

The biological availability of methionine sulphoxide. By GABRIELLE M. ELLINGER and R. PALMER, *Rowett Research Institute, Bucksburn, Aberdeen*

Heat processing of food proteins may impair the biological availability of the methionine they contain, with little effect on the amount of methionine determined by chemical analysis (Ellinger & Boyne, 1965; Miller, Carpenter, Morgan & Boyne, 1965). There is little information regarding the nature of the modification that makes methionine unavailable. One requisite for the product is that it readily converts to methionine (or methionine sulphoxide) during acid hydrolysis; another that it quantitatively oxidizes to methionine sulphone. Methionine sulphoxide is one compound that fits this description. Its labile relationship with methionine during acid hydrolysis is acknowledged; methionine values obtained by ion-exchange chromatography are frequently derived from the sum of methionine and methionine sulphoxide found.

Miller & Samuel (1968) found that under conditions when methionine is the limiting amino acid in the net protein utilization (NPU) test, free methionine sulphoxide may partly replace methionine. In Ford's assay, *Streptococcus zymogenes* responded equally to free methionine and methionine sulphoxide (Miller *et al.* 1965). In our assays, to avoid the possible reduction of methionine sulphoxide when the medium was autoclaved, we added the sulphoxide to the autoclaved medium through a membrane filter. On an equimolar basis L-methionine sulphoxide then had 90% L-methionine activity for *S. zymogenes*.

The problem of nutritional availability is, however, mainly concerned with amino acids in the peptide-bound state. Accordingly, casein was treated with hydrogen peroxide under conditions (Toennies & Kolb, 1939) that favour the oxidation of methionyl residues to sulphoxide but minimize reactions with other amino acids. Hydrogen peroxide was added to a suspension of casein in 0.5 N-HCl:methanol (3:2 v/v) at the rate of 1.2 m-moles/m-mole methionine in the casein. After vigorous stirring for 3 h and settling overnight at room temperature, the casein was washed free from unreacted hydrogen peroxide and dried under reduced pressure at room temperature. A supplement of 1% L-tryptophan was given with oxidized casein in NPU tests. Methionine continued to be the limiting amino acid. Oxidation reduced the NPU of the casein from 71 to 58 (mean for three preparations). The 'available methionine' determined by the *S. zymogenes* assay fell from 2.9 to 2.3 g/16 g nitrogen. Thus it appears that the oxidation of the peptide-bound methionine to the sulphoxide reduces the availability of methionine in casein to growing rats and *S. zymogenes*, while free methionine sulphoxide is nearly completely utilized by *S. zymogenes*.

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***Measurement of muscle protein turnover by constant intravenous infusion of ^{14}C -glycine.** By P. J. GARLICK (introduced by J. C. WATERLOW), *M.R.C. Tropical Metabolism Research Unit, St. Mary's Hospital, London, W2*

***Degradation of human serum albumin by human pepsins.** By W. J. UNGER, J. WATKINS and I. F. STAMFORD (introduced by C. H. GRAY), *King's College Medical School, London, SE5*

An improved diet for carbohydrate preference studies with rats: some criticisms of experimental diets. By HEATHER GREENFIELD, GEORGE M. BRIGGS, R. H. J. WATSON and JOHN YUDKIN, *Queen Elizabeth College, London, W8*

In the belief that in nutritional research the diet should be designed specifically for the experiment, an improved diet for carbohydrate preference tests with rats was formulated to fulfil the following requirements:

(1) Nutritional adequacy within the sphere of present nutritional knowledge, using the National Research Council (1962) requirements for the rat as a guide.

(2) Ability to support good growth and reproduction.

(3) Presentation of all components in one complete formula.

It is believed that this diet may find application in other areas of nutritional research. It has the following percentage composition: casein 24, arachis oil 10, maize starch or sucrose 60, salt mix 4, vitamin mix 2. The vitamin mix provided in 100 g of diet: vitamin A 1300 i.u., vitamin D₃ 122 i.u., vitamin E 7.5 mg (all fed as Rovimixes, Roche Products Ltd), menaphthone 0.1 mg, thiamine hydrochloride 1.0 mg, nicotinic acid 6.0 mg, riboflavine 1.0 mg, calcium-D-pantothenate 4.0 mg, folic acid 0.5 mg, biotin 0.1 mg, pyridoxine hydrochloride 1.0 mg, vitamin B₁₂ 5.0 µg, choline bitartrate 180 mg, ascorbic acid (as antioxidant) 7.5 mg, cellulose (as carrier) 1.76 g. The salt mix (Johnson, Bouchard, Tinoco & Lyman, 1967, modified) provided in g/100 g diet (i.e. in 4 g of salt mix): CaHPO₄ 1.3, CaCO₃ 0.82, KCl 0.82, Na₂HPO₄ 0.74, MgSO₄.H₂O 0.28, MnSO₄.H₂O 0.018, FeC₆H₅O₇.5H₂O 0.0174, ZnCO₃ 0.003, CuSO₄ 0.0015, KIO₃ 0.0001.

A survey of purified diets for rats as published in the *British Journal of Nutrition* in 1967 and 1968 was made with special reference to salt mixtures. This revealed that where details were given or a readily traced reference, adequate levels of minerals were usually being fed. In the remaining cases either no details were given or the reference when eventually traced disclosed insufficiencies of minerals such as zinc, manganese, copper and iodine. Thus, in such cases no true evaluation of the results is possible.

We believe that a more critical attitude to traditional methods of diet construction, particularly among junior research workers, is necessary for higher standards in research in nutrition. Furthermore, we recommend that editors insist more strin-