analyses of insoluble material produced during digestion show that very few if any peptides have been overlooked. Analyses of the enzyme for carbohydrate suggest that little or none is present.

Digestion of the intact subunit with carboxypeptidases revealed a single C-terminal sequence, -Lys-Asn. The amount of asparagine released is compatible with a polypeptide molecular weight described above.

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The Production of Protein for Non-ruminant Feeding by Growing Filamentous Fungi on Barley

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Barley, the chief staple in pig feeding, contains usually 10–12% crude protein ($N \times 6.25$). Because the young pig's requirement for protein is about 16–20% of the diet (Agricultural Research Council, 1967), the deficit is generally made good by adding soya bean or fishmeal. Microbial fermentation in which added non-protein N is converted into protein N at the expense of barley carbohydrate is another way of improving the protein content. We thus sought a non-pathogenic organism giving rapid dominant growth that would ferment barley grain under non-aseptic conditions.

Twenty-three fungi were screened in shake-flask cultures grown for 3 days in a medium containing 5% barley, 0.1% urea and 0.2% KH_2PO_4 . Contents of crude protein (dry basis) in the biomasses ranged from 8% (*Geotrichum candidum*) to 23% (*Aspergillus oryzae*). Comparison of two high-yielding organisms, *A. oryzae* and *Rhizopus arrhizus*, was made in 7-litre stirred cultures. With 1% barley and 0.1% urea, pH 4.0, *Rhizopus* grew at a much lower rate than *Aspergillus*, where mycelial growth stopped at 24h (3.5g/l, dry basis), whereas *Rhizopus* was still growing at 60h (4.0g/l); mycelial N contents were maximal at about 7% during early growth. With $(NH_4)_2SO_4$ as N source both organisms gave higher mycelial and protein yields within 24h (*Rhizopus*, 3g/l; *Aspergillus*, 5g/l). Growth, which ceased at 24h, was limited by carbohydrate; 50% only of the added N was utilized.

With 2% barley, *A. oryzae* gave a mycelial yield of 7.4g/l at 24h with a maximum at 34h of 8.5g/l; the mycelial N content was 7–7.5% and utilization of

carbohydrate and $(NH_4)_2SO_4$ was almost complete. With 3% barley and 0.42% $(NH_4)_2SO_4$ the mycelial yield was 8.5g/l at 24h and 10.5g/l at 34h; the N content was 7.5%. At 34h 90% of carbohydrate and 80% of N source were used up and uptake ceased. With both 2% and 3% barley the concentration of reducing sugar reached a high value during the rapid phase of growth.

A. oryzae has been successfully grown under non-aseptic conditions in 1000-litre culture in a modified pig-feed mixing vessel. With 20kg of barley, 1kg of urea, 2kg of KH_2PO_4 , pH 3.5, in 24h, 9.3kg of biomass was recovered at 31% crude protein (dry basis); this contained (g/16g of N): lysine, 4.4; methionine, 1.2; cysteine, 1.5; leucine, 5.8; isoleucine, 3.2; valine, 4.8; threonine, 3.4; phenylalanine+tyrosine, 6.4; arginine, 5.9; hexosamine, about 7%. The biomass, fed to rats, gave a net protein utilization value of 56; mixed with barley it was readily eaten by pigs, which showed satisfactory weight gains.

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The Purification and Physical Properties of 3-Carboxy-*cis-cis*-muconate Cycloisomerase

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Cell-free extracts of *Aspergillus niger*, grown on a *p*-hydroxybenzoate carbon source, contain an enzyme that catalyses the lactonization of the product of aromatic ring fission (3-carboxy-*cis-cis*-muconate) to 3-carboxymuconolactone (Cain *et al.*, 1968). The reaction is similar to that catalysed by the enzyme muconate cycloisomerase (EC 5.5.1.1), which has been characterized in the pathway responsible for the degradation of catechol in *Pseudomonas putida* A3.12 (Ornston, 1966). The *A. niger* enzyme has therefore by analogy been called 3-carboxy-*cis-cis*-muconate cycloisomerase.

The enzyme was purified by a combination of ammonium sulphate fractionation, DEAE-cellulose chromatography and gel filtration. The 30–50% saturation- $(NH_4)_2SO_4$ fraction of 10.5 litres of cell-free extract was purified first by batch elution on Whatman DE11 anion-exchange DEAE-cellulose at pH 6.5 followed by gradient elution of the enzyme from a column of Whatman DE52 DEAE-cellulose at pH 7.5. A final separation on a column of Sephadex

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