Perspectives in Nutrition

The Study of Intestinal Absorption in Man

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BASIC mechanisms of intestinal absorption and secretion in man and laboratory animal are probably similar. Therefore the value of studies in man which cannot hope to compete with animal studies for elegance of experimental design may be debated. Indeed, some[1] have questioned whether we know enough about the physiology of absorption to make study in human subjects worthwhile. Whatever validity this criticism has for the study of normal intestinal absorption, there can be little question that a number of disease states in man have no counterpart in laboratory animals, and therefore, investigation in human patients afflicted with such conditions becomes mandatory.

Granting that the study of absorption in man is desirable, it may be asked whether this is technically feasible. A number of different technics have been used including tolerance tests, with or without recovery of the ingested test substance in the urine; balance studies; analysis of intestinal lymph; and various intubation procedures. While all of these have certain limitations, some are suitable for specific purposes and may yield valid information.

ORAL ABSORPTION TESTS

With regard to the use of oral absorption tests, three sets of distinctions need to be made. The first is whether or not the test substance is metabolized after absorption. Glucose, triglycerides, fatty acids and amino acids undergo almost complete metabolic transformation, and the results of tests utilizing these substances represent only the net result of the rates of metabolic degradation, renal excretion and intestinal absorption. These tests, therefore, are useless for measuring intestinal absorption and should be discarded. In another category are certain substances which undergo incomplete (D-xylose) or negligible (3-0-methyl-D-glucose) metabolisms and blood levels or urinary excretion of these test materials after oral ingestion are more likely to reflect the extent of their absorption from the intestinal tract.

A second distinction to be made concerning oral absorption tests is whether blood levels or urinary excretion is studied. Blood level measurements, at best, indicate the *rates* of gastric emptying and intestinal absorption. Analysis of the test substance in the urine is feasible if it is incompletely metabolized and if its renal excretion can be reproduced and measured. In this case, the test result will yield a more accurate estimate of the total amount absorbed. However, renal excretion may be affected by available body stores of the material under study as well as by variations in the blood level that occur with different rates of absorption but without alteration in the total amount absorbed. Vitamin absorption tests conducted in this manner probably give unreliable results, except for the Schilling test which employs a radioisotope of vitamin B_{12} and a "flushing dose" of unlabeled vitamin B_{12}. Urinary recovery of two test sugars, D-xylose and 3-0-methyl-D-glucose, provides a useful

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estimate of their absorption since the percentage of absorbed D-xylose which is excreted in the urine is not affected by variations in the xylose blood level and because almost all of the absorbed 3-0-methyl-D-glucose is excreted into the urine regardless of the rate at which it enters the blood.

The third distinction made concerns the completeness of absorption of the test substance. Materials such as D-xylose, carotene and vitamin B_{12}, which are incompletely absorbed in normal subjects, afford sensitive tests of intestinal malabsorption since incomplete absorption is depressed further by any disease of the small bowel which involves the area of intestine in which these substances are primarily absorbed. Substances such as 3-0-methyl-D-glucose, which are completely absorbed in normal subjects, are less sensitive in detecting malabsorption since the capacity to absorb these materials is great and disease of the small bowel must be relatively extensive in order to depress their absorption. However, as a measure of the *rate* of absorption in persons with normal, over-all absorbing capacity, the 3-0-methyl-D-glucose test may prove to be a useful tool.[2]

Blood tolerance tests in man have been refined by the use of the double isotope technic. A radioactive isotope of the test material is given intravenously and intraenterically. Blood disappearance rate and distribution space are calculated from the blood concentrations of the intravenously injected isotope. The specific activity in the blood of the isotope given intraenterically is also determined. From these data, the absorption rate of the isotope injected into the intestine then can be estimated. This technic determines the unidirectional transfer from intestine to blood. In the case of iron[3] this value closely approximates net absorption, whereas no conclusions on net absorption can be drawn from the unidirectional water[4] and sodium[4,5] flux measured this way. The limitations and assumptions on which this method is based have been discussed by others.[3]

Counting of total body radioactivity several days after ingestion of an isotopically labeled test dose has been employed recently for the study of iron absorption.[6] This approach is restricted to substances which are absorbed but subsequently not excreted. Its accuracy in man has not been fully established, and expensive counting equipment is required.

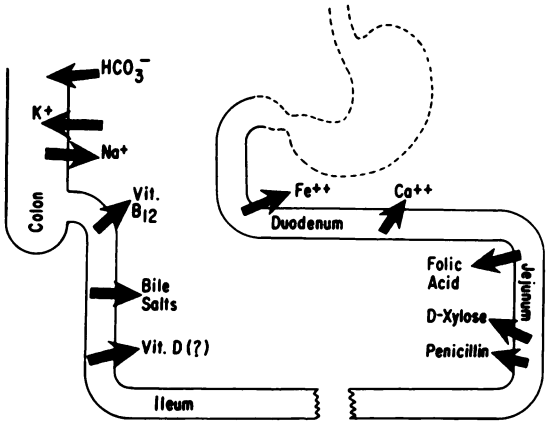

FIG. 1. Schematic diagram indicating the areas in which net transport per unit mucosal surface of certain substrates is significantly higher than in other segments of the intestinal tract. All these substances, except D-xylose, are transported by active mechanisms. The absorption of D-xylose, penicillin and vitamin B_{12} at different levels of the small intestine has been studied in human subjects; the others have not.

BALANCE STUDIES

Balance technics have long been a standard method for evaluating intestinal absorption in man. Without altering intestinal motility and other physiologic events, this method permits the study of *net* absorption or secretion of orally ingested substances over a period of several days. However, apart from the prolonged period necessary to achieve a "steady state," other considerations limit the usefulness of this approach. First, a number of foodstuffs are utilized, altered or synthesized by the intestinal flora which reduces the value of measuring intestinal absorption by the difference between oral intake and fecal excretion. Secondly, secretion of some substances into the gut via gastric, pancreatic, biliary and intestinal fluids may partially or totally mask the absorption of the orally ingested materials. This endogenous secretion is particularly significant with regard to cholesterol, bile acids, calcium, proteins, water and electrolytes. In

some situations this problem can be circumvented by the use of isotopically-labeled test substances. The data from such isotope studies are valid only if corrections are made for the magnitude of the specific activity of the labeled compound which was excreted after having been previously absorbed and secreted back into the intestinal lumen. This technic is discussed by Stanley and Cheng.[7]

Another limitation of the balance technic is that it does not lend itself to the study of the various segments of the intestinal tract. An increasing number of observations, mainly derived from *in vitro* experiments with rodent intestinal sacs, suggest that certain substrates are absorbed at higher rates in some areas of the intestine than in others. The reasons for these regional differences are unknown but may reside in different metabolic requirements of the segments of the intestinal tract[8] or in variations in the amount of specific carrier substances present within the absorbing cells. These cells, however, are structurally indistinguishable along the length of the intestine. Figure 1 summarizes the available data, but it must be emphasized that the data shown in Figure 1 are subject to species variations. For example, calcium transport in the hamster is greater in the ileum than in the duodenum, while the converse is true in other experimental animals that have been studied.

In recent years, the measurement of fecal excretion of I^{131} following ingestion of I^{131}-triolein and I^{131}-oleic acid has been employed for the detection of defects in triglyceride digestion and fat absorption.[9] Advantages of this technic over the ordinary fat balance study are the shorter time period needed and the ease with which I^{131} can be counted compared to the chemical assay for fatty acids in the stool. The test has proved to be of some value to the clinician, but most investigators have found the isotopic procedure to be not very sensitive in detecting steatorrhea as determined by a fat balance study. False negative results are found in 15 per cent or more of patients with steatorrhea. As pointed out by others,[10] the I^{131}-triolein and the fat balance method differ in that the former uses a single lipid given in a single dose, whereas the latter measures fat excretion after a mixture of lipids is given in three daily meals. Exactly comparable results, therefore, should not be expected. However, a number of serious objections have been raised against the use of I^{131} labeled lipids for studying fat absorption in general: (1) The test material may not be pure; 40 to 70 per cent of the radioactivity in commercial I^{131}-triolein was not combined with triolein.[11] (2) The I^{131}-triolein linkage is not entirely stable in small intestinal juice.[12] (3) During absorption and subsequent metabolism, I^{131}-triolein cannot be considered a valid label of nonradioactive carrier fat.[13] (4) The test assumes that fecal fat is of predominantly dietary origin and neglects endogenous fat secretion and the synthesis of fat by the fecal flora.[12]

INTUBATION TECHNICS

Attempts to study absorption from different levels of the small bowel in intact human subjects must, of necessity, involve intestinal intubation procedures, which in themselves may not be physiologic. The possible effects of this manipulation constantly must be borne in mind. One method involves the isolation of a segment of small intestine between the opening of a tube through which the test solution is instilled and an inflated balloon distal to the point of infusion.[14] This technic is open to a number of objections. Complete recovery of the unabsorbed test substance can be questioned, but this could be obviated by the use of a nonabsorbable marker. However, some degree of telescoping of the gut on the inflated balloon may occur so that the true length of the isolated segment of gut is unknown. Furthermore, this method, by definition, utilizes an obstructed loop of intestine, and the results obtained might not apply to an unobstructed segment.

More recently, a combination of the nonabsorbable marker technic with intubation procedures has been described.[15] This involves feeding a test meal containing a water-soluble, nonabsorbable substance, e.g., polyethylene glycol (molecular weight 4,000) to subjects whose small bowel has been intubated with a small plastic tube. Intestinal contents are collected through this tube and the dilution or

concentration of the test meal determined from the concentration change of the nonabsorbable marker. The amount of unabsorbed test substance, as it passes the aspiration site, may then be calculated from its concentration in the collected sample. By feeding the test meal repeatedly, with collections carried out at different levels of the gut, the site of absorption of the various components of the test meal may be determined. This technic is useful for the study of absorption of water-soluble substances; it is advantageous in that the results are not obscured by the metabolic fate of the test substance after absorption. It must be pointed out, however, that at present this method is not valid for the study of fat absorption, since it has been shown that the water- and fat-soluble phases of a meal dissociate in the stomach[16] and are propelled at different rates through the intestinal tract.[17] Unfortunately, nonabsorbable, fat-soluble markers are not yet available.

The described approach recently has been extended to allow direct comparison of the absorptive capacity of different areas of the small intestine. The technic we have used[2,18,19] follows: A solution containing the nonabsorbable marker and the test substance is infused into the small intestine at a constant rate through the proximal opening of a small double-lumen plastic tube. Samples are collected 20 to 60 cm. distally through the other opening of the tube; absorption and secretion within the intervening intestinal segment can be analyzed from the ratio of marker to test substance in the infused and in the aspirated fluid. Since total recovery of the unabsorbed test solution is not required, devices to obstruct the intestine are not necessary. Radioisotopes of water and electrolytes may be added to the test solution in order to study unidirectional flux rates as well as net absorption or secretion. The technic has the advantage that different areas of the gut are directly exposed to test solutions of identical composition. Furthermore, since the rate of delivery of the test solution at different levels of the gut is identical, the effects of variations in intestinal motor activity on absorption are reduced. Figure 2 is an illustration of the use of this principle in the study of water absorption in normal subjects and in patients with

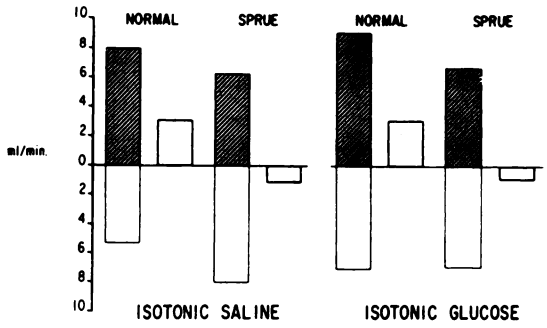

FIG. 2. Absorption of water in normal subjects and in patients with idiopathic sprue from 30 cm. segments of jejunum. The test solution was infused at a rate of 10 ml. per minute. The flux out of the lumen is plotted above the line (cross-hatched boxes), flux into the lumen below the line (blank-boxes), and the net volume change to the side (shaded boxes). If net absorption occurred, the net volume change is plotted above the line. If there was a net increase in intestinal contents, the net volume change is plotted below the line.[12]

idiopathic sprue. These technics require x-ray facilities for localization of the tubes, and a period of three days is usually required to study both the upper and lower portions of the small bowel. Others[20] have employed the same principle but used a single lumen tube which is occluded permanently at one point with perforations on either side of the occluded area. The tube then is allowed to pass from nose to rectum, and the occluded part is manipulated into the area of intestine which is to be studied. The test solution is infused through the end of the tube which enters the nose, and samples are withdrawn from the rectal end. A much longer time is required to carry out this type of study, but there is less likelihood of telescoping of the gut over the tube than with the other technics.

The use of these intubation procedures is restricted to water-soluble substances for reasons mentioned previously. In addition, the magnitude of analytical errors precludes the accurate study of materials which undergo relatively slow net absorption, such as vitamin B_{12}, iron and calcium.

SUMMARY

A variety of technics for studying absorption in man is available. The validity of oral tolerance tests depends on the extent to which the test substance is metabolized, whether

urinary excretion or blood levels are studied, and whether the test substance is completely or incompletely absorbed in normal subjects. Tests utilizing materials which are largely or completely metabolized yield grossly unreliable results and should be discarded in the study of intestinal absorption. Recovery in the urine of partly or negligibly metabolized substances more accurately reflects total intestinal absorption, provided renal excretion is not governed by variations in blood levels. Test substances which are incompletely absorbed in normal subjects are more sensitive indices of intestinal malabsorption than those which are completely absorbed.

In certain instances, such as establishing the presence of steatorrhea, laborious balance technics are indispensable. However, because the time required for such studies is prolonged and the information obtained limited, this approach will probably be used less often in the future. Tests combining intubation procedures and a nonabsorbable marker offer a number of advantages: (1) different levels of the gut can be studied, (2) the results are not obscured by subsequent metabolism of the absorbed material, and (3) endogenous secretion into the intestinal lumen can be measured. One disadvantage is that in some instances the intubation maneuver itself may affect the results.

If the limitations and advantages of these various methods are kept in mind, significant information can be expected to come from their application to the study of human physiology and disease.

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