

## A note on the method of Miller & Payne for prediction of protein value

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The validity of the equation given by Miller & Payne (1961 *a*) for the prediction of protein values from chemical scores rests upon three assumptions: (1)  $NPU_{(op)}$  decreases linearly with increasing content of protein calories in the diet; (2) the regression line extrapolates to the value of zero for  $NPU_{(op)}$  at the same concentration of protein calories ( $P_z = 54\%$ ) for all protein sources; and (3) the amount of protein calories required for maintenance ( $P_m$ ) is given by  $P_m = 400/NPU_{(st)}$ .

The method seems to give good prediction of net dietary protein values (ND-pv) of a variety of protein sources (Miller & Payne, 1961 *a, b*). The prediction equation is, however, credited with more than empirical significance: the value of  $P_z$  referred to above is given a definite biological meaning in relation to the specific dynamic action of protein (Miller & Payne, 1961 *a*) and the prediction equation is derived from a new theory of protein metabolism (Miller & Payne, 1961 *b*). In view of this it seems warranted to examine the biological significance of the three assumptions upon which the prediction equation is based.

Table 1. *Values for the concentration of protein calories (P) of the diet when the regression equation between NPU and P becomes zero ( $P_z$ ), together with  $NPU_{(10\%)}$  calculated from the regression equations*

Protein source	$P_z$	$NPU_{(10\%)}$	Reference
Beef powder	52.6	72	} Miller & Payne (1961 <i>a</i> )
Casein	54.4	62	
Wheat gluten	56.7	37	
Whole egg	43	92	} Forbes <i>et al.</i> (1958)
Casein	51	76	
Groundnut meal	53	58	
Soya flour: no. 1	44	53	} Barnes <i>et al.</i> (1946)
no. 2	43	52	
Wheat gluten	81	30	

The experimental evidence given by Miller & Payne (1961 *a*) seems to substantiate the first assumption, but does not do so convincingly with the second. It is convenient to discuss these two points together since the problems they pose are interconnected.

In Table 1 are given the values for  $P_z$  found by Miller & Payne (1961 *a*) using their own data and those of Forbes, Vaughan & Yohe (1958). Together with each value for

$P_z$  is given a value for  $\text{NPU}_{(10\%)}$  which was calculated from the regression equation of NPU on the content of protein calories in the diet ( $P$ ). This value for  $\text{NPU}_{(10\%)}$  is intended as a measure of protein quality. It is seen that  $P_z$  decreases with improved protein quality of the protein source. In Table 1 are included also results obtained by calculation from those of Barnes, Bates & Maack (1946). The NPUs were calculated from the data of these authors for body-nitrogen gains and their estimates for the maintenance requirements. The calculated value of  $P_z$  for wheat gluten was much higher than those for the soya flours. The data for whole-egg protein could not be used for calculation of the regression equation because NPU was virtually constant at about 90% at four of the seven protein levels tested.

The fact that the regression equation of NPU on  $P$  extrapolates to zero NPU at gradually lowered values for  $P_z$  as the quality of the test protein improves is in accord with *a priori* expectations: the better the protein quality the lower the protein level at which the saturation point for the protein utilization would be expected to be attained, which is substantiated by the data for gain of body protein given by Barnes *et al.* (1946). The saturation level seemed to be at about 9% protein calories for whole-egg protein and at about 13% for the two soya flours. For wheat gluten the body-protein gain increased gradually up to the 38% level, which was the highest tested.

An important question is whether the linear description of NPU in terms of  $P$  possesses biological significance other than that of describing the results reported. An answer may probably be obtained by comparing experimental evidence with inferences drawn from a combination of this description with an equation derived from the original definition of NPU (Block & Mitchell, 1946-7). One formulation of NPU is the following (Njaa, 1959):

$$\text{NPU} = D - 100(U - U')/EC = D - 62.5(U - U')/EfP. \quad (1)$$

In this equation the symbols are:  $D$ , true digestibility (%);  $E$ , food intake (g/rat);  $P$ , protein calories (%);  $fP$ , protein content of diet (%);  $C$ , nitrogen content of diet ( $(fP \div 0.625)$  mg/g);  $EC = I$ , nitrogen intake;  $U$ , urinary nitrogen;  $U'$ , endogenous urinary nitrogen (the three last mg/rat);  $f$ , proportionality factor relating protein content (%) to protein calories (%) on the assumption that protein is incorporated in the diet at the expense of carbohydrate on an isocaloric basis. In diets containing at most 10% of ingredients with no fuel value and at least 5% fat, the minimum value for  $f$  would be 0.96.

The linear regression equations relating NPU to  $P$  are of the following form (Miller & Payne, 1961*a*; Forbes *et al.* 1958):

$$\text{NPU} = b - aP, \quad (2)$$

where  $b$  and  $a$  are constants.

Combination of equations (1) and (2) gives:

$$100U/EC = 62.5U/EfP = (D - b) + aP + 62.5U'/EfP. \quad (3)$$

Equation (3) indicates that the apparent urinary recovery of ingested nitrogen passes through a minimum at the value of  $P_{\min} = \sqrt{(62.5U'/afE)}$  when the food intake

is equalized. This inference is in accord with the observation of Hamilton (1939), who used the paired-feeding technique, that  $100U/EC$  was lower at a level of about 10% protein calories than below and above this level, and with the observation of Njaa (1959), who used equalized food intake, that values for  $100U/EC$  determined at a level of about 6.2, 8.4 and 10.6% protein calories were not significantly different. Equation (3) further indicates that, above  $P_{\min}$ ,  $100U/EC$  would be expected to increase more

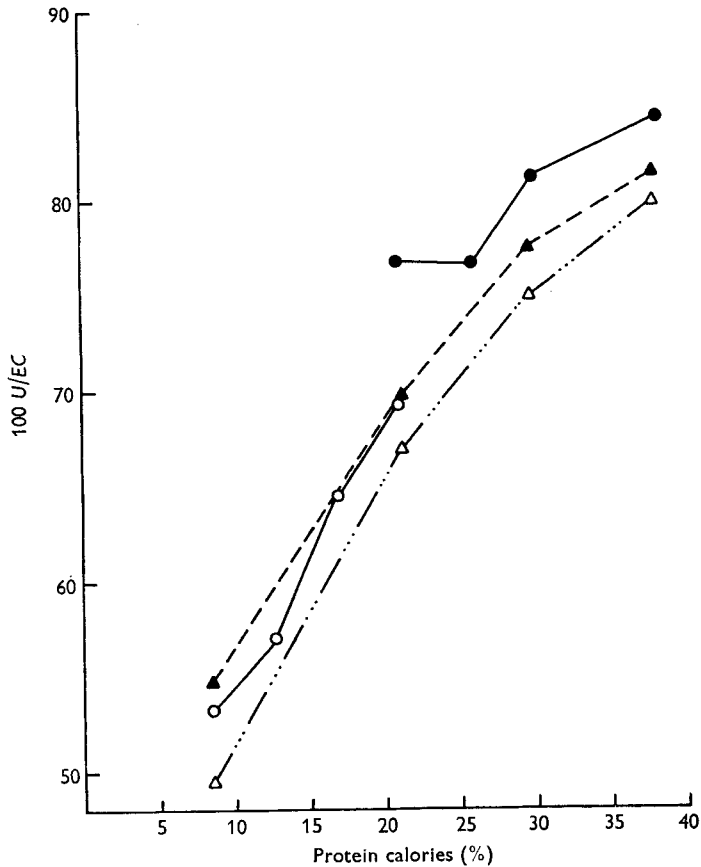


Fig. 1. Apparent urinary recovery of ingested nitrogen ( $100 U/EC$ ). Curve 1,  $\circ$ — $\circ$ , and curve 2,  $\bullet$ — $\bullet$ , were drawn from the data of Forbes *et al.* (1938) related to uniform food intake. Curve 3,  $\blacktriangle$ — $\blacktriangle$ , and curve 4,  $\triangle$ — $\cdots$ — $\triangle$ , were drawn from the data of Forbes *et al.* (1939) and relate to lower and higher food intakes, respectively.

rapidly with increasing  $P$ , but available literature data (Forbes, Voris, Bratzler & Wainio, 1938; Forbes, Black, Thacker & Swift, 1939) indicate that the rate of increase of  $100U/EC$  becomes less as  $P$  increases (Fig. 1). NPUS calculated on the basis of such nitrogen excretion data as those given in Fig. 1 would tend to level off at the higher protein levels if it is assumed that  $D$  and  $U'$  in equations (1) and (3) are independent of the protein level. Levelling off of NPU at high protein levels is in accord with the results of Forbes *et al.* (1958) and of Dreyer (1960). The values of  $b$  and  $a$  obtained would therefore depend upon the levels of  $P$  at which NPUS were determined, but there

is nothing to indicate that the ratio  $b/a$  would remain constant. Moreover, any single determination of  $b/a$  would be subject to large error since  $b$  is determined by extrapolation and  $a$  (the regression coefficient) is based on a limited number of observations.

The possible biological significance of assuming a constant value for  $P_z$  may be assessed from a combination of equations (1) and (2) when NPU is zero. It follows that NPU is zero when  $U - U' = fEDb/62.5a = fP_zED/62.5$ , or when the urinary nitrogen excretion above the endogenous level becomes equal to the amount truly absorbed. The definite meaning of  $P_z$  as the protein content at which this condition is attained requires no assumption about the protein metabolism. However, Miller & Payne (1961*a*) regard  $P_z$  as the quantity complementary to the percentage specific dynamic action of protein. This assumed significance of  $P_z$  seems doubtful because if all the truly absorbed nitrogen were excreted in the urine, no distinction would be possible between protein used for heat production and protein used for other purposes. The reasoning of Miller & Payne (1961*a*) implies that they do not regard  $P_z$  only as an abstraction, but that they really assume NPU to become zero at a definite protein content of the diet ( $P_z = 54\%$ ) for all protein sources. In this connexion it may be mentioned that Njaa & Brækkan (1953) observed good growth over 10 weeks in young rats given a casein diet containing about 57% protein calories.

The third assumption is that the protein calories required for maintenance are given by  $P_m = 400/\text{NPU}_{(\text{st})}$ . It may be tested against the original definition of NPU written in the following form:

$$\text{NPU} = \text{Bal } \% + 100(F' + U')/EC = \text{Bal } \% + 62.5(F' + U')/EfP, \quad (4)$$

where  $\text{Bal } \% =$  nitrogen or protein balance and  $F' =$  metabolic faecal nitrogen.

$\text{NPU}_{(\text{st})}$  is defined as the NPU determined for maintenance only, that is when  $\text{Bal } \% = 0$ . Therefore

$$\text{NPU}_{(\text{st})} = 62.5(F' + U')/EfP_m = 400/P_m. \quad (5)$$

If the excretion of metabolic faecal nitrogen is 2 mg/g food (Mitchell, 1948; Metta, 1960),  $U'$  would be given by

$$U' = 2E(3.2f - 1). \quad (6)$$

According to their body-weight the young rats used in experiments for determination of NPU would probably eat between 6 and 12 g food/day, that is to say, if  $f$  is about unity an endogenous excretion between about 25 and 50 mg/rat daily would be expected. For higher values of  $f$ ,  $U'$  would be correspondingly higher. This is certainly higher than the values conventionally accepted (Njaa, 1959), which in turn would indicate that  $P_m$ , as estimated by Miller & Payne (1961*a*), would be too high. It is noted that Allison, Wannemacher, Middleton & Spoerlein (1959) found that the maintenance level of nitrogen intake is similar for proteins of widely different value. The import of these considerations for the prediction equation of Miller & Payne (1961*a*) is that the value used for  $P_z$  (54%) is probably too low for protein sources with low scores and too high for sources with high scores. For intermediate scores the value of  $P_z$  would probably be approximately correct. The values calculated for  $P_m$  and used in the equation would probably always be too high, but to what extent is difficult

to assess. The effects on the calculated ND-pv would be rather complicated. Partial differentiation demonstrates that when  $P_z$  is underestimated, the effects of erroneous  $P_z$  and  $P_m$  would tend to balance one another, and when  $P_z$  is overestimated both effects would tend towards a too high calculated ND-pv. The picture is, however, complicated by the fact that the relative effects of the two sources of error would be influenced by the protein level of the diet for which the calculation was made. The fact that the calculated ND-pvs reported by Miller & Payne (1961 *a, b*) agree well with the observed values indicates that the balancing effect of the three factors  $P$ ,  $P_m$  and  $P_z$  must be of importance within the range of protein scores tested.

Recently, Miller & Payne (1961 *b*) advanced a new theory for protein metabolism from which an equation of the form of the prediction equation (Miller & Payne, 1961 *a*) was derived.

The explicit assumptions on which the theory is based may certainly be questioned; however, in relation to the present discussion, the implicit assumptions seem to be more important. It is implied that for a protein source the efficiency of utilization,  $b$ , is independent of the protein level (Miller & Payne, 1961 *b*, equations 8 and 9) and that the proportionality factor ( $k$ ) relating the fraction of food protein diverted for energy to the protein content of the diet is independent of the protein source and of the efficiency of utilization (Miller & Payne, 1961 *b*, equation 7). In terms of equation (2) the NPU of a protein source would be given by:

$$\text{NPU} = b - aP = b(1 - kP). \quad (2')$$

Thus the assumed constant efficiency of protein utilization ( $b$ ) would be equivalent to the constant term in the regression equation ( $b$ ). It has been pointed out above that the constant term in the regression equation would vary according to the levels of  $P$  at which the values of NPU were obtained.

It is concluded that theoretical objections may be raised against the basic assumptions made by Miller & Payne (1961 *a*) when developing the prediction equation for ND-pv. The method seems, however, to give good predictions for a great variety of protein sources. It may, therefore, be a useful empirical solution of the prediction problem.

It is further held that NPU is too insensitive a measure of protein metabolism to allow any generalizations about the latter from variations in the former.

#### SUMMARY

1. The assumptions made when predicting net dietary protein values from chemical scores (Miller & Payne, 1961 *a*) were examined.

2. At variance with the assumptions, it was inferred that (1) NPU levels off at high protein levels, (2) the linear regression of NPU on the level of protein calories ( $P$ ) extrapolates to the value of zero for NPU at a lower level ( $P_z$ ) the better the quality of the protein source, (3) the maintenance requirement of protein ( $P_m$ ) is overestimated by  $P_m = 400/\text{NPU}_{\text{st}}$ .

3. The prediction equation is discussed in relation to a new theory of protein metabolism suggested by Miller & Payne (1961 *b*).

4. It is concluded that although theoretical objections may be raised against the prediction equation suggested, it may be a useful empirical solution of the prediction problem. The balancing effect of  $P$ ,  $P_m$  and  $P_z$  is pointed out.

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