XLVIII. THE RELATIONSHIP OF THE METABOLIC NITROGEN OF THE FAECES TO BODY WEIGHT AND TO FOOD INTAKE FOR RATS.

By BURCH HART SCHNEIDER¹.

From the Division of Animal Nutrition, University of Illinois, Urbana, Illinois, U.S.A.

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In the computation of biological values of proteins, investigators are divided in the manner of estimating the so-called "metabolic nitrogen of the faeces." Thomas [1909] and Martin and Robison [1922] thought this loss of body nitrogen to be constant, irrespective of the quantity of food ingested. Mitchell [1924] has considered it to be proportional to the amount of food dry matter consumed. Boas Fixsen and Jackson [1932], who favour the former viewpoint, make the following statement: "Mitchell [1924] argued that the amount of faecal nitrogen of endogenous origin excreted by a given animal was proportional to the weight of dry food ingested and that this ratio varied for different rats. In our experience, however, the results of several series of experiments on the same rat receiving nitrogen-free diets provide no evidence in support of such a relationship, at any rate where adult rats are concerned. On the contrary, in our work the same rat has shown an excretion of faecal nitrogen which is roughly constant in amount, in spite of large variations in the food intake...."

As these observations of Boas Fixsen are not in harmony with those made at this laboratory, the literature has been searched for evidence bearing upon the problem. A summary was made of all rat metabolism data relating to low-nitrogen diets that are available. This summary includes 1160 determinations of metabolic nitrogen (about one-third of which is from the author's unpublished data and the remainder from the literature) with rats of different body weights and consuming different amounts of food. The correlation of metabolic nitrogen and food consumed secured from these data is shown in Fig. 1. The regression line has a significant slope, which measures the rate of increase in metabolic nitrogen as more food is consumed. The rectilinearity of these data throughout most of their range seems apparent. The means of the arrays of the data when grouped follow a straight line except at the lowest and highest food intakes; the significance of these exceptions will be discussed later.

It cannot be stated, however, that all of the metabolic nitrogen excreted is proportional to the food consumed, since the intercept of the regression line on the y axis (zero food intake) is a significantly positive value. This part of the metabolic nitrogen obviously is not related to food intake.

There is, therefore, a constant fraction of the metabolic nitrogen, as well as a fraction which varies with food intake. If for any reason one fraction is much larger than the other, the impression may easily be created that the metabolic nitrogen is all of that fraction, either all constant or all proportional to food intake. Mitchell [1924; 1926] noted a relationship of metabolic nitrogen to body

¹ National Research Council Fellow in the Biological Sciences, 1931-33.

weight, but recognised with his small rats a much greater relationship to food intake. Boas Fixsen with large rats found "no evidence in support of such a relationship."

The correlation coefficient (r_{FM}) between food intake and metabolic nitrogen obtained from these 1160 observations is 0.745 ± 0.009 . Correlation coefficients much nearer unity have been obtained by the author with data from individual rats¹. The correlation coefficient (r_{WM}) between body weight and metabolic nitrogen is 0.502 ± 0.015 , demonstrating that the relationship of metabolic nitrogen to body weight with these data is much less than the relationship of metabolic nitrogen to food consumption. To express adequately the relationship of metabolic nitrogen to food intake or body weight, irrespective of any relationship that may exist between body weight and food intake, it is necessary to make use of partial correlation coefficients. The partial correlation coefficient ($r_{FM,W}$) of food intake and metabolic nitrogen independent of body weight is 0.744 ± 0.009 , while that ($r_{WM,F}$) of body weight and metabolic nitrogen independent of food intake, is 0.499 ± 0.015 , indicating, as with simple correlation, that a closer relationship exists between food intake and metabolic nitrogen than between body weight and metabolic nitrogen.

The fact that the metabolic nitrogen in the faeces is related to body weight as well as to food intake, suggests that the constant fraction of the metabolic nitrogen, represented by the intercept of the regression line on the metabolic nitrogen axis in Fig. 1, varies with the body weight of the rat. Evidence confirming this suggestion may be deduced from these data. At the upper right-hand corner of Fig. 1, it will be noticed that the metabolic nitrogen values associated with the greatest food intakes, which were secured only with the larger rats, are all located above the regression line. Likewise, the points which are above the regression line at its lower end, and cause the data to depart from rectilinearity, were obtained again with the larger rats. In fact, the values obtained from larger rats tend to be above the regression line and those obtained from small rats below the regression line throughout the scale of food intake, the regression line representing the food intake-metabolic nitrogen relationship for the rat of average weight. Over two-thirds of the data from rats above average weight are located above the regression line, while the reverse is true with rats smaller than average.

There are 43 determinations of faecal nitrogen at a daily food intake of 9 g. that merit special study. The points referring to the larger rats are above the others, showing again that the larger the rat the greater is the constant fraction of the metabolic nitrogen. These 43 determinations with constant food intake show a correlation (r_{WM}) of 0.830 ± 0.032 between body weights and metabolic nitrogen values. The tendency for larger rats to have greater metabolic nitrogen excretions at any one level of food intake is evidenced also by the significantly positive partial correlation coefficient ($r_{WM,F} = 0.499 \pm 0.015$) between body weight and metabolic nitrogen.

Thus, it may be considered that the larger the rat the larger the constant fraction of the metabolic nitrogen, and assuming the same slope one may imagine a number of lines parallel with the regression line in Fig. 1, each line representing metabolic nitrogen as a function of food intake for one rat, the height of the line

¹ From data to be published elsewhere, the correlation coefficients between the food intake and the metabolic nitrogen of the same rat fed at from 10 to 19 levels of food intake were as follows for six different rats: 0.981 ± 0.006 , 0.971 ± 0.009 , 0.993 ± 0.003 , 0.964 ± 0.011 , 0.937 ± 0.019 and 0.924 ± 0.023 .

varying with the weight of the rat. Hence, the data of Boas Fixsen, resulting from experiments with large rats (which consumed less food relative to body weight than small growing animals) show comparatively less change in the total metabolic nitrogen when food intake is varied and thus do not reveal the effect of food consumption on the metabolic nitrogen to the same extent as Mitchell's data with smaller rats, excreting correspondingly less of the constant fraction of the metabolic nitrogen and relatively more of the variable fraction related to the intake of food.

Another reason why Boas Fixsen failed to detect a relationship between metabolic faecal nitrogen and food intake pertains to her experimental technique, which does not appear to be sufficiently exacting. Maintaining a constant level of food intake during an experimental period of 4 days does not ensure regular excretion. Unless the same intake is continued for several days before the

Fig. 1.

Fig. 2.

collection period, there is a marked tendency for the amount of faecal dry matter collected at extreme levels of food intake, large or small, to regress toward the mean, *i.e.* toward the amount of faeces from the usual intakes of food prevailing before the experimental collection period. Longer collection periods would also diminish errors of this type. The determination of faecal nitrogen reveals little of quantitative value unless the amount of faeces analysed is excreted as a result of exactly that food intake to which it is ascribed. One cannot be sure of obtaining a quantitatively accurate sample of faecal dry matter in four-day periods without feeding a faeces marker. Distinct demarcation of faeces can be obtained by using alternately ferric oxide and chromic oxide to colour the first feeding of successive periods. Also, nitrogen-free diets, such as have been used by Boas Fixsen [1930], having little indigestible substance, do not favour regular defaecation. Furthermore, the slope of the regression line of metabolic nitrogen on food intake for such highly digestible diets is less than that of the regression line for those having more indigestible dry matter. Thus, the metabolic nitrogen of Boas Fixsen's rats increased on an average only 0.56 mg. per g. of dry food

consumed, while the average for all rats considered was an increase of 1.25 mg. of metabolic nitrogen per g. of food dry matter consumed.

Considering these factors of technique, involving the use of large rats, inaccurate demarcation of faeces, no preliminary periods¹, short collection periods and highly digestible diets, it is not surprising that the total metabolic nitrogen as measured should appear "roughly constant" for any one rat. Nevertheless, an examination of Fig. 1 reveals that the data of Boas Fixsen (indicated by circles) harmonise fairly well with the other 1103 observations. In fact, all of her data show a correlation coefficient between food intake and metabolic nitrogen of 0.481 ± 0.069 , indicating a significant relationship for the pooled data which is scarcely suggested by the data from the individual rats which were fed at eight or fewer levels of food intake.

As a part of the metabolic nitrogen is constant and not proportional to food intake, it might be reasoned that to estimate the metabolic nitrogen in a period of protein feeding a regression equation (instead of the ratio introduced by Mitchell [1924]) should be derived from two levels of feeding of low-nitrogen food. The curves in Fig. 2² show the ratio "mg. of metabolic nitrogen per g. of food dry matter consumed" as a function of food intake. The ratio can be considered to be fairly constant and within the limits of experimental error for all but the lowest food intakes. The solid curve (derived from the data in Fig. 1) is for the "average" rat of the population. With larger rats the curve is to the right and above, its slope extending to greater food intakes. This indicates, of course, that with larger rats greater food intakes are required before the ratio becomes practically constant. The data from smaller rats produce a curve which is to the left and below, reaching a level at lower food intakes. Each curve approaches an asymptote for food intakes infinitely large, that is not influenced by the size of the rat. All curves approach infinity as the food intake approaches zero.

An increase in the amount of indigestible substance in the diet raises the position of the curve, but does not change its form. Hence, in determinations of the biological values of proteins it is important that the dry matter of the standardising low-nitrogen diet be of approximately the same digestibility as that of the protein-containing ration. The curves reveal that the ratio "mg. of metabolic nitrogen per g. of food," although not the most exact statement of the mathematical relationship between food intake and metabolic nitrogen, in practice lies well within the limits of experimental error when more than a certain minimum quantity of low-nitrogen food is ingested daily, and that it affords an appropriate means of computing the metabolic nitrogen in protein-feeding periods.

CONCLUSIONS.

The metabolic nitrogen in the faeces of rats consists of two fractions, one related to the body weight and the other to the intake of dry food.

The ratio of metabolic faecal nitrogen to the intake of dry food assumes a practically constant level for intakes of food above a certain minimum value, which seems to approximate to that required for maintenance of body weight in the case of the usual low-nitrogen diets used in protein evaluation work. Hence, in

¹ [Note by Editor.] The author is mistaken on this point [see Boas Fixsen, 1930, p. 1796].

² Considering that the regression line in Fig. 1 is described by the equation $y = a + bx$ in which y is the metabolic nitrogen, a the y -intercept, b the slope constant and x the food intake, the curves in Fig. 2 were fitted by the equation $y = \frac{a}{x} + b$, y being the ratio of metabolic nitrogen to food intake in the latter case, the other symbols having the same significance.

determinations of the biological values of proteins by Mitchell's technique, the ratio can logically be used in estimating the metabolic nitrogen in the faeces in the protein-feeding periods, if its limitations are born in mind. Furthermore, no better method of estimation appears to be available.

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