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DEVELOPMENT OF AN IMPROVED FORMULATION OF ORAL REHYDRATION SALTS (ORS) WITH ANTIDIARRHOEAL AND NUTRITIONAL PROPERTIES: A "SUPER ORS"[1]

by

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1. INTRODUCTION

Oral rehydration therapy (ORT) is recognized as a powerful intervention for the treatment of dehydration due to acute diarrhoea, an invaluable public health tool, and an essential component of primary health care. The oral rehydration salts (ORS) solution recommended by WHO contains glucose (20 g) and three salts - sodium chloride (3.5 g), trisodium citrate, dihydrate (2.9 g) or sodium hydrogen carbonate (2.5 g), and potassium chloride (1.5 g), to be mixed in one litre of water. This solution has been used in the treatment of acute diarrhoeal illness for over a decade. Its scientific basis rests in the fact that glucose-linked enhanced sodium absorption in the small intestine remains largely intact during acute diarrhoeas of diverse etiology. The composition of the ORS formulation recommended at present is optimum for the rehydration of patients of all ages - infants, children, and adults - with dehydration due to acute diarrhoea of any etiology. Moreover, it has been found suitable for the replacement of ongoing diarrhoeal losses (maintenance therapy) when appropriate amounts of the solution are administered along with other fluids, particularly to infants (52) - e.g., breast milk, diluted cow milk or formula, and water (reviewed in 46, 47, 31 and 32).

ORT has been described as "potentially the most important medical advance of this century" (67). The reasons for this are the following:

(a) ORT can today be used alone to successfully rehydrate 90% of patients with dehydration due to acute diarrhoea who previously would have received routine intravenous (IV) therapy. In patients requiring IV fluids, ORT can be used after the initial deficit has been corrected.

(b) ORT can reduce hospital case-fatality rates by 40-50% (20). This observation is presumed to be related both to the diminished use of IV therapy (often associated with sepsis and overhydration) and to the general improvement in care associated with the use of ORT (ORT requires the active participation of the mother and the health worker).

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2 Diarrhoeal Diseases Control Programme, World Health Organization, Geneva, Switzerland

(c) ORT can reduce diarrhoeal disease hospital admission rates by 50-60% (7). In this way the child and mother are spared the trauma of hospitalization and the costs of treatment of diarrhoea are reduced by as much as 80%.

(d) When ORT is used at home in the early stages of the diarrhoeal episode to prevent dehydration, it can substantially reduce the number of visits to treatment facilities and overall diarrhoea mortality (25).

(e) ORT administered along with active feeding during and after diarrhoea, according to established guidelines, can limit the weight loss often associated with diarrhoea (21).

(f) ORT is one of the least expensive health interventions. The cost of treating a case of diarrhoea with ORT is about US$1 and the cost of preventing a death from diarrhoea with ORT is $200-300 (61).

(g) Finally, ORT is simple and can easily be administered by mothers and other family members. As such, it is a useful entry point for the introduction of other critical interventions for child survival.

There is, however, a drawback in that oral rehydration therapy with the present ORS formulation does not reduce the volume, frequency, or duration of diarrhoea (51, 19, 34 and 54). This raises the practical problem of its acceptance, since a major concern of the mother (and consequently of the health care provider) during diarrhoea is the frequency and volume of the child's stools. This leads to a persistent desire to use "antidiarrhoeal drugs" and to withhold fluids from babies during diarrhoea. It has been estimated that each year about US$350 million worth of antidiarrhoeal drugs are sold by leading western manufacturers alone, of which 33% are sold in developing countries (WHO analysis, unpublished data). Since 1980, the WHO Diarrhoeal Diseases Control Programme has been promoting both basic and applied research to develop and test antisecretory drugs. To date, none of the compounds that appeared promising under experimental laboratory conditions has proved to be of practical value in therapeutic trials.

Since many water-soluble organic molecules - e.g., D-hexoses, neutral amino acids, dipeptides and tripeptides of neutral amino acids, water-soluble vitamins - can enhance the absorption of sodium from the small intestine (water absorption being governed by osmotic forces) (59), it has been hypothesized that optimum exploitation of this phenomenon could lead to the development of an improved ORS formulation that would not only successfully replace the deficit of salts and water in diarrhoea, but also actively induce the reabsorption of endogenous intestinal secretion and thus reduce the volume and duration of diarrhoea - i.e., act as an absorption-promoting antidiarrhoeal drug (33). A nutritional benefit is another potential advantage of this type of improved ORS formulation, primarily because the reduced severity and duration of diarrhoea will make it possible to introduce early a liberal and more effective feeding regime. Two early studies and some recent controlled clinical trials indicate that the development of an improved "Super ORS" formulation along these lines is feasible (see below).

This document examines the feasibility of developing a Super ORS formulation, based on a review of existing knowledge of physiology and relevant research accomplishments. It also indicates further research that could lead to the development of such a solution.

2. SCIENTIFIC BASIS OF ORT AND DEFINITION OF SUPER ORS

2.1 Coupled transport of sodium and organic solutes

As mentioned above, the absorption of a wide variety of organic solutes by the small intestine is closely linked with the absorption of sodium. These mechanisms, elucidated by *in vitro* studies, have been reviewed (60). Of most relevance to the present review are a large number of *in vivo* experiments such as intestinal perfusion studies in animals and human volunteers, which provide semi-quantitative information on the magnitude of the effect of

various organic solutes on the absorption of sodium and water. It should, however, be noted that at the luminal concentrations attained by the organic solutes usually used in ORS formulations, salt absorption also takes place (in vivo) by an additional mechanism called "solvent drag" (15). This mechanism, stated simply, is as follows: as a result of the coupled sodium and organic solute entry process into the enterocytes and thence into the circulation, water osmotically flows in the same direction; the bulk flow of water between and through the enterocytes traps additional sodium and chloride molecules in the flowing stream and increases salt absorption.

There are three groups of organic solutes (mainly nutrients) which are absorbed efficiently and relatively independently of each other by the small intestine and enhance the absorption of sodium and water. They are:

(a) D-hexoses - e.g., glucose, galactose, and their precursors, such as disaccharides and trisaccharides (maltose, maltotriose), oligosaccharide mixtures (several grades of maltodextrins also called glucose syrup solids or corn syrup solids), polysaccharides (e.g., starch from rice, other cereals, and potato).

(b) neutral amino acids - e.g., glycine, l-alanine, leucine, and precursors such as proteins and hydrolysed proteins like casein or lactalbumin (some preparations may contain up to 20% free amino acids).

(c) dipeptides and tripeptides of neutral amino acids - e.g., glycyl-glycine, glycyl-alanine, leucyl-glycine, glycyl-glycyl-glycine, and precursors such as proteins and hydrolysed proteins like casein and lactalbumin (some preparations may contain up to 70% dipeptides and tripeptides).

2.2 "Super ORS" defined

As postulated earlier, optimum exploitation of water-soluble, organic-molecule linked sodium and water absorption may lead to the development of an improved ORS formulation (Super ORS) that can:

- reduce the volume of diarrhoea by inducing reabsorption of the endogenous secretion into the small intestine due to diarrhoea and thus act as an absorption-promoting antidiarrhoeal medicine; consequently, it should also shorten the duration of diarrhoea by reducing the volume of effluent flow into the colon to less than its maximum absorptive capacity;

- correct dehydration and maintain hydration more efficiently so that it reduces the number of treatment failures due to high rates of purging;

- provide a potential nutritional benefit by making it possible to introduce early a more liberal and effective feeding regime as a consequence of the reduced duration and magnitude of diarrhoea. (The effect of the increased nutrient content of the formulation is marginal, and is limited to the first few hours of rehydration therapy, when the currently recommended feeding regime (36) is followed during the treatment of diarrhoea.)

3. OSMOTIC FORCES IN THE GUT LUMEN AND ORS FORMULATIONS

Organic nutrients in ORS that are not absorbed create adverse osmotic effects inside the gut lumen and reduce the efficiency of the solution. The maximum amount of glucose that can be absorbed in acute diarrhoea is around 2% (51,19,34,54); with a greater concentration of glucose, more severe and more frequent malabsorption has been observed, and osmotic diarrhoea may result. When malabsorption of the carbohydrate load leads to osmotic diarrhoea, there is a relatively higher loss of water than of electrolytes and a consequent risk of hypernatraemia (39). The disastrous effect of using a very high carbohydrate concentration (12.5 g of glucose polymer instead of 2 g of glucose per 100 ml) in ORS was demonstrated by a recent study in the United Kingdom (55) in which one infant out of 7 developed osmotic

diarrhoea, hypernatraemia, and convulsion. A most illuminating example of the importance of carbohydrate malabsorption in the etiology of hypernatraemia (besides its association with ORS) is the regular finding of hypernatraemia in breast-fed babies with hereditary glucose-galactose malabsorption (40), in spite of the fact that the sodium concentration of breast milk is very low - about 7 mmol/litre.

It cannot be overemphasized that rapid and complete absorption of the organic components is essential for the success of an improved ORS formulation. Possible ways of minimizing the adverse osmotic effects of an ORS formulation include:

- use of organic nutrients that are absorbed rapidly;
- use of a combination of nutrients that do not compete with one another for absorption - e.g., D-hexoses, neutral amino acids, and dipeptides of neutral amino acids;
- use of polymers of organic molecules (e.g., (a) polysaccharides, oligosaccharides, and disaccharides; (b) proteins, partially hydrolysed proteins, oligopeptides) which may contribute little to the initial osmotic load of the formulation and, if they are able to liberate digestive end products at a rate favourable for their rapid absorption, will give rise to minimal adverse osmotic effects;
- use of a quantity of organic nutrients that does not exceed the intestine's absorptive capacity; of particular relevance is the absolute quantity of carbohydrate used in the formulation.

4. BICARBONATE ABSORPTION AND THE SALTS OF WEAK ORGANIC ACIDS IN THE DESIGN OF SUPER ORS

The bicarbonate or citrate included in ORS, as sodium hydrogen carbonate or trisodium citrate, contributes to faster correction of the acidosis associated with diarrhoeal dehydration, especially in cholera. Bicarbonate is actively absorbed from the small intestine against a steep electrochemical gradient and independently enhances sodium absorption (65,66) even when glucose-linked or amino acid (glycine)-linked sodium absorption is maximized (17). Except for some preliminary observations in actively purging cholera patients (50), this phenomenon has only been studied in healthy volunteers or in normal animal intestine and not as yet in acute diarrhoeal states.

Weak organic acids such as short-chain fatty acids like acetate, propionate, and n-butyrate are rapidly absorbed from both the small (57,58) and the large intestine (reviewed in 11) and enhance the absorption of sodium and potassium; this phenomenon is associated with bicarbonate secretion into the lumen. It should, however, be noted that rapid transit time (as is common in acute diarrhoea) and/or altered bacterial flora (as may result from the use of antimicrobial drugs) may impair this mechanism in the colon.

Sodium citrate has been shown (*in vitro*) to stimulate sodium and chloride absorption by rabbit ileal mucosa both under basal conditions as well as during a secretory state induced by heat-stable enterotoxin (ST_A) of *Escherichia coli*; this effect on ion absorption was dose-dependent and the absorption of citrate was shown to be an active process (45). One study in adult cholera patients demonstrated that replacing sodium bicarbonate in the ORS formulation by the sodium equivalent quantity of trisodium citrate led to a significant decrease in diarrhoea stool output and duration of diarrhoea (Moechtar *et al.*, Indonesia, unpublished data). A study using a smaller number of patients (adults and older children) with cholera showed a similar trend (23). However, in 4 separate studies in infants with diarrhoea this advantage of sodium citrate based ORS was not seen. The relative efficiency of sodium (or potassium) salts of various weak organic acids in stimulating absorption when used in ORS formulations needs further study.

5. ORGANIC NUTRIENTS AND PHYSIOLOGY OF DIGESTION AND ABSORPTION

This review has identified 3 nutrient groups that are absorbed efficiently and independently of one another from the small intestine (see Section 2.1). Exploitation of an optimum combination of these nutrient groups should make it possible to design a Super ORS. As mentioned earlier, more complex nutrient molecules (e.g., starch, protein, maltodextrins, protein hydrolysates, oligopeptides, etc.), which on digestion liberate these nutrient groups, could also be utilized, provided that their rate and completeness of digestion is advantageous. Use of the latter may also give the formulation an osmotic advantage; however, this should be weighed against their rate and completeness of digestion. While protein digestion is efficient from the moment of birth, the digestion of starch may be slow and incomplete in infants up to 6 months of age (8,23,12,68,64,27,26), due to the lack of pancreatic amylase. Some relevant features of the digestion and absorption of these nutrients are reviewed in the sections that follow.

5.1 Carbohydrates

5.1.1 Monosaccharides (e.g., glucose, galactose, and fructose): Of the monosaccharides, glucose remains the most important and forms the basis of the highly successful standard ORS formulation. As discussed earlier, glucose absorption during diarrhoea is rate limiting, and increasing its concentration beyond 2g/100ml increases the frequency and magnitude of its malabsorption and leads to osmotic diarrhoea. Galactose, though an equally strong stimulant of sodium absorption, is not a commonly available sugar; its common source is lactose in milk, which needs to be hydrolysed by the intestinal epithelial cell enzyme lactase - a step that limits the amount that can be absorbed. Galactose and glucose share a common absorption pathway and compete with each other in the small intestine. Fructose (liberated from sucrose hydrolysis and naturally occurring in honey) stimulates sodium absorption (in vivo) when absorbed in the small intestine, but its rate of absorption is slow (about half the rate of glucose)(14).

5.1.2 Disaccharides: They stimulate sodium absorption through the liberation and absorption of monosaccharides. The absorption of glucose from maltose has been shown to be faster than from the equivalent glucose solution, leading to proportionately increased sodium absorption (10,56,30). This is explained by the fact that maltose is broken down at the brush border of enterocytes, and that the liberated glucose is more favourably located near the site for glucose absorption - the so-called kinetic advantage. A similar kinetic advantage was demonstrated for sucrose in vitro (35), but studies in vivo (16) could not confirm this. Potentially, maltose (with or without glucose) could be used to advantage in improved ORS formulations.

5.1.3 Oligosaccharide mixtures: "Glucose polymers" (also known as "corn syrup sugars", "dried glucose syrups", or malto-dextrins) are produced by enzyme/acid hydrolysis of food corn, waxy corn, or potato starch and are widely used in commercial infant formulas. Their bulk price is similar to that of glucose. They are a heterogeneous group of linear chains of glucose residues linked mainly by 1-4 glucoside bonds, numbering from 5 to 100 residues in length. The rationale for their use in place of sugars in infant formulas is to decrease osmolality without changing caloric density. Glucose polymers are available in various grades according to the degree of hydrolysis and are often referred to by the term "dextrose equivalent" (DE); the term refers to the degree of depolymerisation in terms of the total reducing substances, expressed as dextrose, as a percentage of the dry weight. Therefore, the higher the DE number, the greater the percentage of constituents of low molecular weight. Each preparation contains a wide range of lengths of glucose polymers centred around a most prevalent form.

Oligosaccharides of short chain length ($\leq$10 glucose molecules) are hydrolysed efficiently in the mucosal brush border by α-glucoamylases (24. The long-chain oligosaccharides, however, require luminal hydrolysis by pancreatic (and or salivary) α-amylase. The major concern in the use of glucose polymers (as of starch) in infancy is that pancreatic α-amylase, the major enzyme in the hydrolysis of starch and presumably of a large proportion of glucose polymers, is either absent or extremely low in concentration in the first 6 months of life. Salivary amylase and amylase in human milk may compensate for this physiological deficiency.

The digestibility and absorption of glucose polymers in early infancy have been studied only to a limited extent. In young infants (6 months of age and younger), a proportion of ingested glucose polymer usually escapes digestion and absorption in the small intestine and enters the colon (62,28). Up to a limit, the colonic bacteria ferment this carbohydrate into short-chain fatty acids (mainly acetates, propionates, and n-butyrates), which are absorbed by the colon and utilized for energy (the so-called colonic salvage mechanism) (53). Their absorption is at least partly associated with sodium and chloride absorption. Of the various grades of glucose polymers, the ones with a higher proportion of low-molecular-weight constituents (i.e., of shorter chain lengths of glucose molecules) will give the least difficulty as regards digestion and absorption in the small intestine in infants. This advantage (in terms of nutrient and salt absorption) may be lost if the colonic transit time is short (as in the high-output secretory diarrhoea of cholera), if the colonic flora are altered by antibiotics or by the disease itself, or if the amount of carbohydrate entering the colon exceeds the fermenting capacity of the colonic bacteria. In one study in infants aged 3 to 4 weeks, the total absorption and utilization of a glucose polymer given in a milk feed (at 1 g/kg body weight) from the small intestine and from the colonic salvage mechanism was comparable to that from the same dose of glucose (62). Two-to-three-week-old premature infants showed as good a glycaemic response after a glucose polymer tolerance test as after a lactose tolerance test (9). Using a grade of glucose polymer containing predominantly short-chain polymers (e.g., grades having a DE of 24 and above) should minimize the problem of incomplete digestion and absorption in the small intestine in young infants. From a physiological point of view, a mixture of glucose polymers containing exclusively short-chain polymers (less than 10 glucose units) may be an ideal substrate for use in all age groups, particularly young infants, because they are efficiently hydrolysed into glucose by intestinal epithelial gluco-amylases which are fully developed even in the newborn.

5.1.4 Polysaccharides: Polysaccharides from cereals and legumes (mainly amylopectin and amylose) are hydrolysed by pancreatic and salivary α-amylases into short-chain glucose polymers, which in turn are finally hydrolysed by mucosal glucosidases into glucose for absorption. As discussed in section 6.1.3, pancreatic α-amylase is deficient in infants under 6 months of age but may be partly compensated for by salivary α-amylases and possibly mammary amylases in breast-fed infants. Owing to methodological limitations, several studies produced conflicting results which led to controversy regarding the age at which cereals could be introduced in the diet of infants. By using newer techniques, some recent studies have at least partly resolved these conflicts (62,28). Present knowledge on starch digestion and absorption is summarized below.

Based on the breath hydrogen test, it has been shown that nearly all healthy adult subjects fail to absorb an appreciable portion of dietary starch (an estimated 6 to 20%) in the form of common foods - e.g., corn, potato, oats, and wheat - with the exception of rice starch (6,29). Malabsorbed carbohydrate is degraded by colonic bacteria to produce short-chain fatty acids, hydrogen and other products (as discussed in 6.1.3). In an elegant study (62), it was demonstrated that healthy 4-week-old infants, given a dose of 3g/kg/day, partially digested and absorbed corn starch in the small intestine and largely salvaged the rest of it through colonic fermentation and absorption of short-chain fatty acids; the extent to which the cereal was oxidized and hence utilized for energy (as measured by the breath CO_2 derived from oxidation of the ingested carbohydrates) was comparable to that with glucose or a glucose polymer. Despite colonic salvage, in some infants a loss of products derived from the ingested corn starch (at this modest level of intake) was demonstrated in the faeces. It is possible that, if larger doses are given, a greater amount will be lost in the stool. The important role of colonic bacterial flora in the utilization of carbohydrates incompletely digested in the small bowel has also been confirmed in infants (62). It appears that the more favourable results reported in earlier studies on starch digestion and absorption in the small intestine in infants were largely due to methodological inadequacies. It was demonstrated in 6 out of 7 infants aged 2-1/2 to 6 months that hydrolysis of amylopectin (the most abundant form of polysaccharide in cereals and legumes) in the duodenum was incomplete, as a consequence of low levels of α-amylase activity in the duodenal juice after an amylopectin meal (8).

In summary, healthy young infants incompletely digest and absorb starch from cereals in the small intestine. Although the colonic salvage mechanism recovers much of the malabsorbed carbohydrate in a significant proportion of infants, salvage is incomplete and products from unabsorbed starch appear in the stool and increase its bulk by osmotically binding water. In acute diarrhoea it is likely that the colonic salvage mechanism will be less efficient (depending on colonic transit time and the degree of alteration of colonic bacterial flora). Of all the cereals tested (in adults), rice is most efficiently digested and absorbed (6,29).

5.2 Proteins and products of digestion

5.2.1 Neutral amino acids: These are absorbed quickly from the small intestine and enhance the absorption of sodium and water. The absorption of one is influenced by the presence of another amino acid - e.g., one with a higher affinity for the membrane carrier system retards the absorption of another with a lower affinity by competition. Because of this competitive inhibition, the use of more than one amino acid in the formulation does not offer any added advantage. Data are available from human perfusion studies confirming the phenomenon of enhanced absorption of sodium by glycine, l-alanine, and leucine (3,17), and the concentration ranges of amino acids used in these studies are within the proposed optimum ranges for use in oral rehydration formulations. In vivo perfusion studies using the whole length of the jejunum and ileum in the rat showed that the absorption of sodium and water from an electrolyte solution similar to ORS but containing glycine instead of glucose was similar to their absorption from a glucose-containing solution (33).

Certain amino acids, when parenterally administered in large doses, produce adverse side effects (e.g., tryptophane, isoleucine, threonine, methionine) (41). Under similar experimental conditions, the least toxic ones include two non-essential amino acids relevant to the present review - glycine and l-alanine (41). Arginine in small doses protects against the toxic effects of many amino acids (41), but does not itself stimulate sodium absorption from the small intestine in vivo (17). Besides the specific noxious effects of a particular amino acid, there are non-specific effects of large doses of amino acids (or proteins) which include elevation of blood ammonia by rapid deamination of amino acids, hypoglycaemia (e.g., with l-leucine), and disturbance of acid-base balances (41). A judicious choice of type and quantity of amino acids should eliminate or minimize possible adverse side effects. Also, ORS administered over a longer period of time is unlikely to produce the bolus effect of amino acids administered intravenously or intraperitonally.

5.2.2 Dipeptides and tripeptides: The most important new advances that have been made in recent years in our understanding of protein digestion and absorption concern peptide absorption from the small intestine (reviewed in 1,37,38). Certain characteristics of di- and tripeptides of neutral amino acids make them attractive candidates for use in ORS formulations. The digestion of protein in the intestinal lumen and at the brush border produces a mixture of small peptides and amino acids in which peptides predominate. The process of absorption of small peptides involves two mechanisms: (i) entry of di- and tripeptides into the absorptive cells with intracellular hydrolysis, and (ii) hydrolysis at the brush border followed by uptake of free amino acids by the usual pathways; only the free amino acids usually enter the blood. The relative importance of the two modes of peptide absorption varies from one peptide to another and also according to the site of absorption. Some relevant aspects of di- and tripeptide absorption (38) are reviewed below:

- The absorption of amino acids is more rapid from a di- or tripeptide than from the equivalent free amino acids; this has been shown in vivo and in vitro in man and several other species (38). Most of the in vivo studies have been carried out at peptide concentrations ranging between 10 and 100 mmol/litre. In a human volunteer perfusion experiment, the absorption of glycine from a 50 mmol/litre solution of its dipeptide glycyl-glycine was 70% higher than from an equivalent 100 mmol/litre solution of glycine (2). Similar experiments also demonstrated faster absorption of glycine, l-alanine, and leucine from dipeptides like glycyl-leucine (59) and glycyl-alanine (38) as compared with constituent amino acid solutions.

- Di- and tripeptide uptake by the small intestinal mucosa is independent of the uptake of free amino acids (38). The addition of a peptide has been shown to result in a large increase in absorption when the uptake mechanisms for its constituent amino acids are completely saturated. Also, the absorptive capacity of the small intestine is considerably greater for oligopeptide/amino acid mixtures than for mixtures of free amino acids (63,13). A wide range of small peptides appear to share the same mucosal peptide uptake system and they inhibit each other's uptake by competitive inhibition (38).

- The peptide absorption rate is relatively high throughout the whole length of small intestine, while the absorption of amino acids may be reduced in the ileum.

- Peptide absorption is more robust than that of free amino acids under adverse pH conditions (highly acidic pH) and in disease states such as tropical (18) and non-tropical (5) sprue.

- Dipeptides of neutral amino acids such as glycyl-glycine, glycyl-alanine, and glycyl-leucine have been shown to enhance the absorption of sodium (and water) nearly twofold, as compared with their constituent amino acids in equivalent concentrations. Also, enzyme-hydrolysed proteins (e.g., lactalbumin, casein) containing a mixture of peptides (about 72%) and amino acids (20%) induced 70% greater sodium absorption than an equivalent amino acid mixture (13,18); the order of magnitude of sodium absorption is comparable to that induced by a pure peptide (e.g., glycyl-glycine)(2).

- In view of the novel mechanism whereby many di- and tripeptides are absorbed intact by the intestinal mucosa, their use in ORS formulations should minimize the problem of osmotic water loss into the lumen which would occur if they were initially hydrolysed into amino acids before being absorbed.

5.2.3 Food proteins: Studies in human volunteers have shown that meal protein is digested 60% in the upper small intestine and 40% in the distal small intestine, the whole process taking more than 4 hours to complete (4), and that the ileum is important for the completion of protein digestion and absorption. Protein digestion and absorption are generally efficient even in malnourished children, but may be seriously impaired by pancreatic insufficiency. The brush border membrane of the intestinal mucosa, however, is efficient in the hydrolysis of peptides with 3 to 6 amino acid residues. For use in ORS formulations, other factors such as availability and cost, storage properties, and packaging requirements need to be considered. Also, the use of intact proteins or large peptides in early infancy may pose allergic problems (see Section 6.2).

6. STUDIES ON IMPROVED ORS FORMULATIONS

The studies that have been conducted to date to improve the absorption efficiency of ORS formulations are summarized in the Table. The limited number of approaches tested include the use of (a) a combination of glucose and a neutral amino acid (glycine) (45,48); (b) a cereal (rice) powder containing complex carbohydrate and protein (which on digestion should liberate glucose and oligosaccharides, small peptides, and amino acids) (49,42); and (c) a combination of cereal (rice) powder and an amino acid (glycine) (Patra, F.C., Mahalanabis, D., Jalan, K.N. - unpublished data).

6.1 "Super ORS" containing defined additives

Early observational studies in adults and older children with cholera and non-cholera diarrhoea (44) indicated that a combination of glucose and glycine (110 mmol per litre each) in ORS could substantially reduce the stool volume and duration of diarrhoea. A recent, double-blind, controlled clinical trial of glucose and glycine (111 mmol/litre) based ORS in infants and small children with non-cholera diarrhoea (48) confirmed that the stool volume could be reduced by about 50% (Table), while the duration of diarrhoea and volume of ORS required were reduced by 30% as compared with controls receiving the standard WHO ORS

formula. It may be noted that when glucose is packed together with an amino acid, they react with each other, leading to a deterioration of the product. This problem may be overcome by using appropriate packaging techniques.

6.2 Natural food-based ORS

Starches and proteins in cereals, when broken down by the digestive processes in the intestinal tract, release glucose, amino acids, and short-chain peptides, all of which are organic solutes which enhance the absorption of sodium and water. Thus, added cereals can convert a complete electrolyte solution into a Super ORS in the same way as the more defined additives (e.g., glycine, l-alanine, glycyl-glycine, maltodextrin, etc.). They may also provide more calories as they can be used in larger quantities with little osmotic penalty.

It was demonstrated (43), using retrospective controls, that a rice powder-based ORS (30 g per litre) was as effective as sucrose-based ORS (40 g per litre) in adults and older children with cholera. In a subsequent controlled clinical trial in infants and small children (3 months to 5 years of age) with non-cholera diarrhoea (49), it was demonstrated that ORS containing cooked rice powder (50 g/litre) could significantly reduce the volume (by 49%) and the duration (by 30%) of diarrhoea (Table). A subsequent clinical trial in adults and older children (mostly over 2 years) predominantly with cholera (42) confirmed that ORS with rice powder (80 g/litre), in adults and children respectively, could reduce the volume of diarrhoea stool by 28% and 24% and the quantity of ORS required by 27% and 30% (Table). The latter study also showed that, at least for cholera, increasing the rice powder content much above 50 grams per litre does not appear to render the solution any more absorption-efficient. A more recent clinical trial in infants (4 months to 2 years) used a combination of cooked rice powder (50 g/litre) and glycine (111 mmol/litre) in ORS and showed that adding glycine to rice did not further improve its absorption efficiency (Patra, F.C., Mahalanabis, D., Jalan, K.N. - unpublished data). Thus, in the studies conducted so far, rice-based ORS has achieved a reduction in total diarrhoea stool of up to 28% in adults and older children with cholera and up to 49% in infants and young children with non-cholera diarrhoea.

The constraints of cereal-based ORS which need further research include:

(a) need for cooking prior to use. Rice powder needs to be cooked for at least 7-10 minutes and whole rice for a longer period before it can be used in preparing ORS. This requirement introduces logistic and operational constraints which need to be further examined.

(b) fermentation and bacterial overgrowth once the solution is made. A solution prepared from rice has a life at the bedside of only about 8 to 12 hours (particularly in hot and humid conditions) due to fermentation and bacterial growth, as compared with about 24 hours for standard ORS; further research is needed on the useful life of a prepared rice-based solution under various conditions of temperature and humidity to permit the formulation of appropriate recommendations for its use.

(c) incomplete digestion of starch in very young infants. Pancreatic amylase is deficient in early infancy for a period of up to 6 months. Because of this problem and possible allergic/immunological reactions due to proteins in some cereals, their use in young infants (3 months and younger) can be considered controversial, particularly in fully breast-fed babies; it may also interfere with breast-feeding and deprive exclusively breast-fed infants of the benefits of this practice.

To conclude, improved ORS formulations tested so far are highly encouraging and advances in this area compare favourably with the progress made to date in the development of antisecretory drugs.

7. DESIGN ASPECTS OF SUPER ORS - RESEARCH ISSUES

An optimum combination of organic nutrients is the key to a successful Super ORS. An appropriate choice of sodium or potassium salts of weak organic acids may further assist in increasing the absorption efficiency of ORS (see Section 4). As discussed in Section 2.1, 3 groups of nutrients have been identified which are absorbed relatively independently of one another. The use of one from each of the 3 groups, or even one from each of 2 groups of nutrients in the ORS formulation could markedly enhance its absorption efficiency (as already shown with the glucose-glycine ORS formulation). It should be noted that increasing concern has been expressed about the introduction of foreign proteins (e.g., in cereals, legumes, and animal foods) in infancy, especially in fully breast-fed infants before they are at least 3 months old. The use of synthetic amino acids and dipeptides (as discussed) obviates this problem.

In view of the fact that formulations such as glycine-fortified glucose ORS have already demonstrated a 50% reduction in diarrhoea stool output compared with controls treated with standard glucose ORS solution, a 70-80% reduction in diarrhoea may be arbitrarily set as an achievable target for newer absorption-promoting ORS formulations.

7.1 Organic nutrients under consideration for use in Super ORS

7.1.1 Glucose and glucose polymers: Glucose is the best studied nutrient in ORS formulations. The use of a precursor such as maltose or a glucose polymer may give ORS formulations an osmotic and kinetic advantage as regards absorption. The use of a glucose polymer containing predominantly short-chain polymers should minimize or even eliminate the problem of digestion in very young infants and still retain its advantage. The stability of short-chain glucose polymers when packaged with an amino acid and/or a peptide requires further research.

7.1.2 Synthetic amino acids: Amino acids should be used that are rapidly absorbed, stimulate sodium absorption, have minimal side effects in relatively large doses, and are easily available at low cost. Two neutral amino acids (glycine and l-alanine) are suitable for use in ORS formulations. L-alanine is absorbed more rapidly than glycine, but at higher concentrations glycine retains its efficiency as a stimulant of sodium absorption, whereas l-alanine becomes relatively less efficient. Also, glycine is the least expensive of all the amino acids.

7.1.3 Dipeptides: Three peptides (glycyl-glycine, glycyl-alanine, and glycyl-leucine) have been well studied in humans. Leucine can produce hypoglycaemic side effects, but the first 2 of these synthetic peptides could be used to advantage in ORS formulations in combination with a D-hexose and an amino acid. Glycyl-glycine is not hydrolysed in the brush border and is relatively slowly hydrolysed inside the enterocytes as compared with glycyl-alanine, so that there would be less competition (through back diffusion of the resulting amino acids) for absorption of the amino acids used in the formulation. Therefore, glycyl-glycine should be a good choice for use in ORS. If glycyl-alanine is used, its more rapid hydrolysis by enterocytes and partial back diffusion of the amino acids released into the intestinal lumen need to be considered when adding another amino acid to the formulation.

7.1.4 Natural food sources, their scope and limits: As discussed, the absorption efficiency of rice-based ORS is unlikely to be improved further. One other possible approach would be to combine a cereal with a legume (e.g., lentil, "mung" bean) or with a protein source such as soya or cow milk (e.g., casein, lactalbumin, etc.). The problems of starch digestion and of allergical/immunological reactions to some food proteins in early infancy have already been discussed (see Section 6.2). Nevertheless, they may be useful in older infants, children, and adults, particularly as a basis for home therapy. It may be noted that the food industry has long experience in manufacturing precooked cereal powder for use as a weaning food - the so-called "baby cereals". The feasibility of using such pre-cooked cereal preparations in ORS should be evaluated.

7.2 Suggested formulations for clinical trials

While our knowledge about the secretion/absorption processes in health and during diarrhoea will continue to grow, controlled clinical trials should be promoted with "improved ORS formulations" based on existing knowledge as discussed above. The control groups should be treated with the standard WHO-recommended ORS formulation and follow the recommended guidelines for feeding during diarrhoea (36). The electrolyte composition of the ORS to be evaluated should be the same as in the control solution. The organic nutrient contents should belong to 3 functional groups - i.e., (a) glucose or glucose polymer, (b) neutral amino acids such as glycine or l-alanine, and (c) dipeptides such as glycyl-glycine or glycyl-alanine. Separate studies should also be conducted using natural foods as the organic nutrient content of ORS.

Some suggested organic nutrient contents of ORS formulations for testing in clinical trials are:

(i) Glucose + glycine. This formulation has already been shown to be highly efficient though the solution tested was grossly hyperosmolar (442 mosm/litre). Further studies with a reduced concentration of one or both of the organic components may further improve the efficiency of this formulation. Such formulations are at present undergoing clinical trials in Indonesia, Peru, Thailand, and USA.

(ii) Glucose polymer + glycine. Use of a glucose polymer (e.g., dextrose equivalent 25 or 40) at 20 g/l in place of glucose in this formulation may improve the absorption efficiency while retaining the nutrient density by reducing the osmolality, and by offering some kinetic advantage for absorption of glucose. Such formulations are undergoing clinical trials in Egypt and Nigeria.

(iii) A combination of (a) a dipeptide (glycyl-glycine or glycyl-alanine) - e.g., at 30 mmol/l; (b) an amino acid (glycine or l-alanine) - e.g., at 50 mmol/l; and (c) glucose or a glucose polymer (as under paragraph ii) at 90-100 mmol/l equivalent of glucose. For reasons discussed in Section 7.1.3, when glycyl-alanine is used the amount of a free amino acid used in the formulation may need to be appropriately reduced. Also, the combination of a dipeptide and a glucose polymer will allow the use of a relatively higher nutrient density with some kinetic advantage for absorption and without causing an adverse osmotic effect; however, their digestion and absorption characteristics during acute diarrhoea will need to be studied. These formulations are undergoing clinical trials in Burma, Costa Rica, Egypt, India, and the Philippines.

(iv) Cereals and other food sources. In view of the fact that rice starch is more easily digested than other food starches and rice protein is hypoallergenic, careful metabolic studies to evaluate rice-based ORS in infants under 3 months of age with diarrhoea should be promoted. Studies of such components of ORS formulations should be extended to potato and other major cereals in addition to rice in the age-groups 3 months and above. In addition, suitable combinations of natural foods - e.g., (a) cereal plus lentils or "mung" beans, (b) cereal plus a food protein such as soya protein or milk protein - should be evaluated in terms of absorption efficiency as well as nutritional advantage in appropriate age groups. Rice powder-based ORS formulations are undergoing clinical trials in Bangladesh, Egypt, India and Senegal, and field studies in Bangladesh; "rice-water" and salt solutions are being examined in clinical and field studies in Indonesia. Formulations based on maize, millet, and sorghum powder are being evaluated in clinical and field studies in Kenya. Formulations based on "mung" beans and "green plantain" are undergoing clinical trials in India and the United Republic of Tanzania, respectively.

SUMMARY OF FINDINGS DOCUMENTING IMPROVED ABSORPTION EFFICIENCY OF ORS FORMULATIONS

	Studies (Reference)	Organic components used in ORS	Study population and type of study	Outcome measurements and and comments
1.	Nalin et al. (44)	Glycine and glucose (8.4g/l and 20g/l respectively)	- Adults with cholera-like diarrhoea - Non-randomized, observational type of study with objective outcome measurements	- Reduced volume of diarrhoea stool (70%) - Shorter duration of diarrhoea (28%) - Better net absorption (gut balance)
2.	Patra et al. (49)	Cooked rice powder (50 grams per litre)	- Children aged 3 months to 5 years - Randomized, controlled clinical trial with objective outcome measurements (standard WHO-ORS formula in controls)	- Reduced volume of diarrhoea stool (49%) - Shorter duration of diarrhoea (30%) - Reduced ORS requirement (30%) - Comparable failure rate
3.	Patra et al. (48)	Glycine and glucose (8.4g and 20g/l respectively)	- Children aged 3 months to 5 years - Randomized, double-blind controlled clinical trial with objective outcome measurements (standard WHO -ORS formula in controls)	- Reduced volume of diarrhoea stool (50%) - Shorter duration of diarrhoea (30%) - Reduced ORS requirement (30%) - Comparable failure rate
4.	Molla et al. (42)	Cooked rice powder (80 grams per litre)	- Adults and children with cholera and non-cholera diarrhoea - Randomized, controlled clinical trial with objective outcome measurements.	- Reduced volume of diarrhoea stool in both adults and children (28% & 24% respectively) - Reduced ORS requirement (27% & 30% respectively) - Comparable failure rate
5.	Patra et al. (unpublished data)	Cooked rice powder and glycine (50g and 8.4g/l respectively)	- Children aged 3 months to 5 years - Randomized, controlled clinical trial with objective outcome measurements	- Reduced volume of diarrhoea stool (40%) - Reduced ORS requirement (36%) - Comparable failure rate - Magnitude of improvement is no better than in study No.2 (ORS with cooked rice powder alone as organic component)

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