Quantitation of Gastrointestinal Protein Loss

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Abstract

Plasma proteins leak into the gastrointestinal tract under physiological conditions. This may assume enormous proportions in certain pathological conditions associated with the stomach, intestines, intestinal lymphatics and capillaries, resulting in severe hypoproteinemia. Quantitation of this leakage has been achieved by using plasma proteins or non-protein foreign colloids and macromolecules labelled with radioactive isotopes. The requirements of an ideal label have been listed and the advantages/disadvantages of different tracer labels have been reviewed. No label presently available fulfils all the requirements of an ideal tracer. In spite of this, a wealth of information has been acquired by gastroenterologists to unravel the mystery of hypoproteinemia of unknown cause.

Plasma proteins constantly leak into the secretions of the gastrointestinal tract. This leakage seems to be largely independent of the molecular size, and hence all plasma proteins appear to be affected by this process. In addition, all levels of the digestive tract are involved. This is a major factor in the pathogenesis of the hypoproteinemia associated with gastrointestinal disorders.

In various conditions, collectively known as 'exudative gastroenteropathies', this seepage of proteins may become grossly elevated. These conditions include diseases of the stomach or intestine, and disorders of the intestinal lymphatic circulation, venous return, or capillary permeability.

Quantitation of Plasma Proteins

The detection and quantitation of this plasma protein loss presents certain peculiar problems. Whereas in nephrosis the protein loss can be easily measured in the urine, the quantitation of enteric protein loss has been complicated by the rapid catabolism of proteins leaked into the gastrointestinal lumen and subsequent reabsorption of the constituent amino acids, resulting in only a negligible fraction left for excretion in the feces. The problem of quantitation of leakage has been tackled from different directions.

It is possible to analyze the intestinal contents and fecal extracts using sensitive immunological methods, but these beside being laborious and time-consuming are only qualitative or, at most, semiquantitative tests. The tests using radioactivity-tagged plasma proteins, colloids or other macromolecules have proved to be superior. Interest in this field has grown steadily and the problems have been discussed at various international symposia.

The Radioactive Label

The requirements of an ideal label may be stated as follows: (i) In the case of proteins, the labelling should not alter their metabolic behaviour in the body, making it feasible to simultaneously quantitate the rate of intestinal protein loss, endogenous catabolism and synthesis (in the steady state). (ii) The label should not be absorbed from the intestinal tract, to avoid underestimation. (iii) The label should appear in the intestinal tract only in the tagged form, attached to the macromolecules, to circumvent overestimation. (iv) The label should be easy to trace and quantitate in the feces, besides being safe for the patient. It should be inexpensive and easily available.

No label, as yet available, fulfils all the above requirements. The following survey will further exemplify these points.

The preferred plasma protein has been albumin, which has been labelled with various isotopes: 125I-albumin, 51Cr-albumin, 55Nb-albumin and 111InTc-albumin. Ceruloplasmin is another plasma protein labelled with 64Cu.

125I-albumin: The classical procedure of labelling albumin with 125I is McFarlane's iodine monochloride method, which yields an unsatisfactory preparation. In the case of hypoproteinemia due to gastrointestinal protein loss, the 125I-albumin catabolic rate will be higher than in normal. However, such an increased degradation rate may also be observed in nephrosis, skin diseases with exudation, some endocrine diseases like Cushing's syndrome and thyrotoxicosis, and acute stress situations such as coronary infarction, pneumonia or trauma. Fortunately, all these other causes can be excluded on clinical basis.

In normals the fecal excretion of radioactivity averages 0-14 per cent of the injected dose of 125I-albumin, representing 0-63 per cent of the total label leaving the body, while in cases of protein-losing gastroenteropathies the fecal activity may be as high as 10-50 per cent. Actual protein loss cannot be quantitated, because the label is reabsorbed after protein degradation and excretion through the kidneys. To prevent this reabsorption, Jeejeebhoy and Coghill advocated oral administration of an anion exchange resin, Amberlite IRA-400 (CP), every 4 hours for 7-10 days, following
an intravenous injection of 30-100 μCi (1-11-3.7 MBq) of \(^{131}I\)-albumin. Using this technique they found the daily fecal \(^{131}I\) activity in normals to be less than 2% of the total body activity.

Though the resin has been shown to bind nearly 80% of the radioactivity of the digested \(^{131}I\)-albumin (both in vitro and in vivo) after its oral administration along with \(^{131}I\)-albumin, this test has been shown to give unreliable results due to the fact that free iodine stemming from swallowed saliva and gastric secretion (both active excretors of iodide ion) is also bound to the resin.\(^{31}\) Besides, the absorption of non protein-bound \(^{131}I\) from the small intestine is very rapid.\(^{22}\) This fact, in association with discontinuous intake of resin, makes quantitative binding of iodide doubtful.\(^{23}\)

\(^{99m}Tc\)-albumin: This label fails to meet the first requirement, ie it alters the metabolic behaviour of albumin. Albumin can be labelled with \(^{99m}Tc\) by the methods described by Waldmann.\(^{14,24}\) Chromic ion is largely unabsorbed. Although the protein carrier, may be digested, \(^{99m}Tc\) is recoverable to an extent of 93-95% per cent when fed orally, with any activity appearing in the urine. The recommended dose is 30-50 μCi (1-11-1-85 MBq). Feces are collected for 96 hours and in normal subjects the fecal radioactivity amounts to 0.1-0.7 per cent of the dose. However, because of the very drastic conditions used for labelling, the labelled albumin cannot be used for turnover data. Hoffer et al\(^{33}\) have performed biochemical and metabolic studies and have determined the degree of denaturation of \(^{99m}Tc\)-albumin. They have demonstrated that while most of the chromatographed albumin was present as a monomer fraction, considerable amounts of radioactivity were retained in two additional fractions. One was an aggregated fraction of relatively high specific activity, and the other was a non-protein fraction. This, probably, is the cause of an unphysiologically high catabolic rate.

Instead of using in vitro labelling, some investigators have achieved in vivo labelling of plasma proteins by injecting \(^{51}Cr\)Cl in sterile isotonic solution.\(^{25,26}\) \(^{51}Cr\)-albumin and \(^{51}Cr\)-albumin have been shown to behave identically as regards plasma disappearance curves of \(^{51}Cr\) and fecal \(^{51}Cr\) excretion.\(^{28,29}\) Of the plasma proteins, transferrin, a beta-1 globulin (mol wt 90,000), seems to have stronger affinity towards \(^{51}Cr\) than albumin and other plasma proteins. Thus \(^{51}Cr\) seems to be transferred from albumin to transferrin in vivo. Consequently, the plasma disappearance curve of \(^{51}Cr\) is more an indicator of transferrin turnover than that of albumin.\(^{30}\) This radiopharmaceutical is no longer commercially available.

\(^{99m}Tc\)-albumin: Though \(^{99m}Tc\) can label plasma proteins when injected as an oxalate,\(^{11}\) specific electrolytic labelling to albumin can be obtained in vitro.\(^{31,32}\) \(^{99m}Tc\) is unabsorbed through the oral route.\(^{12}\) After an injection of 15-20 μCi (555-740 KBBq) 

When properly labelled, \(^{99m}Tc\)-albumin has been shown to behave like \(^{131}I\)-albumin in vivo.\(^{13}\) Compared to \(^{51}Cr\)-albumin, \(^{99m}Tc\)-albumin seemed to be a better tracer since protein loss and metabolic turnover data could be obtained simultaneously. Furthermore, unlike \(^{51}Cr\), a majority of \(^{99m}Tc\) label remains attached to albumin in vivo. However, in some patients, all activity has been shown to persist for a long time in blood and finally gets accumulated in bone and connective tissue.\(^{25}\) Thus, it could present radiation hazards to the patient if the radioactivity is not exhausted fast enough in feces.

In the past we have used this radiopharmaceutical successfully in a few patients.\(^{15}\) At present it is not commercially available.

\(^{99m}Tc\)-albumin: Recently this radiopharmaceutical, in conjunction with scintillation camera, has been used to diagnose protein-losing enteropathy.\(^{16}\) In a single patient study, it has been claimed to be relatively stable in vivo. Serial abdominal imaging after intravenous injection of 20 μCi (740 MBq) of \(^{99m}Tc\)-albumin is comparatively easy to perform and results can become available in 24 hours. It may also be possible to demonstrate sites of protein loss with this technique, provided the claim about the in vivo stability of \(^{99m}Tc\)-albumin is substantiated and there is no blood loss into the bowel. It is, however, obvious that this radiopharmaceutical cannot provide information about the albumin turnover.

\(^{64}Cu\)-cereuloplasmin: The possibility of using \(^{64}Cu\)-cereuloplasmin, an alpha-2 glycoprotein (mol wt 160,000), as a tracer to evaluate gastrointestinal protein loss has been explored.\(^{17,24}35\) Since copper is present in this protein (8 atoms per molecule), \(^{64}Cu\) is a natural label giving an essentially native protein. The procedure for introducing \(^{64}Cu\) in cereuloplasmin molecule has been described by Sterrenburg.\(^{16}\)

Copper has been shown to be poorly absorbed from the gastrointestinal tract. Waldmann et al\(^{34}\) recovered 82-100 per cent of the orally fed dose in the stools of dogs, rats and humans. However, this is true only if the dose fed is large. In tracer dose, the relationship of initial absorption to dose has been shown to be linear.\(^{37,38}\) Therefore, to minimize recirculation of radioisotope, the intestinal copper pool has to be increased by giving 10 mg of copper sulphate orally, three times a day, along with the tracer dose of 20 μCi (740 KBq) in 100 mg cereuloplasmin. Under such conditions of the test the normal fecal clearance of \(^{64}Cu\) on an average is 2-9 per cent of intravascular pool per day, whereas patients with gastrointestinal protein loss show values up to 40 per cent.

\(^{64}Cu\)-cereuloplasmin fulfils the first three requirements of an ideal label. However, \(^{64}Cu\) is a cyclotron-produced isotope. Hence, the cost of production of \(^{64}Cu\) is prohibitive. Furthermore the physical half-life is short (61-8 h).

Methods using Labelled Colloids and Macromolecules

\(^{131}I\)-polyvinylpyrrolidone (\(^{131}I\)-PVP) has been the most important artificial polymer studied.\(^{9,36}\) The other colloid used is dextran labelled with \(^{99m}Tc\) (Imferon),\(^{11,42}\) while \(^{131}I\)-labelled dextran\(^{3}\) has not been very successful.

Since these substances are not proteins, it is obvious that they will not meet requirement (1). Besides, in a

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few cases side effects, characterized by so-called "colloid reaction", have been observed following intravenous injection. 

131I PVP: Commercially available PVP has a molecular weight ranging from 15,000 to 110,000, with an average range of 30,000 to 40,000. The labelling procedures have improved over the years. When fed orally, 131I-PVP is neither absorbed from the intestine nor retained in the intestinal enzymes and bacterial flora. The usual dose is 10-25 μCi (370-925 kBq) or 5 μCi (185 KBq) per g per cent total plasma proteins. In normals only 1-0% per cent of the dose is excreted in total 96 h in stool specimen.

Though it has been used with some success it is not devoid of disadvantages. PVP is not a normal metabolite and contains a very wide range of molecular weights. Some preparations seem to be partially absorbed from the gastrointestinal tract. To some extent the label seems to get detached in the intestinal lumen. Furthermore, since the intravenously administered radioactivity is rapidly excreted by the kidneys, contamination of the feces with small amounts of urine yields false-positive values. This is especially important in children.

Fe-iron-dextran: The iron-dextran complex has an average molecular weight of 180,000. On oral feeding, Anderson et al.41 recovered 80-100 per cent of injected activity in 5 days' stool sample. With a dose of 0-1-0-2 μCi (3-7-7.4 KBq) per kg body weight, normals excrete up to 8% per cent of the dose.42

The advantage of using Fe-iron-dextran is that this tracer measures protein loss over a short time, because of its short mean life in plasma.42 In addition, no urinary excretion of 55Fe takes place, thus preventing radioactive contamination of stool specimen with urinary activity. Besides, 55Fe (1-1 Mev) has an energy spectrum different from that of 131I (0-36 Mev). This enables the separation of the two isotopes, making it feasible to study simultaneously the turnover of plasma protein by an 131I-labelled protein in the same patient.

None of the radioactive tracers so far employed in the quantitation of gastrointestinal protein loss studies meets all the requirements of an ideal label mentioned above. In recent years there has not been any major breakthrough in this field. In spite of these difficulties, a wealth of information has been acquired by gastroenterologists to unravel the mystery of hyperproteinaemia of unknown cause.

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