XCI. A STUDY OF THE EFFECT OF OVERFEEDING ON THE PROTEIN METABOLISM OF MAN

III. THE PROTEIN-SAVING EFFECT OF CARBO- HYDRATE AND FAT WHEN SUPERIMPOSED ON A DIET ADEQUATE FOR MAINTENANCE

BY DAVID PATON CUTHBERTSON AND HAMISH NISBET MUNRO

From the Institute of Physiology, University of Glasgow

(Received 13 February 1937)

IN a previous paper we have drawn attention to the fact that retention of N and S by the adult organism readily occurs when excess protein-containing food-stuffs are added to an apparently adequate diet [Cuthbertson *et al.* 1937]. The degree of retention appeared to bear a relationship not to the increment in protein *per se* but to the total increment in the energy value of the diet.

The protein-sparing effects of carbohydrate and fat have frequently been compared and the difference in their actions has been shown in various ways. As far as the writers know, Voit's experiments on the dog constitute the only evidence available on the relative effects of the addition of carbohydrate or fat to an apparently adequate diet [Voit, 1869]. The experimental conditions were not exactly reproduced in his experiments and so it is not possible to compare accurately the relative effects of these two principles.

According to Voit & Korkunoff [1895] not more than 15 % of the previously metabolized protein can be spared by such additions. The consensus of opinion is that the carbohydrate of the food cannot be replaced by an isocaloric amount of fat without having an adverse effect on the N balance. As Cathcart [1922] has pointed out, "isodynamic" (equal in energy) is not the same as "isotamieutic" (equal in sparing effect). The latter term covers all phases of cellular activity. If the replacement of the carbohydrate by fat is such as to affect not more than half the total calories, the effect is but slight on the N balance, but if this is exceeded then an unfavourable influence is exerted on the N balance.

Maignon [1933] has recently adduced evidence to show that the relative effects of carbohydrate and fat on protein utilization by the rat are dependent on the ratio of the protein to fat or protein to carbohydrate in the diet fed. With rations of average protein content, the N balance was approximately the same in both cases; on the other hand with rations containing a high proportion of protein the N balance was in all seven experiments in favour of fat. When the ration contains a high proportion of fat or carbohydrate, carbohydrate may prove to be superior to fat.

In order to determine the role played by the general rise in energy value of the diet above the energy requirement in their earlier experiments, the writers were the subjects of a further series designed to elucidate this factor.

EXPERIMENTAL

The procedure adopted was somewhat similar to that used in the previous paper. Experiment 1 was carried through in the latter half of April and beginning of May, Experiment 2 in July and Experiment 3 in September, 1936.

Exp. 1. Subject 1 (subject 5 of previous paper). Table I, Fig. 1.

The basal diet differed from that previously adopted in having no cheese present, otherwise the quality was the same and the quantity only slightly different. During an initial period of three days, weight was lost slightly and the diet had to be adjusted by incorporating more bread to balance this tendency. The caloric value of the basal dietary was approximately 3400 as calculated from Plimmer's tables (75 g. protein, 130 g. fat and 460 g. carbohydrate). The "first class" protein moiety amounted to 19 g. of milk proteins. The actual N intake was 15.06 g./day as determined by analysis of the composite diet. The additions of beef, lactose and butter which were made subsequently were equivalent to the protein, carbohydrate and

Fig. 1.

Table I. *Subject 1*

Day of exp.	Body weight kg.	Daily diet	Total Cal.	Urine			Av. daily faecal N g.	S : N of retained material
				Vol. ml.	Total N g.	Total S g.		
4	86.70	Basal adjusted to maintain body wt.	3400	—	—	—	1.765	1 : 33.8
5	86.55			—	—	—		
6	86.15			—	—	—		
7	86.75			1565	12.07	—		
8	86.45	Basal + 160 g. lean topside beef	3600	1500	12.42	0.855	1.491	1 : 21.6
9	86.90			1570	12.50	0.880		
10	86.93			1605	14.68	1.093		
11	87.05			1710	15.95	1.099		
12	86.95	Basal + 160 g. beef + 46 g. lactose + 35 g. butter	4057	1480	15.92	1.103	2.317	S : N of material stopped being retained
13	86.90			1880	15.45	1.086		
14	87.30			1670	16.35	1.118		
15	87.30			1925	16.11	1.114		
16	87.10	Basal reduced in carbohydrate and fat but not protein	2627	1475	15.43	1.049	1 : 19.00	
17	87.33			1660	15.00	1.034		
18	87.50			1990	16.24	1.058		
19	87.93			1900	15.78	1.044		
20	87.42	Ordinary diet	—	1810	17.02	1.153		
21	87.00			2100	18.92	1.232		
22	86.95			1800	18.98	1.262		
23	87.19			2040	19.08	1.178		
24	87.20			1600	18.95	1.230		
25	87.30			—	—	—		

Rise in body weight at end of 5 days extra beef = 0.40 kg.

Further rise in body weight at end of 5 days extra beef + lactose + butter = 0.63 kg.

Fall in body weight at end of 5 days reduced intake = 0.73 kg.

fat contents of a litre of milk. Water equivalent to that present in a litre of milk was added to the drinking water, a control observed in all these experiments.

When N equilibrium was established 160 g. lean topside beef were added to the diet for a period of 5 days, then 46 g. lactose and 35 g. butter were superimposed for a further 5-day period and finally, the protein content being kept constant, the diet was reduced from 4057 Cal. to 2627 Cal./day for a final period of 5 days by the removal of 900 Cal. fat (butter) plus 530 Cal. carbohydrate.

The additional food was consumed in two equal portions at lunch and at supper. The N content of the beef was variable (5.4–5.9 g./100 g. beef) but as in the previous experiments with beef an average value was selected in the final computation.

On superimposing beef alone the excretion of total N and S in the urine gradually rose, but even after 5 days it fell distinctly short of the level to be expected if N and S equilibrium had been established. The daily output of N during the first 3 days was greater than that observed in an earlier experiment in which beef+lactose+ butter were superimposed for an equivalent period. This experiment however had to be abandoned owing to illness.

After 5 days of extra beef intake, the addition of 46 g. lactose and 35 g. butter caused a depression of N and S elimination which tended to reach a constant value after 3 days, i.e. the existing retentions of N and S were still further increased. The increment in the energy value of the diet caused a still greater saving of protein, or it may be that preferential oxidation of carbohydrate and fat had delayed the catabolism of the retained N and S compounds—presumably protein.

As the course of N excretion appeared now to be following the same type of curve noted with Subject 6 of the previous paper when he consumed extra beef, lactose and butter over a period of 15 days, it was decided to withdraw sufficient non-protein calories to bring the total intake to a value below the subject's actual requirement at that metabolic plane. Food equivalent to 1430 Cal. and composed of butter, lactose, cane sugar and apple jelly was removed from the diet. Body weight which had been increasing during the addition of the beef and more rapidly still under the influence of the additional carbohydrate and fat now fell rapidly, and coincidentally the N and S excretions rapidly rose, so that after 2 days in place of a very definite daily retention of N and S there was now a very small loss which continued for the few remaining days of the experiment, the elimination of the retained N and S material taking place at an even slow rate, distinct from the more rapid catabolism of the other retained materials, presumably mainly fat, as evidenced by the initial rapid fall in weight. It was apparent that immediately the necessity to get rid of superfluous food material was removed the normal intake of protein could be dealt with, and that the stored protein was slowly eliminated at a relatively uniform rate. This last observation may help to explain the fact that in the somewhat similar experiment on this Subject 6 in the preceding paper, the basal level of N excretion was not reached following the resumption of the basal diet. In this case there was also a slight but steady loss of N.

The reduction in the calorie value from 4057 to 2627 Cal. was drastic but when the latter diet was consumed over a period of 5 days the fall in body weight had not reached the basal value. Indeed it tended to remain constant after falling for the first 2 days, suggesting that a new metabolic plane had been reached.

The S : N ratio of the material retained during the first 5 days of additional intake was 1 : 33.8 and during the period of extra carbohydrate and fat 1 : 21.6, the S : N ratio of the beef being 1 : 18.55. The values for the retained material are comparable with our recalculated values obtained from Wilson's data [1931]. No account was taken of the faecal loss in these calculations. To calculate the S : N ratio of the material which ceased to be retained, the total urinary outputs of N and S for the preceding 5-day period of beef+lactose+butter were determined, viz. 78.56 g. N and 5.299 g. S. These values were deducted from the total urinary N and S excretions on the low caloric diet, viz. 92.95 g. N and 6.055 g. S. The S : N ratio of the material which was no longer excreted was therefore 1 : 19.0, a value practically identical with the S : N ratio of the beef ingested and quite distinct from the S : N of the total output for this final period which was 1 : 15.35.

Analyses of four separate specimens of lean topside beef gave a mean S : N ratio of 1 : 18.55 (1 : 18.0–19.2). This is higher than the usually accepted ratio of 1 : 14–15 for beef. In determining the total S, Denis's modification of Benedict's method was used following preliminary treatment with conc. HNO_3 , the reagents being S-free (Kahlbaum) and all heating done by electricity. The loss of N in cooking the beef for three-quarters of an hour was not greater than 1 % and is not taken into account.

The increase in weight during the period of overfeeding was approximately 1.03 kg., and the retention of N, 24.89 g. On the basis of 1 kg. body substance (i.e. mainly muscle) equivalent to 30 g. N the expected increase would have been 0.83 kg. Considering the many factors which affect body weight and the doubtful generality of Rubner's basis, there appears to be a fair correspondence again between the calculated and actual gains in weight in this and other experiments. This correspondence however is probably more apparent than real.

This experiment confirmed our growing belief that the retention phenomena which we had observed in the milk, beef and soya bean experiments described in the previous paper were due to the "protein-saving" effect of the excess energy content of the diet, for here we have found that when protein and/or carbohydrate and fat are added to the diet so that the total energy value of the diet is in excess of the organism's requirement, N and S storage take place—at least for a time. When the caloric value is cut down below maintenance or probably even to maintenance requirement the storage rapidly ceases. There is probably then a slow elimination of the retained N and S.

We have purposely used the term protein-saving in place of protein-sparing for in these experiments there is a direct banking of material, presumably protein.

The next question which required to be determined was the relative effectiveness of carbohydrate and fat as protein-savers.

Exps. 2 and 3. Subjects 1 and 2 (5 and 6 of previous paper). Figs. 2 and 3, Tables II and III

A preliminary experiment was carried out by Subject 1, the basal diet being similar to that used in previous experiments with the exception that the milk was reduced to 240 ml./day and slight alterations made in the bread intake. The diet was calculated to have an energy value of approximately 3200 Cal. (protein 66 g.—9 g. first class; fat 113 g. and carbohydrate 460 g.). Body weight was maintained at a constant level for a week prior to the superimposition of the extra food. When a constant urinary N excretion was achieved carbohydrate equivalent to 780 Cal. was added, the addition being made in the form of

"lemon sago" composed of sago, sugar, syrup and lemon juice. This pudding was divided into two equal portions and consumed at lunch and supper. Some difficulty was experienced in consuming this material on an already satisfying diet. The superimposition of additional carbohydrate effected a saving of 3.68 g. N in the 3-day period and, on removing it, retention ceased, there being a

Fig. 2.

Fig. 3.

Table II. *Subject 1. N- and S-saving effects of superimposed carbohydrates and fat on a diet adequate for maintenance*

Day of exp.	Body weight kg.	Daily diet	Total Cal.	Urine			Av. daily faecal N g.
				Vol. ml.	Total N g.	Total S g.	
0	87.55	Ordinary	—	—	—	—	
1	87.15	Basal	3200	—	—	—	
2	87.00			—	—	—	
3	87.15			—	—	—	
4	87.28			—	—	—	
5	87.10	Basal + carb.	3980	2020	11.53	0.822	2.000
6	87.00			1490	10.96	0.797	
7	87.12			1400	10.99	0.753	
8	87.11			2055	10.13	—*	2.723
9	87.65	Basal	3200	1855	9.56	0.747	
10	87.40			2770	9.57	0.743	
11	87.32			1830	10.02	0.744	1.467
12	87.08			2020	11.36	0.801	
13	87.05	Basal + fat	3900	1660	11.43	0.814	
14	87.40			1580	11.61	0.788	
15	87.60			1770	11.07	0.730	
16	87.48			2540	10.88	0.763	
17	87.15	Basal	3200	1920	10.72	0.753	
18	87.43			2230	11.42	0.774	

* Urine accidentally contaminated with H_2SO_4 .

Table III. *Subject 2. N-saving effects of superimposed fat and carbohydrate on a diet adequate for maintenance*

Day of exp.	Body weight kg.	Daily diet	Total Cal.	Urine			Av. daily faecal N g.	S : N material saved
				Vol. ml.	T.N. g.	T.S. g.		
3	63.30	Basal	2890	2257	10.86	—	0.84	
4	62.95			2040	11.01	0.772		
5	63.25			1810	11.06	0.821		
6	63.45			1700	10.55	0.766		
				Mean	10.87	0.786		
7	63.45	Basal + fat	3590	1450	10.01	0.719	1.06	1 : 14.0
8	63.40			1665	10.25	0.749		
9	63.40			1290	9.78	0.724		
10	63.30			1327	9.73	0.723		
11	63.40	Basal	2890	1168	9.49	0.697	1.03	1 : 11.6
12	63.45			1830	9.54	0.645		
13	63.30			1880	9.67	0.693		
14	63.30			1410	9.81	0.704		
15	63.05	Basal + carb.	3670	1630	10.00	0.724	1.13	1 : 14.2
16	63.15			1175	10.52	0.784		
17	63.15			1210	10.56	0.752		
18	63.15			1435	11.35	0.774		
19	63.25	Basal + double previous carb.	4450	1840	10.08	0.694	1.13	1 : 13.0
20	63.60			1400	9.41	0.719		
21	63.80			1735	9.20	0.668		
22	63.65			1500	8.57	0.629		
23	64.15	Basal	2890	1640	8.69	0.630	1.13	1 : 13.8
24	64.65			1860	8.18	0.540		
25	64.80			1825	7.08	0.532		
26	64.85			1730	7.15	0.499		
27	64.10	Basal	2890	2230	8.27	0.643	1.13	1 : 13.8
28	64.20			1690	9.19	0.664		
29	63.95			1980	10.14	0.701		
30	63.85			1820	10.11	0.730		
31	63.15			2040	9.74	0.796		
32	63.90			1885	11.16	0.765		
33	—			1950	10.95	0.729		

lag of a day before the basal value was reached and apparently passed. It appeared as if there was a slow liberation of the previously stored N.

On the third day of resumed basal diet, additional fat in the form of 90 g. fresh butter—700 Cal.—was added. This was easily taken and caused a definite but less marked sparing action. Owing to a limitation of time for the conduct of this experiment it was not possible to prolong the basal period preceding the addition of fat. Strictly speaking time should have been allowed for a return to the basal N excretion. It is perhaps legitimate to take the average N excretion of the last two days of this second basal period as the base line for the assessment of the N-saving effect of the extra fat rather than the average basal value of the first period. If this be taken the retention within the three days of extra fat intake was 0.64 g. N. This saving effect of fat appeared to persist into the first day of the final basal period. That is, there was a lag before basal conditions were again reached. If we take the four days of N retention in each period we find that carbohydrate equivalent to 780 Cal. spared 4.64 g. N (0.59 g. per 100 Cal.) and fat equivalent to 700 Cal. spared 1.48 g. N (0.21 g. per 100 Cal.). The ratio of the N saved by carbohydrate

N saved by fat per 100 Cal. was 2.81.

Owing to the accidental contamination of one of the day's collections of urine with H_2SO_4 the relative significance of the S retentions cannot be assessed accurately. The data which are available, unsatisfactory as they are, indicate that the S-saving effects of carbohydrate and fat were not so widely different when assessed over this relatively short period. The retentions of N and S in each period of surfeit feeding were accompanied by increments in body weight which were in part reduced by a diuresis which occurred in each instance on the third day of extra food.

To rule out the possibility that this difference in N-saving power might be due to the carbohydrate effect not having passed off before the superimposition of the additional fat, the experiment was repeated by Subject 2 but the order of superimposition was reversed.

Subject 2's basal dietary had an energy value of approximately 2890 Cal. (protein 66 g.,—22 g. first class; fat 140 g. and carbohydrate 320 g.), the ingredients being similar in quality and approximately in quantity to those selected in previous dietaries. No reduction in milk intake was made in this case. The additional fat was butter but the selected carbohydrate glucose. The additional butter was all taken at breakfast and to simulate the slow digestion and absorption of fat, the additional glucose was taken in small lots hourly over a period of 3 hours. The total daily intake of water was kept constant. The addition of 90 g. butter raised the energy value of the diet from 2890 Cal. to 3590 Cal. and caused definite retentions of N and S amounting to 6.42 and 0.459 g. respectively. The S:N ratio of this stored material was 1:14.0. Resumption of basal conditions did not cause an immediate return to normal. There was a lag of three days before the normal levels of urinary N and S were attained. The S:N ratio of the material stored during this period was 1:11.6. Although marked by initial fluctuations in body weight, this retention was accompanied by an increase in weight, for on resumption of basal conditions a slight fall in weight resulted.

By an error in Exps. 2 and 3, in place of an isocaloric equivalent of sugar, slightly larger supplements were given (approximately 12%). In Exp. 3, in place of 185 g. glucose, 210 g. were superimposed for 5 days, an addition of 780 Cal./day. A greater N- and S-saving effect was noted with glucose than with butter even when allowance was made for the slight difference in caloric value. The N-saving effect of the carbohydrate over 5 days was 8.40 g. (1.08 g./100 Cal.), that of fat for an equivalent period 5.09 (0.73 g./100 Cal.). The S-saving effect of the carbohydrate for the same period was 0.59 g. (0.076 g./100 Cal.), that of fat 0.318 g. (0.045 g./100 Cal.). The ratio $\frac{\text{N or S saved by carbohydrate/100 Cal.}}{\text{N or S saved by fat/100 Cal.}}$ was for N 1.48 and for S 1.69 for the period under consideration. Corresponding to this increased power of carbohydrate over fat to save N and S, was its faculty of causing a greater weight increase. The S:N ratio of the material retained was 1:14.2—a value similar to that noted during the addition of fat.

At the end of 5 days the intake of glucose was doubled, thus raising the total caloric value of the diet to 4450 Cal., a value far in excess of the subject's requirement for no additional energy expenditure was incurred. The additional glucose was taken at hourly intervals between 2 and 6 p.m. and was continued for 3 days. A further saving of N and S was effected amounting to 10.20 g. N and 0.787 g. S for the period. The first addition of glucose depressed the urinary excretions of N and S to levels 2.18 g. and 0.156 g. respectively below their basal outputs. The second addition reduced the levels of N and S excretion to values 3.72 and 0.287 g. below their respective basals. This second addition of glucose

caused the body weight to rise still higher. At the end of this period it had risen by about 1.7 kg. The S : N ratio of the stored material was 1 : 13.0 at this stage. It appeared possible to spare respectively 34 % and 36 % of the previously metabolized N and S.

Resumption of basal conditions caused a rise in N and S output and a fall in body weight, the basal values being reached in about 5 days. This rapid decline in weight was not accompanied by a contemporaneous liberation of the stored N and S nor was it mainly due to the freeing of water from the organism. The material catabolized by the organism during this period must probably have been largely fat. The experiment unfortunately could not be continued longer so that it was not possible to determine the fate of the retained N and S. In view of our previous experiments it is highly probable that these would have been slowly liberated.

DISCUSSION

The sparing effect of carbohydrate and fat on the loss of protein which occurs on an insufficient diet is understandable, but the fact that an increment in the energy value of the food beyond the needs of the organism also causes a retention of N and S requires an extension of our conception of the metabolism of protein, carbohydrate and fat.

When the adequately fed organism is confronted with the necessity of disposing of food ingested in excess of its energy requirement it burns what it can and stores what it cannot. An alimentary glycosuria may result if huge excesses of carbohydrate are given. No glycosuria was noted in our experiments. Although observations on the basal metabolic rate during the earlier milk experiments on Subject 2 indicated no elevation of the basal metabolism this cannot be taken as indicative of the absence of an increment in the oxidative processes over the normal during the 24 hours. If 24-hour metabolic observations can be made the present writers believe that such work will reveal a definite increase in the oxidative processes but one insufficient to account for the disposal of all the excess potential energy of the food.

With regard to the storage mechanism we know definitely that it is associated with a retention of N and S, and with an increment in body weight. The retention of N in the experiments in which there was a direct increase in the protein intake corresponded approximately to the increment in body weight if it is assumed that 30 g. N are equivalent to 1 kg. body substance, i.e. mainly muscle (Rubner's basis). This equivalence may not have any real value and did not hold in the last experiment when the supplements were non-protein. We believe that the optimum ratio for the proximate principles in an adult dietary are precisely those selected by the mass of the people, viz. 10 % of the energy requirement from protein (approx. 1 g. per kg. body weight) 25–35 % from fat and the remainder from carbohydrate. If, and only if, the total energy requirement of the organism be exceeded and the substance in excess of its normal content be protein, does storage of some of this protein take place. In the case of protein there is good evidence to indicate that the metabolic plane is gradually raised so that after a time further storage of N ceases. On the other hand if the substance in excess be carbohydrate the glycogen stores of the liver, muscle and other tissues are soon filled up, for the capacity to store glycogen is limited. With a continued flooding of the glucose into the blood, carbohydrate oxidation rises more and more, while the rate of glycogen deposition falls off. It is at this point probably that fat formation must be set up in order to dispose of the continued supply of carbohydrate. The capacity for fat storage is enormous. With the

rise in carbohydrate oxidation and the necessity for its disposal there occurs a diminished deamination of amino-acids for the non-protein moiety is no longer required for fuel purposes, consequently there occurs N and also S retention, the unrequired amino-acids being stored as new protein or attached as Roche [1934, 3] would have us believe to already existing protein. When it is the fat moiety which is increased, there occurs a rise in the rate of fat oxidation and storage also readily occurs. Again there is not the same demand for protein for energy purposes so that deamination diminishes and storage of the amino-acids takes place. In this case however the effect on protein metabolism is not so marked, perhaps owing chiefly to the facility for fat storage and also because the increase in fat oxidation will require for its completion an increment in carbohydrate metabolism and so necessitate the utilization of more of the non-N residue of the amino-acids.

The superimposition experiment overcomes the difficulty of measuring the cutaneous losses for it can reasonably be assumed that such alteration as may occur in the amount of N excreted by this path on changing the diet will not alter appreciably the relationship between excess intake and excess output. This factor and the question of defective digestion and absorption from the gut can be ruled out of account as contributing to the observed phenomena.

It is of interest to consider the possible nature and site of this retained N and S.

The nature and site of the retained N

There is no evidence that any of the catabolites which we associate with the breakdown of protein is retained in the tissues.

Cathcart & Leathes [1905] showed that during active absorption from the intestine there was a definite amount of N stored in the liver. This was purely a temporary phenomenon but the work of Pugliese [1904] has demonstrated that the liver is extensible, increasing in weight and total N content during feeding, decreasing during fasting. Gautier & Thiers' [1928] experiments on the frog confirm this. Luck [1936] has recently shown that mammalian liver yields four fractions, viz. globulin II, euglobulin, pseudoglobulin and albumin and in rats maintained on a low-protein diet these were respectively 5.07, 4.58, 1.06 and 0.86 g. per 100 g. liver. On a high-protein diet each of the above fractions was increased by 50–60 %. There was not only hyperplasia or hypertrophy but also an increased content of protein per unit of tissue. All the liver proteins therefore participate equally in the function of storage.

Here we have definite evidence of a N reserve. The essential character of such a reserve is its variability. The work of Terroine [1933] has demonstrated that the organism possesses two great groups of substances (1) the necessary elements of all the tissues in equilibrium amongst themselves and presenting fixed ratios one to the other, the concentration of which rather than their nature is not modified by any external influence, and (2) the reserve elements, variable in quantity and quality, whose presence effects necessary differences in the total composition of the organism. Just as the amounts of fat and glycogen in the organism can be increased or diminished by appropriate dietary measures, so ought also the N content.

Terroine *et al.* [1924] and others have demonstrated that starving animals are richer in N than well nourished, owing to the disappearance of the fat reserves, and this fact has to be taken into account in experiments designed to differentiate the *élément constant* from the *élément variable* for N. It is well known that the N wastage through "wear and tear" is very little and even if one ceases to cover

it for a fairly long time little real damage is done to the organism. It is not surprising therefore if the storage of nitrogenous matter as a true reserve is dwarfed in the analysis of the total carcass or specific organ.

During the fattening of mature domesticated animals there is a gain in protein and mineral matter in addition to fat. A certain amount of this gain in protein must be accounted for by growth of the epidermis and its appendages and by an actual increase in the cells of the adipose tissue. The classical experiments of Lawes & Gilbert [1859] were probably the earliest examples of an investigation designed to ascertain the nature of the material stored. The average age of the cattle selected was 4 years and the composition of the increase was as follows: water 24.64; ash 1.47, protein 7.69 and fat 66.20%. Armsby [1917] has summarized the literature on this aspect of nutrition. It may be noted in passing that balance experiments also substantiate a retention of protein, sometimes of quite a considerable order. In Kellner's experiments protein varied from 8.06 to 26.25% of the organic matter retained. It is doubtful if the epidermal and adipose tissues can account for all the N stored during fattening.

If the N is stored in the tissues as protein, then it is not impossible that the constitution of the total protein should change, for it is unlikely that the stored material should necessarily possess the same molecular structure as the proteins entering into the active protoplasmic mass of the tissue. The experiments of Luck [1936] do not substantiate this conjecture at least so far as the liver is concerned. Roche [1933] has found in rats that the N loss on a N-free diet comes from a partial disintegration of the molecules of muscular protein, which substantiates MacCollum & Steenbock's earlier hypothesis [1912]. The monoamino-acids are increased, the diamino-acids greatly decreased, cystine, histidine and arginine remained constant, while tryptophan, tyrosine and lysine diminished [Roche, 1934, 2]. In the absence of food protein, the N requirement was met by the partial mobilization of muscle protein and not of a special reserve protein [Roche, 1934, 1]. Can we extend this idea to the question of retention or loss of N in the period preceding the establishment of a new equilibrium? Is it also to be explained by the integration or disintegration of existing protein units? In this process does the liver, or muscle tissue, play the dominant role? Addis *et al.* [1936, 1, 2] have investigated the quantities of protein lost by the various organs and tissues of rats during 2-day and 7-day fasts. After a 2-day fast the livers lost 20% of their original protein content, the kidney, heart and all the other organs and tissues combined lost 4%. During a 7-day fast the liver lost 40% of its protein, the prostate and seminal vesicles 29%, the heart, kidneys, drawn blood and alimentary tract 18 to 28%, the muscle, skin and skeleton 8% and the brain 5%. Of the total protein lost in 7 days the muscle, skin and skeleton contributed 62%, the liver 16%. The protein reserves of the organism are relatively enormous and if they are capable of waxing and waning in molecular complexity even to a very slight extent N retention and N loss become explicable. Rubner [1902] and others have proved that the fraction of the ingested protein which is absorbed and retained exercises no specific dynamic action, a fact which may be held to indicate that this deposited N is for a time in a stable state.

What might have proved a useful line of enquiry into the nature of the retained protein has been partially dispelled by Wilson's work [1931]. It seemed reasonable to argue that the S : N ratio of the material catabolized on changing from a high to a low diet should indicate the nature of the retained material provided that its S : N ratio was different from that of the tissue protein. Wilson found that the organism can store protein with varying S : N ratios, the higher the S content the more unstable the retained material.

If the elements of the tissues are degraded or integrated in the same proportion as they occur in the tissues then all the nitrogenous constituents of the metabolic N—the urea, amino-acids and ammonia from the protein and from the other components of the tissues, purine bodies and derivatives, creatine and creatinine—ought, according to Terroine & Boy [1931], to rise or fall simultaneously. On the other hand if there had been a storage of protein, then degradation of this reserve will result in constituents of protein origin. Their experiments to settle this point indicated that the catabolites which are excreted in the urine when the N excretion drops from that of a normal diet to the endogenous level are of protein origin.

Although the increase in caloric intake in the majority of our experiments was some 25 %, it proved about the limit for comfort during protracted experiments where the energy expenditure was maintained at a relatively constant level.

It is fairly obvious that the processes of retention which occur in the normal organism faced with an ingestion of excess food cannot go on indefinitely. In the absence of a rise in the metabolic plane sufficient to utilize the excess energy the condition which limits the process is simply failure of appetite. The normal adult organism as soon as possible catabolizes the stored material as evidenced by the rapid reduction in weight which occurs when the additional intake is removed. This is not so certain in the later stages of adolescence for further growth is still possible and it is of interest that the S : N ratio of the material stored then closely resembles that lost in starvation.

Lack of appetite, as a factor limiting the process of overfeeding, was demonstrated in an interesting fashion by some observations on another subject not previously described in this series. During a prolonged period of daily observation of his weight, this subject attempted to superimpose 500 ml. raw milk per day without, at least consciously, reducing his usual diet. Body weight did not rise but fell. An independent observer of this experiment noted that the subject had unconsciously reduced his intake. This was substantiated by the fact that in a previous trial with 1000 ml. additional milk per day the intake of other food had obviously had to be reduced.

Our observations may shed some light on the effect of work on protein metabolism. If the energy intake is in excess and the work done insufficient to utilize the excess, a rise in the output of N would be expected, a rise greater than that due to a general increment in "wear and tear". On the other hand if work is superimposed on a dietary which is just adequate to meet the new energy requirement, then no rise beyond perhaps a slight increment due to wear and tear would be anticipated. Experiments to elucidate the effect of work are in process of being formulated.

SUMMARY

1. When the intake of potential energy in the diet of the writers was raised so that it exceeded the requirement necessary to maintain body weight and N equilibrium, an increase in body weight, coupled with definite retentions of N and S, took place during the experimental periods considered.

2. The addition of glucose, equivalent to 780 Cal., to a diet of 2890 Cal. caused reductions in the daily urinary output of N and S of 2.18 g. and 0.156 g. respectively. Doubling this intake of glucose caused ultimately a total reduction in the daily outputs of N and S of 3.72 g. and 0.287 g., thus saving daily respectively 34 and 36 % of the previously metabolized N and S.

3. The ratio $\frac{\text{N/or S-saving effect of carbohydrate}}{\text{N/or S-saving effect of fat}}$ per 100 Cal. additional intake was for one subject, 1.48 for N and 1.69 for S. For the other subject the ratio for N was 2.81. These values indicate that under the experimental conditions adopted carbohydrate had greater N- and S-saving effects than fat.

4. The nature and site of the retained material is discussed.

In conclusion we wish to express our thanks to Prof. E. P. Cathcart, for his helpful criticism and to Prof. G. M. Wishart for his kind co-operation in this work.

A grant was made from the D. C. Andrews' Research Fund in aid of this investigation.

REFERENCES

- Addis, Poo & Lew (1936, 1). *J. biol. Chem.* **115**, 111.
 ——— (1936, 2). *J. biol. Chem.* **115**, 117.
 Armsby (1917). *The Nutrition of farm Animals*. (Macmillan & Co., New York.)
 Cathcart (1922). *Biochem. J.* **16**, 747.
 ——— & Leathes (1905). *J. Physiol.* **33**, 462.
 Cuthbertson, McCutcheon & Munro (1937). *Biochem. J.* **31**, 681.
 Gautier & Thiers (1928). *Bull. Soc. Chim. biol., Paris*, **10**, 537.
 Lawes & Gilbert (1859). *Philos. Trans.* **149**, 493.
 Luck (1936). *J. biol. Chem.* **115**, 491.
 McCollum & Steenbock (1912). *Res. Bull. Wisc. Agric. Exp. Stat.* No. 21, 53.
 Maignon (1933). *Nutr. Abst. Rev.* **3**, 345.
 Pugliese (1904). *J. Physiol. Path. gén.* **6**, 193.
 Roche (1933). *Bull. Soc. Chim. biol., Paris*, **15**, 1290.
 ——— (1934, 1). *Bull. Soc. Chim. biol., Paris*, **16**, 257.
 ——— (1934, 2). *Bull. Soc. Chim. biol., Paris*, **16**, 270.
 ——— (1934, 3). *Skand. Arch. Physiol.* **69**, 75.
 Rubner (1902). *Die Gesetze des Energieverbrauchs bei der Ernährung*. (Deuticke, Leipzig and Vienna.)
 Terroine (1933). *Métabolisme de l'azote* (Les Presses Universitaires de France, Paris).
 ——— & Boy (1931). *C.R. Acad. Sci., Paris*, **193**, 1034.
 ——— Brenkmann & Feuerbach (1924). *Arch. int. Physiol.* **22**, 233.
 Voit (1869). *Z. Biol.* **5**, 329.
 ——— & Korkunoff (1895). *Z. Biol.* **32**, 58.
 Wilson (1931). *J. Physiol.* **72**, 327.

Note added 6 April 1937. Since this paper was written the interesting experiments of Larson and Chaikoff [*J. Nutrit.* 1937, **13**, 287] have appeared. Their experiments on the effect of additional carbohydrate on the N metabolism of the dog confirm our work. They noted that the maximum N-saving effect occurred when the additional carbohydrate was ingested at the same time as the other food or within one hour of it. The interval during which the extra carbohydrate was able to exert an effect was limited to 4 hours before and to 4 hours after the ingestion of the daily meal.