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SHORT-TERM RESPONSES TO THE INGESTION OF FOOD

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Problems caused by variation in food intake in experiments on protein and nucleic acid metabolism

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The aim of this review is to survey reports of the rapidity of response of an animal's metabolic processes to changes in the quantity and quality of its diet, and to emphasize the relevance of these findings to the design of investigations of the effects of nutritional changes.

Resolution of food intake, lighting and time-of-day effects on metabolic patterns

Over short periods, the effects of food intake on an animal's metabolic processes are often closely linked with diurnal rhythms regulated by both time of day and lighting conditions. To resolve the effects of these three factors is not easy but Potter, Baril, Watanabe & Whittle (1968) have probably come nearest to solving some of these problems by using rats kept under controlled feeding and lighting regimens. Windowless rooms were used and the normal 12 h light and dark cycle was reversed. Access to food was allowed *ad lib.* for only 8 h out of 24 h or 48 h, commencing at the start of a dark period. Use of the '8+16' regimen to investigate duirnal variations in cycloleucine concentration in rat liver, following injection of the [^{14}C] labelled compound, revealed a clearly defined diurnal cycle the presence of which was completely obscured when rats had *ad lib.* access to food. It seems likely that in similar investigations the use of a controlled feeding regimen may help to reduce the random variations associated with *ad lib.* feeding schedules. The '8+40' regimen has the further advantage that it includes two periods of light and dark only one of which is complicated by food intake. Comparisons between the two periods allow an assessment of the relative importance of control by food intake or lighting. By use of this regimen, Potter *et al.* (1968) showed that liver cycloleucine concentration and liver serine dehydratase (*EC* 4.2.1.13, L-serine hydro-lyase) activity each gave a single peak which was related to food intake. In contrast, tyrosine

transaminase (*EC* 2.6.1.5, L-tyrosine:2-oxoglutarate aminotransferase) showed two peaks of activity per 48 h cycle, one associated with food intake but the other occurring during the 40 h fast period and presumably stimulated by the lighting condition. The regulation of tyrosine transaminase by food intake was further demonstrated by Fuller & Snoddy (1968). They showed that the peak of enzyme activity which normally occurs during the hours of darkness, the period of maximum food intake on an *ad lib.* regimen, shifted to the middle of the light period if access to food was restricted to 4 h at the start of this part of the cycle.

Time-course of induction of enzymes

Berlin & Schimke (1965) have emphasized the often neglected point in comparative studies of the response of a series of enzymes to stimuli that the magnitude of the response of each enzyme is a function of its half-life. For comparative purposes it is insufficient merely to consider changes in activity or concentration. After hydrocortisone treatment, although tryptophan oxygenase (*EC* 1.13.1.12, L-tryptophan: oxygen oxidoreductase) activity was markedly increased within a few hours, arginase (*EC* 3.5.3.1, L-arginine amidinohydrolase) activity was only marginally higher after 4 d. An enzyme with a short half-life, tryptophan oxygenase (half-life, 2·5 h), requires a relatively high basal rate of synthesis to maintain its basal activity whereas the converse applies to arginase (half-life, 96 h). The rate of accumulation of the enzymes following hydrocortisone treatment depended on both the basal rate of synthesis and on its percentage stimulation. When these considerations were taken into account, Berlin & Schimke (1965) found that, despite very different rates of accumulation of the enzymes, the percentage stimulation was virtually the same in each instance.

Sanchez & Swendseid (1969) failed to show any effect of excess dietary methionine on cystathionase (*EC* 4.2.1.15, L-homoserine dehydratase) activity when they assayed the enzyme after 3 d on the diet. Daniel & Waisman (1969) examined the time-course of induction of enzymes during feeding of a similar dietary excess of methionine. It was found that whereas serine dehydratase activity greatly increased within 1 d of feeding the excess methionine, cystathionase activity increased slowly reaching significantly higher levels only after about a week. Clearly, the interpretation of the effects of excess dietary amino acids on hepatic enzymes requires careful attention to the time-course of induction of the enzymes and, in view of the work of Berlin & Schimke (1965), information on the half-life of the enzyme concerned is necessary if the observed rates of accumulation of the enzyme are to be related to changed rates of its synthesis.

Use of enzymes as indicators of protein nutrition

Hurvitz & Freedland (1968) have shown that the activities of serine dehydratase, tyrosine transaminase and glutamic:pyruvic transaminase (*EC* 2.6.1.2. L-alanine: 2-oxoglutarate aminotransferase) in liver all respond to the level of protein in the diet and also to the administration of hydrocortisone. Waterlow & Stephen (1969) have

suggested that these and similar enzymes might be used as indicators in the protein nutrition of an animal. However, the points raised above suggest how careful one would have to be to ensure adequate control of such factors as environmental conditions, feeding schedules, hormone balance and period of enzyme induction before changes in enzyme activity could be used as a measure of protein quality or quantity.

Plasma amino acids as indicators of amino acid requirements

As early as 1953, Richardson, Blaylock & Lyman (1953) reported that changes in dietary amino acids caused alterations in plasma amino acid pattern. However, well over 10 years later, methods for assessing the amino acid status of a diet based on the pattern of tissue amino acids are still not standardized. Zimmerman & Scott (1965) fed chicks for 7 d on diets containing different amounts of lysine and investigated their effects on plasma amino acid concentrations. They found that if the dietary lysine was inadequate, as assessed by the growth of the chicks, the lysine content of the plasma remained unchanged. However, diets supplying lysine in more than adequate amounts caused the plasma lysine concentration to increase almost in proportion to the dietary excess of the amino acid. In contrast, McLaughlan & Illman (1967) found that, for each of several essential amino acids, the plasma amino acid concentration was proportional to the dietary content of that amino acid even when this supplied less than the animal's requirement. These workers were using rats rather than chicks as the experimental animals but they suggested that the difference between their findings and those of Zimmerman & Scott (1965) was caused by feeding the experimental diets for only 3 d since in previous experiments (McLaughlan, 1964) extending over 7 d they had found amino acid patterns similar to those of Zimmerman & Scott (1965). McLaughlan & Illman (1967) used a 3 d period within which they claimed that plasma amino acid pattern reflected the dietary supply of amino acids and compared the plasma amino acid concentrations obtained by varying the dietary concentration of a particular amino acid with that obtained for the same amino acid in fasting rats. They assumed that the dietary requirement for an amino acid was the concentration capable of maintaining a plasma amino acid concentration the same as that of a fasted rat. Their rats were fasted for 16 h and it is questionable in view of the studies of Gan & Jeffay (1968) whether this assumption was correct. These workers have investigated the effects of different periods of fasting on the proportion of tissue amino acids derived from the plasma. For liver, they showed that 50% of the intracellular free lysine of a fed rat was derived from the plasma but that after a 1 d fast only 10% was still derived from the same source despite the total lysine concentration remaining unchanged. The effect of fasting was apparently to increase the supply of amino acids from liver protein catabolism and this persisted for at least 2–3 d after which the percentage of free lysine derived from the plasma gradually returned to that of a fed animal. The effects with muscle were much less pronounced and showed a gradual decline in the free lysine derived from plasma from 60% in the fed animal to 30% after 7 d starvation. These differences with duration of fasting and between tissues in the origins of tissue amino acids were

almost certainly reflected in the pattern of plasma amino acids since in fasting animals these will largely be derived from the catabolism of tissue proteins. Therefore, if plasma amino acids are to reflect the animal's requirements for the amino acids the choice of the duration of starvation before sampling is critical. McLaughlan & Illman (1967) chose a 16 h period but Harker, Allen & Clark (1968), using a range of dietary protein concentrations and starvation times of 3, 6, 9 and 12 h, concluded that 6 h starvation gave values which most nearly represented the true requirements of the animals.

Use of plasma amino acids as indicators of dietary protein quality

Even with an accurate knowledge of amino acid requirements the direct assessment of the protein quality of a diet by analysis of its amino acid composition is often invalidated by variations in biological availability of amino acids when the diet is based on intact proteins. A commonly employed method of assessment has been to compare the plasma amino acid patterns obtained from feeding a standard diet with those from the experimental diet (McLaughlan, 1963). However, after a single meal of a protein-containing diet, the changes in the plasma concentration of amino acids vary with time. In this instance it is important that the plasma amino acid pattern reflects not the animal's requirements but the amino acids it is receiving from the diet. Smith & Scott (1965) have suggested that problems arise in this type of experiment because of the use of standard proteins with amino acids of unknown availability and, also, uncontrolled feeding allows variations in both the time between feeding and sampling and the food intakes of the experimental and standard diets. In an attempt to overcome these problems Dean & Scott (1966) fed to one group of chicks a reference diet containing free amino acids in the proportions shown to be optimal for growth. This group was compared with others that were given free amino acid diets imbalanced in one or more amino acids. By use of free amino acids they hoped to overcome variations in availability and, to control feeding differences, they fed small quantities of diet at twelve consecutive 30 min intervals in an attempt to produce a steady state of plasma amino acids. The quantities of diet at each feed were small enough to be completely consumed so that food intake was equalized in both quantity and periodicity. All chicks were killed 30 min after the last feed ensuring a constant interval between sampling and killing. By use of these strict controls they were able to deduce from the plasma amino acid patterns which amino acids were present in deficiency or excess in the experimental diets and even the order of inadequacy of them in the diets. When similar methods were tried with diets based on intact test proteins (Smith & Scott, 1965), a comparison of the plasma amino acid patterns of chicks fed the test diets with those on the reference free amino acid diet suggested deficiencies in the test diets which agreed well with previous knowledge of the quality of the proteins used. It seems, therefore, that the strict controls applied in this instance may be of great value in interpreting protein quality from plasma amino acid patterns.

Control of experiments in which ad lib. *food intake is affected*

Where the voluntary food intake of experimental animals is affected by changes in the quality of the diet, the inequalities of food consumption can produce metabolic changes not related direct to the changes in diet composition. Feeding zinc-deficient diets to rats caused a cessation of growth within 5 d and at the same time a reduction in *ad lib.* food intake to approximately a maintenance level (Chesters & Quarterman, 1970). In studies of the effects of Zn deficiency on DNA synthesis after growth had ceased, pair-fed control animals were used to allow for any effects of reduced food intake on the incorporation of [^{3}H]thymidine into DNA (Williams & Chesters, 1970). The incorporation into DNA was not significantly different between Zn-deficient and pair-fed control groups. In a second experiment, thymidine incorporation was investigated during the initial 5 d of the deficiency before food consumption was affected. When compared with control rats given the Zn-supplemented diet *ad lib.*, the incorporation in the Zn-deficient rats was markedly reduced despite their food intake being the same as that of the controls. It seems, therefore, that Zn deficiency does inhibit thymidine incorporation into DNA but that, once growth is affected by the deficiency, the reduction in *ad lib.* food intake is sufficient to produce a similar inhibition of thymidine incorporation in pair-fed controls. Effects of food intake on DNA synthesis have also been reported by Phillips & Wachtel (1968) who showed a prolonged inhibition of DNA labelling in rat kidney following overnight starvation.

Table 1 presents results from another experiment (J. K. Chesters, unpublished results) where the effects of Zn deficiency are apparently obscured by comparison with pair-fed rats. The increase in ornithine decarboxylase (*EC* 4.1.1.17, L-ornithine

Table 1. *Ornithine decarboxylase activity of liver of Zn-deficient and control rats 18 h after partial hepatectomy*

(Mean values with their standard errors for groups of six rats)

		Ornithine decarboxylase (mμ moles/mg protein per 30 min)	
Duration of Zn-deficiency	Type of control	Zn-deficient activity	Control activity
4 d	*Ad lib.*	0·128 ± 0·022	0·284 ± 0·047
11 d	Pair-fed	0·135 ± 0·029	0·158 ± 0·029

Methods based on Russell & Snyder (1968).
The *ad lib.* fed control group was significantly different from all other groups. No other significant differences were found.

carboxy-lyase) following partial hepatectomy (Russell & Snyder, 1968) was inhibited by Zn deficiency. However, because of a similar inhibition as a consequence of reduced food intake, the effect of Zn deficiency was only apparent in the initial period when *ad lib.* fed controls were appropriate and not once growth had ceased and pair-fed controls were used. In these experiments the use of pair-fed controls,

although warranted by the circumstances, tended to obscure genuine nutritional effects.

Beaton (1963), studying the effects of cold exposure on the differences in nitrogen retention of rats fed low and high protein diets *ad lib.*, showed that environmental temperatures of 2° reduced the differences in retention compared with rats at 22°. However, the proportional reduction in intake on the low compared with the high protein diet was less at 2° than at 22°. When comparisons were made of rats given the high protein diet but pair-fed with those on the low protein diet, the differences in nitrogen retention were apparent at both 2° and 22°. That pair-feeding may not provide the answers to all problems of experimental design is, however, illustrated by the work of Grace & O'Dell (1970). They found that magnesium-deficient rats had a reduced liver polysome content when compared with *ad lib.* or pair-fed rats but not when compared with rats whose food intake was restricted to produce a rate of weight gain similar to that of the Mg-deficient animals. It is clear that the choice of a control animal for an experiment in which the nutritional change being investigated inhibits *ad lib.* food intake is very difficult. Possibly, in some instances, force-feeding or interval feeding (Leung, Rogers & Harper, 1968) may be of help in equating intakes and thus allowing a separation of the effects of restriction of food intake from direct effects of nutritional changes.

Conclusions

In view of the rapidity with which changes in the quality and quantity of food intake can affect the biochemical processes of an animal it is essential that great care be exercised in designing investigations of nutritional effects on an animal's metabolism. Particular attention should be paid to control of environmental conditions, feeding patterns and total food intake and, where effects are observed, the time-course of the response would normally merit investigation.

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Movements of ammonia following intraruminal administration of urea or casein

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In considering the immediate responses to the ingestion of food in the ruminant we have not only to define the composition of the meal but also the state of the rumen fluid into which the ingested food is added. Every meal is modified by microbial action in the rumen. The host animal absorbs metabolites first from the rumen and then from the omasum, abomasum and intestines as a result of the digestion of the rumen contents passing through the alimentary tract. The ration fed over the previous few days determines the pH of the rumen contents, its chemical composition, and the distribution of bacterial and protozoal species present. Into this predetermined fluid arrives a meal together with saliva. The rumen contents before the meal, the chemical and physical attributes of the meal and the salivary response it provokes are all variables which contribute to the immediate movement of metabolites.

In this paper we wish to consider the absorption of ammonia, a product of microbial digestion of protein and the end-product of urea hydrolysis. Ammonia toxicity can arise from feeding urea or ammonium salts but there is no evidence of ammonia poisoning from protein feeding even when the concentration of ammonia in rumen liquor reaches values of over 200 mg ammonia-nitrogen per 100 ml (Briggs, Hogan & Reid, 1957). The difference between the two systems must lie in the relative importance of the rates of production and the routes of absorption of ammonia and the effect of different conditions upon them. In the discussion which follows, the term ammonia will be used to mean total ammonia; NH_4^+ refers to ammonium ion and NH_3 to free ammonia.

McDonald (1948) focused attention on the importance of ammonia production in the rumen and the ready absorption of ammonia from the rumen of the anaesthetized sheep, and his observations were confirmed by Chalmers, Cuthbertson & Synge (1954). The first systematic investigation of ammonia metabolism was that of Lewis, Hill & Annison (1957) using surgically prepared animals. They showed that, in conscious sheep, ammonia is absorbed from the rumen into the portal system and found a close correlation between changes in total ammonia concentration in the rumen and ammonia in portal blood. There was no evidence for any mechanism regulating absorption of ammonia and the authors concluded that ammonia transference was affected by simple diffusion through the rumen epithelium. Two observations made in this paper merit attention; firstly, 'leakage of ammonia through