are grateful for this presentation as another incisive and well-executed study that sheds new light on surgical nutrition. Intravenous nutrition as compared with enteral nutrition is associated with mucosal atrophy, decreased DNA and protein content in the mucosal cells, decreased intestinal hormone production, decreased mucosal blood flow, increased permeability to small molecular weight substances, increased translocation of endotoxin and microbes, and an increased incidence of infection. You now show a decreased transport of most amino acids except glutamine with intravenous hyperalimentation in man. In a more global sense can you postulate that the decreased amino-acid transport is associated with the difficulties that are associated with reinstitution of enteral feeding and with diarrhea? And is it possible that your findings might be the result of endotoxin translocation as has been demonstrated to occur in humans as well as animals on TPN rather than a simple lack of luminal substrates, keeping in mind that translocated endotoxin can alter villous blood flow and stimulate cytokine production? Finally, is there any evidence that the reduced blood flow associated with intravenous hyper-alimentation can influence the brush-border nutrient transporters?

DR. J. RAYMOND FLETCHER (Mobile, Alabama): I rise to congratulate the authors for providing me with the manuscript for their presentation well in advance of the meetings and the quality of the work that these folks have been doing for some period. The purpose of their study was stated very well and presented well by Dr. Copeland. They utilized very sophisticated methodology as Dr. Fischer implied. I am not an expert in that particular area, but I still would like to comment about it. I will make one comment about the study and then I have several questions. The authors are leaders in this field and are to be commended for their contribution to this complex area. The present study certainly reflects their capability in performing these studies. My questions are as follows. How truly uniform is the patient population that has been studied? Dr. Copeland implied that these patients were normal patients, but it seems to me from the manuscript that these patients did have some reason to have surgery, and how do we know that in those particular patients who have carcinoma of the colon or other types of problems that are requiring them to have a small bowel resection, that they have normal GI tract function? Exactly how were the cohorts of patients selected and studied? The authors state that the patients were randomized but do not provide us with the information in the manuscript which groups were included in each data. From Dr. Copeland's presentation it would appear that all patients in the two groups were compared to the other group. I always have some questions about studies that show that one can operate on an animal or a human, take out the tissue, grind it up, pulverize it, subject it to abnormal temperatures, study it at temperatures that it does not function at in the body, then say that this represents the function of these cells or tissue *in vivo*. So I have concerns about that. Dr. Copeland alluded to the fact that glutamine was not present in the TPN yet the glutamine transport was unchanged. I wish they would speculate a little bit more detail about the role that glutamine may have in GI tract metabolism and function as we have all been led to believe. The number of patients appear to be adequate for valid comparison and these findings will continue to unravel the complexities of GI tract function and will lead to better treatment of our patients.

DR. WILEY W. SOUBA (Closing Discussion): Let me first thank the discussants for their helpful comments and also thank the Association for the privilege of closing. In the interest of time and the fact that we had four discussants, I will keep my comments short and try to address the specific questions. Dr. Grant asked whether this effect was truly due to nutrient absence and bowel rest or some abnormal nutrient composition. It appears that it is most likely due to nutrient absence and it fits with the hypothesis put forth about a decade ago which shows that nutrients regulate their respective transport activity. In other words, if you were to feed an animal a high glutamine diet, you could increase the glutamine transport activity above that seen in the controlled, normally fed patients. Whether ingestion of nonabsorbable bulk would affect transport is unclear. To my knowledge that work has not been looked at in animals or humans. The question about the selectively of the transport, i.e., is it down in jejunum as well as ileum, is an important one. We have data from jejunum from one patient that's in the manuscript and we recently have data from a second jejunal sample. It too exhibits decreased transport of all nutrients studied, least so for glutamine, which is a very sort of tantalizing question that we are not sure we can explain. We have not evaluated TPN for more than 1 week. Other people have looked at morphometries in patients receiving TPN for 3 weeks and surprisingly in humans have shown virtually no change in villous height or villous number. There is biochemical atrophy, but unlike rats, the human intestine does not appear to become atrophic on long-term TPN. We appreciate and respect Dr. Grant's work on glutamine-enriched TPN in animals. We concur with their studies. We have not done any work to date on glutamine-enriched TPN in patients, although we are approved by the FDA to do so. Dr. Fischer asked about uptake and the possibility that it is an uptake between the two routes of entry. In other words, there is uptake in these cells across the basolateral membrane, blood-derived nutrients and uptake from the nutrients, and we suspect that if transport of one goes up, the transport of the other goes down. That has been shown for several nutrients by other groups. We have taken measures to ensure that we have brush-border membrane vesicles. In other words, these are vesicles from the brush border of the enterocytes. The way we ensure that, and again they are not 100% pure but they are pure enough, we think, that this is a reflection of luminal apical membrane transport, as we look at the activities of certain enzymes that are selective for the brush border, specifically gamma-glutammyl transpeptidase and alkaline phosphatase. They were enriched 16- to 20-fold in the vesicles compared to the crude homogenate. At the same time there is impoverishment of sodium potassium ATPase, which is a marker that is selective for the basolateral

membrane. So the fact that its activity is down in the vesicle suggests that we have a paucity of basolateral membrane markers. We agree with you that one of the puzzling questions raised by the study is, why is glutamine transport maintained but alanine transport is down if they're transported by the same carrier protein? We cannot answer that question, except to say that alanine may also be transported by the system A protein which is diminished as shown by methyl AIB transport. And in the intestine, system L that transports leucine and system Y+ that transports arginine both of which are sodium-independent carriers are not linked, and they both exhibit diminished transport. The selectivity of glutamine or its uniqueness also as a fuel. Dr. Fischer, we agree on that, but also as your group has shown it plays an important role in modulating protein synthesis and nucleotide biosynthesis. Dr. Alexander, we do not think this is endotoxin or blood flow or cytokines as nutrient absence in cultured enterocytes show similar kinds of change. And Dr. Fletcher, we tried as best as possible to use normal people that would not ordinarily receive total parenteral nutrition. This was a study approved by the Institutional Review Board. Although some of these patients did have bladder cancer and tumors of the ascending colon, none of them had advanced disease. None of them had weight loss and when we compared transport from their intestine to intestine obtained from donors, it was not different unless they were on TPN. And again, we think the vesicle model is a useful one since transport in vesicles does reflect transport seen in cultured enterocytes and in everted intestinal sacs.