The use of hydroxyproline analyses to predict the nutritional value of the protein in different animal tissues

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1. The amounts of available cystine and tyrosine in the protein of different animal tissues showed a close correlation with the level of hydroxyproline, and may be estimated from hydroxyproline values by regression equations.

2. Estimates of 'chemical score' have been calculated from the content of hydroxyproline determined in a series of samples for which net protein utilization (NPU) for rats had also been determined. Chemical scores calculated as percentages of the total 'essential + semi-essential' amino acid content of each material correlated closely with NPU, whereas scores calculated as percentages of total amino acids did not. 'Methionine + cystine' were calculated to be first limiting amino acids in every sample.

The nutritive value of the proteins of different animal tissues which have the same proportion of connective tissue proteins appears to be almost constant. This follows from the close correlation that has been found between the amount of particular available essential amino acids and the amount of hydroxyproline in tissue proteins of slaughter animals, independent of species, age or sex of the animals (Dvořák & Vognarová, 1969*a*, *b*). This may be explained by collagen being the main variable component of animal tissue proteins (Vognarová, Dvořák & Böhm, 1968). Collagen is exceptional among the animal tissue proteins in containing a high level of hydroxyproline but only low levels of most essential amino acids. Therefore it has seemed worth while to investigate whether the level of hydroxyproline in tissue proteins can be used indirectly as a measure of their nutritive value. The regression equations already obtained (Dvořák & Vognarová, 1969*a*) make it possible to obtain an estimate for the content of each essential amino acid merely from knowledge of hydroxyproline and protein content of most tissues, at least of slaughter animals, but the calculation of chemical scores requires estimates of the levels of the semi-essential amino acids, cystine and tyrosine, as well as the fully essential ones. The work reported here was designed first to study the relationship between the levels of these two amino acids and that of hydroxyproline, and then to test the usefulness of 'estimated chemical scores' worked out from a knowledge of hydroxyproline levels alone, as predictors of the net protein utilization (NPU) of materials as determined in feeding experiments with rats.

EXPERIMENTAL

Materials

Animal tissues and organs were taken from the slaughter-house. Each sample represents a mixture of samples from at least seven animals. They were then dehydrated and defatted by three extractions with ethanol, then ethanol–diethyl ether (1:1) and at least three more times with diethyl ether alone at room temperature.

Residual solvents were then removed by spreading the material on filter-paper at room temperature; they were then ground.

As a sample with minimum content of hydroxyproline, the muscle psoas major from a bull, 2 years old, was used, from which all visible portions of extra- and intra-muscular connective tissue were removed. The hydroxyproline content proved to be 0·220 g/16 g nitrogen (or −0·657 on a logarithmic basis).

Two sets of materials were used in this work, one for cystine and tyrosine, and another for rat-feeding studies.

Methods

Hydroxyproline was determined by the procedure of Serafini-Cessi & Cessi (1964), after previous hydrolysis of the samples in 6 M-HCl at 110° for 24 h in sealed tubes. N was determined by a semi-micro-Kjeldahl method (Block, 1960).

Available cystine and tyrosine were assayed microbiologically, after exhaustive enzymic hydrolysis of the proteins by papain, leucine aminopeptidase and prolidase, as previously described (Dvořák, 1968). *Leuconostoc mesenteroides*, P-60, ATCC 8042, was used for microbiological determination: its cultivation and the composition of media were as described by Block (1960). To 5 ml of basal medium, portions of enzyme hydrolysate were added in duplicate, corresponding to 50, 100, 150 and 200 μg N of the original sample. After incubation for 72 h at 38° the growth of micro-organisms was measured by titration with 0·05 M-NaOH. Results were evaluated by the method of Wood (1945) and expressed as g/16 g N. All assays were replicated at least three times.

NPU was determined by the shortened method of Miller & Bender (1955). Male Wistar rats, of 35 g live weight, were distributed four animals to one cage and then acclimatized for 1 week, on a diet whose composition was similar to those used during the assays.

The test diet consisted of dehydrated and defatted animal tissue (as the source of test protein) 6, 8 or 10%, butter 15%, mineral mix 5%, agar 3%, glucose 15%, maize starch 5%, and rice starch 37–41% (to total 100%). Vitamins were mixed with maize starch. To 1 kg of starch were added: 0·06 g thiamin hydrochloride, 0·2 g riboflavin, 0·04 g pyridoxine hydrochloride, 1·2 g calcium pantothenate, 4·0 g nicotinic acid, 4·0 g myoinositol, 0·04 g pteroylmonoglutamic acid, 0·001 g cyanocobalamin and 12·0 g choline chloride. Fat-soluble vitamins were mixed with butter (per kg): 2400 i.u. retinol acetate, 180 i.u. vitamin D and 7·8 mg α-tocopherol acetate. Mineral mixture contained per kg: 46 g NaCl, 94 g NaH_2PO_4, 258 g K_2HPO_4, 146 g $CaHPO_4$, 32 g ferric citrate, 351 g calcium lactate and 72 g $MgSO_4$.

All diets were kneaded to a paste with the addition of a 2·5% solution of agar. From this, pellets were formed and dried for 2 h at 50°, then at room temperature in the air in darkness. Such a diet was prepared a week before use. In the control, N-free diet, tissue proteins were replaced by corresponding amounts of rice starch.

Feeding experiments were continued for 10 d. The N content of the rats in each group was determined in triplicate after they had been dried and ground. The results of experiments carried out at different protein levels are not strictly comparable, but it is hoped that within the range of levels used this effect will be small.

Table 1. *Available cystine and tyrosine in animal tissue proteins*

Tissue		Hydroxyproline (log g/16 g N)	Cystine (g/16 g N)	Tyrosine (g/16 g N)
Fillet	Veal	−0·140	1·48	3·13
	Beef	−0·402	1·60	3·79
Round	Veal	0·161	1·26	2·97
	Beef	−0·239	1·37	3·28
	Pork	−0·159	1·59	3·38
Loin	Veal	0·091	1·16	2·99
'low'	Beef	−0·141	1·38	3·19
'high'	Beef	0·029	1·28	2·78
	Pork	−0·117	1·48	3·57
Shoulder	Veal	0·147	1·16	2·96
	Beef	0·225	0·94	2·46
	Pork	−0·039	1·35	3·15
Neck	Veal	0·339	0·88	2·73
	Beef	0·227	1·17	2·33
	Pork	0·123	1·23	2·96
Flank	Veal	0·428	1·01	2·33
	Beef	0·298	0·98	2·66
Rib	Veal	0·415	0·88	2·43
	Beef	0·214	1·08	2·59
	Pork	0·002	1·30	2·79
Shank	Veal	0·483	0·89	2·20
	Beef	0·555	0·88	2·26
Trotters	Pork	0·409	0·98	2·35
Tongue	Veal	0·306	1·09	2·50
	Beef	0·221	1·09	3·00
	Pork	0·353	0·90	2·40
Heart	Veal	0·014	1·12	2·87
	Beef	0·155	1·13	2·59
	Pork	0·080	1·39	2·91
Liver	Veal	0·007	1·18	3·01
	Beef	0·005	1·15	3·05
	Pork	−0·304	1·71	3·70
Kidney	Veal	0·123	1·02	2·70
	Beef	0·266	1·10	2·44
	Pork	0·052	1·20	2·87
Spleen	Veal	0·162	1·19	2·75
	Beef	0·245	1·00	2·59
	Pork	−0·056	1·23	3·41
Lung	Veal	0·311	0·86	2·13
	Beef	0·529	0·71	2·08
	Pork	0·374	0·92	2·40
Chitterlings	Beef	0·814	0·56	1·56
Udder	Beef	0·684	0·68	2·04

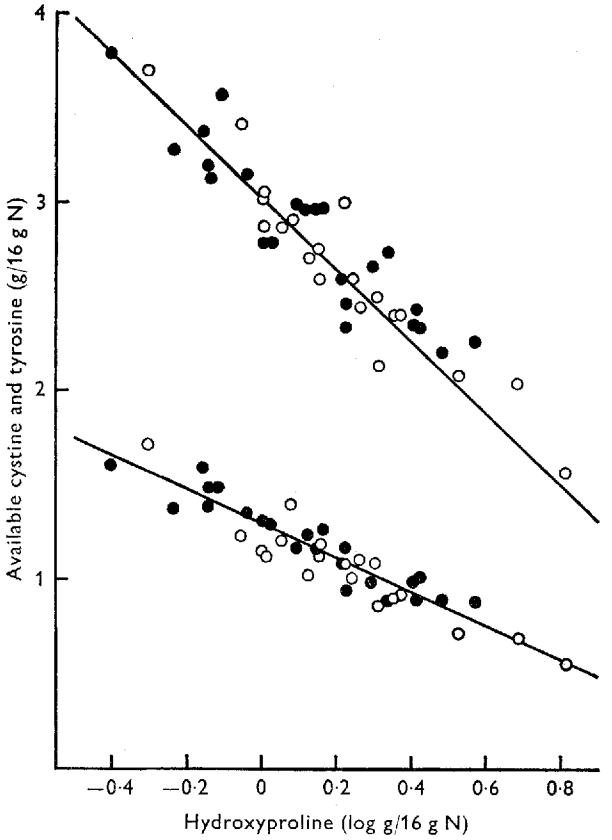

Fig. 1. Relationship between available cystine and tyrosine, and hydroxyproline in animal tissue proteins. Upper relationship, available tyrosine; lower relationship, available cystine. ●, values for muscle tissue; ○, values of other tissues and organs.

RESULTS

The results of the amino acid assays are given in Table 1. The levels of available cystine ranged from 0·56 to 1·71 g/16 g N and of tyrosine from 1·56 to 3·79 g/16 g N.

Generally, the contents of available cystine and tyrosine were linearly related to the content of hydroxyproline, expressed on a logarithmic basis. These relationships are shown in Fig. 1, and may be expressed by the following regression equations:

$$\text{for cystine} \quad Y = 1{\cdot}282 - 0{\cdot}902x; \quad s_{xy} = \pm 0{\cdot}091; \quad r = -0{\cdot}932,$$

$$\text{for tyrosine} \quad Y = 3{\cdot}037 - 1{\cdot}704x; \quad s_{xy} = \pm 0{\cdot}168; \quad r = -0{\cdot}935,$$

where Y = the amount of available cystine or tyrosine, in g/16 g N; x = the amount of hydroxyproline, in log g/16 g N; s_{xy} = standard deviation of the mean of regression straight line; and r = correlation coefficient.

NPU determinations

The results are set out in Table 2 and a scatter diagram relating NPU values and hydroxyproline values on a logarithmic basis is included in Fig. 2.

Table 2. *Estimated chemical score and net protein utilization* (NPU) *of animal tissues*

Test material	Hydroxyproline, (log g/16 g N)	'Estimated chemical score'	Protein, % in the test diet	NPU
Beef tenderloin	−0·657	85·6	8·9	89
Beef tenderloin	−0·402	81·8	8·1	84
Pork liver	−0·335	81·0	8·0	83
Beef round	−0·239	79·5	7·3	82
Beef liver	−0·130	77·8	5·4	77
Pork shoulder	0·000	75·3	5·5	79
Pork neck	0·130	70·4	8·4	77
Beef shoulder	0·230	70·0	7·1	75
Pork shoulder	0·253	67·3	8·2	75
Pork belly	0·403	65·3	5·8	68
Pork belly	0·403	65·3	5·1	60
Pork from the head	0·795	50·6	5·4	49

DISCUSSION

'Chemical score' of tissue proteins as estimated from the content of hydroxyproline

Hen's egg protein was used as the reference standard for calculation of 'chemical scores' and the following analytical values (FAO, 1970), were assumed for it: isoleucine 6·29; leucine 8·82, lysine 6·98, methionine 3·36, cystine 2·43, phenylalanine 5·74, tyrosine 4·16, threonine 5·12, tryptophan 1·49 and valine 6·85 g/16 g N. These values correspond to a total content of 'essential + semi-essential' amino acids equivalent to 51·24 g/16 g N.

For the first method of calculating 'chemical scores', the ratio A:E is given by the expression $(A_x \times E_e)/(A_e \times E_x)$ (FAO, 1970), where A_x and A_e are the available amounts of a particular amino acid in the protein investigated and in hen's egg protein, respectively, in g/16 g N; E_x and E_e represent the total amount of 'essential + semi-essential' amino acids in the protein investigated and in hen's egg protein, respectively. 'Chemical score' A:E is given by the lowest of these ratios.

The best estimates of the levels of particular essential amino acids in tissue proteins with different amounts of hydroxyproline were found in previous work (Dvořák & Vognarová, 1969*a*), to come from the following regression lines:

for isoleucine, $Y = 5{\cdot}107 - 2{\cdot}447x \quad s_{xy} = \pm 0{\cdot}211,$
for leucine, $Y = 6{\cdot}902 - 2{\cdot}996x \quad s_{xy} = \pm 0{\cdot}194,$
for lysine, $Y = 8{\cdot}032 - 2{\cdot}578x \quad s_{xy} = \pm 0{\cdot}169,$
for methionine, $Y = 2{\cdot}205 - 1{\cdot}537x \quad s_{xy} = \pm 0{\cdot}163,$
for phenylalanine, $Y = 4{\cdot}023 - 1{\cdot}279x \quad s_{xy} = \pm 0{\cdot}150,$
for threonine $Y = 4{\cdot}177 - 1{\cdot}478x \quad s_{xy} = \pm 0{\cdot}123,$
for tryptophan, $Y = 1{\cdot}128 - 0{\cdot}772x \quad s_{xy} = \pm 0{\cdot}082,$
for valine, $Y = 5{\cdot}089 - 1{\cdot}816x \quad s_{xy} = \pm 0{\cdot}181,$

where Y = the content of available amino acid, in g/16 g N; and x = the amount of hydroxyproline, in log g/16 g N.

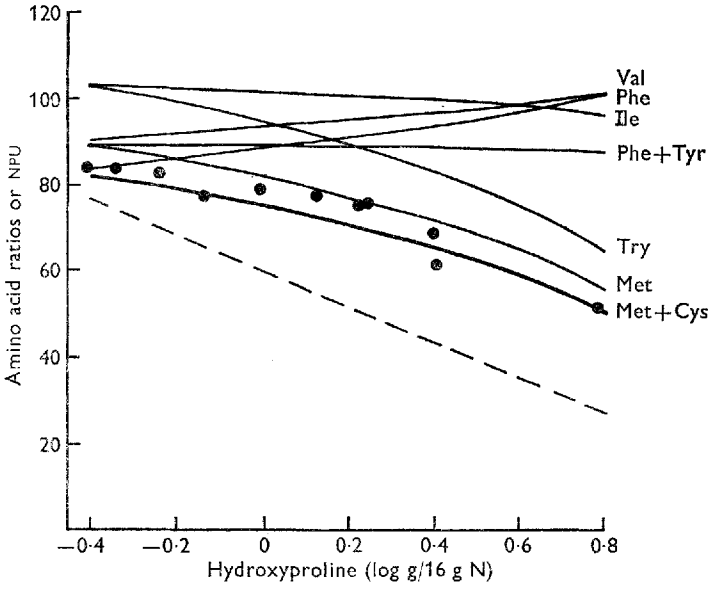

Fig. 2. Relationship between amino acid ratios A:E, A:T, net protein utilization (NPU) and hydroxyproline in animal tissue proteins. ——, ratios A:E; thick line represents ratios A:E of limiting amino acids; – – –, ratios E:T of methionine+cystine as limiting amino acids; ●, NPU values tabulated in Table 2.

Thus it follows that the estimate of total 'essential+semi-essential amino acids' (g/16 g N) for a given value of x is $41{\cdot}0-17{\cdot}5x$. The resulting estimates of A:E for animal tissue according to their 'x' content for individual essential amino acids and pairs of 'essential+semi-essential amino acid', all expressed as percentages of the corresponding values for egg protein, are set out in Fig. 2. Values for lysine, threonine and leucine are omitted as they are all approximately 100% or over. It is seen that, over the whole range, the apparently limiting amino acids are 'methionine+cystine'. It is seen from Fig. 2 that the 'estimated chemical scores' calculated in this way are good predictions of the actual NPU values, plotted on the same figure, and further unpublished experiments indicate that 'cystine+methionine' are, in fact, the limiting factors in these test materials.

Since the combined regression equation for 'cystine+methionine' is $Y = 3{\cdot}487-2{\cdot}439x$, the predicted 'chemical score', expressed as

$$\frac{A_x}{E_x}\times\frac{E_{egg}}{A_{egg}}\times 100 = \frac{3{\cdot}487-2{\cdot}439x}{41{\cdot}0-17{\cdot}5x}\times\frac{51{\cdot}24}{5{\cdot}79}\times 100 = \frac{123{\cdot}3\,(1{\cdot}43-x)}{(2{\cdot}34-x)}.$$

The corresponding calculations of 'chemical score' A:T expressed as % of the total crude protein ($N\times 6{\cdot}25$) in test material and egg respectively give the A:T relationship with 'x' shown in Fig. 2. Cystine+methionine again appear as the first limiting amino acids throughout but these values appear to underestimate the actually determined NPU values considerably.

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