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TESTING OF PROTEIN-RICH FOOD MIXTURES FOR WEANING

by

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No protein-rich weaning food mixture may be accepted for human feeding unless it has been rigorously tested The testing includes two stages preclinical and clinical

PRECLINICAL TESTING[1]

A new mixture proposed for possible use as a weaning food must be tested for safety and its nutritive value must be evaluated.

Safety The first requisite for the safety of supplementary food mixtures is the choice of raw materials These must be of good quality If they include products of vegetal origin which contain naturally an antimetabolite, it must be inactivated, removed or destroyed In soybean, for example, the antitryptic factor is inactivated by heat In cottonseed, gossypol may be inactivated by heat or it can be extracted by the use of selective solvents or azeotropic mixtures, if glandless cottonseed is not available Some legumes contain also haemagglutinins, haemolysins, or factors toxic for the nervous system Raw materials subject to microbial or fungic contamination and spoilage must be examined for the presence of pathogenic organisms (e g Salmonellae, Shigella, Staphylococci and Clostridia) or for the toxic substances they produce, as well as for the possible presence of mycotoxins, such as aflatoxins For aflatoxins, every effort should be made to keep their level in food mixtures as low as possible The Protein Advisory Groups (PAG) have tentatively recommended, in 1969, that their concentration should not exceed 30 mg per kg (30 parts per million), but this important question will be reviewed shortly by PAG, and a new recommendation as to permissible levels of aflatoxin may ensue Microbial and fungic contaminations can be avoided either by care in the selection of the raw materials or by appropriate methods of storage

Whenever materials used in the manufacture of supplementary food mixtures have been treated with pesticides or fungicides, it is necessary to ascertain that the treatments have been conducted according to "good agricultural practice" If it is impossible to obtain accurate information concerning the treatments used, special analyses should be carried out on representative samples to determine the levels of pesticide or fungicide residues When solvents are used in the processing (as is often the case for oilseed meals), residues should be eliminated as completely as possible and analyses should be carried out to make sure that the quantities that are left of the solvents themselves, or of any derivatives, are below toxic levels.

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[1] For details see FAO/WHO/UNICEF Protein Advisory Groups (PAG) Guideline No 6 Guideline for preclinical testing of novel sources of protein.

The safety of protein-rich food mixtures to be used as weaning foods depends also on their bacteriological quality which has to be verified not only during the testing of the product but also regularly during the industrial production and the storage period. PAG are now working on this question and are preparing a guideline for the testing of final products for the following categories of micro-organisms

aerobic bacteria (standard plate count)
faecal coliforms
coagulase-positive staphylococci
Salmonella

The PAG guideline will also include sampling plans for these micro-organisms. For unusual ingredients, an evaluation of safety in animals, including multi-generation studies, may have to be envisaged

In fact, the extensiveness of the testing for safety will vary according to the composition of the mixture considered, the origin of its ingredients and the nature of the process employed for its production The same applies also to the testing for nutritive value.

Nutritive value At the stage of the preclinical testing, the nutritive value of a protein-rich food mixture, may be evaluated chemically and biologically.

Chemical evaluation This will include a determination of the proximate composition, i.e. moisture, total nitrogen, fat (ether extract), ash, crude fibre and carbohydrate (by difference). The nitrogenous components will by hydrolyzed and the amino-acid spectrum determined by chromatography. The interpretation of the amino-acid profiles is based on comparison with good proteins such as whole egg or milk, or with the FAO reference pattern Both the protein content and the amino-acid corposition should be expressed on a N x 6,25 basis. Useful information concerning the effect of heat on the product may be obtained not only by determination of available lysine but also by spectrophotometric determination of products of the Maillard (browning) reaction, or, in the case of leguminous proteins, of the heat-labile antitryptic factor.

Biological evaluation Three different procedures are available. The most commonly used involves the determination of the protein efficiency ratio (PER) in which the average net gain in body weight per unit weight of protein consumed (N x 6,25) is compared with that observed for the reference protein, usually casein PER values are customarily adjusted to an assumed value of 2.5 for casein. The diets are fed ad libitum to groups of 10 weanling male rats weighing 40-50 g at 20-23 days for a period of 28 days.

The method based on the estimation of the net protein utilization (NPU), in which the total body nitrogen of both a protein-fed and a protein-free group of animals are determined, is less frequently employed. In this method the nitrogen retention is expressed in relation to the weight of protein or nitrogen consumed. Generally the tests are performed in groups of four to six rats 28 to 30 days old which are fed the test or control diets for a 10-day period

For a more precise evaluation of the nutritive value of a food mixture nitrogen balances are measured. This technique allows for the differentiation between the digestibility of the nitrogen source tested and the proportion of nitrogen retained for tissue anabolism. Usually three to five-day collection periods follow a similar adjustment time.

The chemical analyses and the biological procedures give valuable information as to the nutritive value of the mixture tested but they cannot be assumed to provide an absolute measure of its nutritive value for man. This will be evaluated owing to the clinical testing which can be carried out only if the preclinical tests give every indication that the product is suitable for human tests.

CLINICAL TESTING[1]

Clinical, or human, testing includes mainly tests for acceptability and tolerance of the product, its effect on growth and nitrogen balance experiments for the determination of its nutritive value.

Acceptability and tolerance tests They are designed to provide information on short-term acceptability and tolerance of the intended recipient to the product. They are carried out in institutions or in closely observed samples of population. When it is possible to conduct several such tests, at least one should take place in the country in which it is intended to introduce the product. To eliminate the influence of mild disease processes, it is useful to establish a control group.

The sample of children tested should consist of no less than 20 subjects of the age or ages for which the mixture is developed The test should last for at least four weeks during which the product, prepared in the form of a suitably flavoured gruel or incorporated into a local recipe, should be given to the subjects in a quantity covering more than 50 per cent of the protein requirements of 97 per cent. of such individuals. Whenever possible, an ad libitum feeding test should also be carried out to find out the maximum quantity which is well accepted and tolerated per meal and per day. Children should be left to feed themselves or should be helped in such way that the reaction of the attendant to the product does not influence their own The food should of course never be forced on the subjects

Refusal to eat the preparation is considered an indication of poor palatability and the acceptability is evaluated by such criteria as liking, disliking, indifference to the product, and average quantity consumed per child per meal per day in the ad libitum test Tolerance is judged by noting persistent gastro-intestinal upsets, vomiting, diarrhoea, loss of appetite, flatulence, etc

Careful records must also be kept of any allergic reactions.

Growth test. The measurement of growth can include the determination of body weight or body height gains Measurement of height has certain advantages because it is less variable than weight but differences in the latter can be detected over shorter periods, it is why weight measurement is more frequently used.

Since growth is faster and protein requirements higher in young age, and since, in testing a new protein-rich food mixture, it is important to find out which is the lower age at which the product supports adequate growth, the tests should be carried out first in children six months to one year old (products for infants should be tested in younger infants). The subjects selected should be above the third percentile in height and should have weight for height above 95 per cent. of ideal, based on standards for well-nourished children

Under carefully controlled conditions the test should last from at least two to four weeks for infants about one year old, and from three to six months for somewhat older subjects The number of children to observe will depend on their age and co-operativeness, the duration of the test and the extent to which various interfering factors can be eliminated. Valuable information may be obtained with as few as five infants in a study in an institution. Ideally, a closely matched control group should also be studied

In infants, weights should be measured every day, in older children at least every one to two weeks The test protein should be the sole source of protein in the diet and the latter should supply adequate intake of calories, of vitamins and of minerals. The total protein intake should at least conform to the recommended intakes of FAO/WHO [2] The control

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[1] For details see PAG Guideline No. 7 Guideline for human testing of supplementary food mixtures.

[2] FAO/WHO (1971) Report of an ad hoc joint FAO/WHO Expert Committee, Requirements of energy and proteins, Wld Hlth Org. techn. Rep. Ser. (In press).

'oup should receive milk or egg as the source of protein with comparable levels of protein
:d calories.

Nitrogen balance measurements They can be carried out only if facilities for prepara-
on and weighing of diets, precise control of food intake, complete collection of urine and
ieces, execution of biochemical analyses, minimizing of cross-infections, close and
ntinuous supervision of the subjects, etc., are available For this test, children six
) 36 months, fully recovered from malnutrition, are convenient subjects, but this does not
'clude children outside this age range It is extremely important that the subjects have
) infection, as even mild infections induce a stress response which increases urinary
.trogen loss. As for control, the best plan is to use each subject as his own control,
:th consecutive tests on control and experimental diets

The calories supplied must be equal in all balance periods and water, vitamins and
ineials should be fed in adequate and constant amounts It is recommended that the protein
itake does not exceed 300 mgN (slightly less than 2 g protein) per kg per day. If the
linical condition of the child justifies feeding a lower quantity (e g. 1 5 g protein per kg
:r day), difference in protein value will appear more clearly.

Before each balance an adaptation period is necessary whenever the level of nitrogen or
:e source of protein is changed. A three-day period is generally sufficient in infants,
ve days or more may be needed in older children. Collections of urine and faeces should be
:tained for a minimum of six days (e g two three-day and three two-day periods) In boys,
:parate collection of urine and faeces makes it possible to measure apparent digestibility
well as apparent biological value. Generally it is not necessary to measure the basal
"endogenous" urinary and faecal nitrogen loss to determine true digestibility and biological
.lues, for practical purposes, the figures available in the literature are accurate enough.

For clinical as for preclinical testing, the extensiveness of the tests will depend on
ie nature, origin and processing of the ingredients of the mixture studied. Products made
well-known staples and processed in conventional ways may need no more than acceptability
id tolerance testing whilst unconventional and new products may have to go through all the
:sts described here Whatever may be the case, when preclinical and clinical testing will
ive given satisfactory results we shall know that the product considered can be used as a
:aning food The next step will be to carry out marketing trials and these will be
:scribed elsewhere