Panel Discussion

DR. DAVID ADLERSBERG* (New York, New York): Dr. Butterworth has been quoted before as being interested in the study of the ultra structure of the jejunum in normal patients, in normal control subjects and also in patients with the malabsorption syndrome. Dr. Butterworth has worked with the San Juan team in Puerto Rico for many years and then at Walter Reed Hospital. At present he is in Birmingham and was kind enough to join us. Dr. Butterworth, may I ask you to tell us something about this work?

DR. CHARLES E. BUTTERWORTH, JR. (Birmingham, Alabama): I think the many interesting papers and comments have borne testimony to the many facets of sprue, and to the interest that many of us have in this subject.

The appearance of the jejunal specimen in sprue has already been covered by Dr. Adlersberg, but I would like to discuss biopsy specimens.

An electron photomicrograph made by Dr. Hartman at the Walter Reed Hospital, demonstrated a goblet cell in the act of discharging its mucus, the brush border, and of course the nuclei and the interdigitated cell borders, which place each cell in intimate contact with it neighbor. The brush border itself is made up of microvilli. (In studying biopsy specimens we find that in normal people there are about 60 microvilli in a cross section of a cell, making approximately 3,600 per cell. These measure approximately a tenth of a micron in diameter and micron in length.)

In (untreated tropical sprue) you will see the columnar cell nuclei. Microvilli are not apparent. There is a vacuole where the brush border should be. These may also be seen in light microscopic sections. (In untreated tropical sprue the brush border seems to be grossly distorted if not entirely absent.)

If we remember that there are about 3,600 microvilli per cell it will give us a fair indication of the order of magnitudes of difference. We can calculate that the little top of the microvillus would have a surface area approximately 0.008 square micron, and that using the formula for the surface area of a cylinder, we can find that the sides would have a surface area of 0.314 square micron, for a total surface of 1 microvillus of approximately 0.322 square micron. This feature of the cells will endow the cell with a surface area that is approximately twenty times greater than the surface area would be if the surface were flat and plane. And further, we might state that if the microvilli are shortened by one-half their length, the surface area would be reduced by one-half, just as surely as if one-half the intestine has been resected. I would like to place these preliminary observations before you to indicate the importance which may be present in the factor of cell surface for the absorption mechanism.

DR. ADLERSBERG: This is certainly an intriguing and important approach to the problem. I do not know whether this picture is reversible. Could you say anything about that? Did you see any changes brought about by therapy with gluten-free diets or steroids?

FROM THE FLOOR: We have had an opportunity to study two patients, both before and after the institution of a gluten-free diet, and the changes in the brush border were even more striking than those described by Dr. Butterworth. In the patients with sprue there were practically no microvilli whatever before they were given a gluten-free diet. After that, fat absorption and all clinical evidences of sprue disappeared. The brush border appeared perfectly normal.

DR. ADLERSBERG: Dr. Victor Herbert has developed a very interesting technic of studying the presence or absence of the intrinsic factor in the gastric juice. I would like him to tell us something about that.

DR. VICTOR HERBERT (Boston, Massachusetts): Our work has been concerned with mechanisms of vitamin B_{12} absorption and has been carried out primarily with rat and human liver and intestine *in vitro* and *in vivo*.

We have found that the initial uptake of vitamin B_{12}, by either intestinal mucosa or by liver cell surface, is apparently a physical phenomenon, it is not metabolic, and it is calcium-dependent and reversible by EDTA.

We have used this simple system, working mainly with rat liver slices or homogenates, as an "assay" for intrinsic factor, and so far it has been uniformly successful in our hands in patients with achlorhydria. We have used gastric juice from patients with pernicious anemia, from patients with the malabsorption syndrome, and from normal persons with achlorhydria. We find that gastric juice from patients with pernicious anemia has no effect on vitamin B_{12} uptake by liver slices or liver homogenates, whereas gastric juice from patients with the malabsorption syndrome or from normal persons markedly enhances such uptake.

We hope that this will prove, in fact, to be an assay for intrinsic factor. We cannot call it such, because intrinsic factor has never been purified. We do not even know what intrinsic factor is. We believe, as most people do, that it is probably a mucoprotein, but we have to agree with Dr. Glass and with others that it may be nothing more than a prosthetic group.

We might mention that the calcium effect may play a role in the intestinal malabsorption of vitamin B_{12}

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* Deceased.

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in certain patients with malabsorption syndrome. We reported, at the last symposium of the National Vitamin Foundation, that sodium phytate, a calcium chelating agent, would markedly inhibit vitamin B_{12} absorption in normal persons. In the Scandinavian literature Grasbeck and co-workers have reported a similar phenomenon observed with EDTA.

Furthermore, they made a striking observation, which was reported in *Lancet*, in a few patients with steatorrhea, especially in one such patient. This patient, who was almost totally unable to absorb vitamin B_{12}, when given calcium lactate orally, absorbed vitamin B_{12} in completely normal fashion. The same phenomenon to a slight degree was noted in their other patients. They concluded that the mechanism of vitamin B_{12} absorption was in fact calcium-dependent, and that the inhibition of vitamin B_{12} absorption in at least some patients with the malabsorption syndrome, is due to the fact that the fatty acids tie up calcium in the intestine, precipitate as calcium soaps, and are excreted in the stool, thereby making it impossible for vitamin B_{12} to be absorbed.

DR. ADLERSBERG: The group at Cornell, as you know, has been interested for many years in metabolic and balance studies, somewhat similar to those presented by Dr. French. Dr. Sleisenger, would you like to discuss this?

DR. MARVIN H. SLEISENGER (New York, New York): I appreciate very much the invitation to discuss the upshot of seven years' work.

The group at The New York Hospital has treated non-tropical sprue or adult celiac syndrome for a number of years, particularly with the elimination of gluten and gliadin from the diet.

I think is it extremely important to emphasize the concept which Dr. Cooke placed before us of calling this syndrome adult celiac disease. I have now come to the point where I am willing to say that this disease represents a gluten enteropathy. Three years ago, when I presented our metabolic data in Atlantic City on a group of such patients, I was appropriately hesitant and conservative about it. The corroboration of our results by other groups with this form of therapy now permits greater certainty of this concept. The importance of the role of gluten in non-tropical sprue is further emphasized by several observations: (1) Elevated excretion of urinary 5-hydroxy indole acetic acid in patients with non-tropical sprue in relapse, or even mildly symptomatic on steroid therapy with fall to normal levels upon successful institution of dietotherapy. (2) Observations of Girdwood that ingested folic acid is improperly metabolized in non-tropical sprue as evidenced by reduced excretion of urinary metabolite. The defect apparently continues despite correction of anemia in these patients with gliadin elimination. (3) Findings of Weijers and van de Kamer that, after feeding gliadin to celiac children, there is an abnormal rise in blood glutamine. (4) The evidence for disturbance of ascorbic acid metabolism in non-tropical sprue.

Dr. Adlersberg stated that during remission patients still had diminished absorption of fat and fat-soluble vitamins. This has not been the case in patients treated with the gluten-gliadin-free diet. I presume his patients were receiving adrenocorticosteroid therapy. This has also been the experience, as you know, of the Mayo Clinic group and of others who have studied metabolism of these patients during steroid therapy.

That the difference between tropical and non-tropical sprue is superficial, I would disagree. I think the weight of the evidence, both clinically and metabolically, not to speak of biochemically, is that we are dealing with two different entities that have the same or similar clinical appearance.

A brief report of the results of a metabolic study made in 1955 on a patient with non-tropical sprue follows:

The patient was a thirty-seven year old woman, who had had a celiac disease from the time she was two years of age. During the control period the patient absorbed only 54 per cent of fat which was administered. Although the patient was in positive nitrogen balance, the urinary creatinine excretion was low, and the patient weighed only 74 pounds. She was then placed on a gluten-gliadin-free diet, and within a matter of three days there was some increase in fat absorption, although not, I am sure, great enough to be statistically significant. Nitrogen retention also began to increase; and in a further period of three days, fat absorption rose to 68 per cent, and there was further increase in positivity of nitrogen balance. The patient was studied again two months later, at which time fat absorption was 94 per cent, and although positivity in the nitrogen balance was reduced, the ratio between the urinary excretion of nitrogen and its fecal excretion was reversed toward the normal. At the same time, the patient's urinary creatinine, a reflection of nitrogen metabolism, had more than doubled and she had gained 35 pounds.

On administering a diet which contained wheat, oat or rye products, the patient began within a short period to have steatorrhea.

We may summarize in one individual the biochemical changes noted in twenty-four of our twenty-six patients. This was a young woman in whom a rather severe picture of the so-called malabsorption syndrome developed due to adult celiac disease. This young woman was cachectic, weighed a little over 39 kg., and was having foamy diarrhea. Blood albumin was only 1.9 gm. per 100 ml. and prothrombin time was elevated. The serum cholesterol also was very low. She was placed on a gluten-gliadin-free diet, and despite loss of weight due to mercurial diuresis, this patient gained about 15 pounds during the six weeks that the diet was given. At the end of this time the albumin was normal, as were all the parameters which depend upon normal fat absorption such as serum calcium, carotene, cholesterol and plasma prothrombin time.

Last year, at the annual meeting of the Association

of American Physicians in Atlantic City, we reported on twenty patients with non-tropical sprue, seventeen of whom had responded to dietotherapy. We now have twenty-six patients and in all but two, therapy has been successful. Of these two, one is suspected of having another disorder, and the other is not adhering to the diet closely.

The diarrhea, which was moderate to severe in all instances, remitted; the change to normal usually occurred within seventy-two hours, but occasionally it required several weeks. The weight loss, which had been remarkable in these patients, was corrected in all instances. The weight increases were so dramatic that about 25 per cent of these patients eventually restricted their caloric intake.

The duration of sustained remission, without any other form of therapy, is now twelve months longer than the longest one, so that it is almost six years.

I think the biochemical implications are more important than the clinical results. Some other work has been carried out by Dr. Kowlessar in our laboratory which is quite interesting; he may have an opportunity to present that work to you.

DR. ADLERSBERG: I am struck by the fact that of your seventeen patients sixteen were female and one male. Is this correct?

DR. SLEISENGER: Of our total of twenty-six patients, twenty-three are female.

DR. ADLERSBERG: Is this not a very high ratio of females?

DR. SLEISENGER: Yes.

DR. ADLERSBERG: Dr. Kowlessar, would you like to continue this discussion?

DR. O. DHODANAND KOWLESSAR (New York, New York): Like most investigators, one becomes interested in the reason why things occur in medicine, i.e., the abdominal distention, the nausea and diarrhea that one sees frequently in patients with non-tropical sprue. Because of this, we began a study of the urinary excretion of 5-hydroxy indole acetic acid in non-tropical sprue, and these are the data at the present time.

In our patients with non-tropical sprue, prior to dietotherapy, one can see levels of 5-hydroxy indole acetic acid ranging from 11 to 17 mg. per twenty-four hours. In our normal subjects, the values ranged from about 1 to 6 mg. per twenty-four hours. These patients also had abnormal fat excretion at this time, and were not considered in remission.

Other diarrheal states with steatorrhea that we have had the opportunity to study include ileojejunitis, regional enteritis and pancreatitis with pancreatic steatorrhea. Only our patients with malignant carcinoid and those with symptomatic non-tropical sprue had elevated levels of 5-hydroxy indole acetic acid. The significance of elevated levels of urinary 5-hydroxy indole acetic acid is under investigation.

DR. ADLERSBERG: There are a number of questions here, which are extremely interesting. Dr. Cooke, what is known about the interrelations between vitamin B_{12} and folic acid, iron and folic acid, iron and vitamin B_{12} absorption? Can you answer that in one minute?

DR. W. TREVOR COOKE (Birmingham, England): I do not think I can answer in a minute but I can add a certain amount of confusion to the problem. If you follow the blood levels of folic acid of a patient with vitamin B_{12} deficiency and administer a dose of vitamin B_{12}, there is a gradual increase of folic acid levels rising to a maximum ten to twenty days later, the increase being as much as tenfold. If you also measure the urinary excretion of folic acid following test doses of folic and administer vitamin B_{12} there is a fall in the urinary excretion of folic acid (*Clin. Sc.*, 17: 693, 1958). A rise of folic acid blood levels is seen following severe hemorrhage or in response to the administration of hematinics. If an injection of 5 mg. of folic acid is given to a patient with pernicious anemia in relapse, insufficient to cause any adequate hematological response, only about 1 mg. is usually excreted in the urine. However, a further dose of 5 mg. will show the patient to be fully saturated. It is clear as far as pernicious anemia is concerned that deficiency of folic acid is not the primary defect. Also if the levels of folic acid in the marrow of patients with pernicious anemia and with adult celiac disease are considered they are not outside the realms of normal although the concentrations are rather on the low side; but when taken in conjunction with the increased volume of bone marrow in these patients then the absolute amount of folic acid is considerable

My colleagues and I believe that there must be an upset in folic acid metabolism and we have demonstrated this elsewhere. We do not know how vitamin B_{12} affects it; ascorbic acid is involved for vitamin B_{12} administration to patients with megaloblastic anemia restores the fasting ascorbic serum levels to normal and also the rate of disappearance of ascorbic acid from the plasma. We have not investigated whether folic acid administration will do the same.

FROM THE FLOOR: What do you mean by your folic acid?

DR. COOKE: Pteroylglutamic acid activity.

FROM THE FLOOR: I supposed you were measuring the net effect of about six different substances there, which may have varying metabolic effects.

DR. COOKE: I will accept that; but I do not think anybody can get any closer at the moment. All I can say is that the net total of the things do what we show there, pteroylglutamic acid activity.

DR. ADLERSBERG: Here is another question. "Does Dr. Cooke relate folic acid blood levels to body build if his standard dose of 5 mg. is used? This might explain some of his different expressions, percentagewise."

DR. COOKE: The answer is no. I do not think it makes the slightest bit of difference in the technics used, and you cannot relate it to any body build in our tests.

There is one further point that should be made and that is tissue unsaturation which may be found in

any malnourished patient as judged from urinary excretion of folic acid following intramuscular injection and which does not present clinical manifestations of folic acid deficiency, is not the same as folic acid malutilization which is an entirely different problem.

FROM THE FLOOR: How do antibiotics work in malabsorption?

DR. FRANK H. GARDNER (Boston, Massachusetts): I do not know. We see more and more of these patients respond to therapy with antibiotics; and a large number of patients with tropical sprue have responded, as measured by decreased fecal fat excretion. I am anxious to see some of these patients given massive amounts of ascorbic acid, because I wonder if we actually are not seeing a true intraluminal bacteria competition for vitamin C. If we could eliminate the bacterial metabolic needs *per se* without antibiotics, could we not study the problem by leaving the bacterial flora intact and provide adequate nutrition, so that it would not be competitive for nutrients?

FROM THE FLOOR: In other words, your explanation would be based on the same principles as have been used to explain the effect of antibiotic therapy in the blind loop syndrome, if I am correct.

DR. GARDNER: Yes,

DR. A. LEONARD LUHBY (New York, New York): My colleagues and I have been interested in the metabolism of amino acids which are dependent upon folic acid. This work was encouraged by Dr. Jukes and Dr. Broquist a few years ago.

We have studied particularly the degradation of formiminoglutamic acid to glutamic acid. This step requires folic acid. The absence of folic acid interferes with the normal degradation of formiminoglutamic acid to glutamic acid. The formiminoglutamic acid then accumulates and is excreted in the urine. Rats made folic acid-deficient by diet plus sulfonamides excrete excessive amounts of urinary formiminoglutamic acid. The quantity is in direct proportion to their folic acid deficiency. We have found that many patients with sprue and malabsorption syndromes also excrete considerable quantities of formiminoglutamic acid in their urine. If this can be taken as a criterion of folic acid deficiency, and there seems to be good evidence that this is so, then study of the urinary excretion of formiminoglutamic acid provides a direct biochemical means of assessing the folic acid nutriture of patients with sprue and malabsorption syndromes. A report of our findings has appeared (*Am. J. Clin. Nutrition*, 7: 397, 1959).

DR. ADLERSBERG: There are two questions we could probably answer together. Is there any feeling by the panel that the patient with sprue may do well to follow other than a gluten-restricted diet? What is the experience of the panel with gluten-free diets in conditions other than non-tropical sprue? Would you start, Dr. Cooke?

DR. COOKE: As a matter of clinical practice, I find it difficult at times to make a patient adhere to a gluten-free diet. I am always reminded of the number of patients that I have had for sixteen or seventeen years, who have had only a fortnightly injection of a crude liver extract, and yet have maintained good health, with all their biochemical abnormalities going strong, and leading a hard working life. When you have patients like that it is difficult to persuade them to abandon their hematinics and take to diet. If they are not feeling well then they should go on a gluten-free diet.

As far as the other conditions, secondary steatorrheas, are concerned, we have not made any exact studies but I have the impression that some diminution of the amount of diarrhea does result. In fact, if you are on a gluten-free diet you will find yourself becoming very constipated.

DR. ARTHUR B. FRENCH (Ann Arbor, Michigan): For some reason, our patients do not seem to have the trouble staying on a gluten-free diet that other people's patients do. We have a set of recipes that we make available to them, but I know that Dr. Cooke and others have similar recipes that their patients use.

Our diet is very strict. The reason for our success in keeping patients on the diet is not a less strict diet. Actually, in England most of the groups, I believe, do permit oats, which we do not permit. The reactions which have been shown to oats are relatively milder than those to rye, barley and wheat. Perhaps it does not make too much difference whether they have oats or not; but our patients do adhere very closely to the diet. We have had no trouble with keeping patients on the diet except for one girl, who had very striking psychologic problems, and for whom there were obvious reasons why she preferred to be in the hospital, and one other patient.

DR. ADLERSBERG: What about the use of gluten-free diets in other diarrheal conditions?

DR. FRENCH: In secondary sprue, there will be an occasional response, and certainly we have little else to offer these patients. Use steroids and see if they work, and certainly a gluten-free diet should be tried in any patient in whom nutrition is a problem. I would emphasize that in many of these patients it is not nutrition that is the problem.

For instance, in lymphoma or in tuberculosis or regional enteritis, in most cases the malabsorption is easily demonstrable but it is not the limiting factor in these patients. There we should work toward modifying the underlying condition rather than the malabsorption. But when nutrition is the limiting problem, I think a gluten-free diet should be tried, and if it does not work, it can be discontinued.

DR. GARDNER: We placed everybody on a gluten-free diet because we wanted to evaluate absorption. I think we ought to be quite frank and say that the clinical results are good, and that we want to study certain histochemical and hematinic reactions. I am sure that if I did not want to do this, I would not put them on a gluten-free diet unless they were unable to be maintained with the usual measures that have worked in the past.

DR. ADLERSBERG: What I have been impressed with during the past year or so is that patients with ulcerative colitis and also ileitis who had no evidences of malabsorption occasionally responded very strikingly to a gluten-free diet. Dr. Sleisenger, have you had any experience with the gluten-free diet in conditions other than celiac disease of the adult or non-tropical sprue?

DR. SLEISENGER: We have had some experience, but it is not enough to take any strong stand. We have treated several patients with regional enteritis and ulcerative colitis with this diet in, as Dr. Gardner would put it, a university hospital. Despite our enthusiasm, it did not work. Another patient, in whom there was a decrease in steatorrhea, turned out eventually to have a lymphoma. However, I would remind you that experimentally it is possible to induce not only diarrhea but also steatorrhea in animals by giving them gluten in the diet. And the fact that the elimination of gluten may ameliorate other diarrheal states does not detract from the specific role that a glutamine polypeptide may play in the adult celiac syndrome.

I would strongly disagree with Dr. Gardner about the choice of treatment in non-tropical sprue. Except in the most desperately ill patient, the choice of therapy is the gluten-gliadin-free diet. I think that the "usual measures" cannot in the long run be very dangerous, with the exception of prolonged steroid therapy.

I am sure you will have an opportunity to clarify this, for I think we should realize that even if a patient is receiving small doses of corticosteroids, 5 to 10 mg. daily, over a period of years there can be exceedingly detrimental and dangerous effects, particularly with reference to the skeletal system.

DR. ADLERSBERG: I certainly would disagree with your last statement. We have treated a considerable number of these patients on normal diets, which is a terrific advantage, with small doses of steroids over a period of many years. If you really do it with minimal doses, there is absolutely no danger, provided you watch your patient carefully. With doses of 5 or 7 mg. of prednisone daily or correspondingly small doses of Decadron,® say, 0.075 mg. per day, we have not seen any severe complications, even after several years of treatment.

One more question. Why does the absorption defect in tropical sprue persist in 50 per cent of patients after treatment has caused their symptoms to disappear? Do you think that symptoms disappear first and that if these patients are followed up long enough the results of the absorption tests will become normal, too?

DR. GARDNER: No, I do not think that the fat balance and other absorption studies will revert to normal in a patient who has had sprue long enough to have a severe megaloblastic anemia develop, and then responds hematologically. Certainly some of the patients studied in the Puerto Rican series, who have been under therapy for many years, still have abnormal tolerance tests and excess fecal fat.

In fact, I believe one of the first patients described by Dr. R. Suarez in the early use of folic acid was in 1947. Twelve years later she still had defects in absorption, despite having had an initial and sustained hematological response. I think if you can review the bowel biopsy reports that Dr. Butterworth and Perez-Santiago originally published on treated patients with sprue, you can understand that the jejunal mucosa in those patients was still abnormal despite years of therapy.

End of Symposium