atom Cr/carboxyl group. With increasing basicity the size of the chromic complex increases, so that the carboxyl groups which can form stable compounds will now be associated with several chromium atoms. The more isolated groups remain ineffective, and we see that the ratio of fixed chromium to total carboxyl approaches unity. The absence of an upward inflexion from the curve for highly acetylated collagen may imply that the largest chromic complexes cannot be firmly held without the help of more than one stabilizing group. When all the amino and nearly all the hydroxyl groups have been acetylated, stabilization of the bound chromium is dependent entirely on the carboxyl and, perhaps, guanidino groups.

Normal collagen, on the other hand, contains about 2·8 amino or hydroxyl groups for every free carboxyl, and there is a much higher probability that every chromic complex reacting with carboxyl will also be able to co-ordinate with one or other of the former. More chromium will be fixed even from solutions of low basicity, and at high basicities the rapidly growing polynuclear complexes will come within the range of additional co-ordinating groups and will be firmly held. This double effect of large particle size in the chromic salt coupled with greater availability of amino and hydroxyl groups can be expected to cause the sharp upward sweep of the curves in Fig. 2.

The fact that several groups must be acetylated to block the entry of one chromium atom does not, of course, imply that all are attached to that atom. It is purely a probability effect resulting from the fact that all groups can be expected to be equally susceptible to acetylation but that only some of them are placed near carboxyl groups. If it is assumed that the amino and hydroxyl groups of collagen are randomly distributed, that all are equally effective in co-ordinating chromium and that all groups of the same kind are equally easily acetylated, then, at least at moderate basicities, the fixation of chromium should be linearly related to the degree of acetylation, as in Fig. 3. The lower curve of this figure shows that similar considerations apply to reactions with anionic chromium complexes.

SUMMARY

1. Acetylation of either free amino or free hydroxyl groups of collagen causes a marked diminution of its ability to take up either cationic or anionic chromium complexes from solution.

2. For chromic sulphate solutions, this effect has been studied over a wide range of basicities and for differing degrees of acetylation. It is most pronounced at high basicity and increases almost linearly with the degree of acetylation.

3. The experimental results suggest that, while the initial reaction between the chromic complex and collagen occurs at the carboxyl groups of the latter, the co-ordination of amino or hydroxyl radicals is essential to the formation of a stable compound.

4. In the basicity range normally employed in industrial chrome tanning, approximately one collagen amino or hydroxyl group in every three appears to be involved in the reaction.

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The Influence of Energy Intake on Protein Metabolism

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Although it is well known that protein metabolism is influenced by energy intake, the underlying biochemical mechanism remains obscure. We thought that it might be profitable to study animals under circumstances in which changes in energy intake fail to affect protein metabolism and to compare them with animals under conditions in which the normal relationship holds. Examination of the literature suggested that rats receiving low and high levels of protein intake provide the necessary contrast. Several reports indicate that reduction of the intake of a protein-free diet may not alter the nitrogen balance of rats (Mitchell, 1924; Treichler & Mitchell, 1941; Vars & Gurd, 1947), at least until intake represents less than half the voluntary consumption (Swanson, 1951). On the

other hand, addition of carbohydrate or fat to diets containing adequate amounts of protein results in an improvement in the nitrogen balance of the rat (Forbes, Bratzler, Thacker & Marcy, 1939; Forbes & Swift, 1944; Lathe & Peters, 1949).

In using these observations as a basis for study, it seemed desirable to establish the range of energy intake over which protein metabolism is affected at adequate levels of protein intake and at the same time the range over which the nitrogen balance is unaffected when the animal is receiving a protein-free diet. We have therefore carried out studies of the nitrogen balance and of the protein content of the liver of rats receiving diets rich in protein or deficient in protein, in combination with various levels of energy intake.

METHODS

The influence of energy intake on nitrogen balance and on the protein content of the liver was studied in four series of experiments. Two were carried out using diets containing protein and two with diets free from protein, and at each of these levels of protein intake the variations in energy intake were produced in one series of animals by changes in dietary carbohydrate and in the other series by changes in dietary fat. Two separate experiments were carried out under each of these four dietary conditions; as the results were similar, they have been combined in Exps. 1–4. In addition, a confirmatory experiment (no. 5) was carried out in which only the liver was studied.

Animals. Male albino rats about 5 months old were used. After fasting the stock overnight, sufficient rats weighing between 230 and 270 g. were selected for a single experiment. These were then distributed between the various experimental groups according to the randomized block technique (Snedecor, 1946). This statistical procedure permits one to reduce the effect of differences in initial body weight as a factor in the analysis of the results. The mean initial weights of the rats in Exps. 1–5 were 251, 255, 251, 253 and 258 g., respectively. Their body surface areas were computed from the formula $S = 12{\cdot}54 + W^{0{\cdot}60}$, where S is the surface area and W is the body weight (Lee, 1929).

Diets and general management. The rats were housed under thermostatic conditions (23–25°) in individual metabolism cages. Food was given twice daily in heavy ointment jars and was moistened with water to prevent scattering. At 9 a.m., 2 g. of a vitamin-mineral-roughage mixture (Munro, 1949) were given to all animals. At 5 p.m., rats on the protein-containing diet each received 2·8 g. of casein (Glaxo), 1·0 g. of potato starch, 1·0 g. of glucose and 0·5 g. of fat; in the case of the animals on the protein-free diet, the casein was replaced by an equivalent amount of carbohydrate (half starch and half glucose). These basic diets provided daily about 800–900 kg.cal./sq.m. of body surface area and carbohydrate or fat was added to them to bring the energy intake up to any desired level. The source of additional energy was incorporated in the morning meal, in order to ensure that we were dealing with the effect of energy intake *per se* and not with the interaction effect occurring when protein and carbohydrate are fed at the same meal (Munro, 1949).

At first all rats received an energy intake of approximately 1200 kg.cal./sq.m./day for a period of 7 days, in order that they might become accustomed to the diet. This level of energy intake was obtained by adding 3·5 g. of glucose (Exps. 1, 3 and 5) or 1·5 ml. of olive oil (Exps. 2 and 4) to the appropriate basal diets. During this preliminary period many of the rats on the protein-containing diet showed a slight loss of weight. However, comparison of rats losing weight with those gaining weight during the 7-day period does not suggest any influence of these changes in body weight upon the subsequent response of protein metabolism to variations in energy intake. As might be anticipated, there was a considerable loss of weight during the preliminary feeding period when the diet was free from protein. At the end of this period the energy intake of different animals was altered by addition to, or subtraction from, the diet of carbohydrate or fat fed in the morning meal. In this way different energy levels were provided under otherwise identical conditions. Because of the preliminary week of training to the diet, there was no difficulty in persuading animals to consume diets providing up to 1700 kg.cal./sq.m. of body surface area. Each rat was maintained for 4 days at the new level of energy intake. During this period urine and faeces were collected in Exps. 1–4 by the procedure described by Cuthbertson, McGirr & Robertson (1939), the faeces being marked with ferric oxide. At the end of this period the rats were killed by exsanguination under ether anaesthesia and the livers taken for determinations of protein, nucleic acids and phospholipins. In Exp. 2, the other viscera (stomach, intestines, heart, lungs, kidneys, testes and bladder) were also removed and analysed for nitrogen.

Analytical procedures. Nitrogen analyses on food, urine, faeces and tissues were carried out by a semi-micro-Kjeldahl method, using metallic Hg as the catalyst. The adequacy of the procedure was established by comparison with results obtained using the micromethod of Hiller, Plazin & Van Slyke (1948), which has been thoroughly checked by these authors and by comparison with results obtained on a food mixture with micro-Dumas determinations of N on the same sample.

The livers were analysed for protein, nucleic acids and phospholipins by a slight modification of the Schmidt-Thannhauser (1945) procedure. Each liver was weighed on a chilled watchglass and coarsely minced with scissors. About 0·5 g. of this was accurately weighed into 5 ml. of ice-cold 10% (w/v) A.R. trichloroacetic acid (TCA), ground with a glass rod and centrifuged. The precipitated material was washed twice with 10% TCA and then successively extracted with 5 ml. portions of 80% (w/v) ethanol, absolute ethanol, thrice with an ethanol-$CHCl_3$ mixture (3:1) at 75° for 0·5 hr. and finally with ether. The extracts were combined and the P content determined by the Allen (1940) procedure (lipid P). The residue, containing the nucleic acids and protein, was digested for 18 hr. in N-NaOH at 37°. A portion of the digest was neutralized and TCA added to give a final concentration of 10% (w/v). This precipitated the deoxyribonucleic acid; the precipitate was washed twice with 5% TCA and the supernatant and washings were combined for P estimation (ribonucleic acid P). The precipitate was dissolved in N-NaOH and the P content estimated (deoxyribonucleic acid P). Finally, the N content of the alkaline digest was determined as described above; from this the protein N was obtained by subtracting the contribution of

the nucleic acids to the N contained in the alkaline digest (ribonucleic acid P + deoxyribonucleic acid P × 1·69). The results of the ribonucleic acid and phospholipin analyses will be reported separately.

Statistical analyses. The relationship between energy intake and N balance or liver protein on the different diets was obtained by calculating the regression coefficients, using the analysis of variance technique to determine the significance of the results (Snedecor, 1946). In no instance did the regression lines deviate significantly from linearity.

RESULTS

The first four experiments show (Tables 1 and 2) that there was a linear relationship between energy intake and change in body weight. It is noteworthy that the influence of energy intake on body weight was greater in the two experiments with protein-containing diets than in the two experiments in which the diets were free from protein. This difference, which is statistically significant in the case of carbohydrate but not of fat, is presumably due to the considerable effect of energy intake on nitrogen balance when the diet contains protein, resulting in changes in body protein as well as body fat.

The influence of energy intake on nitrogen balance was found to be dependent on protein intake. In the case of additions of carbohydrate to the diet (Fig. 1), nitrogen balance was affected in a strictly linear fashion by the increments in energy intake when the

Table 1. *Changes in body weight and nitrogen output caused by alterations in energy intake over a 4-day period*

(Groups of four rats in Exps. 1, 3 and 4; groups of five in Exp. 2.)

Exp. no.	Diet	Source of energy variation	Daily energy intake (kg.cal.) Intake/rat	Daily energy intake (kg.cal.) Intake/sq.m. body surface area*	Body weight change (g.)	N intake and ouput during the 4-day period: Intake (mg.)	Urine (mg.)	Faeces (mg.)
1	Protein-containing	Carbohydrate	28·2	850	−13·1	1425	1510	141
			37·5	1100	− 6·0	1425	1419	149
			47·0	1370	+ 1·4	1425	1271	168
			56·3	1660	+ 8·4	1425	1161	164
2	Protein-containing	Fat	28·2	830	− 9·8	1495	1476	124
			42·1	1230	− 0·7	1495	1425	119
			56·1	1610	+ 5·2	1495	1270	145
3	Protein-free	Carbohydrate	29·3	900	−18·5	13	304	117
			38·6	1180	−16·6	13	249	132
			48·1	1450	− 9·5	13	240	127
			56·7	1710	− 6·5	13	227	141
4	Protein-free	Fat	29·3	920	−13·1	13	186	130
			38·3	1190	− 9·0	13	197	130
			47·3	1460	− 7·1	13	214	135
			56·3	1730	+ 0·5	13	194	152

* Surface area calculated from mean body weight during the 4-day period according to the equation of Lee (1929).

Table 2. *Statistical analysis of the effect of changes in energy intake on body weight, nitrogen balance and liver protein*

(The original data are given in Table 1 and Figs. 1–3. The regression coefficients represent the change produced over a 4-day period by an increment in energy intake of 1000 kg.cal./sq.m. of body surface area.)

Exp. no.	Diet	Source of energy variation	Regression coefficients: Body weight (g.)	N balance (mg.)	Liver-protein N (mg.)
1	Protein-containing	Carbohydrate	+26·6*	+410*	+33·0*
2	Protein-containing	Fat	+19·3*	+237*	+46·1*
3	Protein-free	Carbohydrate	+15·7*	+69†	−11·4‡
4	Protein-free	Fat	+15·8*	−41‡	−4·1‡

The probability that the regression coefficient is different from zero is shown as follows:

* Statistically highly significant ($P < 0·01$).

† Although the linear regression coefficient is statistically significant ($P = 0·05 - 0·01$), the regression coefficient based on log. energy intake is more highly significant ($P < 0·01$).

‡ Not statistically significant ($P > 0·05$).

The regression coefficients in Exps. 1 and 2 are significantly different from those obtained in Exps. 3 and 4, except in the case of changes in body weight caused by addition of fat to the diets.

animals were receiving adequate amounts of protein. On the other hand, the same additions of carbohydrate had a much smaller effect on nitrogen balance when the diet was devoid of protein. Moreover, on the latter diet there was a closer relationship between nitrogen balance and the logarithm of energy intake than between nitrogen balance and energy intake itself (Table 2). This suggests a curvilinear relationship, as shown in Fig. 1, and implies that additions of carbohydrate to a protein-free diet become progressively less effective in influencing nitrogen balance as energy intake increases. In the case of additions of fat to the basal diets (Fig. 2), there was again a linear effect on nitrogen balance at adequate levels of protein intake. Fat added to the protein-free diet caused no significant change in N balance. Thus we find a similar positive influence when energy in the form of carbohydrate or fat is added to diets adequate in protein, whereas in the case of diets free from protein there is a failure of carbohydrate to influence nitrogen balance above energy intakes of about 1200 kg.cal./sq.m. and a failure of fat over the whole range studied. Comparison of the regression coefficients (Table 2) shows that the influence of protein level is highly significant in the case of both carbohydrate and fat. These changes in nitrogen balance are essentially due to alteration in urinary output; in all four experiments there was a slight tendency for faecal nitrogen output to rise with increasing energy intake (Table 1).

The effect of energy intake on the total amount of protein in the liver is also significantly influenced by protein intake (Fig. 3 and Table 2). At adequate levels of protein intake (Exps. 1 and 2), the amount per liver was positively influenced by increments in energy intake, whether produced by addition of carbohydrate or of fat. In the case of protein-free diets, on the other hand, there was a slight though not significant tendency for the amount of protein in the liver to decline as energy intake rose (Exps. 3 and 4). These results have been confirmed in Exp. 5, in which animals at adequate and at low levels of protein intake were studied simultaneously. Addition of carbohydrate to these diets to bring the energy intake up from about 850 to 1600 kg.cal./sq.m. resulted in an increase in liver protein on the high-protein diet and a fall in liver protein on the protein-free diet (Table 3). The difference in behaviour at the two levels of protein intake was again statistically highly significant.

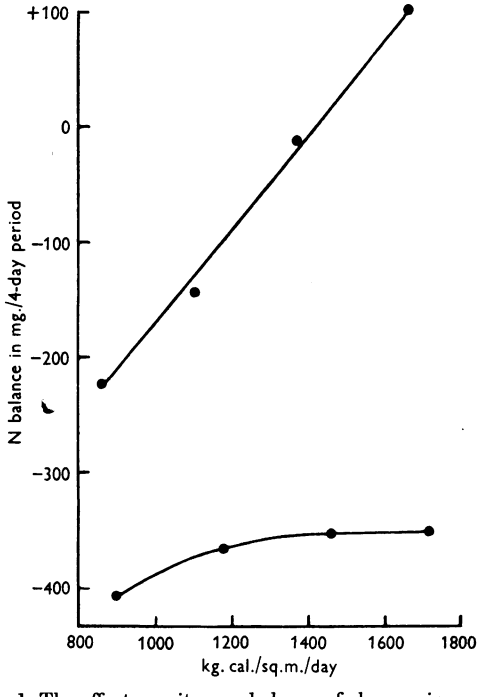

Fig. 1. The effect on nitrogen balance of changes in energy intake produced by alterations in dietary carbohydrate. Upper regression line, protein-containing diet (Exp. 1). Lower line (drawn freehand through points), protein-free diet (Exp. 3). Each point is the mean result obtained with four rats.

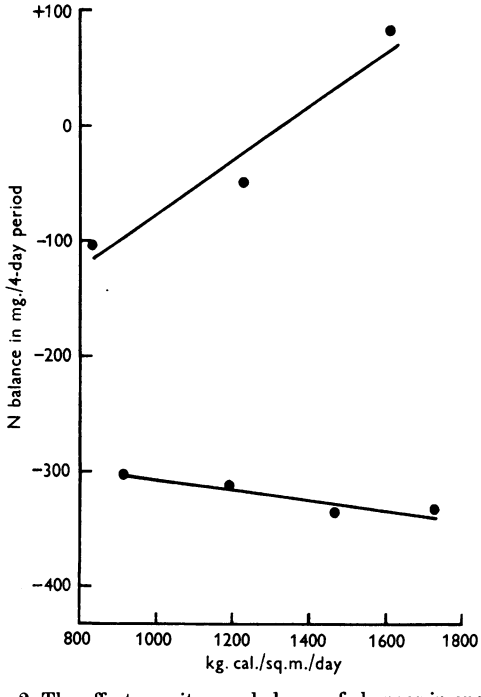

Fig. 2. The effect on nitrogen balance of changes in energy intake produced by alterations in dietary fat. Upper regression line, protein-containing diet (Exp. 2). Lower regression line, protein-free diet (Exp. 4). Each point is the mean result obtained with five rats (Exp. 2) or four rats (Exp. 4).

Exp. 2 provides some information about the partition within the body of changes in nitrogen balance brought about by alterations in the energy content of protein-containing diets. In addition to determinations of liver protein (Fig. 3), the total nitrogen content of the liver and of the other viscera was estimated. The data show (Table 4) that the

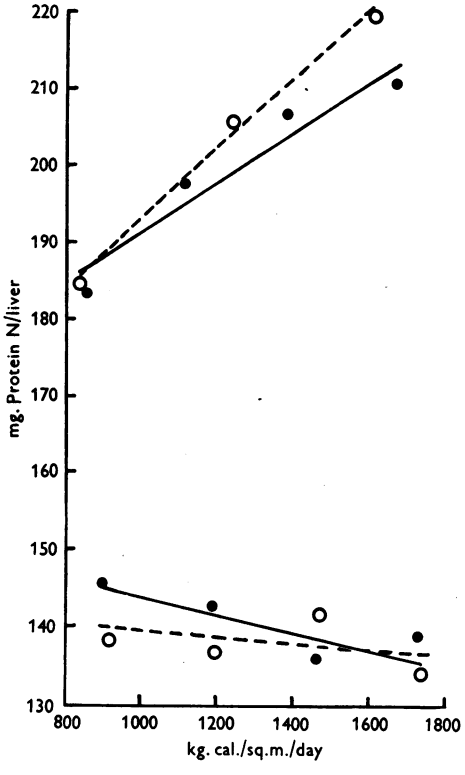

Fig. 3. Changes in liver-protein nitrogen produced by variations in energy intake from carbohydrate (●—●) or from fat (○--- ○). The upper two curves represent the regression lines obtained when the diet contained protein (Exps. 1 and 2). The lower two regression lines were obtained when the diet was free from protein (Exps. 3 and 4). Each point is the mean result obtained with four or five rats.

effect of energy intake on the total nitrogen content of the liver did not account for more than a quarter of the change occurring in nitrogen balance. Energy intake did not have a significant effect on the nitrogen content of the other viscera and it may be concluded that the major part of the change in nitrogen balance occurred through alterations in the amount of nitrogen in the carcass.

DISCUSSION

Exps. 1 and 2 illustrate the relationship between nitrogen balance and energy intake which obtains when the diet provides adequate amounts of protein. In these experiments energy intake was varied from sub-maintenance levels up to levels which caused appreciable increments in body weight and a positive nitrogen balance. Over this range the relationship of nitrogen balance to energy intake was a linear one without evidence of a break at or near nitrogen equilibrium (Figs. 1 and 2). Protein metabolism is thus in a state of dynamic equilibrium with energy

Table 3. *The response of liver protein to changes in energy intake (carbohydrate) at different levels of protein intake*

(Exp. 5, ten rats per group. Analysis of variance demonstrates a significantly different response to changes in energy intake at the two levels of protein intake ($P<0.01$ for interaction). The regression coefficients represent the change produced by an increment in energy intake of 1000 kg.cal./sq.m. of body surface area. Both coefficients differ very significantly from zero and from one another ($P<0.01$).)

Diet	Daily energy intake (kg.cal./sq.m.)	Liver-protein N per rat (mg.)	Regression coefficients (mg.)
Protein-containing	820	191	+28·4
	1570	213	
Protein-free	880	142	−12·3
	1650	133	

Table 4. *The effect of adding fat to a protein-containing diet on the nitrogen content of the liver and other viscera, compared with the changes produced in the nitrogen balance of the whole rat*

(The regression coefficients represent the change produced by an increment of 1000 kg.cal./sq.m. body surface area. Exp. 2, five rats per group.)

Daily energy intake (kg.cal./sq.m.)	N balance per 4-day period (mg.)	Total liver N per rat (mg.)	Total viscera N per rat (mg.)
830	−105·0	240·9	460·9
1230	− 49·5	271·9	480·1
1610	+ 80·3	287·7	471·9
Regression coefficients	+237*	+60·3*	+14·5†

* Statistically highly significant ($P<0.01$).
† Not statistically significant ($P>0.05$).

intake, even when the animal is in nitrogen equilibrium, and any variation in energy level will lead to a corresponding alteration in nitrogen balance. Experiments on human subjects reported by Rubner (1903) and Neumann (1919) are compatible with our findings. Each of these authors studied a subject on a sub-maintenance diet to which was added increasing amounts of fat or carbohydrate. These additions caused a progressive improvement in nitrogen balance through equilibrium to a considerable nitrogen retention. Inspection of their data suggests that the relationship of nitrogen

balance to caloric intake was approximately linear over the range studied. Rosenthal & Allison (1951) describe experiments on dogs in which successive reductions in caloric intake caused a decline in nitrogen balance from positive at high-energy levels to negative at low-energy intakes. It is apparent from their data, however, that quite large changes in energy intake had little effect on nitrogen balance when the animals were near equilibrium. There is no obvious reason for this difference in behaviour of the dog from that of man and the rat.

Exps. 3 and 4 show that the relationship between energy intake and nitrogen balance is altered when protein is omitted from the diet. When carbohydrate intake was changed on a protein-free diet, nitrogen balance was little affected until energy intake fell below 1200 kg.cal./sq.m. of body surface area (Fig. 1). In the case of changes produced by varying fat intake, no significant alteration in nitrogen balance occurred over the whole range of caloric intake studied (Fig. 2). The difference in response to energy in the form of carbohydrate and energy in the form of fat may be related to the composition of the diet given prior to varying the energy intake. In the case of Exp. 3, the energy intake during the preparatory period was made up to 1200 kg.cal./sq.m. by adding carbohydrate to the basal protein-free diet, in order that the lowest energy intakes might be produced by removing this carbohydrate. In Exp. 4, fat was added to the basal diet during the preparatory period, for a similar reason. The fat content of the preparatory diet may have influenced protein metabolism during subsequent undernutrition. Samuels, Gilmore & Reinecke (1948) have observed that nitrogen output during starvation is reduced when the preceding diet contains a high proportion of fat, and Swanson (1951) has noted that restricting the intake of a protein-free diet has a less serious effect on nitrogen balance when the diet contains a high proportion of fat.

Essentially the same picture as that obtained from nitrogen balance studies was obtained from the study of liver protein in these experiments (Fig. 3 and Table 3). Liver protein varied in amount with energy intake in the case of the protein-containing diets, in agreement with Campbell & Kosterlitz (1948) who used the non-glycogen non-lipid solids as an estimate of liver protein, but increments in the energy content of the protein-free diets failed to raise the amount of liver protein. It will be noted in Exp. 3 that there was no change in the amount of liver protein corresponding to the slight improvement in nitrogen balance when carbohydrate was added to the protein-free diet.

These experiments on the relationship between energy intake and protein metabolism may at first sight seem most readily explicable on the hypothesis that the amount of dietary protein used for energy-yielding purposes is inversely proportional to the amount of energy provided from other dietary sources. Thus a decreasing energy intake would result in more and more dietary protein being utilized for supplying energy and less for protein synthesis. If this were so, one would expect the addition of a given amount of protein to the diet to be less effective in causing protein deposition in the body when energy intake was low, since a greater proportion of the added protein would be utilized to provide energy. However, studies on dogs (Allison & Anderson, 1945; Allison, Anderson & Seeley, 1946) have demonstrated that for a given increase in protein intake the same change occurs in nitrogen balance over a considerable range of energy intakes. A similar interpretation can be placed on the observation by Campbell & Kosterlitz (1948) that the amount of protein (non-glycogen, non-lipid solids) in the liver of the rat is related to protein intake by the same regression coefficient at different levels of energy intake.

An alternative explanation of our data is in terms of the factors affecting protein synthesis. Protein synthesis depends on the supply both of amino-acids and of energy, either of which can be a limiting factor in the rate of synthesis. In the case of protein-free diets, energy intake may influence nitrogen balance up to a certain point (e.g. 1200 kg.cal./sq.m. of body surface area in Exp. 3) by promoting re-utilization of amino-acids circulating in the blood, but eventually the limited nature of this source of amino-acids will prevent further improvements in nitrogen balance as energy intake rises. When protein is included in the diet this limitation no longer obtains and protein synthesis proceeds at a rate dependent on the level of energy-yielding metabolites in the tissues. In Exp. 3 nitrogen balance was influenced by changes in energy intake up to 1200 kg.cal./sq.m., but over this range the protein content of the liver did not alter significantly. This indicates that some tissues are less sensitive than the liver to the limitation imposed by a protein-free diet.

A final point which emerges from these experiments is the bodily distribution of changes in nitrogen balance produced by varying the energy content of a diet containing protein (Table 4). Changes in the total nitrogen of the liver accounted for about a quarter of the alteration in nitrogen balance. Since the nitrogen content of the other viscera was not significantly altered, the main change must have taken place in the carcass, i.e. muscle, skin, bone and adipose tissue. Of these, muscle seems the most likely tissue to be involved in such metabolic changes. It is unlikely that the protein of adipose tissue was significantly altered by deposition or removal of fat, since similar changes in

the fat content of the body must have occurred when the energy content of the protein-free diet was altered. Although the changes in liver nitrogen account for only a small proportion of the total change in nitrogen balance, the effect on liver composition is considerable. If we take the nitrogen content of the liver at an energy intake of 1200 kg.cal./sq.m. as our reference standard, then a change in energy intake of 1000 kg.cal./sq.m. caused an alteration of 23 % in the nitrogen contained in the liver. The nitrogen in the carcass of a 250 g. rat amounts to about 6 g., so that the change in nitrogen balance per 1000 kg.cal. not accounted for by the liver would represent only 3 % of the carcass nitrogen. The relative magnitudes of the change in the liver and in the carcass resemble those found by Addis, Poo & Lew (1936) when they altered the protein content of the rat diet. Calculations based on their data show that the liver contained 53 % more protein after 5 days of a high-protein diet than on the 10th day of a protein-free diet, whereas the carcass protein had changed by only 4 %. The ratio of the change in liver protein content to carcass protein content is of the same order of magnitude as the relationship found by us for the effect of changes in energy intake, and this strengthens our belief that the influence of protein intake and of energy intake on protein metabolism operates through a common mechanism, namely their effect on protein synthesis.

SUMMARY

1. Studies of nitrogen balance and the protein content of the liver were made on rats receiving diets either rich in protein or deficient in protein, in combination with various levels of energy intake (from about 850 to 1700 kg.cal./sq.m. of body surface area) which were obtained by adding carbohydrate or fat to standard basal diets.

2. When the diet provided adequate amounts of protein, increments in energy intake, produced by adding either carbohydrate or fat to a sub-maintenance diet, caused a linear improvement in nitrogen balance. Protein metabolism must thus be in a state of dynamic equilibrium with energy intake over the range studied. The total amount of protein contained in the liver also responded to increasing energy intake in a linear fashion. Changes in energy intake caused a smaller percentage change in the N content of the carcass than in the N content of liver.

3. When the diet contained no protein, addition of fat to bring energy intake up from 900 to 1700 kg.cal./sq.m. failed to influence nitrogen balance. Addition of carbohydrate produced some improvement up to 1200 kg.cal./sq.m. but not thereafter. In neither case was the amount of protein in the liver significantly altered.

4. It is suggested that, when the supply of amino-acids circulating to the tissues comes solely from endogenous sources, this becomes a limiting factor in the rate of protein synthesis at quite low levels of energy intake. When the diet supplies adequate amounts of protein this limitation no longer obtains.

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