J. Physiol. (1948) 107, 383–398 612.398.145:612.351

THE ASSAY OF THE NUTRITIVE VALUE OF A PROTEIN BY ITS EFFECT ON LIVER CYTOPLASM

By ROSA M. CAMPBELL and H. W. KOSTERLITZ

From the Department of Physiology, University of Aberdeen

(*Received* 1 *July* 1947)

Addis and his collaborators were the first to examine in some detail the relationship between dietary protein intake and the protein content of the liver. Addis, Lee, Lew & Poo (1940) found that liver protein increased with rising casein intake. Yuen, Poo, Lew & Addis (1940) showed that serum albumin, serum globulin or yeast protein were less effective in raising liver protein than colostrum protein, casein and lactalbumin which, in turn, were inferior to liver and kidney proteins.

Kosterlitz (1944) showed that the quantity of cytoplasm present in the liver cell was dependent on the quality and quantity of dietary protein, and suggested that the estimation of labile liver cytoplasm may lend itself to a rapid assessment of the biological or nutritive values of proteins. Since that time, various procedures which may achieve this have been examined in detail with successful results (Kosterlitz, 1944–6; Kosterlitz & Campbell, 1945, 1946). Harrison & Long (1945) also followed up this suggestion and fully confirmed and extended the data given in our previous papers. Their method was based on the regeneration of protein of rats' livers following a 48 hr. fast.

METHODS

Analytical methods. These have already been described in a previous paper (Kosterlitz, 1947).

Experimental procedures. For each experiment 4 or 5 female rats, 3·75–4·5 months old, were selected from the colony. Their average body weight was 221 g., ranging between 181 and 260 g. The mean body weight of each group of 4 or 5 rats varied between 210 and 230 g. If two proteins were compared with each other, litter mates were equally distributed between the two test diets; if a protein was compared with casein, for which a regression line had been constructed, not more than 2 rats of any one litter were placed on the test diet, in order to prevent the results from being unduly biased by a particular litter.

Three different procedures were employed. In procedure 1 the rats were transferred from the stock diet directly to the test diet which was given for 7 days. The basal diet consisted of 2% agar, 3% salts, 25% sucrose, 60% potato starch and 10% lard (Kosterlitz, 1947). The lard was supplemented with vitamins A, D, and E, and the B vitamins were given partly in the form of the pure chemical compounds and partly as liver concentrate. In procedure 2 the rats were given the

protein-free basal diet for 4 days, in order to deplete the liver of its 'labile' cytoplasm, and were then given the test diet for 4 days. Finally, procedure 3 had the same general arrangement as procedure 2, but no liver concentrate was given and 0·5 ml. containing the following water-soluble vitamins was given daily: 5 μg. biotin (Merck), 5 μg. folic acid (Folvite, Lederle), 20 μg. synkavit dicalcium phosphate (Roche), 30 μg. aneurin, 30 μg. pyridoxin, 50 μg. riboflavin, 100 μg. calcium *d*-pantothenate, 100 μg. nicotinic acid, 100 μg. *p*-aminobenzoic acid, 1 mg. inositol and 17·3 mg. choline hydrochloride.

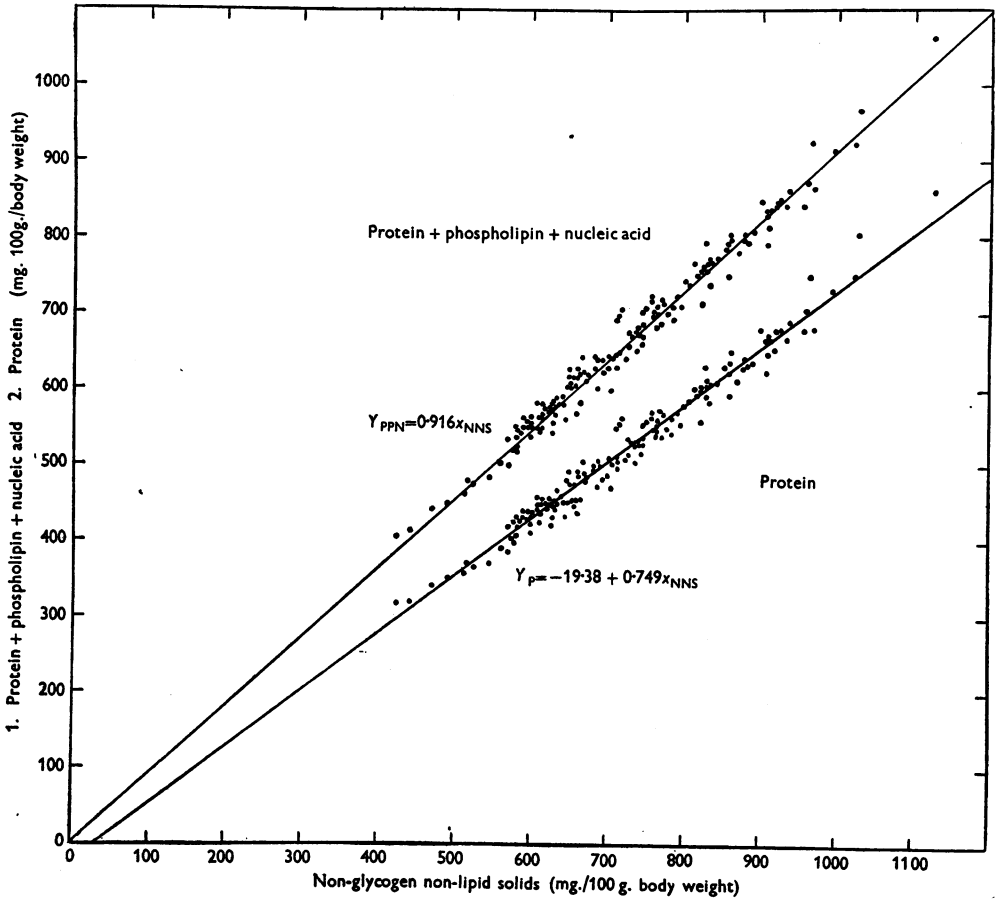

Fig. 1. Regression of protein + phospholipin + nucleic acid (y_{PPN}) and of protein (y_P) on non-glycogen non-lipid liver solids (x_{NNS}).

Estimation of 'labile liver cytoplasm'. The amount of cytoplasm present in the liver may be determined by estimating the protein, phospholipin or ribonucleic acid contents (Kosterlitz, 1944, 1947). The methods available for these estimations, although not difficult, are rather time-consuming. Harrison & Long (1945) used the total N content of the livers as an estimate of their protein contents, but this is open to certain objections. By determining the non-glycogen non-lipid solids (NNS) of the livers, values are obtained which, in female rats 4 months old, show a very high correlation with the protein + phospholipin + nucleic acid contents (PPN) and only a slightly less high correlation with the true protein values (P) (Fig. 1). The variable x_{NNS} accounts for

97·9 % of the variance of y_{PPN} and for 97·2 % of the variance of y_P. The standard deviation of Y_{PPN} predicted for values of $x_{NNS} = 725 \pm 100$ varies between 1·42 and 1·79 and that of Y_P between 1·35 and 1·71. Thus, in female rats of the strain used in these experiments, NNS are an excellent predictor for protein and for protein + phospholipin + nucleic acid. Labile NNS represent the difference between total and non-labile NNS; the latter are constant for rats of a given age and sex.

The estimation of NNS is technically relatively simple: the glycogen and the total fatty acids (including unsaponifiable matter) are deducted from the total solids. The total solids can be determined with a high degree of accuracy and any errors made in the estimations of glycogen and total fatty acids have little effect on the final results since, under the conditions of these experiments, total fatty acids and glycogen account for only a small part of the total solids, viz. 10–15 and 10–25 % respectively. Further, with this method, information is obtained regarding the effects of various dietary proteins on the fatty acid and particularly the glycogen contents of the livers.

Calculation of results. All results are given for 100 g. initial body weight; this is calculated as the mean of the weights found on the first day of the test and the 2 days before it.

The nutritive value (NV) of a protein was computed as follows. From the regression equation for casein, the amount of casein N was calculated which would have produced the same quantity of (total) NNS as did the test protein N actually fed. The NV of the test protein was then mg. casein N/mg. test protein N. If the mean daily food intake was below 7 g./100 g. body weight, a correction of +46 mg. NNS/g. food deficit had to be applied (cf. Table 2). As will be shown later in this paper, a reduction in calorie intake causes, by itself, a decrease in NNS. It should be noted that the term 'nutritive value' (NV), as used in this paper, corresponds to the 'net utilization value' and not to the 'biological value' of the balance-sheet method (Block & Mitchell, 1946–7). NV and 'net utilization value' are calculated in terms of ingested protein N, and 'biological value' in terms of protein N actually absorbed.

Statistical treatment of the results. The variances of NNS within groups receiving the same type of diet did not differ significantly among themselves, with the exception of the groups receiving the stock and flour diets. The variances within the groups other than cereal and stock diets were pooled to give a combined estimate of 1348·2, obtained with 173 degrees of freedom. The variance within the groups receiving flour diets by procedure 1 and 2 was 2359 (36 degrees of freedom) and within the groups receiving flour diets by procedure 3, 504 (16 degrees of freedom). So far, no explanation can be offered for these differences in variability. The variance within the group receiving the stock diet was 4329 (19 degrees of freedom), very significantly larger than the variance of the non-flour diets. This was possibly due to the fact that the food intake of the rats on the stock diet was not controlled.

If not expressly stated otherwise, the symbols used in the regression equations of this paper are as follows: Y stands for predicted (total) NNS (mg./100 g. body weight), x for the logarithms of the daily protein N intakes (mg./100 g. body weight), and t for days on the protein-free diet.

RESULTS

Changes in the composition of the liver caused by varying the intake of protein

In the experiments in which varying quantities of casein were given by procedure 1, the mean daily food intake varied between 6·65 and 7·3 g./100 g. body weight with an overall mean of 7·0 g. In order to save space, the results are not given in detail; they may be summarized as follows. The weight and the water content of the livers did not show any significant changes when the casein N intake varied between 50 and 280 mg./100 g. body weight, while both values increased when the casein N intake was between 380 and 760 mg./100 g. body weight. The protein and phospholipin contents, and to a less extent the nucleic acid content, increased with rising casein N intake. There were no

significant changes in the glycogen and neutral lipid contents of the livers. The phospholipin indices (phospholipin × 100/protein + phospholipin + nucleic acid) fell slightly, and the protein indices rose slightly, when the protein intake was above 200 mg. N/100 g. body weight, but otherwise showed no significant changes. The nucleic acid indices decreased with increasing protein intake; this is in agreement with the view that only ribonucleic acid takes part in these changes (Kosterlitz, 1944, 1947; Campbell & Kosterlitz, 1947). Substitution of part of the carbohydrate of the diet by fat did not lead to any important changes in the composition of liver tissue. Apart from a slight increase in the neutral lipid content, the results obtained by feeding 5·75 g./100 g. body weight of a 30% casein diet containing 40% lard were not significantly different from those obtained by feeding the diet containing only 10% lard.

Diets deficient in tryptophan led to considerable losses in protein, phospholipin and nucleic acid (labile cytoplasm) from the liver. If appetite was markedly depressed, the liver weight fell simultaneously as in the case of acid-hydrolysed casein. If, on the other hand, the rats ate comparatively well, as, for example, in the case of zein, increased amounts of liver glycogen were deposited and the liver weight was only slightly diminished. Deficiency of lysine was very much less effective in decreasing labile liver cytoplasm than was tryptophan deficiency.

It has been shown previously (Kosterlitz, 1944, 1947) that rats fed on a protein-free diet for 4 days lost practically all labile liver cytoplasm. Addition of 0·6% *dl*-methionine or of 0·38% *l*-tryptophan did not prevent this. There was, however, an unexpected increase in the glycogen and neutral lipid contents of the livers when methionine was added to the basal protein-free diet. When tryptophan was added, the neutral lipid content increased but the glycogen content was smaller than in the rats fed on the basal protein-free diet only. The liver weights showed corresponding changes.

Disappearance of labile liver cytoplasm in protein deficiency

Further observations on the relation of NNS to time in female rats transferred from the stock diet to a protein-free diet indicate that the loss of NNS during the first few days is better described by a simple exponential function of time than by the combined exponential and linear function of Campbell & Kosterlitz (1946). The recalculated equation is

$$Y = 620 + 254\ (0{\cdot}33)^t,$$

the first term on the right representing non-labile NNS (mg./100 g. body weight) and the second the amount of labile NNS after t days on the protein-free diet (Fig. 2). It follows that the labile NNS falls to 9·1 mg./100 g. body weight after 3 days and to 3 mg./100 g. body weight after 4 days on the protein-free diet.

Diets deficient in single essential amino-acids, or in calories, were less efficient in removing labile NNS than was a protein-free diet (Fig. 2). If rats had lost their labile NNS by being fed on a protein-free diet, 2 days of refeeding with a 20% casein diet almost completely restored their labile NNS (Fig. 3). For this reason, in procedure 2, rats were first fed on a protein-free diet for 4 days in order to free their livers of labile NNS, and then given the test diet for 4 days. No experiments were specially designed to ascertain whether, in

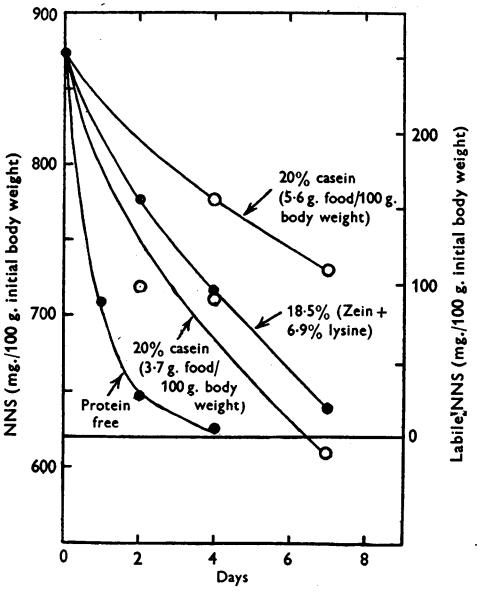

Fig. 2.

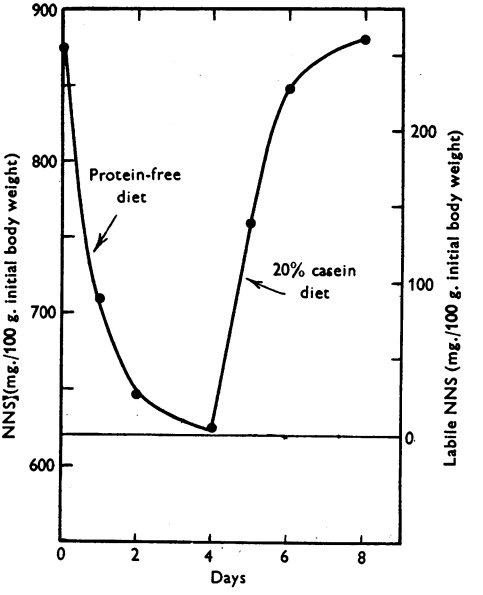

Fig. 3.

Fig. 2. Rate of disappearance of labile liver cytoplasm. The curves were drawn by eye with the exception of that for the protein-free diet, which fits $Y = 620 + 254\,(0{\cdot}33)^t$. Each point represents the mean of at least 4 rats.

Fig. 3. Rate of breakdown and formation of labile liver cytoplasm. Each point represents the mean of at least 4 rats.

procedure 1, the duration of 7 days on the test diet was sufficient to ensure equilibrium of labile NNS. However, as will be shown later, the regression coefficients for casein obtained by procedures 1 and 2 did not differ significantly, and in the tests with flour proteins no difference was found between the two procedures.

Although, on theoretical grounds, procedure 2 may have been expected to be more sensitive than procedure 1, no such difference was found. From a practical point of view, procedure 2 was superior to procedure 1 in that less protein was required for the test and, in the case of deficient and unpalatable diets, the rats were more likely to eat their daily ration.

Quantitative relationships between casein intake (at different levels of food intake) and NNS

Casein was chosen as a standard for the evaluation of the nutritive values of proteins. Although it is not a complete protein, it is readily available in sufficiently pure grade.

When the NNS (mg./100 g. body weight) of rats fed on different quantities of casein were plotted against the logarithms of the daily casein N intakes (mg./100 g. body weight) straight lines were obtained (Fig. 4). The regression lines found by procedures 1 and 2 did not differ significantly.

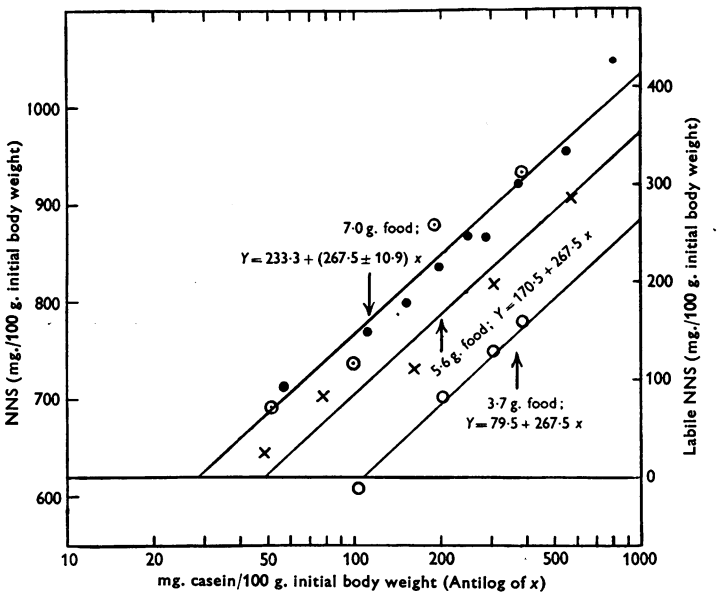

Fig. 4. Regression lines for casein for different calorie intakes. Each point represents the mean of at least 4 rats. ●, 7 g. food/100 g. body weight (procedure 1); ⊙, 7 g. food/100 g. body weight (procedure 2); ×, 5·6 g. food/100 g. body weight (procedure 1); ○, 3·7 g. food/100 g. body weight (procedure 1).

When the daily food intake was lowered from 7·0 to 5·6 or 3·7 g./100 g. body weight, the regression coefficient did not alter, but the NNS values obtained for a given casein intake fell with decreasing food intakes. The three lines thus obtained differed very significantly as regards vertical distance. If the NV of casein at 7·0 g. food intake is taken as 1·00, the apparent NV's at 5·6 and 3·7 g. food intake are 0·58 and 0·26 respectively. On the other hand, at 8·5 g. food intake the apparent NV is the same as at 7·0 g. so that 7·0 g. may be taken as the minimal food intake which will assure full utilization of the proteins (Table 1).

TABLE 1. Effect of feeding 5 and 10% casein diets at the rate of 26 or 30 Cal./100 g. body weight on the non-glycogen non-lipid liver solids (procedure 1)

Diet	No. of rats	Mean daily intake of Food (g.)*	Mean daily intake of Casein N (mg.)	Non-glycogen non-lipid liver solids (NNS) (mg.)	Values calculated from regression line (mg.)
5% casein	4	7·3	55	731±19·8	699±7·4
5% casein	4	8·45	61	691±19·8	710±7·0
10% casein	3	7·45	101	755±22·9	769±5·5
10% casein	4	8·5	118	781±19·8	788±5·1

* All estimations are in g. or mg./100 g. initial body weight.

The distances between the three regression lines suggest that the effect of the reduction in food intake on labile NNS may be a linear one. The decrease in NNS between 7·0 and 5·6 g. food amounted to 62·8 mg./1·4 g. or 44·8 mg./g. food deficit, and that between 5·6 and 3·7 g. was 91 mg./1·9 g. or 47·8 mg./g. food deficit. An approximate correction can thus be applied in the evaluation of the nutritive values of the proteins by adding for 1 g. mean food deficit/100 g. body weight, 46 mg. NNS/100 g. body weight to the mean NNS value obtained in the test.

For the purpose of calculation of the calorific value of diets, the coefficient of digestibility was determined for potato starch by the method of Mayerhofer (König, 1910) and found to be 68·9±2·6% (10 estimations). By allowing for 100 g. potato starch with 18·2% moisture 242 Cal., for 100 g. sugar 400 Cal., for 100 g. lard 925 Cal., and for 100 g. casein (N=13·35%) 348 Cal., the following calorific values were obtained for 7 g. diet: protein-free 23·8, 5% casein 24·4, 10% casein 24·6, 20% casein 25·3. Therefore, 24–25 Cal./100 g. body weight was considered to be the minimal calorie requirement for a full utilization of the proteins fed. (In a preliminary communication (Kosterlitz & Campbell, 1946) the minimal calorie requirement was erroneously given as 27–30 Cal./100 g. body weight.) It is not possible to give an estimate of the calorific value of the high-casein diets as the digestibility of the casein has not been determined. In any case, any decrease in the digestibility of casein would probably be offset by the smaller amount of poorly digestible potato starch in the high-casein diets.

Effects of nitrogenous constituents of liver extract and potato starch

In procedures 1 and 2, small quantities of amino-acids may be introduced by the liver concentrate. Therefore, 12 animals were fed by procedure 3 in which the water-soluble vitamins were given as pure chemical compounds. At the same time, the potato starch was replaced by sucrose. The concentrations of casein in the diet were 10, 23 and 54% (Fig. 5). The regression coefficient of 327·7±41·8 was not significantly different from that of the casein lines obtained previously. The difference in vertical distance between this line and that for

casein with liver extract and potato starch is $31{\cdot}2 \pm 11{\cdot}6$, which is significant at the 1% level. These results suggest that amino-acids in the liver concentrate and possibly also the potato starch raise the nutritive value of casein above its true figure.

The nutritive values (NV) of various proteins

Egg albumin (procedure 1). In the case of egg albumin, sufficient values (16) were available to construct a regression line and compare it with that obtained for casein (Fig. 5). The regression coefficient was found to be $253{\cdot}0 \pm 29{\cdot}4$, which

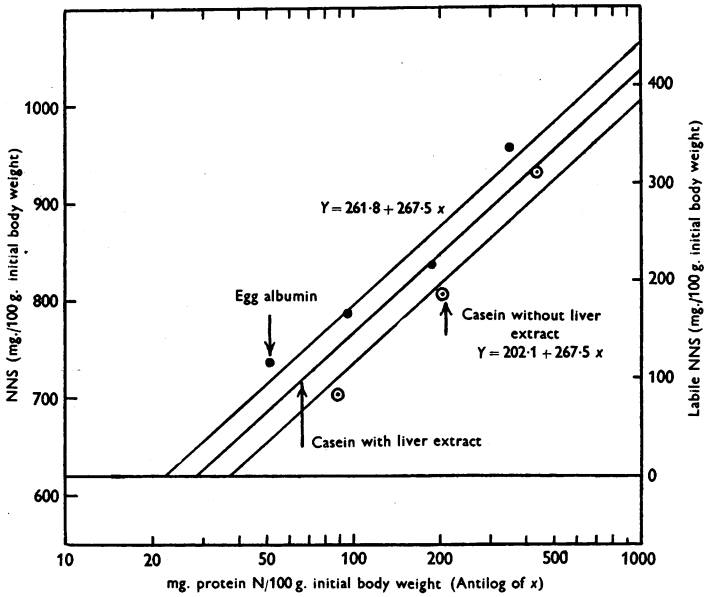

Fig. 5. Regression lines for egg albumin, casein with and without liver extract. Each point represents the mean of at least 4 rats.

did not differ significantly from that of casein, 267·5. The difference in vertical distance was $28{\cdot}5 \pm 10{\cdot}24$, which was significant at the 1% level. The calculation of the nutritive value gives NV = 1·28, indicating that egg albumin is significantly superior to casein.

Zein (procedures 1 and 2). The results with zein, zein supplemented with either tryptophan or lysine or with both, are summarized in Tables 2 and 3. Insufficient data are available to construct regression lines in these cases. Zein was tested at the 18 and 55% levels of intake, and NV's of 0·29 and 0·22 respectively were obtained. Since the difference between the two NV's was not significant, a pooled NV of 0·25 was obtained. Supplementation with lysine alone did not affect the NV, yielding a value of 0·23. Supplementation with both tryptophan and lysine raised the NV to 0·89, which is not significantly different from that of casein. Supplementation with 1·82% *l*- or *dl*-tryptophan

TABLE 2. Estimation of non-glycogen non-lipid liver solids and assessment of the nutritive values of zein, with and without the addition of tryptophan and lysine (procedure 1)

Diet	No. of rats	Change in weight during exp. (g.)	Mean daily intake of: Food (g.)*	Mean daily intake of: N (mg.)	Total solids (mg.)	Glycogen (mg.)	Total fatty acids (mg.)	Non-glycogen non-lipid liver solids (NNS): Found (mg.)	Non-glycogen non-lipid liver solids (NNS): Corrected for inadequate food intake (mg.)	Daily casein N required to produce effect of test protein (mg.)	Nutritive value of test protein (significance of difference from casein = 1)
18·5% zein	4	−10	6·65	183	978	152	146	680±18·1	696	53·7	0·29 (SS)†
55·5% zein	4	−14	6·6	533	999	116	116	767±18·1	785	115·7	0·22 (SS)
18·5% zein supplemented with 6·9% *l*-(+)-lysine hydrochloride	4	−17	6·45	178	871	118	115	638±18·1	663	40·4	0·23 (SS)
18·5% zein supplemented with 1·82% *l*-tryptophan	4	−7	7·0	192	1065	166	139	760±18·1	—	93·1	0·49 (SS)
18·5% zein supplemented with 1·82% *l*-tryptophan and 6·9% *l*-(+)-lysine hydrochloride	4	−3	7·0	191	1028	65	133	830±18·1	—	169·8	0·89 (NS)

* All estimations are in g. or mg./100 g. initial body weight.

† (NS), not significant; (SS), significant at the 1% level.

TABLE 3. Assessment of the nutritive value of zein supplemented with 1·82% *dl*-tryptophan (procedure 2)

Concentration of zein supplemented with 1·82% *dl*-tryptophan in diet	No. of rats	Changes in weight during experiment (g.)	Mean daily intake of: Food (g.)*	Mean daily intake of: N (mg.)	NNS (mg.)	Nutritive value of test protein (significance of difference from casein = 1)
4·6	4	$\frac{-7}{+2}$†	$\frac{7·5}{7·45}$	$\frac{3·3}{56}$	676±18·1	0·82 (NS)‡
9·3	5	$\frac{-9}{+1}$	$\frac{7·35}{7·35}$	$\frac{3·5}{104}$	748±16·2	0·81 (NS)
18·5	5	$\frac{-13}{+7}$	$\frac{7·4}{6·9}$	$\frac{3·3}{188}$	758±16·2	0·51 (SS)
30·0	4	$\frac{-4}{-3}$	$\frac{7·35}{5·9}$	$\frac{3·4}{266}$	812±18·1	0·84 (NS)

* All estimations are in g. or mg./100 g. initial body weight.

† The values above the horizontal line were obtained during the protein-free period and those below the line during the test period.

‡ (NS), not significant; (SS), significant at the 1% level.

alone raised the NV to 0·5 when the diet contained 18·5% zein. On the other hand, at zein levels of 4·6, 9·3 and 30%, supplementation with 1·82% *dl*-tryptophan raised the NV to approximately 0·8. Because of the small number of animals used in the tests at the different levels of supplemented zein intake, the difference between 0·5 and 0·8 is just significant at the 5% but not at the 1% level. If, in Table 3, all NV values are pooled, a combined value of 0·736 is obtained which is very significantly lower than the NV of casein. If only the values for 4·6, 9·3 and 30% zein, supplemented with tryptophan, are pooled for the estimation of NV, a value of 0·822 is found which is just significantly lower (at the 5% level) than that of casein. Further, the values given in Table 2 for zein supplemented by tryptophan alone and for zein supplemented by tryptophan and lysine differ very significantly in favour of the latter. From these results it may be concluded that tryptophan deficiency causes a very marked lowering of NV, while lysine deficiency has a very much smaller but probably still significant effect.

Soya bean proteins (procedure 1). The isolated soya bean proteins had a surprisingly low NV of 0·38, which was only slightly raised to 0·48 by supplementation with 1·2% *dl*-methionine (Table 4). Because of the small number of animals used, this difference is not statistically significant. The smallest difference in NV which would have been significant is 0·14.

Protein hydrolysates (procedure 1). An acid hydrolysate of casein which had a tryptophan content of only 0·3% had a NV of 0·42 (Table 4). This could be raised by supplementation with *dl*-tryptophan. Three different levels were used: 0·38 and 0·77% *dl*-tryptophan were definitely insufficient, while the hydrolysate supplemented with 1·54% tryptophan had a NV of 0·79 which did not differ significantly from the NV of casein. Another acid hydrolysate of casein supplemented with 1% *dl*-tryptophan had a NV of 1·09. It would appear that a tryptophan content of 1·2–1·5% is required in the hydrolysate, a figure which agrees well with the values obtained by the analysis of intact casein. In enzymatic hydrolysis of casein tryptophan is not destroyed; the only enzymatic hydrolysate which was tested had, however, a slightly lowered NV (0·72). A commercial enzymatic digest of meat had the same NV as casein.

Proteins of flours of different extractions (procedures 1, 2 and 3). The flours tested were samples of the 'K' series of flours used by Chick, Copping & Slack (1946). They were milled from a grist of mixed wheat. Their nitrogen contents were equalized by adding potato starch in the first and second series of experiments and by adding sucrose in the third series. Further, in the experiments with whole wheat meal, the daily food intake was raised from 7·5 to 7·85 g./100 g. body weight in order to allow for the low content of utilizable calories in whole wheat (McCance, Widdowson, Moran, Pringle & Macrae, 1945). The content of utilizable calories was well above the minimum requirements, viz. 28·5 Cal./7·5 g. diet in the case of the 70, 80 and 85% extraction flours and

28·5 Cal./7·85 g. diet in the case of the whole wheat flour. In procedure 1, the weights of the rats remained constant; in procedures 2 and 3 the recovery in body weight during the test period was not significantly affected by the different extraction rates of the flours.

TABLE 4. Assessment of the nutritive values of various proteins and protein hydrolysates (procedure 1)

Protein tested	No. of rats	Change in weight during experiment (g.)	Mean daily intake of Food (g.)*	Mean daily intake of N (mg.)	NNS (mg.)	Nutritive value of test protein (significance of difference from casein = 1)
Soya bean proteins	4	− 6	7·1	196	734	0·38 (SS)†
Soya bean proteins supplemented with 1·2% *dl*-methionine	4	0	6·7	184	741	0·48 (SS)
Acid hydrolysate of casein	4	−23	5·2	144	624	0·42 (SS)
Acid hydrolysate of casein supplemented with 0·38% *dl*-tryptophan	4	−10	6·6	180	736	0·48 (SS)
Acid hydrolysate of casein supplemented with 0·77% *dl*-tryptophan	4	− 3	7·05	193	776	0·55 (SS)
Acid hydrolysate of casein supplemented with 1·54% *dl*-tryptophan	4	− 2	6·2	171	766	0·79 (NS)
Commercial acid hydrolysate of casein supplemented with 1% *dl*-tryptophan	4	− 1	7·5	204	861	1·09 (NS)
Commercial enzymatic hydrolysate of casein	5	− 7	7·3	102	733	0·72 (S)
Commercial enzymatic hydrolysate of meat	5	+ 3	7·1	195	840	0·95 (NS)

* All estimations are in g. or mg./100 g. initial body weight.
† (NS), not significant; (S), significant at the 5% level; (SS) significant at the 1% level.

No significant differences were found between the NNS values obtained by procedures 1 and 2; therefore the results of the first and second series of experiments were pooled (Table 5). None of the results differed significantly from any other, although the differences between the 70 and 85% extraction flours ($33 \pm 17{\cdot}2$ mg. NNS/100 g. body weight) almost reached significance at the 5% level. The minimum difference in NV which could have been considered significant was 25·9%. Thus the difference in NV between the 70 and 85% extraction flours can be taken to be less than 25·9%. The relatively low sensitivity of the flour tests was due to a high variance of error ($S^2 = 2359$) which was significantly greater than that for the non-flour experiments ($S^2 = 1348$).

In the third series of experiments in which 70 and 100% extraction flours were compared, procedure 3 was employed, in which the whole liver concentrate was replaced by biotin, folic acid, *p*-aminobenzoic acid and vitamin K. The difference of $5{\cdot}0 \pm 10{\cdot}6$ between the two flours was not significant; because

of the low variance of error ($S^2 = 504$) in this series, a difference of 22·5 mg. NNS/100 g. body weight would have been significant. Thus, a difference in NV of 17·6% would have been detectable.

TABLE 5. Assessment of the nutritive values of the proteins of flours of different extraction rates

Extraction rate of flour (%)	No. of rats	Mean daily intake of Food (g.)*	Mean daily intake of N (mg.)	NNS (mg.)	Nutritive value of flour proteins (significance of difference from casein = 1)
		First and second series combined			
70	15	$\frac{7\cdot4\dagger}{7\cdot45}$	$\frac{3\cdot4}{130}$	755±12·5	0·70 (SS)‡
80	10	$\frac{7\cdot25}{7\cdot35}$	$\frac{3\cdot3}{129}$	770±15·4	0·79 (NS)
85	17	$\frac{7\cdot45}{7\cdot4}$	$\frac{3\cdot4}{130}$	788±11·8	0·92 (NS)
		Third series			
70	9	$\frac{7\cdot3}{7\cdot4}$	$\frac{0\cdot4}{124}$	762±7·5	0·77(SS)§
100	9	$\frac{7\cdot4}{7\cdot85}$	$\frac{0\cdot4}{126}$	767±7·5	0·79(SS)§

* All estimations are in g. or mg./100 g. initial body weight.

† The values above the horizontal line were obtained during the protein-free period and those below the line during the test period.

‡ (NS), not significant; (SS), significant at the 1% level.

§ The nutritive values of 70 and 100% extraction flours, third series, would be raised to 1·00 and 1·02 respectively by using for calculation the regression line for casein obtained by procedure 3.

DISCUSSION

Two methods have been widely used for the evaluation of the nutritive values of proteins. The first determines, in growing rats, the maximum ratio of increase of body weight to protein consumed, known as 'protein efficiency ratio' (Osborne, Mendel & Ferry, 1919). The second method (balance-sheet method) measures the percentage of absorbed nitrogen retained for maintenance and, in young rats, for growth, known as the 'biological value' (Mitchell, 1924).

The method described in this paper has, in the first instance, been designed for the evaluation of the nutritive value of proteins in adult rats. A comparison may therefore be made with the balance-sheet method. In Mitchell's method the determination of the endogenous urinary nitrogen and of the faecal metabolic nitrogen may cause considerable difficulties and, if not done with great caution, vitiate the results. On the other hand, the standard deviation of the results is small in experienced hands; Mitchell, Burroughs & Beadles (1936) state that they can determine the biological values with a standard error of only 1·2. Statistical evaluation of results obtained from other laboratories shows that this low coefficient of variation is not always found.

In the method described in this paper, the standard deviation of the actual estimations of the NNS is reasonably small, and corresponds to a value of

$\pm 9{\cdot}2$ for the standard error of a mean estimated from 16 animals. This is only 1–1·5% of mean NNS values in the usual range of 700–900 mg./100 g. body weight. On the other hand, because of the logarithmic relationship between protein intake and NNS, the coefficient of variation of the NV values is considerably greater, viz. 7·4% for a mean obtained from 16 estimations.

A reduction of this relatively high variability may possibly be obtained by replacement of the liver concentrate with synthetic biotin and folic acid. This was adopted in procedure 3 and seemed to have the desired effects in the flour experiments. Further, it may be possible that the ratios of protein/deoxyribonucleic acid may have a smaller variance of error than NNS; the estimation of these ratios would, however, require rather complicated chemical techniques. Lastly, in the male rat the regression coefficient for NNS on log casein intake is about 75% greater than in the female (Campbell & Kosterlitz, 1946), so that it may be preferable to use male rats.

The relatively low sensitivity of the new method is its only serious drawback. Its advantages are the following: The technique is simple and rapid; this has also been stressed by Harrison & Long (1945). No assumptions have to be made, as, for example, in the 'balance-sheet' method, with regard to the constancy of the excretions of endogenous nitrogen; the statistical treatment of the results is therefore uncomplicated. The effect of an insufficient calorie intake on the nutritive value of a protein can be studied quantitatively. The test can be made at any level of protein intake above minimum requirement while the biological value determined by the balance-sheet method decreases with rising protein intake. Thus, it has been found to be advantageous to use protein concentrations between 10 and 20%. From a practical point of view, the new method is particularly suitable for the 'screening' of proteins or protein preparations when a rapid assay is required without need for great accuracy.

Harrison & Long (1945), following up the suggestion made by one of us with regard to the assay of the nutritive values of proteins (Kosterlitz, 1944), fasted rats for 48 hr., and then fed them for 4 days on a diet containing the protein to be tested. During this period, male rats weighing 250–280 g. were given 12 g. of a diet containing 26% fat on the first day and 10 g. on each of the subsequent 3 days. Thus, the calorie intake was considerably lower than the 24–25 Cal./100 g. body weight which may be considered as the minimum for the full utilization of proteins. For this reason, Harrison and Long's method is not quite as sensitive as our method, and diets containing 10% casein or 10% lactalbumin did not cause any 'regeneration' of liver protein. In principle, however, the results obtained by Harrison & Long and those by ourselves are in good agreement.

The nutritive values obtained with the new method agree fairly well with those obtained with the older methods. Gelatin has a very low NV (Kosterlitz,

1947). Tryptophan deficiency causes a serious fall in the NV. Acid-hydrolysed casein, which is deficient in tryptophan, has a low NV which can be raised to normal by the addition of about 1% *dl*-tryptophan.

Absence of lysine from a protein causes a decrease of the NV which appears to be just significant. According to Neuberger & Webster (1945) lysine is required for the maintenance metabolism of the adult rat, a view which is not shared by Mitchell (1947). Frazier, Wissler, Steffee, Woolridge & Cannon (1947) found that weight recovery of protein-depleted adult rats is impossible in the absence of lysine. The reduction of labile liver cytoplasm in lysine deficiency suggests that absence of this amino-acid from the diet of the adult rat interferes with its protein metabolism, although a considerable amount of labile liver cytoplasm is present even in the complete absence of lysine. It is of interest that the same amount of NNS is found in lysine deficiency whether the rats are transferred directly from the stock diet to the test diet or are first given a protein-free diet and then the test diet. In the latter case labile liver cytoplasm is built up in the absence of dietary lysine which therefore has been obtained from other tissues of the body.

No significant difference could be detected between flours of 70, 80, 85 and 100% extraction. This finding is in good agreement with those of Henry & Kon (1945); although these authors found a decrease in the biological values of the proteins of bread with decreasing extraction rates, the 'net utilization' values (= biological value × true digestibility × 10^{-2}) indicate no difference between the various extraction rates. Our results on the adult rat are not necessarily in disagreement with the results obtained by Chick, Copping & Slack (1946) who, in growth experiments, found a superiority of the high extraction flours.

A comparison of the NV's of some of the proteins tested in this paper shows a fairly good agreement with the 'net utilization' values obtained by Mitchell's method (Table 6). For this purpose, the NV's have been multiplied by 68, the 'net utilization' value of casein.

TABLE 6. Comparison of net utilization values obtained by the balance-sheet method with nutritive values obtained by the liver cytoplasm method

	NV	NV × 68	Net utilization*
Egg albumin	1·28	87	83 (growing rat)
Casein	1·0	68	68 (growing rat)
Whole wheat	0·79	54	61 (growing rat) 69 (adult rat)
White flour	0·73	50	52 (growing rat)
Soya bean flour	0·38	26	72 (growing rat) 49 (adult rat)

* Block & Mitchell, 1946–7; Mitchell, 1947.

The effect of reducing calorie intake on the nutritive value of a protein is of considerable interest. It has, of course, been known for a long time that, unless sufficient calories are supplied in the diet, part of the protein will be utilized for

energy requirements. Nevertheless, 'labile' NNS are still present in calorie deficiency, although with decreasing food intake more and more protein N is required to obtain the same effect. That the regression coefficient is not significantly affected by the reduction in calories means that the response of the liver to increasing protein intake is only quantitatively but not qualitatively altered. It was found that about 24 Cal./100 g. are required for a 4-month old female rat to utilize fully the protein of the diet. Reductions of the calorie intake by 20 and 47% caused a fall of the NV from 1·0 to 0·58 and 0·26, respectively; this indicates that a relatively small deficiency in calories will lead to serious protein deficiency. From the practical point of view, these findings suggest that in conditions of shortage of both protein and calories, attention should first be paid to a sufficient supply of calories. On the other hand, if, for pathological reasons, the ingestion of the normal quantities of food should become impossible, large quantities of protein will have to be administered; thus, if in the rat the food intake is reduced by half, the protein concentration will have to be raised eight times in order to obtain the quantity of NNS found when the food intake is normal.

Under the conditions of the experiments described in this paper, in which adequate quantities of choline were supplied, only minor changes were found in the neutral lipid contents of the livers, while there were great variations in the glycogen contents. The glycogen contents were often but not always raised when the NV of the test protein was low, for example in the case of zein or zein supplemented with tryptophan only. If lysine was also added, the glycogen content was lowered to values usually found in casein or stock diets. No such excessive deposition of glycogen was found when soya bean proteins of a low NV were fed. It is possible that these differences between the proteins may be due to variations in the glycogenic properties of the constituent amino-acids.

SUMMARY

1. A new method is described for the assay of the nutritive value of proteins in adult rats. It is based on the fact that the amount of labile cytoplasm present in the liver depends on the quantity and quality of dietary protein. Labile liver cytoplasm can be estimated by a determination of protein, phospholipin and nucleic acid or of the non-glycogen non-lipid liver solids (NNS).

2. The quantity of NNS varies directly with the logarithm of the protein intake. The method can therefore be used at all levels of protein intake above minimum requirement. Regression equations are given for casein with normal and reduced calorie intake, and for egg albumin.

3. A reduction in calorie intake lowered the amount of NNS without altering the regression coefficient. Decreases of 20 and 47% in calorie intake reduced the nutritive value of casein from 1 to 0·58 and 0·26 respectively. The implications of these findings are discussed.

4. Nutritive values (NV) of various proteins have been determined and found to be in fairly good agreement with those obtained by the older methods. If the NV of casein was taken to be 1·0, zein had a NV of 0·25 which was not altered by the addition of lysine. Addition of tryptophan to zein caused a marked improvement, and the NV of zein supplemented by both tryptophan and lysine was not significantly different from that of casein. Acid hydrolysates of casein had a low NV due to tryptophan deficiency; addition of 1% *dl*-tryptophan restored the NV to its original level. The NV of egg albumin of 1·28 was significantly higher than that of casein. No significant differences were found between the proteins of flours of 70, 80, 85 and 100% extraction rate.

We wish to thank Dr D. N. Lawley, who kindly carried out most of the statistical analyses, for his valuable help and advice throughout this investigation. We are indebted to the Medical Research Council for grants for scientific assistance and expenses (to H.W.K.). It is gratefully acknowledged that the following materials have been placed at our disposal: wheaten flours of different extraction rates by Dr H. Chick and Dr T. F. Macrae, *l*-(+)-lysine dihydrochloride and *dl*-methionine by the Protein Requirements Committee of the Medical Research Council, *l*-(+)-lysine hydrochloride, *dl*-tryptophan and soya bean proteins by Ashe Laboratories, Ltd. (Dr W. E. Gaunt), liver concentrates and *dl*-tryptophan by Glaxo Laboratories, Ltd. (Mr A. L. Bacharach and Mr F. A. Robinson), folic acid by Lederle Laboratories, Inc. (Dr S. M. Hardy), biotin by Merck & Co. Inc. (Dr R. T. Major) and Synkavit dicalcium phosphate by Roche Products, Ltd.

REFERENCES

Addis, T., Lee, D. D., Lew, W. & Poo, L. J. (1940). *J. Nutrit.* **19**, 199.
Block, R. J. & Mitchell, H. H. (1946–7). *Nutr. Abstr. Rev.* **16**, 249.
Campbell, R. M. & Kosterlitz, H. W. (1946). *J. Physiol.* **105**, 33 *P*.
Campbell, R. M. & Kosterlitz, H. W. (1947). *J. Physiol.* **106**, 12 *P*.
Chick, H., Copping, A. M. & Slack, E. B. (1946). *Lancet*, **1**, 196.
Frazier, L. E., Wissler, R. W., Steffee, C. H., Woolridge, R. L. & Cannon, P. R. (1947). *J. Nutrit.* **33**, 65.
Harrison, C. & Long, C. N. H. (1945). *J. biol. Chem.* **161**, 545.
Henry, K. M. & Kon, S. K. (1945). *J. Soc. chem. Ind., Lond.*, **64**, 227.
König, J. (1910). *Chemie der menschlichen Nahrungs- und Genussmittel.* **3**, pt. 1, 441. Berlin: Julius Springer.
Kosterlitz, H. W. (1944). *Biochem. J.* **38**, *Proc.* xiv; *Nature, Lond.*, **154**, 207.
Kosterlitz, H. W. (1944–6). Unpublished reports to the Protein Requirements Committee of the Medical Research Council.
Kosterlitz, H. W. (1947). *J. Physiol.* **106**, 194.
Kosterlitz, H. W. & Campbell, R. M. (1945). *J. Physiol.* **104**, 16 *P*.
Kosterlitz, H. W. & Campbell, R. M. (1946). *Nature, Lond.*, **157**, 628.
McCance, R. A., Widdowson, E. M., Moran, T., Pringle, W. J. S. & Macrae, T. F. (1945). *Biochem. J.* **39**, 213.
Mitchell, H. H. (1924). *J. biol. Chem.* **58**, 873.
Mitchell, H. H. (1947). *Arch. Biochem.* **12**, 293.
Mitchell, H. H., Burroughs, W. & Beadles, J. R. (1936). *J. Nutrit.* **11**, 257.
Neuberger, A. & Webster, T. A. (1945). *Biochem. J.* **39**, 200.
Osborne, T. B., Mendel, L. B. & Ferry, E. L. (1919). *J. biol. Chem.* **37**, 223.
Yuen, D. W., Poo, L. G., Lew, W. & Addis, T. (1940). *Amer. J. Physiol.* **129**, 685.