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Nitrogen balances

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Duncan (1958), Hegsted (1964) and Martin (1966) noted that published balances of certain nutrients can be so positive as to imply larger retentions than those calculable from carcass analyses. Nevertheless balances of chromium sesquioxide (Cr_2O_3) (Chanda, Clapham, McNaught & Owen, 1951) and of borate (Owen, 1942–4), which we quantitatively recovered from the diet (Fig. 1) are not subject to this criticism. Fig. 2, which records graphically information from the paper of Owen, Smith & Wright (1943), who demonstrated that urea can be substituted for protein, shows the output of nitrogen by cows as a function of their N intake. In these experiments the diet was of concentrate meal and hay which required separate 2-day analyses, and the output was urine plus faeces plus milk. Refusals of meal or hay were measured. In Fig. 2 the heights of some of the ordinates above the line of equilibrium, indicate retentions of N which might at first sight seem unacceptably large. Two questions, therefore, arise: (1) how large a positive balance is physiologically admissible, and (2) if the balances are inadmissibly large, from what technical imperfections could they have arisen?

Question (1)

The 1943 experiment was done during the war when cattle food was rationed, and on a farm where the ration coupons were returned in order to find out whether wartime self-sufficiency was feasible. Before the experiment, therefore, these cows had been undernourished. Moreover, as a result of many years of N balance experiments on milch cows, the Institute had recommended a lower N allowance than was customary (Owen, 1941). Fig. 3 is a similar graph from a later experiment
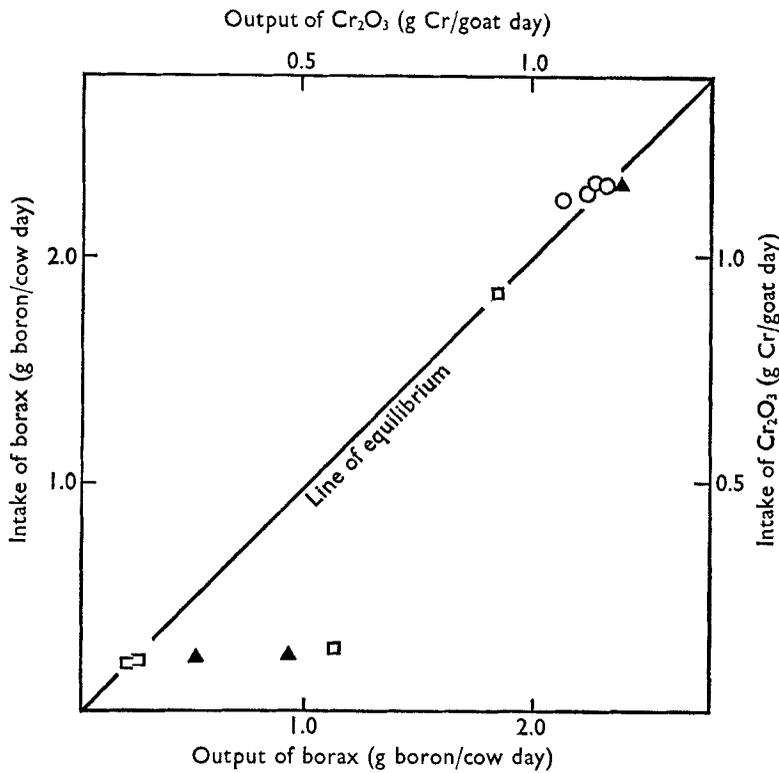
Output of Cr_2O_3 (g Cr/goat day)

Fig. 1. (1) Complete recovery of Cr_2O_3 added to the diet of four goats (○). (Chanda *et al.* 1951). (2) Recovery of borate added to the concentrates of two cows (□) and (▲) (Owen, 1944). The smaller amounts represent recovery of boron found by analysis of the untreated rations but the two points at the higher intakes indicate complete recovery of added borate. This experiment and those illustrated in Figs. 2 and 3 were done with the same apparatus.

(Owen, 1948*a,b*) with cows of the same breed which had not been subjected to the exigencies of wartime rationing. In Fig. 3, N balances are mostly positive but by no means as much so as those in Fig. 2. The sizes of some of the balances in Fig. 3 were diminished by subcutaneous injection of thyroxine, though the effect of thyroxine on the two cows G and H which were deliberately more heavily fed was to change both the intake and output of N in proportion so that these last two cows remained in balance but at a higher metabolic turnover. In the cows not heavily fed, thyroxine increased the milk yield partly because it increased digestion of the diet and partly because it increased drafts from body stores.

The N balances in 1943 were very large compared with those of 1948, but the cows used in 1943 had been so undernourished before the experiment that in periods 1 and 2 their N balances were very large. However in period 3, when the only dietary change was the removal of urea, retention of N declined abruptly and three of the cows' N balances were negative. When urea was replaced in cows C, D and E there were once more large positive balances which remained when urea was later replaced by protein. Thus the diminutions of N balance occasioned by the removal of urea
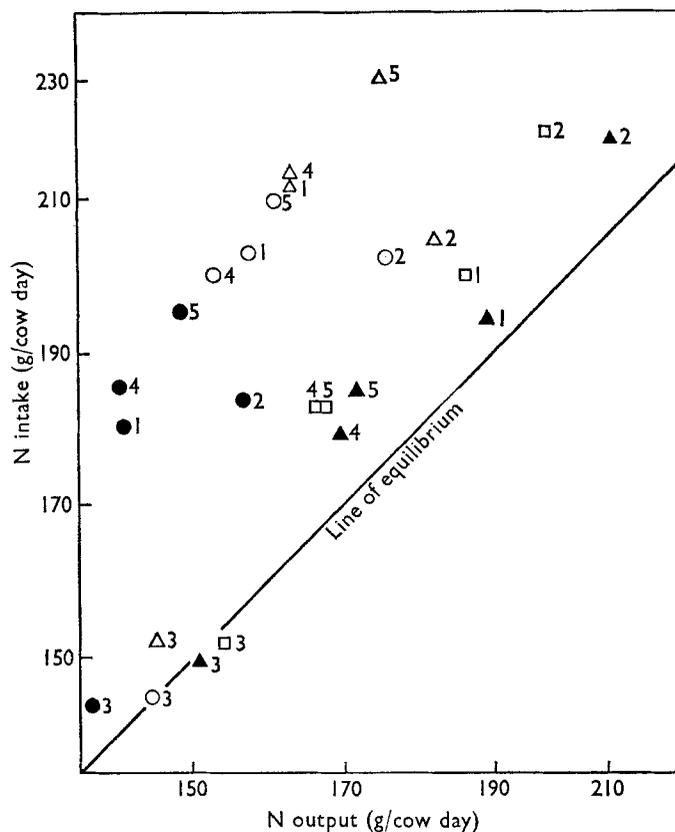
Fig. 2.　Nitrogen balances of Ayrshire cows in milk. Cows C (●), D (○), E (△), F (▲), G (□) (Owen *et al.* 1943) were receiving diets in which protein was replaced by its N and energy equivalent of a mixture of urea and potato starch intimately mixed with the concentrates. Protein (1) was given first, followed by urea plus starch (2). Urea was then removed from the diet (3) and later replaced in the diet once again (4). In the final period protein again replaced urea plus starch (5). All the urea and protein diets gave positive balances of N, but when urea was removed three cows showed small negative balances. The output of N is the sum of the N contents of milk, urine and faeces.

in 1943 were larger than the diminutions caused by thyroxine in the 1948 experiments, in spite of the fact that thyroxine is a potent katabolic agent in man and animals.

There is a problem as to why the 1943 cows lost weight in period 1 when their N balances were strongly positive. As Owen *et al.* (1943) stated at the time, this phenomenon had not been observed by previous investigators at the Institute who were using the same metabolism apparatus with the same breed of cows. It is a reasonable assumption, therefore, that the large positive N balances accompanying small changes of body-weight were due to the undernutrition of the 1943 cows before they were subjected to the experiment. The average initial weight of the 1943 cows was 477 kg; that of the 1948 cows was only 419 kg, which is consistent with the supposition that the 1943 cows had become somewhat oedematous as a result of the strict rationing to which they had been subjected, so that their weight change in period 1 was the difference between the increase resulting from protein retention and the decrease
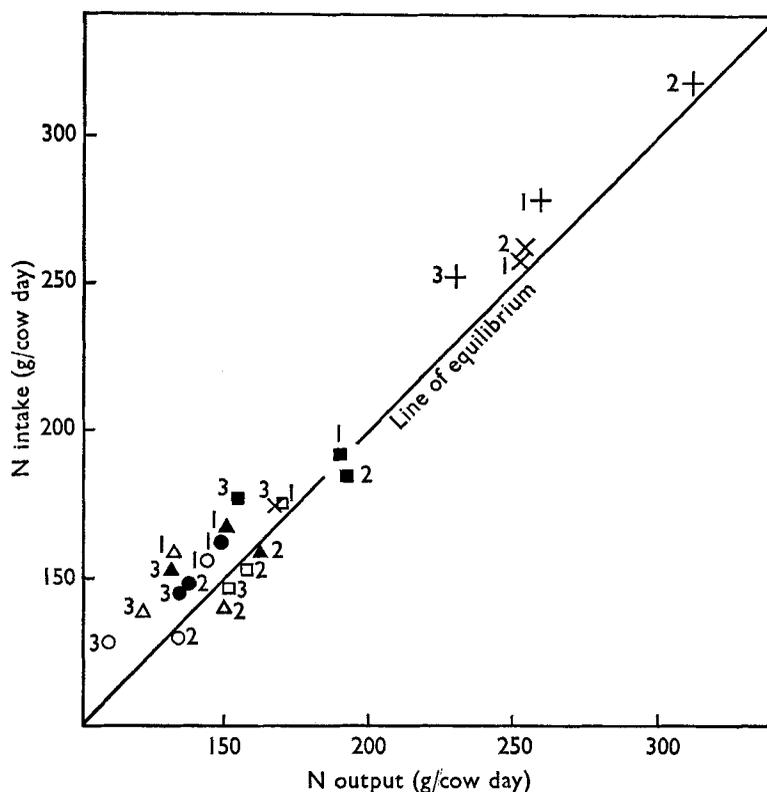
Fig. 3. Intakes of nitrogen of eight lactating Ayrshire cows (Owen, 1948*a*) as functions of their N outputs. There were three periods 1, 2 and 3. During period 2, all the cows except B (●) and E (□) were given thyroxine. Cows A (○), C (△), D (▲) and F (■) went into negative N balances when thyroxine was injected but returned to positive N balance when thyroxine was discontinued. In the more heavily fed cows G (+) and H (×) thyroxine did not make the balances negative.

through loss of oedema fluid (Passmore, 1967; Isaksson & Sjögren, 1967). It is a commonplace of obesity clinics that overweight people when medically treated can lose a stone or two of weight without losing the expected amount of protein. They lose, together with fat, salt water from oedema fluid (Isaksson & Sjögren, 1967). Moreover, the weight of the skeleton can vary independently of the weight of the soft tissues in the adult animal (Owen, 1940; Duncan, 1967). Glycogen stores, muscle mass and N in the central nervous system are also subject to variation. For these reasons changes of N balance are not calculable from changes of body-weight.

It would be useful, in balance studies, to measure sodium and thiocyanate spaces to see what part losses and retentions of water play in changes of body-weight. These losses or retentions could be large, for, in protein-calorie deficiency experiments with pigs (Platt, Heard & Stewart, 1964), changes from 62 to 80% of water in the body were found, and these large increases of water content which were accompanied by large changes of fat content were diminished by dietary protein and increased by dietary carbohydrate. Excretion of a water load was delayed and the water retention

was associated with retention of sodium chloride. By using krypton (Hytten, 1964) one could make direct estimates of body fat. A concentration of techniques such as would reveal simultaneous changes of weights of water, fat, glycogen, bone salts, protein and nucleic acids would help to remove ambiguities in the interpretation of balance experiments with adult animals, but even then cumulative errors might exceed those of the balance experiment itself.

As regards the question of the admissible level of N balance due to protein or nucleic acid storage, Mitchell (1962–4) states that man can store 9 g N/day. Body-weight, for body-weight this corresponds to a retention of 59 g/day or more for a cow weighing 450 kg, which is of the same order as the largest of the daily balances found by Owen *et al.* (1943), which ranged from −2·3 to +49·2 g N/cow. Also of the same order of magnitude was the loss of N by Deuel in experiments on himself (reported by Mitchell, 1962–4) on a protein-free diet for 31 days. Munro (1964) thinks that
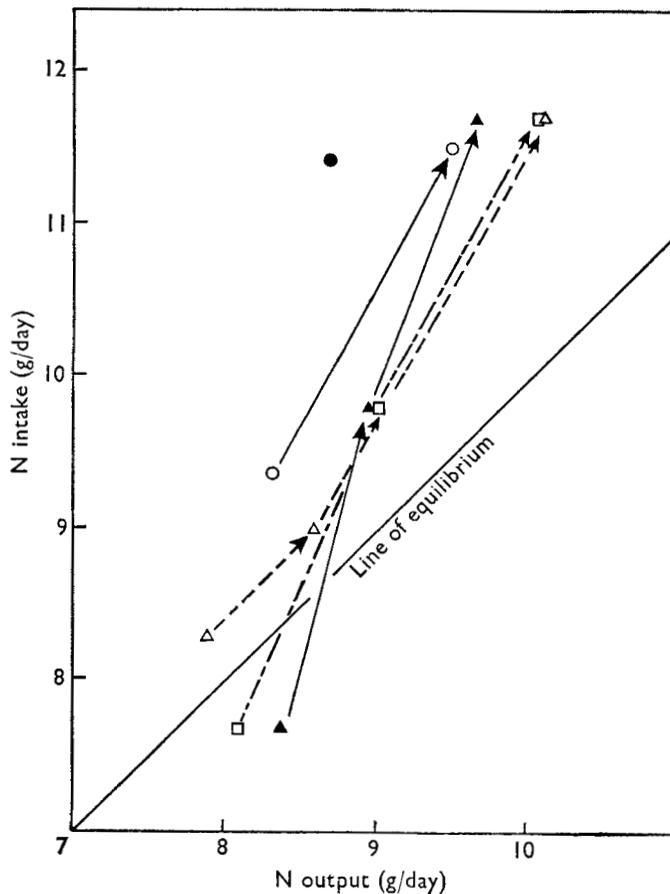

Fig. 4. Men (of average age 68), selected by dietary survey, were given diets at three increasing levels of N as shown (Owen *et al.* 1939). As the intake increased, balances of N increased thus showing that elderly men can store N. Subjects 6 and 7 were in negative balance at the lowest N intake, and subjects nos. 3 (○), 4 (●), 5 (△), 6 (▲) and 7 (□) were on balance for 15, 9, 27, 24 and 24 days respectively. The diets were entirely of foods in common use.

Deuel overestimated his loss of N. However, it can be calculated from the rat experiments of Kosterlitz (1947) that on a weight-for-weight basis a 450 kg cow might lose 81 g N/day for 5 days. A regain of such an amount is larger than the largest positive balance of Owen *et al.* (1943). Moreover, the loss which Kosterlitz reported does not take into account the further asymptotic loss which follows it in all animals, including man, in which it has been studied (Kosterlitz & Campbell, 1945–6; Munro, 1964). Munro (1964) has discussed both historically and critically the loss and regain of N by various animals. Martin (1966) measured losses of N as ammonia in the excreta or breath of urea-treated sheep. These would amount in a cow, on a weight-for-weight basis, to 1 mg N/cow daily. From Martin's results, losses from the skin would correspond to about 1 g N/cow daily. From these various considerations it is apparent that the largest positive balances found by Owen *et al.* (1943) were not necessarily excessive.

In experiments (Owen, Irving & Lyall, 1939; Owen, 1952) with human subjects at at least two and sometimes three levels of intake, calcium and phosphorus balances went from negative to positive with increasing intake. N balances of some of these subjects are shown in Fig. 4. There were no positive balances which could be regarded as unrealistic on the basis of the foregoing discussion. Thus the greatest ratio of intake of N to output of N was 1·31 (average 1·11) which is much the same as the greatest ratio of intake of N to output of N in the 1943 experiment with cows (1·32) (average 1·09). These values should be compared with the greatest ratio in the 1948 cow experiment of 1·16 (average 1·05). The similarity between the ratios of intake to output of N in the cows in 1943 and the men in 1939 is noteworthy since the technical conditions in the two experiments were necessarily so different. The probable reason for the similarity is that all the patients were from Orr's (1937) least privileged group but were promoted in dietary privilege by the conditions of the experiment so that like the 1943 cows they were changed by the experiment from frugal to luxus conditions.

In the 1948 experiments with cows (Fig. 3) thyroxine had the unexpected effect of increasing the retention of P while increasing the output of Ca to such an extent that the faecal Ca exceeded the intake, whereas urinary Ca was unchanged and N balance became much smaller. These simultaneous effects can only mean that thyroxine increased the demand of the soft tissues for phosphate, presumably to increase the level of high-energy substances such as adenosine triphosphate and creatine phosphate in the body (Chanda & Owen, 1951), so that in the two cows G and H which were liberally fed, P from bone passed into the soft tissues and the Ca from the bone being in excess of lactational or soft-tissue requirements, was defaecated; the ratio of Ca:P in bone is 1·8:1 whereas in milk it is almost 1 (Owen, 1948*b*; 1952).

Vitamin D produces citraturia in rats (Bellin, Herting, Cramer, Pileggi & Steenbock, 1954), which seems to be a natural provision for enabling the body to excrete Ca while conserving P (Owen, 1952). Thus Shepherd & Macy (1939) found that vitamin D, when increasing the P and Ca balances of children, decreased their N balances thus showing precedence in these circumstances of bone over soft tissue. Another aspect of this is the increased metabolic rate engendered by rachitic diets

which favour the soft tissues by abstracting P from bone. Not only protein but also the P-containing ribonucleic acid and phospholipid can be stored in labile liver cytoplasm (Kosterlitz & Campbell, 1945–6; Davidson & Waymouth, 1946–7). Doubtless the labile forms of nucleic acid such as the Nissl substance of the neurones are also subject to storage and depletion and may account for the loss of N from the brain of the protein-deficient rats of Addis, Poo & Lew (1936a,b,c). The foregoing discussion shows that it is not necessary to suppose that if the balance of one element is positive the balance of other elements should also be positive.

Question (2)

There are assumptions implicit in balance experiments which may not always be technically justified. These are: (1) that methods of estimation are adequate, (2) that no bias exists in the analyses, (3) that samples of food and excreta are representative, (4) that refused food has the same composition as the food eaten, (5) that a day's food corresponds to a day's output of excreta, (6) that an adult animal ought to be in equilibrium unless it is under some physiological stress, e.g. pregnancy, lactation, hormone treatment, heat, cold, or a deliberately unbalanced diet.

In balance experiments with elements, such as carbon, hydrogen, oxygen, nitrogen, sulphur, selenium, tellurium, fluorine, which can give rise to volatile compounds, other assumptions are made. Thus for N it is supposed (7) that no N is lost in volatile forms and (8) that no atmospheric N is fixed by the animal.

As to assumption no. 1, errors may arise if a supposedly pure precipitate such as calcium oxalate, for example, does not have the composition that theory would require or is contaminated by another constituent of food or excreta. Thus silica in Ca oxalate or in magnesium ammonium phosphate in the analysis of straw could give high results for Ca, Mg or P, if the precipitate is weighed. A non-gravimetric method avoids this difficulty. Properly representative samples are necessitated by assumptions nos. 2, 3 and 4, even if such samples entail the modifying of the experiment so that its relevance to usual feeding practice is diminished. Thus Owen et al. (1943) had to reject the balances obtained from cows A and B because the diet of these two cows contained swede turnips of which the representative sampling of either the parts eaten or the parts refused proved impracticable.

Assumption no. 5 holds only very approximately for goats owing to variation of times of defaecation and owing to different rates of passage of fibrous foods and meal along the gut (Owen, Darroch & Proudfoot, 1959). Assumption no. 6 has already been discussed in connexion with protein and water storage. As to assumption no. 7, Martin (1966) measured the loss of ammonia from sheep in calorimeters. It seems from his studies that ammonia can settle on surfaces, presumably as carbonate, and be subsequently slowly released. Owen (unpublished) found that drying of goat faeces caused a loss of 10% of their N which could be prevented by prior spraying with 100% (w/v) citric acid in water. Faeces which are wet, such as those of man and cow, are best weighed wet from stoppered containers to minimize errors due to evaporation of water or N compounds. Peters & Van Slyke (1932) recommend the solution of human faeces in strong sulphuric acid for sampling. If digestion of

solid food is incomplete, faeces which have not been homogenized will give poor duplication.

Ruminants carry an unusually large and variable 'fill' which may be 20% of their weight, and this gut content is in continual fermentation. Whether any N fixation (Hungate, 1966) or denitrification occurs in the gut does not seem to have been tested. Nitrate in the rumen is readily reduced to nitrite by bacteria, and there seems to be no reason why nitrite should not come into contact with urea from the saliva and so lead to denitrification. Probably the amount of such denitrification is negligible but assumptions (7) and (8) would be justified only after it has been shown that such reactions do not take place.

With regard to assumption no. 3, Prien & Frondel (1947) have listed twelve urinary sediments whose compositions show that filtering of urine could lead to low recoveries of N, P, Ca, Mg, Na or S. Acidification can redissolve some of these precipitates but could bring down uric acid, so substituting one difficulty for another. Phosphate clouds in human urine and magnesium ammonium phosphate in rat, ruminant and human urine are common hazards. Only strong hydrochloric acid will in the author's own experience readily redissolve magnesium ammonium phosphate which adheres very tenaciously to the sides of the urine container and is readily precipitated in cold weather in rat and goat urine. In hot weather, possible loss of ammonia from fermentation of urea is another hazard. Sampling errors may also arise from failure to separate urine from faeces but homogenization of the latter should prevent such errors from affecting the balances.

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