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Microbiological evaluation of protein quality with *Tetrahymena pyriformis* W

2. Relative nutritive values of proteins in foodstuffs

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Conditions for the growth of *Tetrahymena pyriformis* W on intact proteins and the principle of a method for the determination of relative nutritive values have been described (Fernell & Rosen, 1956). The relative nutritive values of a number of partially purified proteins were in general accord with chemical scores and with protein ratings for the growing rat. These studies on the potentialities of *T. pyriformis* for the microbiological evaluation of proteins were continued with direct application of the method to foodstuffs, such as oilseed meals and animal-protein concentrates, without preliminary separation of the protein. Since relatively little was known about the physiology and biochemistry of ciliates cultured on intact proteins, the choice lay initially between a detailed investigation of one, or possibly two, protein sources, and a less detailed survey of a wide range of protein types in foodstuffs. We have tended to follow the latter course on the grounds that it might reveal limitations of the criterion adopted for comparing protein qualities.

In this second stage it was important not only to take into account the effect on the practical procedure of substantial quantities of non-protein material in foodstuffs, but

also to ensure that the assay be made specific for protein quality. It is fundamental in microbiological assays to reduce non-specific stimulation to insignificant levels. Means of achieving this, by regulating the level of known dietary constituents included in the basal medium, were studied. The results of an initial survey of growth on selected meals underlined the need to study the balance of nutrients given to *T. pyriformis*. Particular attention was directed to glucose:protein relationships and their effect on the count:ammonia N ratio, used to determine relative nutritive values. It was followed by a comprehensive survey of a wide range of representative foodstuffs yielding results that agree well with accepted ratings of food proteins.

EXPERIMENTAL

General. The basal media and experimental techniques were similar to those already detailed (Fernell & Rosen, 1956). Additions and modifications were as follows:

Protein sources. Casein was the 'low-vitamin' grade manufactured by Genatosan Ltd. Other protein sources were commercial meals, with the exception of the samples of acetone-dried cod and herring muscle, groundnut-germ meal (prepared by cold hexane extraction of hand-separated groundnut germ) and the overheated groundnut meal (prepared by prolonged autoclaving of a commercial meal in the laboratory).

Solvent extraction of test materials. The protein source was subjected to three successive extractions with diethyl ether at room temperature, with thorough washing on the filter after each extraction. For the survey, the defatted products were similarly extracted with cold 95% ethanol. The residual solvent after filtration was removed by drying in thin layers at 37°.

Grinding of test materials. Grinding was by hand (pestle and mortar) for small quantities or in a Fowler End Runner Mill for larger amounts. The meals were ground to pass a 200-mesh B.S. sieve for the initial growth studies on meals and to pass a 72-mesh B.S. sieve for the comprehensive survey on foodstuffs. Resistant particles, such as bone and shell, were reduced, after drying in a vacuum oven at 60° for 1 h, to the finest size possible and returned to the sample.

Modifications of basal medium. A few changes of the basal medium detailed previously (Fernell & Rosen, 1956, Table 1) were made during the studies reported here. These were:

Component	μg/ml. final medium
K_2HPO_4	875
KH_2PO_4	875
Biotin	0·0625
DL-α-lipoic acid	0·02

Glucose was included in the medium at various levels, as specified in the text. For the survey of protein quality, made at 1 mg N added/ml., the glucose level was 1·5%.

Suspensions of test foodstuffs. These were wetted to a fine paste, diluted to a little above the required concentration, adjusted to pH 7·1 and allowed to stand overnight. This procedure afforded an opportunity for an equilibrium to be reached and minimized changes of pH on subsequent autoclaving for sterilization. After final adjustment of the pH (7·1) and concentration of the suspensions, the procedure was as previously described (Fernell & Rosen, 1956).

Relative nutritive values. These were determined from ranges of N added or from single levels. The values reported in the survey were obtained from analysis of the pooled contents of cultures grown in quadruplicate.

RESULTS

Composition of the basal medium

General. The effect on organism response of varying the levels of groups of basal medium nutrients was studied by use of 'vitamin-free' casein, a protein source on which very little growth occurred in the absence of the purine and pyrimidine, vitamin and mineral groups of the basal medium.

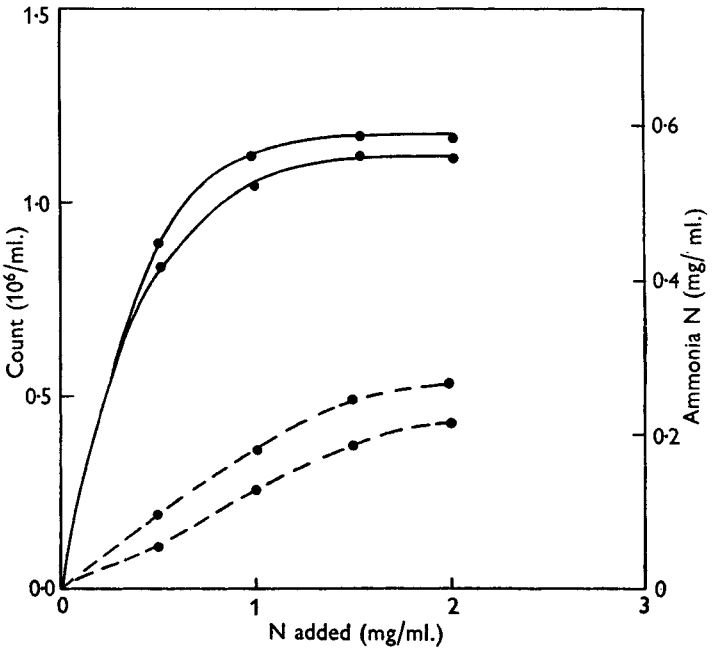

Fig. 1. Effect of fivefold increase in phosphate (lower curve of each pair) on the growth and ammonia production of *T. pyriformis* on 'vitamin-free' casein. ——, count; - - - -, ammonia N. Count: ammonia N, 2·85 at lower level of phosphate; 3·85 at higher level of phosphate; both calculated from integrals for the range 0–2 mg N added/ml.

Phosphates. In the early work on purified proteins the pH of the cultures after 4 days ranged from 4·8 to 8·0. Such variations could conceivably alter proteolytic efficiencies and affect comparisons of protein quality. Preliminary trials with five, ten, twenty-five and fifty times the phosphate level of the basal medium showed that growth was inhibited completely by the twenty-five and fiftyfold and substantially by the tenfold increases. At five times, growth was good and the final pH of cultures on casein, groundnut, soya-bean, egg albumen and herring proteins was much less divergent, lying between 6·3 and 7·5. The effect on population and ammonia production of the fivefold increase in phosphate is illustrated in Fig. 1.

Elevated phosphate increased the count:ammonia N ratio from 2·85 to 3·85 owing to a greater depression of ammonia (*c.* 25%) than of population (*c.* 5%). In view of

the definite advantage in pH control, the higher level of phosphate was adopted and used for all investigations reported hereunder.

Vitamins. Added vitamins in the basal medium were included at 0·001, 0·1, 1 and 10 times the levels in the final medium (see p. 157). Count- and ammonia-response curves are given in Fig. 2 for the range 0–2 mg N/ml.

Tenfold increase of vitamins gave a slight stimulation of count (Fig. 2), but did not appreciably affect the count:ammonia N ratio (Table 1). Lower levels of vitamins resulted in restricted growth, and it is particularly noteworthy that the efficiency of

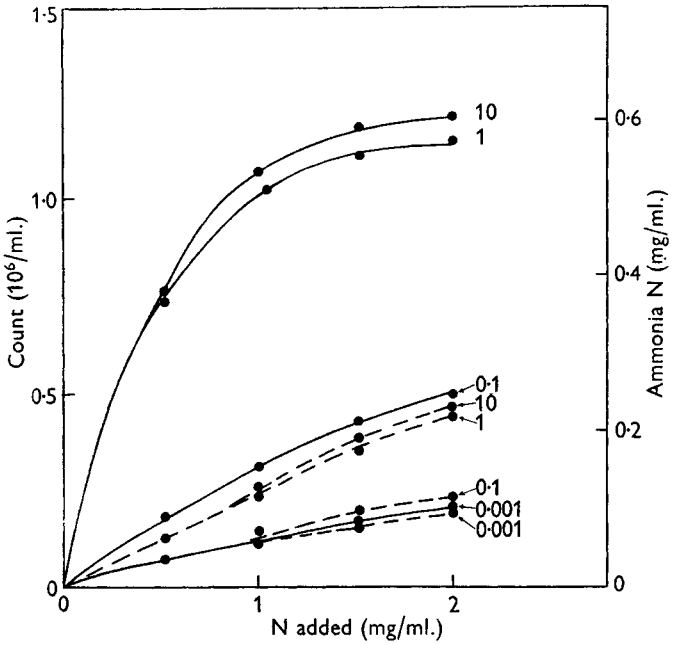

Fig. 2. Effect of changes in the level of vitamins in the basal medium on the growth and ammonia production of *T. pyriformis* on 'vitamin-free' casein. ——, count; - - - -, ammonia N. The figures at the ends of the curves give the relative levels of vitamins (see above).

Table 1. *Effect on count:ammonia N integrals of different levels of basal-medium constituents for growth of* Tetrahymena pyriformis *on 'vitamin-free' casein (with 4% glucose)*

Constituent of basal medium	Level*	Count:ammonia N integral
Vitamins	10	4·06
	1	3·95
	$\frac{1}{10}$	2·45
	$\frac{1}{1000}$	1·08
Minerals B, C†	1	3·72
Minerals B	5	3·91
Minerals C	5	3·80
Purines and pyrimidines	10	4·09
	1	3·95
	$\frac{1}{10}$	2·02

* As a multiple of the level in the basal medium.
† See Fernell & Rosen (1956), Table 1.

utilization of the proteins, as judged by the count:ammonia N criterion, fell sharply in the absence of an adequate supply of vitamins (Table 1).

An additional test on vitamins, in which they were separated into four groups, was carried out as follows:

(1) Known to be absolutely required
Pantothenic acid
Nicotinamide
Pyridoxin hydrochloride
Pyridoxal hydrochloride
Pyridoxamine hydrochloride
Riboflavin
Folic acid
Thiamine hydrochloride

(2) Reported as non-essential for growth on amino-acids
Choline chloride, inositol, *p*-amino-benzoic acid

(3) Suspected stimulant
Biotin

(4) Absolutely required
DL-α-lipoic acid

Levels of these groups were increased two-, four- and eight-fold simultaneously or individually while the other three groups were held at the normal level. Slight stimulation of growth (*c.* 5% increase of count at the 2·0 mg/ml. level) was observed on doubling the levels of (3) and (4), but further increases had no effect. The levels of biotin and lipoic acid in the basal medium were therefore doubled.

Purines and pyrimidines. Levels of 0·1, 1 and 10 times those in the basal medium were tested and the results are quoted in Table 1. It was thus confirmed that the levels used previously were satisfactory.

Minerals. Fivefold increases of the salt components B and C of the basal medium did not affect response, as evidenced by the count:ammonia N ratios in Table 1.

Initial growth studies on protein-containing meals

The behaviour of the organism on suspensions of groundnut meal, groundnut-germ meal and dried whole egg was investigated and compared with growth on a solution of 'vitamin-free' casein, with 4% glucose for all levels of nitrogen.

The meals were defatted with diethyl ether before assay in order to remove free fatty acids, some of which have been reported to inhibit the growth of *T. pyriformis* (Kidder & Dewey, 1951). The meals were ground to pass a 200-mesh B.S. sieve. Nevertheless, difficulties in filling the haemocytometer with fixed culture fluid containing more than 3 mg N added/ml. imposed a restriction on the level used for assays of foodstuffs.

In Fig. 3 are drawn the count- and ammonia-response curves for these four proteins. Table 2 contains the count:ammonia N integrals for the ranges 0–1, 0–2 and 0–3 mg N added/ml. and also ratios calculated from individual values at the 1·0, 1·5, 2·0 and 3·0 mg/ml. levels.

Count:ammonia N ratios for the ranges 0–1, 0–2 and 0–3 mg N added/ml. fell progessively and to a different extent for each protein source. Relative nutritive values therefore depended markedly upon the range selected for comparison, groundnut germ being superior to casein for 0–3 but inferior for 0–1 mg N/ml. Variation in the count:ammonia N integrals and relative nutritive values can be expected from an inspection of the relative shapes of the count:ammonia N response curves (Fig. 3).

It was clearly important, in choosing optimal conditions for protein-quality assays, to investigate further the factors responsible for the marked dependence of count: ammonia N ratio on the level of N added.

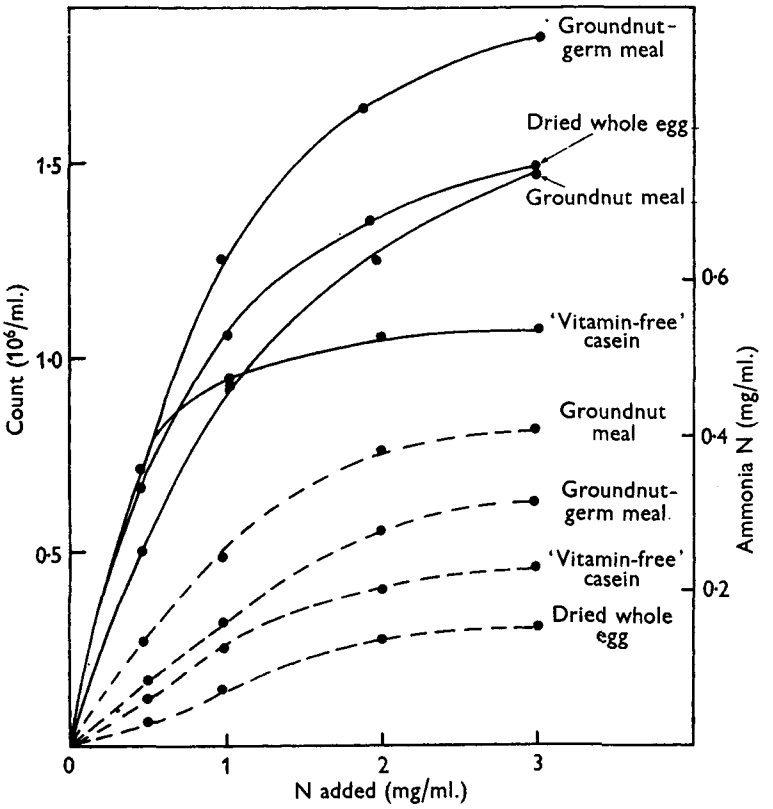

Fig. 3. Growth and ammonia production of *T. pyriformis* on dried egg, 'vitamin-free' casein, groundnut meal and groundnut-germ meal in the presence of 4% glucose. ——, count; - - - -, ammonia N.

Table 2. *Count:ammonia N ratio (C:A) and relative nutritive values (R.N.V.) for* Tetrahymena pyriformis *of 'vitamin-free' casein, dried whole egg, groundnut meal and groundnut-germ meal in a medium containing* 4% *glucose for all levels of N added*

	For ranges of N added (mg/ml.) of:						For single levels of N added (mg/ml.) of:							
	0–1		0–2		0–3		1		1·5		2		3	
Protein source	C:A	R.N.V.	C:A	R.N.V.	C:A	R.N.V.	C:A	R.N.V.	C:A	R.N.V.	C:A	R.N.V.	C:A	R.N.V.
'Vitamin-free' casein	5·8	100	3·8	100	3·1	100	7·2	100	5·8	100	5·2	100	4·7	100
Dried whole egg	8·4	145	6·3	166	5·6	181	15·0	208	11·3	195	9·6	185	10·0	213
Groundnut meal	2·1	36	1·7	45	1·5	48	3·5	49	3·3	57	3·4	65	3·7	79
Groundnut-germ meal	4·7	81	3·6	95	3·3	106	7·9	110	6·5	112	5·9	114	5·7	121

The ratios are calculated throughout the paper as count in millions:ammonia N in mg/ml.

Glucose:protein relationship

Previous work had shown that increase in glucose suppressed ammonia production by *T. pyriformis* (Fernell & Rosen, 1956). This suppression was presumed to result, at least in part, from a sparing action of glucose on the use of amino-acids as an energy source. In the earlier work and in the initial studies on meals, glucose had been invariably included in the media at a level of 4%. In the next series, the supply of glucose and protein was balanced so that the N added:glucose ratio in the medium was constant for all levels of N added.

For convenience, $\left(\frac{\text{N of protein source in mg/ml.}}{\text{Glucose added in mg/ml.}}\right) \times 100$ is referred to as the N:G ratio, and the range studied was from 3·75–30, the extremes representing conditions of excess and deficiency of glucose. The results with this series are given in Table 3.

Table 3. *Count:ammonia N integrals for* Tetrahymena pyriformis *at various N:glucose ratios for 'vitamin-free' casein, dried whole egg and groundnut meal*

N:glucose ratio	Range of N added (mg/ml.)	Count: ammonia N integrals		
		Dried whole egg	'Vitamin-free' casein	Groundnut meal
30	0–1	2·2	2·4	1·6
	0–2	2·6	2·4	1·6
	0–3	2·6	2·4	1·7
15	0–1	3·6	3·8	2·2
	0–2	4·2	3·4	2·2
	0–3	4·1	3·6	2·5
7·5	0–1	9·0	6·0	2·2
	0–2	8·2	4·8	2·4
	0–3	7·8	4·6	2·6
3·75	0–1	12·8	7·2	2·4
	0–2	12·6	7·4	3·0
	0–3*	—	—	—

* Complete inhibition of growth.

With an N:G ratio of 3·75, growth was completely inhibited at the 3 mg/ml. level of N added. This finding is to be expected, since a glucose concentration of 8% is involved.

When the responses were plotted, it was seen that the ammonia-response curves were similar in shape to those of the corresponding count responses; the marked discrepancies in curvature seen in Fig. 3 were no longer manifest.

Inspection of the data in Table 3 reveals that the count:ammonia N (C:A) integrals for ranges 0–1, 0–2 and 0–3 mg N added did not diverge to the same extent as in the previous experiment, when a fixed glucose level was employed (cf. Table 2).

It was therefore concluded that glucose:protein imbalance was largely responsible for the discordant count:ammonia N ratios observed previously at different levels of N given.

It is instructive to consider the count:ammonia N integrals plotted against the N:G ratio (Fig. 4). Relative nutritive values are indicated at various points on the curves and illustrate the convergence of protein qualities under conditions of glucose

deficiency. Under these conditions the pH of the cultures rose progressively during growth to 7·8–8·4, well away from the optimal of 7·0–7·5. Although differences between proteins are accentuated at low N:G ratios (e.g. 3·75), the inhibitory effects of high glucose concentrations (>5%) place a restriction on the range of N that can be given.

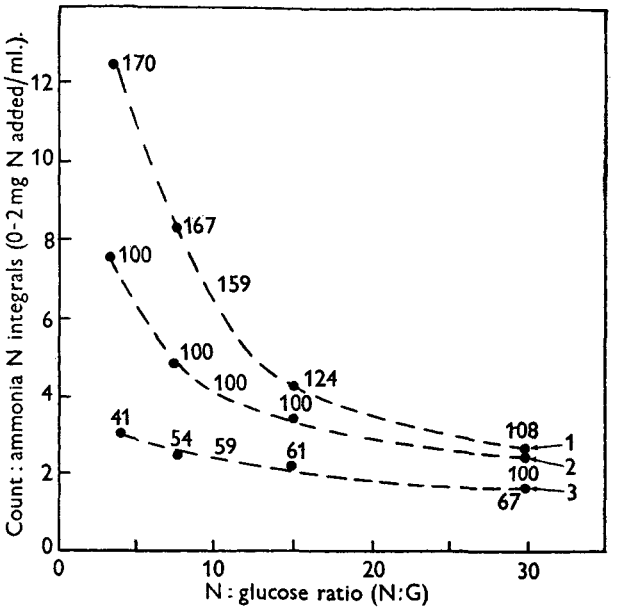

Fig. 4. Effect of variation in the N:glucose ratio (N:G) on count:ammonia N integrals (0–2 mg N/ml.) and on relative nutritive values for *T. pyriformis*. 1, dried whole egg; 2, 'vitamin-free' casein; 3, groundnut meal. Figures on curves give the relative nutritive values.

Survey of protein quality of foodstuffs

Table 4 gives the count:ammonia N ratios for three proteins calculated at the 1·0, 1·5 and 2·0 mg N/ml. levels, and the data offer evidence to support the use of a standard level of N added for assay purposes, thereby reducing the number of analyses. The 1 mg N/ml. level was selected, since it yielded adequate amounts for analysis and substantial populations; though greater populations can be obtained at higher dietary levels, the percentage incorporation of food N into organism tissue is considerably reduced. High conversions to tissue in the organism are desirable, so as to ensure that relative nutritive values are representative of the total protein of the food.

A survey was carried out at 1 mg N/ml. of a wide range of foodstuffs, with glucose at 1·5%, corresponding to an N:G ratio of 6·7. All protein sources save five were ether- and alcohol-extracted to reduce the possible influence of phosphatides, sterols and other lipids. For these sources 0·5% or less of the total N was removed in the alcohol extraction, though dried egg was an exception, as 2% was extracted from it.

As fractions of some of the meals tested were extremely hard and could not easily be ground, a 72-mesh B.S. sieve was substituted for the 200-mesh used previously. A separate test on groundnut meal showed no difference in response for 40–200 mesh.

The results of the survey are set out in Table 5. Noteworthy features were as follows:

(*a*) Vegetable proteins were inferior to animal proteins for *T. pyriformis*.

(*b*) The relative positions of the proteins in the vegetable- and animal-protein groups were in general accord with their chemical scores and ratings for rats.

(*c*) The high nutritive value of groundnut germ should be noted.

(*d*) The deleterious effect of excessive heat on groundnut meal was demonstrated.

(*e*) The whale solubles were inferior to the whale-meat meals as protein sources.

(*f*) Ether–alcohol extracted dried whole egg was inferior to the ether-extracted material.

(*g*) Dried herring muscle, prepared by acetone extraction (*c.* 50% aqueous in the first wash) was inferior to a commercial herring meal. This finding did not apply to acetone-dried cod muscle in relation to white-fish meal.

(*h*) The organism failed to grow on blood meal and on grass-protein concentrate.

(*i*) These results on meals agreed well with our earlier values on partially purified proteins.

Table 4. *Count:ammonia N ratios for* Tetrahymena pyriformis *at single levels of N added at various N:glucose ratios for 'vitamin-free' casein, dried whole egg and groundnut meal*

N:glucose ratio	Level of N added (mg/ml.)	Ratio, count:ammonia N		
		Dried whole egg	'Vitamin-free' casein	Groundnut meal
30	1·0	2·5	2·3	1·5
	1·5	2·6	2·2	1·6
	2·0	2·8	2·4	1·7
15	1·0	4·6	2·8	1·9
	1·5	4·5	2·6	1·9
	2·0	5·0	2·6	2·2
7·5	1·0	7·7	4·7	2·4
	1·5	7·6	4·7	2·6
	2·0	8·5	4·6	2·8
3·75	1·0	11·8	6·7	2·8
	1·5	12·0	7·1	3·4
	2·0	12·0	6·7	3·5

DISCUSSION

Application to foodstuffs, and survey of relative nutritive values

The procedure adopted for assaying foodstuffs was similar in principle to that used for earlier work on the partially purified proteins. The range of dietary N that can be added was smaller, since at levels greater than 3 mg/ml. suspensions were difficult to pipette and an even filling of the haemocytometer cell was achieved only with difficulty. Fine grinding of the samples was advantageous, though response on groundnut meals passing 40, 72 or 200 mesh was not dependent upon particle size. Alcohol washing reduced the relative nutritive value of whole dried egg, but did not have a great effect on other protein sources. The nature of the egg factor is being further investigated; in the present work samples were alcohol-washed before assay. The organism failed to grow only on blood meal and grass-protein concentrates, perhaps owing to the

Table 5. *Relative nutritive values for* Tetrahymena pyriformis* *of proteins in defatted protein concentrates compared with those of partially purified proteins, chemical scores, and net protein utilizations for growing rats (all expressed relative to casein at* 100)

Protein concentrate	Relative nutritive value	Relative nutritive value of partially purified protein (Fernell & Rosen, 1956)	Chemical score†	Net protein utilization (growing rat) †	Net protein utilization (growing rat) ‡
'Vitamin-free' casein	100	100	100	100	—
Bean meal	41	—	—	—	—
Cottonseed meal	78	81	64	78	78
Groundnut-germ meal	98	—	—	—	—
Groundnut meal	50	51	45	82	71
Groundnut meal§	52	—	—	—	—
Groundnut meal (overheated)	36	31	—	—	—
Sesame meal	52	53	67	96	—
Soya-bean meal	80	83	87	106	93
Dried whole egg	124	—	—	137	152
Dried whole egg§	180	—	—	—	—
Herring muscle¶	93	—	—	—	—
Herring meal	130	130	—	—	—
Pilchard meal	127	—	—	—	117‖
Whale-meat meal	148	129	—	—	—
Whale-meat meal§	139	—	—	—	—
Fin-whale meat meal	144	—	—	—	—
Condensed whale solubles§	72	—	—	—	—
Spray-dried whale solubles§	61	—	—	—	—
Cod muscle¶	100	—	—	—	—
White-fish meal	99	—	—	—	113‖
Scottish fish meal	120	—	—	—	97‖
Blood meal Grass meal Grass-protein concentrate	Little or no growth				

* Count:ammonia N for casein (at 1 mg N/ml., 1·5 % glucose) = 5·4.
† Block & Mitchell (1946–7).
‡ Miller & Bender (1955).
§ Assayed without alcohol extraction.
‖ Bender (1954).
¶ Prepared from fresh muscle by acetone extraction and dried at room temperature.

extreme insolubility of these products at pH 7·1. Recently it has been observed that *T. pyriformis* thrives well on media containing cereals, though no assays have yet been made.

With two exceptions, an increase in the levels of the groups of nutrients in the basal medium did not lead to increased response, as judged by count or count:ammonia N ratio. Levels of biotin and α-lipoic acid were doubled, since this multiplication stimulated growth slightly. This medium, with the addition of higher levels of phosphate (see below), was adopted for general use with foodstuffs, though it must be remembered that its efficacy in eliminating non-specific stimulation by known non-protein essential food constituents was demonstrated for 'vitamin-free' casein.

The restriction in growth by suboptimal levels of the vitamin groups was consistent with the deprivation of an essential food factor as in higher animals, and the lower count:ammonia N ratio implied a reduced efficiency in using dietary protein, perhaps owing to changes in metabolism caused by impairment of enzyme mechanisms in which B-vitamins function as coenzymes.

The inclusion of phosphates at five times the level in the basal medium markedly reduced departures of the pH from the initial value of 7·1, as had been manifest in the work on purified proteins. Close control of pH was regarded as vital to the assay; phosphate levels below five times were less effective in their buffering capacity, whereas higher inclusions led to serious restriction of growth. Though the elevated phosphate reduced growth only slightly, it depressed ammonia production appreciably, thereby raising the count:ammonia N ratio. The mechanism of this effect of phosphate, when present in excess of physiological requirements, is not understood, but it may derive from osmotic influence, since it has more recently been observed that the count:ammonia N ratio is also increased by the inclusion of sodium chloride above 0·2%. A change in nitrogenous end-product to urea does not appear to be involved. Further studies are in progress on this aspect.

In the initial studies of the growth of *T. pyriformis* on foodstuffs containing whole protein the marked fall in count:ammonia N integrals with increasing level of N added was analogous to response characteristics on partially purified proteins. It was shown that this was due at least in part to the use of 4% glucose throughout. When the ratio of food N to glucose was constant, this anomaly was much reduced, and confidence in the selection of a standard level of N added for the comparison of protein qualities was thereby increased. A significant and striking observation from the standpoint of comparative protein biochemistry of *T. pyriformis* and higher animals was the convergence of relative nutritive values, due to wasteful utilization of protein under conditions of carbohydrate restriction.

A wide range of different materials was included in the survey of the protein quality of foodstuffs. A general superiority of animal over vegetable sources was manifest and individual ratings agreed well with net utilization characteristics of isolated proteins for growing rats. The inferiority of whale solubles compared with whale-meat meal and that of groundnut compared with soya-bean meal, within the animal and vegetable groups, respectively, are a testimony to the ability of *T. pyriformis* to discriminate between proteins of different qualities. A novel observation was the high rating for groundnut germ, recalling the superiority of wheat germ over whole-wheat protein. It will be interesting to see whether the difference between groundnut protein and germ protein emerges from the results of other assay methods.

Present status of the assay

The demonstration that relative nutritive values for *T. pyriformis* were similar to those for growing rats, and the fact that many well-known facets of protein biochemistry (such as proteolytic inhibition, amino-acid supplementation, protein-carbohydrate and protein–vitamin interrelationships) are displayed by *T. pyriformis*, encourage the belief that microbiological assays of intact proteins will prove of value

in nutritional research, particularly by effecting economies in expensive and time-consuming feeding trials on monogastric animals. Examples of potential applications to current problems are attempts to distinguish between protein damage and gossypol toxicity in cottonseed products, to differentiate in animal-protein concentrates between protein damage and vitamin destruction and to investigate the supplementary relationships of cereal and other proteins.

Simplification of the assay and economies in analysis have been achieved through assaying protein qualities at a single level of N added. Despite the presence of food particles, which tend to increase the difficulty of counting organisms, microscopic count has been retained as the primary manifestation of growth. There is not yet available, in our opinion, any less tedious alternative that can be applied in the presence of food particles and can be related more directly to protein synthesis.

The precision of the assay has not yet been assessed. An indication of the variability of response is available from results of fourteen separate determinations of the count:ammonia N response integral (0–2 mg N/ml.) for 'vitamin-free' casein with 4% glucose, which gave a mean value of 3·70 and a coefficient of variation of 8·7%.

In the application of *T. pyriformis* to the assay of protein quality, it is desirable to seek cultural conditions under which growth is governed primarily by the availability and efficient utilization of essential amino-acids. This condition of growth is particularly important, since morphological and physiological differences between the micro-organism and higher animals, and the inability to effect quantitive separation of ciliates from food residues, rule out criteria based on N balance or retention studies. Progress towards the ideal of limitation of growth by protein quality has been made in the course of these studies. It has been demonstrated that inadequate aeration, restricted supply of vitamins, purines and pyrimidines and glucose, proteolytic inhibition and high concentrations of glucose, phosphates and sodium chloride can restrict growth. The relative importance and interaction of these limiting factors have only been partly clarified. Unknown stimulants may also be important in that whole egg, defatted with diethyl ether, supports at 1–2 mg N/ml. a population some 20–25% greater than the alcohol-extracted egg. Another problem in population restriction arises with 'vitamin-free' casein, for which an increase in N added from 1 to 5 mg/ml. only effects a 15% increase in numbers, whereas under these conditions other proteins support double or triple populations. Further studies are needed for a more precise identification of factors stimulating growth in *T. pyriformis*.

Balancing of N and glucose in the diet reduced appreciably differences in count: ammonia N ratio at different levels of N added. Smaller discrepancies were still apparent, and consideration of the influence of hydrolysis and of glucose utilization at successive stages during growth may be necessary to explain fully the effects observed. However, the criterion of count in relation to ammonia as an index of the efficiency of protein utilization has been shown to differentiate satisfactorily between proteins. Its further value in the microbiological investigation of thermal damage to proteins and mutual supplementation effects is being examined.

An important aspect of future applications of the assay is the selection of conditions whereby near complete hydrolysis of protein can be achieved. In the survey reported,

at a level of 1 mg N added/ml., proteolysis in 4 days amounted to 60–80%. There is a possibility that the protein utilized was not fully representative. For more critical investigations digestibilities of 90% or more are desirable, in the same range as those commonly reported for higher animals. This is particularly important for the investigation of protein mixtures, in so far as unequal digestion of the components in mixtures of lower digestibility might well distort supplementary relationships. Increased digestion can be achieved at lower levels of N added (e.g. 0·5 mg/ml.) or by extending the growth period to 5 days.

Detailed comparison of the results of the biological evaluation of proteins for individual species depends upon a knowledge of digestive and absorptive mechanisms and amino-acid requirements. It is well known that conflicting results for ruminant and monogastric animals are due to a large extent to fundamental differences in primary digestive processes. In the face of the potential advantages of a microbiological assay of protein quality, it is encouraging that the relative nutritive values accord well with results for the growing rat. Very little is known, however, about the quantitative amino-acid requirements of *T. pyriformis* or about factors controlling the relative ingestion of particulate and dissolved nutrients and the relative participation of intra- and extra-cellular proteolytic enzymes of the organism when it is given protein-containing foods. In conclusion, therefore, it must be stressed that the further significance of comparing *T. pyriformis* assays with those on higher animals cannot be assessed until these gaps in our knowledge of the species have been filled.

SUMMARY

1. *Tetrahymena pyriformis* has been used for the microbiological assay of protein quality in a range of twenty different vegetable and animal foodstuffs without prior separation of the protein. Only blood-meal and grass-protein concentrate failed to support growth.

2. The basal medium was selected to eliminate non-specific stimulation by known nutrients when the organism was grown on 'vitamin-free' casein.

3. Differing responses at various dietary levels were considerably reduced by the use of a fixed ratio of N to glucose for all levels of N given.

4. A convergence of relative nutritive values occurred when proteins were assayed in the presence of inadequate amounts of glucose.

5. A survey of nineteen foodstuffs was made, the results agreeing with those of rat assays.

6. These exploratory studies indicate the value of microbiological assays of intact proteins as an adjunct to feeding trials on higher animals.

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The effect of complete hepatectomy on the utilization by rats and rabbits of intravenously administered aqueous dispersions of carotene

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It is now well established that carotene, administered intravenously as an aqueous dispersion, can be converted into vitamin A by rats and rabbits (Bieri & Pollard, 1954; Bieri, 1955; Kon, McGillivray & Thompson, 1955; McGillivray, Thompson & Worker, 1956). The site of conversion has, however, not as yet been determined. It was originally suggested by Mattson, Mehl & Deuel (1947) that parenterally administered carotene might be utilized after being secreted into the intestine by way of the bile. Bieri & Pollard (1954), however, could find no decrease in conversion by rats in which the bile duct had been completely ligated, or in rats from which the small intestine had been removed, and concluded that rats can effectively convert injected carotene at a site other than the small intestine. In further experiments the same authors showed that nephrectomized rats, and rats in which up to 75% of the liver tissue had been removed, formed about as much vitamin A after intravenous injection of carotene dispersions as intact controls. Though it appeared probable from these experiments that the utilization of carotene was independent of the liver, there was still the possibility that sufficient functional liver tissue remained to effect conversion. For this reason it was thought desirable to repeat the experiments in the complete absence of the liver. The results of preliminary experiments involving complete hepatectomy in rats have already been reported (McGillivray *et al.* 1956). The present paper covers subsequent work in this laboratory with hepatectomized and hepatectomized-eviscerated rats and with hepatectomized rabbits.

EXPERIMENTAL

General. The methods and materials were essentially the same as those described by McGillivray *et al.* (1956).

Rats. The rats used in these experiments were inbred albino animals of the Wistar strain from the Massey College small-animal colony, maintained on the basal diet of