ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Vol. 44 Nº 2, 1994

Dietary fibre, what it is and how it is measured

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SUMMARY. Carbohydrates are the major component of the human diet and are an important source of energy. The World Health Organization recommends that 50-70% of ingested carbohydrates should be in the form of polysaccharides such as starch. A small proportion of dietary carbohydrate is in the form of non-starch polysaccharides (NSP) (Dietary Fibre). Dietary Fibre is a medically important component of the diet since epidemiological evidence links it with the etiology of various diseases. Scientists have engaged in trying to understand the mechanism by which dietary fibre prevents disease. This article highlighst the lack of consensus on its chemical definition and the advantages and disadvantages of the two main methods used to measure it. These are the enzymic gravimetric method (AOAC) that measure fibre as the weight of residual matter following enzymic treatment of the food; and the enzymic chemical method that identifies and measures fibre from its chemical components. The latter method, proposed by Englyst and Cummings measures dietary fibre as NSP and gives detailed information about its components. This is important for interpreting epidemiological and physiological studies. The precise and confident measure of the different components of carbohydrates is important in Latin America. It will allow a coherent, scientific and rational approach to the role of carbohydrates in health.

RESUMEN. Fibra Dietaria: que es y como se mide. Los hidratos de carbono (HC) conforman la mayor parte de nuestra dieta y son una importante fuente de energía. La Organización Mundial de la Salud recomienda que del 55 al 75% de la ingestión total de energía la proporcionen los HC, pero del 50 al 70% deberían ser en la forma de polisacáridos tales como el almidón. Una pequeña proporción de los HC la constituyen los polisacáridos no amiláceos (NSP) a los cuales nos referiremos como «fibra dietaria». La importancia médica de este componente de la dieta ha conllevado a científicos de diversas partes del mundo a tratar de entender los mecanismos por los cuales la «fibra dietaria» tiene efectos fisiológicos particulares en el ser humano que previenen el desarrollo de padecimientos tales como cáncer y enfermedades cardiovasculares. Pero aún no existe un consenso internacional en su definición química y esto ha sido la mayor barrera para entender el papel que ésta juega en la dieta. En este artículo se exponen las dificultades para definir el término «fibra dietaria» así como las ventajas y desventajas de los dos principales métodos utilizados en su medición: el gravimétrico enzimático que mide la «fibra dietaria» como el peso residual de materia después que el alimento ha sido tratado, y el método químico enzimático que identifica y mide la «fibra dietaria» como sus componentes químicos. Ejemplo de este último es el propuesto por Englyst y Cummings que mide fibra dietaria como NSP (polisacáridos diferentes al almidón) y proporciona información detallada acerca de NSP pro medio de análisis en cromotografía líquida de gases la cual es importantes en la interpretación de estudios fisiológicos y epidemiológicos. La medición precisa y confiable de los diferentes componentes de los HC por medio de métodos químicos enzimáticos es importante para América Latina. Esto permitirá un abordamiento coherente, científico y racional al análisis del papel que los hidratos de carbono juegan en la salud.

INTRODUCTION

Carbohydrates are quantitatively the major component of our food and represent about 50% of the daily dry matter intake of food. They are important as an energy source and play an important role in the body as glycoproteins and glycolipids in determining cellular structures and cell-surface characteristics, including the specificity of some receptor sites. In developed countries, carbohydrates provide about 45% of total energy intake with nearly half of this energy coming from sugar, whereas in developing countries up to 75% of dietary energy is obtained from carbohydrates (1). The World Health Organization recommends that carbohydrates provide 55-75% of total energy intake (2), with 50-70% of carbohydrate in the form of polysaccharides such as starch, with oligosaccharides and simple sugars like glucose, fructose and sucrose making up the difference. A small proportion of dietary carbohydrate is in the form of non-starch

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polysaccharides (NSPs) (dietary fibre) (Table 1) (3) and these constitute around 5% or less of the total diet. NSPs do not provide carbohydrates to the body but are a source of energy via their fermentation products, i.e. the volatile fatty acids that are absorbed and thus contribute to the body's fuel supply. NSP levels can range from less than 1% in cornflakes to up to 50% in brans, with a wide range for cereals, fruits and vegetables (4). Small as this fraction of NSPs appears to be, its significance for humans has become increasingly apparent. Epidemiological evidence has emerged that relates fibre intake to the etiology of diseases such as appendicitis (5), diverticular disease (6-8), constipation (9,10), diabetes (11-15), obesity (16), stroke (17) and ischemic heart disease (18-21). Evidence also indicates that diet rich in fibre may be protective against cancer (22) of the breast (23), ovary and endometrium, and the majority of individual case-control epidemiological studies suggest that foods rich in fibre are protective against colorectal cancier, although this association is with vegetables, rather than cereal consumption (24). There is circumstantial evidence that fibre-depleted diets favour the development of cholesterol-rich gall stones, dental disease, and probably other disorders such as haemorrhoids, other common anal conditions and varicose veins (25).

Dietary fibre is therefore a medically important component of the diet (21). Scientists from different parts of the world have engaged in the dificult task of trying to understand the mechanisms by which dietary fibre has a particular physiological effect in humans for preventing the appearance of disease. Analysis of fibre in a wide range of foods has been undertaken worldwide to assess how much and which type of fibre individuals eat. A number of methods, gravimetric or chemical, have been developed, each with distinctive features that account for the differences in the results obtained by different approaches (26) to the measurement of the «fibre» fraction.

TABLE 1
NON-STARCH POLYSACCHARIDES (NON ALPHA-GLUCAN POLYSACCHARIDES)*

Class	Description	Solubility at pH 7.0	Monomers	Occurrence
Cellulose	Unbranched beta 1-4 glucan	Insoluble	Glucose	Very widely distributed especially leafy vegetables, peas, beans, and rhubarb
Non-Cellulosic Polysaccharides	Diverse Mixture:			
	Pectins	Soluble	Galacturonic acid	Mainly fruit and vegetables
	Glucans	Soluble	Glucose	Oats, barley, rye
	Arabinogalactans, arabinoxylans	Partly soluble	Arabinose, xylose, galactose, glucose	Wheat, rye, barley
	Gums: gum arabic sterculia	Soluble Soluble	Galactose, Rhammose, galactose, arabinose	Plant gums used as food aditives.
	Mucilages: ispaghula	Soluble	Arabinose, xylose	Seed mucilage of *Plantago ovata*
	Storage: inulin, guar	Soluble Soluble	Fructose Galactose, mannose	Jerusalem artichokes: to a lesser extent in other root vegetables.
	Fungal: chitin	Insoluble	Amino sugars	Mushrooms and other fungi, exoskeletons of crustacea e.g. shrimps and prawns.

* Department of Health U.K. (1991) (3)

Definition of Dietary Fibre

Despite the many reviews of the term dietary fibre (27-29) there is no international consensus on its chemical definition and this has been a major barrier to the understanding of its roles in the diet. The term «dietary fibre» was introduced by Hipsley in 1953 and described as material derived from the plant cell wall in foods (30). In 1972 Trowell defined dietary fibre as "the skeletal remains of plant cells that are resistant to digestion by enzymes of man" (31), a definition that he later refined by restricting the term dietary fibre to the sum of polysaccharides and lignin that are undigested by the endogenous secretions of the human digestive tract (32).

Definition of Non-Starch Polysaccharides

Although the introduction of this idea signified an advance in thinking, criticism of this definition arose (25,33) since it focused attention on the indigestibility of dieary fibre and ignored other physiological properties, such as the effect of fibre on the digestion and absorption in the small intestine of other nutrients. It also implied that dietary fibre is unavailable for metabolic use (34), even though it has been known for a long time that most forms of fibre are degraded by bacterial enzymes inthe lower bowel, releasing short-chain fatty acids (e.g.acetic, propionic and butyric acids) that are absorbed and metabolized (35). This fibre cannot be defined as unavailable carbohydrates.

New concepts in starch digestion have also been emerging (36-38), with studies showing that a considerable amount of starch escapes digestion in the small intestine. Starch becomes resistant to alpha amylase digestion during the cooling stages of food processing or by cooling starch after cooking to produce «resistant starch». During cooking caramelization also occurs, and Maillard polymers are formed (39). All these later findings show that the simple issue of indigestibility is not enough to allow a dietary component to be classified as dietary fibre. Thus, the 1976 definition by Trowell, Southgate et al. wich excluded many indigestible compounds from the definition and aimed to measure DF as the cell-wall polysaccharides (NSP) plus lignin has some merit and has led to the demand for an analysis based on chemical criteria.

Cummings and Englyst suggested by 1978 (40,41) that fibre should be measured as non-starch polysaccharides (NSP) in plant foods and later argued against the inclusion of lignin in the measurement of dietary fibre, since lignin is not a carbohydrate and its physiological significance (in animal studies) is very different from that of NSP. Lignin is also quantitatively a minor component in the human diet and is difficult to dermine (42). It has been proposed that lignin should be considered and measured separately, since the linking of values for NSP and lignin together is arbitrary from a physiological perspective. To measure both together tehrefore may invalidate an assessment of the true significance of both components (43). More recently it has also been proposed that the term «dietary fibre» should become obsolete for scientific purposes since it leads to considerable confusion with endless arguments about what should or should not be included in the definition (44). Since NSPs form the major fraction of «dietary fibre» whatever definition is used, then by selecting NSPs as the basis for the new definition it is possible for these components to be chemically identified and measured with reasonable precision. The plan cell-wall NSPs form the skeletal component of plant cells and this is the component of the diet that, in practice, has been linked to effects when assessed in physiological and epidemiological studies. We therefore conclude that the definition of fibre should now be based on the cell-wall component of the non-starch polysaccharides in food.

The Analysis of Dietary Fibre

Crude Fibre

The first measurements on fibre were probably made in the last century using the Weende System (named after a town in Germany) for the analysis for crude fibre. This method was developed by Einhoff in 1806 for predicting forage digestibility in animal foodstuffs (42). It is a gravimetric method, i.e. one based simply on measuring the residual weight of material after the feed has been hydrolyzed first in dilute acid and then in alkali, then dried and weighed; this residue is called «crude fibre». As well as removing starch, sugar, protein and minerales from the food, various amounts of cellulose, hemicellulose and lignin are also solubilized by this reatment so a very variable measure of plant cell-wall material is obtained. However, this method had an established place in animal nutrition as a very simple and approximate method of predicting the fermentability of forages. The persisting use of crude fibre values in human food tables is however extraordinary. These values have no place in the modern approach to dietary fibre and are not relevant to human nutrition, since deficiencies in this method have been recognized for over a hundred years. Cummings, in his review (27), lists the main problems of the method as:

1. It is not a good predictor of the nutritive value of a feed for man.
2. The value obtained is extremely method-dependent and insensitive at the low levels of crude fibre found in many human foods.
3. Cell-wall constituents are underestimated; for example, the crude-fibre content of wholemeal flour is 2% while the actual amount of total cell-wall constituents is approximately 11 to 12%. With the development of more precise methods for measuring the components of the plant cell wall, it is now known that the crude-fibre method recovers approximately 50-80% of the cellulose, 10-50% of the lignin and 20% of the hemicelluloses.

4. A more important deficiency of the method, however, is that it recovers a variable and unpredictable proportion of the total cell-wall constituents of a plant.

Neutral Detergent Fibre

A modern and simple approach to the gravimetric determination of fibre is that of Goering and Van Soest (45). They developed the Neutral Detergent Fibre method for animal foodstuffs but now use it in the human field as well. A version of this method is the standard American Association of Cereal Chemists (AACC) method for dietary fibre (46). The sample is wieghed, defatted and dried briefly. Buffered neutral sodium dodecyl sulpahte (a detergent) is then added and the sample is boiled. Starch is gelatinized and solubilized by this procedure. After 30 minutes more solution is added together with the enzyme alpha amylase and incubated before the solutions is again boiled for a further 30 minutes. The residue consisting of cellulose, hemicellulose and lignin is then filtered. A second amylase treatment is given to remove any remaining starch, and the residue is then washed, dried and weighed. This residue contains the fibre and mineral fractions of the plant. The residue is then ashed and the loss in weight is a measure of dietary fibre content. Once recognized problem is that the neutral detergent solution also solubilizes the pectins present in the sample, so the method has limited use for those human foods with appreciable amounts of pectin material.

AOAC Prosky Method

Further methods for dietary fibre analysis have been developed. The methods described rely on a similar approach to that of Van Soest, namely attempted solubilization of the starch by enzymes, which then leaves the plant cell-wall fraction. The method proposed by Prosky and others (47,48) is a gravimetric approach that allows the separation of fibre into soluble and insoluble forms, but does not identify the types of monosaccharide involved. The original method was based on the work of Asp et al (49). The aim of the method was to measure cell-wall material. The sample is first gelatinized in buffer solution before being treated with Termamyl, a thermally stable amylase that partly hydrolyzes the starch. An acid pepsin is used to hydrolyze the protein and an incubation with pancreatin completes the hydrolysis stage. The dietary fibre is then separated by precipitation and filtration, leaving free sugars and solubilized protein in solution. The dietary fibre residue is washed, dried and weighed, and then any residual protein is determined in one replicate and ash content in the other. The fibre is then taken as the weigh of residual material once an allowance has been made for the estimated weight of the protein and ash components.

The current AOAC method differs from the Asp procedure because a milder treatment with a neutral protease is used instead of acid pepsin to remove protein. An amyloglucosidase enzyme is then used to hydrolyze the starch to glucose. The rest of the procedure is exactly the same. Disadvantages of the method include the long filtration times for some types of food and the need to correct for the protein and ash content of the residue. The gravimetric procedure requires that all the components from different plants react to the enzyme treatment and filtration in the same way. However, it is now known that the residue includes, in addition to cell-wall material, some starch retrograded during food processing and other substances such as tannins and co-precipitated non-carbohydrate materials. The inclusion of retrogrades starch and other sustances formed during food processing as part of the measured residue gives rise to higher values for the dietary fibre content, so the AOAC value can readily be manipulated by industrials processing. From the anaytical viewpoint it also uses techniques that have long been superceded by modern methods of carbohydrate chemistry. Furthermore a gravimetric residue containing NSP, starch and non-carbohydrates in unknown proportions is impossible to interpret. Nevertheless this method remains popular with industrialists and their associated scientists.

Englyst analysis of Non-Starch Polysaccharides

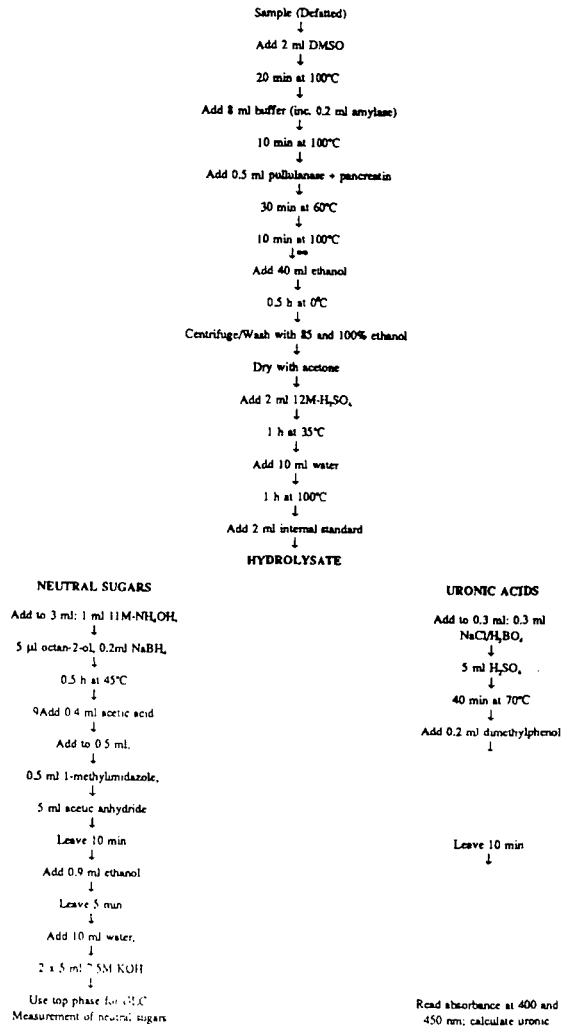
A more sophisticated approach to dietary fibre measurement is one in which the chemical components of the dietary fibre are identified and measured. Methods of this type have evolved from the principles laid down by Southgate in 1969 (50). His analyses of the non-available carbohydrates of foods are the basis for the dietary fibre data in the British food tables, i.e. McCance and Widdowson's Tables of Food Composition (51). The food sample is extracted with 80% methanol to remove free sugars, then with ether to remove fat. It is then treated with alpha amylase to remove starch. Soluble and insoluble dietary fibre residues are separated. Each is then analyzed by sequential acid hydrolysis into constituent sugar residues giving data on cellulosic and non-cellulosic sugars, hexoses, pentoses and uronic acids. The procedure is very detailed and was originally quite lengthy.

The method of Englyst (41) was evolved from the principles of the Southgate method and uses gas-liquid chromatography to separate, identify and quantify the monomeric sugar residues of the non-starch polysaccharides. Englyst has also devised methods for the separate measurement of resistant starch (43) and defined three forms of resistant starch. These resistant starches have a variety of effects in the colon and need to be considered separately fom the components of NSP which form the plant cell walls.

The scheme for the non-starch polysaccharide method is shown in Fig. 1. The sample, defatted if necessary, is dispersed in dimethylsulphoxide. This breaks up the hydrogen bonding in resistant starch, making all the starch available to attack by alpha amylase enzymes. Buffer and the enzymes pancreatin and pullulanase are added, and the solution is incubated overnight to hydrolyze the starch to glucose. The non-starch

polysaccharides are precipitated in 80% ethanol and recovered by centrifugation. The non-starch polysaccharide residue is washed to remove all soluble sugars then dried. The dried residue is then hydrolyzed in acid, which breaks up the polysaccharides into their constituent monosaccharides. This method has been developed further to speed up the procedure and a faster version is currently in use. By the use of a thermostable amylase, the starch hydrolysis step has been cut from 16 hours to 1 hour, and the time for acid hydrolysis of the dietary fibre to release the constituent monosaccharides has been decreased by increasing the strength of the acid used for hydrolysis (52).

FIGURE 1
Determination of total, soluble and insoluble dietary fibre by the Englyst procedure



* Total NSP=Neutral Sugars + Uronic Acids
Soluble NSP = Total NSP - Insoluble NSP
** For measurement of Insoluble NSP, replace the 40 ml ethanol with 40 ml pH7 buffer and extract for 20 min at 100°C and centrifuge

The amounts of monosaccharides released from the NSP can then be determined in either of three ways:

a. Simply by colorimetry, measuring the color produced by reacting the sugars with a color reagent. This method is rapid and gives values for total, soluble and insoluble non-starch polysaccharides. It has recently been modified slightly to reduce the production of color by non-sugar components in the sample (53).
b. By gas-liquid chromatography for neutral sugars and colorimetry for uronic acids. This method identifies and quantifies the individual monosaccharide species released from NSP.
c. By high pressure liquid chromatography using an ion exchange column and electrochemical detection of the separated monosaccharides (54).

By changing the reagents used in the method the amounts and constituent sugars of a variety of components are obtained: total non-starch polysaccharides, insoluble fibre, soluble dietary fibre (by difference), cellulose, non-cellulosic polysaccharides, uronic acids and resistant starch.

DISCUSSION

Different approaches to the measurement of dietary fibre have been developed and these are divided into two main methods: the enzymatic gravimetric methods and the enzymatic chemical methods.

The gravimetric methods measure dietary fibre as the residual weight of material after the food has been treated with starch and protein-degrading enzymes and after correction for the ash and nitrogen content of the residue. An example of such a method is that propsed by Prosky et al (48) which includes in the measurement part of the starch that is retrograded as the result of food processing. Lignin and substances measured as lignin are also included and, on this basis, the Prosky or AOAC methods claim that they are methods that reflect the physiological principles of indigestibility. Unfortunately as yet there is little evidence for this claim. The use of physiological enzymes and the weighing of the residue is considered to leave NSP plus resistant starch in the final sample. This approach partly explains why, for example, the AOAC method gives a fibre content of cornflakes which is much higher than when NSP is measured directly by the Englyst method: the extra weight is thought to reflect the retrograded starch plus Maillard products produced during the processing of the cornflakes. The increased residue after processing explains why food manufacturers, who often seek to claim extra health benefits for their foods because of their fibre content, prefer the AOAC method with its higher results to the NSP measures. By manipulating the processing of the starch, e.g. by heating and cooling, they can increase the resistant starch content of the food and therefore claim, with the AOAC method, extra fibre in their products.

The AOAC method is still being refined as more sources of the physical variability in the filtration process or in the hydrolysis are found. However, the claimed physiological principle is becoming suspect now that it is clear that many compounds escape digestion and therefore will be included in this AOAC method. Yet, in practice, all the supposed advantages of fibre feeding seem to relate to the plant cell-wall NSPs and it is far from clear that the other compunds contribute to the beneficial effects of these plant foods. Thus, feeding experiments with fibre (55) clearly showed the value of NSP in determining faecal bulking and the epidemiological relationships between fibre-rich foods and disease relte best to the NSPs of cell-wall origin. Those who seek to establish the physiological validity of the AOAC method will find it difficult because the method canot be used on physiological fluids, e.g. ileal luminal contents or faeces, because of the ready interference with the physical filtration of the sample by additonal mucus and other endogenous secretions. Given these problems it seems best to work with chemical methods and chemically defined fractions.

The enzymatic chemical methods identify and measure dietary fibre as its chemical components. An example of this method is that proposed by Englyst and Cummings (36). They measure dietary fibre as non-starch polysaccharides where starch is completely removed. The method gives detailed information about dietary fibre by GLC analysis, which is important in the interpretation of physiological and epidemiological studies. However, these detailed analyses of the constituent sugars are not always necessary for routine work or for food labelling. In this case the more rapid colorimetric method could be used since good agreement has been shown between the analytical values for a wide range of foods measured by GLC and colorimetry (56), particularly with the latest small change to reduce the time for color development (53). The GLC procedure is also valid for the analyses of gut content and faecal samples.

A classification of carbohydrates has already been proposed by Englyst, Kingman and Cummings (43) (Table 2). For nutritional purposes starch in foods may be classified into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). The RS may be further divided into three categories according to the reason for its resistance to hydrolytic attack.

TABLE 2
IN VITRO NUTRITIONAL CLASSIFICATION OF STARCH

Type of starch	Example of occurrence	Probable digestion in small intestine
Rapidly digestible starch (RDS)	Freshly cooked starchy food	Rapid
Slowly digestible (SDS) starch	Most raw cereals	Slow but complete
Resistant starch (RS)		
1. Physically inaccessible starch.	Partly milled grains and seeds	Resistant
2. Resistant starch granules	Raw potato and banana	Resistant
3. Retorgraded starch	Cooled, cooked potato, bread and corn-flakes	Resistant

This issue of resistant starch has recently been the subject of a concerted action with the agroindustrial research programme of the commission of the European Communities (57), where resistant starch is defined as «the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals». New efforts are now being made to assess the resistant starch intake in various countries. This calls for accurate and separate measures of NSP and starch as well as accurate measures of food intake. In Latin America where diets are changing rapidly there is therefore a need to move away from the use of the AOAC Prosky method for assessing so-called fibre intakes until a more coherent, scientific and rational approach can be made to the analysis of the role of dietary carbohydrates in health.

CONCLUSION

It is becoming clear that dietary fibre is of great interest to nutritionist and doctors but the problems of defining and measuring dietary fibre are so great that the many different

approaches and changes to the definition and analysis have brought great confusion. Whilst a simple gravimetric method accepted by the AOAC for measuring fibre is very popular, a more rigorous scientifically-based approach is now emerging. Dietary fibre should be classified as a dietary carbohydrate and the cell-wall non-starch polysaccharides are those which contribute 90% or more of the skeletal remains of the plants which is the essence of the definition. There are now simple and robust methods for measuring this fraction of indigestible carbohydrates. These methods, developed by Englyst, have been rigorously tested, evaluated in trials and published in considerable detail. Future research will now be helped by using specific measures of dietary carbohydrates to quantify accurately intakes of dietary fibre and also allow a better understanding ot the importance of other malabsorbed carbohydrates which should not be included in the definition of fibre.

ACKNOWLEDGMENTS

Our thanks are due to the Ministry of Health in Mexico (Department of International Relations), the Scottish Office Agriculture and Fisheries Department, the British Council and the Nestlé Company in Mexico for their support.

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Recibido: 17-12-1993
Aceptado: 08-04-1994