XV. THE RELATIONSHIP OF CARBOHYDRATE METABOLISM TO PROTEIN METABOLISM

I. THE ROLES OF TOTAL DIETARY CARBOHYDRATE AND OF SURFEIT CARBOHYDRATE IN PROTEIN METABOLISM

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EARLIER observations showed that the provision of excess of energy in the human diet is attended by a storage of N and S, the storage being more marked when the surfeit is in the form of carbohydrate than when in the form of fat [Cuthbertson & Munro, 1937]. Simultaneously with these observations, it was demonstrated by Larson & Chaikoff [1937] in one-day superimposition experiments on dogs, that this storage was only provoked by carbohydrate ingested within 4 hr. before or after the protein meal. Moreover, the closer the proximity in time of the surfeit carbohydrate to the meal, the greater was the quantity of N stored.

These effects of surfeit carbohydrate on protein metabolism prompted us to investigate the function of the total carbohydrate of the ordinary adequate diet, as it affects N metabolism, especially with regard to the relative times of ingestion of the protein and the carbohydrate. The present investigation of protein metabolism is divided into four sections:

(I) The effect of complete separation as regards time of ingestion of all, or practically all, the carbohydrate and the protein of an adequate diet (Exps. 1-5).

(II) The effect of separation of only a part of the protein from the carbohydrate in such a diet (Exp. 6).

(III) The effect of taking surfeit carbohydrate at a time separated from that of the ingestion of the protein (Exp. 7).

(IV) The effect on N balance of varying the number of meals in a fixed diet (Exp. 8). (This is intended as a control for certain aspects of the other experiments.)

EXPERIMENTAL

The analytical procedures were similar to those referred to in a previous paper [Cuthbertson & Munro, 1937]. The fluid intake was kept as constant as possible throughout these experiments.

I. *Separate ingestion of dietary protein and carbohydrate*

Exps. 1 and 2. In preliminary experiments, two adults, A. H. S. and H. B. C. (Table I), were put on diets adequate for maintenance, containing 80 g. protein and 2740 cal. in the case of the former, and 97 g. and 3400 cal. in the case of the latter. During Period I this food was divided for both subjects into four equal mixed meals, taken at 8.30 a.m., 1 p.m., 4.30 p.m. and 10 p.m. respectively (Tables II and III). Their body-weights remained constant under this regime.

Table I. *The experimental subjects*

Subject	Age yr.	Height cm.	Wt. kg.	Exp. no.	Diet			Calories
					Protein g.	Carbo- hydrate g.	Fat g.	
A. H. S.	30	168	64	1	80	367	101	2740
H. B. C.	24	184	80	2	97	455	127	3400
H. N. M.	22	164	61.5	3	84	400	61	2500
			61.25	6	116	390	87	2880
			64.4	7	76	320	140	2930
			63.9	8	76	320	140	2930
D. P. C.	38	184	83	4	66	408	172	3500
			83.7	5	76	408	174	3560

Moreover, if one allows 1.3 g. as covering the daily faecal and cutaneous losses of N (which were not estimated), the average daily urinary N values for this period (10.82 g. for A. H. S.; 14.27 g. for H. B. C.) agree well with their N intakes of 12.56 and 15.5 g. respectively, confirming the attainment of N equilibrium in each subject.

In Period II, this arrangement of meals was altered so that all the protein and a part of the fat of the diet were divided between the meals at 8.30 a.m. and 4.30 p.m., while the remainder of the fat and practically all the carbohydrate were consumed at 1 p.m. and 10 p.m. (Tables II and III). This almost complete separation of protein from carbohydrate resulted in a marked loss of N, which obtained throughout the period of observation and amounted on the average to 1.77 g. daily for A. H. S. and 2.02 g. for H. B. C. (Figs. 1 and 2). It will be noticed that during no part of the day was there any lack of energy-yielding food material (Tables II and III).

In the case of H. B. C. a return to the previous dietary was made in Period III, and this effected an immediate cessation of the N loss. Under the arrangement of meals in Period II the protein-containing meals were reduced in number from 4 to 2 (Tables II and III); that this procedure was not of itself productive of the marked negative N balance will be subsequently demonstrated in Exp. 8.

Exp. 3. This experiment followed along the lines of the previous two, but differed in that the procedure was reversed; the daily urinary collection was separated into two fractions, from 8 a.m.–8 p.m. and 8 p.m.–8 a.m., and account was taken of urea, preformed sulphate and creatinine as well as N excretions. The subject H. N. M. (Table I) partook of a diet containing egg-white, cheese, casein, arrowroot, sugar, butter and apples (84 g. protein, 61 g. fat and 400 g. carbohydrate, in all, some 2500 cal.).

In Period I, all the protein and part of the fat were taken at 9.30 a.m. and 12.45 p.m., while the rest of the fat and all the carbohydrate were spread between 4.30 and 7.30 p.m. (Table IV). During this period of separation of carbohydrate from protein, the daily N intake was 13.36 g., while the urinary output averaged 14.80 g. (Fig. 3), a considerable loss.

In Period II, the carbohydrate and fat of the previous two later meals were taken instead along with the protein and fat at 9.30 a.m. and 12.45 p.m.; the hours of protein ingestion were thus unchanged from Period I, whilst the carbohydrate was now associated with the protein. This produced an immediate fall in urinary N output, down to an average over the period of 12.67 g. (intake 13.36 g.), which is presumably only slightly in excess of N equilibrium. The difference in N output between Periods I and II amounted to 2.13 g. daily.

Table II. *Dietary of subject A. H. S., Exp. 1*

	Period I				Period II			
	8.20 a.m.	1 p.m.	4.30 p.m.	10 p.m.	8.30 a.m.	1 p.m.	4.30 p.m.	10 p.m.
	Same food at all meals (g.)				g.	g.	g.	g.
Lean beef			71.25		142.5	—	142.5	—
Egg			37.5		75	—	75	—
Lettuce			10		20	—	20	—
Apple			50		100	—	100	—
Butter			15		—	30	—	30
Arrowroot			35		—	70	—	70
Sugar			56		—	112	—	112
Calorie distribution at each meal:								
Protein		81			166	—	166	—
Carbohydrate		367			46	688	46	688
Fat		236			240	232	240	232

Table III. *Dietary of subject H. B. C., Exp. 2*

	Periods I and III				Period II			
	8.30 a.m.	1 p.m.	4.30 p.m.	10 p.m.	8.30 a.m.	1 p.m.	4.30 p.m.	10 p.m.
	Same food at all meals (g.)				g.	g.	g.	g.
Lean beef			85		170	—	170	—
Egg			50		100	—	100	—
Lettuce			10		20	—	20	—
Apple			50		100	—	100	—
Butter			15		—	30	—	30
Arrowroot			42		—	84	—	84
Sugar			71		—	142	—	142
Calorie distribution at each meal:								
Protein		100			200	—	200	—
Carbohydrate		455			46	864	46	864
Fat		296			360	232	360	232

Fig. 1. Exp. 1. Subject A.H.S.

Fig. 2. Exp. 2. Subject H.B.C.

Figs. 1 and 2. Effect on urinary N excretion of separately ingesting the total protein and the carbohydrate of an adequate diet. P=protein. C=carbohydrate. F=fat.

Table IV. *Dietary of subject H. N. M., Exp. 3*

	Period I				Period II	
	9.30 a.m. cal.	12.45 p.m. cal.	4.30 p.m. cal.	7.30 p.m. cal.	9.30 a.m. cal.	12.45 p.m. cal.
Protein	172	172	—	—	172	172
Carbohydrate	—	—	820	820	820	820
Fat	116	116	168	168	284	284

Fig. 3. Exp. 3. Subject H.N.M.

Fig. 4. Exp. 4. Subject D.P.C.

Figs. 3 and 4. Effect of separately ingesting the protein and carbohydrate of an adequate diet.

Urea and also NH_3 excretion closely paralleled that of total N. The preformed sulphate-S also took the same trend as the N, the excretion in Period I in excess of that in Period II averaging 0.129 g. S daily. The creatinine N output, however, remained relatively constant throughout. A study of the day (8 a.m.—8 p.m.) and night (8 p.m.—8 a.m.) urines indicates that the N content of each was equally affected by the change-over from Period I to Period II. On the other hand, only the day excretion of preformed sulphate was affected, the night excretion remaining relatively constant. It was not possible to record body-weights during the whole course of this experiment.

Exp. 4. This was confirmatory of the previous experiment. The subject D. P. C. (Table I) took a diet (Table V) containing 66 g. protein and 3500 calories,

which were so distributed as to enable him to eat meals consisting only of protein and fat at 8.10 a.m. and 1.15 p.m., the meals at 4.45 p.m. and 10.30 p.m. containing only carbohydrate and fat. During Period I the average daily urinary N output was 11.07 g. (Fig. 4), which with a faecal N of 1.29 g. daily (derived from Exp. 5) indicates a marked loss when compared with the intake of 10.52 g. N.

Table V. *Dietary of subject D. P. C., Exp. 4*

	Period I				Period II			
	8.10 a.m. g.	1.15 p.m. g.	4.45 p.m. g.	10.30 p.m. g.	8.10 a.m. g.	1.15 p.m. g.	4.45 p.m. g.	10.30 p.m. g.
Cheese	60	60	—	—	60	60	—	—
Butter	20	20	35	35	50	50	5	5
Egg	125	150	—	—	125	150	—	—
Lettuce	25	—	25	25	25	50	—	—
Apple	—	—	100	100	—	—	100	100
"Oxo"	—	$\frac{1}{2}$ cube	—	—	—	$\frac{1}{2}$ cube	—	—
Sugar	—	—	146	146	48	48	50	50
Arrowroot	—	—	60	60	60	60	60	60
Calorie distribution at each meal:								
Protein	130	140	—	—	130	140	—	—
Carbohydrate	—	—	816	816	384	384	432	432
Fat	493	521	293	293	493	521	38	38

The association in Period II of a considerable portion of the carbohydrate of the evening meals with the protein-containing meals of the earlier part of the day (Table V) resulted in a sharp drop in the N output to an average value of 9.27 g. daily on the last 2 days of this period (Fig. 4). N equilibrium was virtually obtained by this normal distribution of the food. The division of the urine into day (8 a.m.—8 p.m.) and night (8 p.m.—8 a.m.) portions showed that the fall in N was equally distributed between them. The total S output took a similar trend to the total N, the major change in it occurring in the day, however. These results are confirmatory of the findings in Exp. 3.

Exp. 5. A further experiment was also performed on D. P. C. (Table I), its main object being to determine whether this effect of carbohydrate in promoting the proper utilization of dietary protein were of a quantitative nature or not. A subsidiary consideration was the observation of the changes in body-weight over more prolonged periods of time than the previous separation experiments permitted, and under more uniform conditions.

The diet was similar to that of the preceding experiment (except that 25 g. diabetic bread was included) and contained some 3560 cals. and 76 g. protein; it was so disposed that protein and fat were the constituents of the meals at 8.10 a.m. and 1.15 p.m., and carbohydrate and fat of meals at 4.45 p.m. and 10.30 p.m. On this diet the urinary N averaged 12.80 g. for the 4th and 5th days of Period I (Table VI), the faecal N being 1.29 g. daily. If one allows 0.3 g. for cutaneous loss of N, there is thus a considerable negative N balance over the intake of 12.17 g. N.

On the 6th and 7th days (Period II) 25 g. cane sugar were transferred from the latter two meals of the day and associated with the protein meals at 8.10 a.m. and 1.15 p.m. (half to each meal). A definite reduction in the urinary output, averaging 0.91 g. N and 0.085 g. S daily, took place. In Period III a return to the regime of Period I produced an average daily urinary N over 4 days of 12.58 g.; excretion of S also rose. The transfer of 25 g. cane sugar was again made in

Table VI. *Exp. 5, subject D. P. C. The effect of separately ingesting the protein and carbohydrate in the diet and the effect of varying the amount of carbohydrate associated with the protein*

			Urine						
Period	Days of exp.	Body wt. kg.	Vol. ml.	N (g.)			S (g.)		
				Total	Day	Night	Total	Day	Night
I	1	83.70	—	—	—	—	—	—	—
	2	83.70	—	—	—	—	—	—	—
	3	83.68	2150	13.08	—	—	1.006	—	—
	4	83.68	2090	12.81	7.42	5.39	1.005	0.576	0.429
	5	83.50	1770	12.78	7.65	5.13	1.005	0.612	0.393
II	6	83.20	2210	12.02	6.84	5.17	0.952	0.589	0.363
	7	83.40	1970	11.76	6.57	5.185	0.889	0.532	0.357
III	8	83.50	2290	12.57	7.00	5.57	0.957	0.572	0.385
	9	82.95	2005	12.63	7.56	5.07	0.949	0.577	0.372
	10	82.85	2220	12.38	6.79	5.59	0.991	0.561	0.430
	11	82.80	1965	12.73	7.41	5.32	0.965	0.601	0.364
IV	12	82.75	2280	11.70	6.47	5.23	0.936	0.534	0.402
	13	82.85	2065	12.68	6.51	6.17	0.984	0.529	0.455
V	14	82.73	2180	12.58	7.11	5.47	0.917	0.512	0.405
VI	15	82.72	2270	11.81	7.14	4.67	0.893	0.558	0.335
	16	82.40	2300	11.79	6.72	5.07	0.861	0.488	0.373
	17	82.56	2200	11.54	6.72	4.82	0.893	0.522	0.371
VII	18	82.20	2575	11.71	6.63	5.08	0.861	0.504	0.357
	19	82.65	1770*	11.57	6.46	5.11	0.794	0.422	0.372
	20	82.67	2550	10.78	6.04	4.74	0.840	0.424	0.416

* Accidental loss of part of day urine. Figures inserted have been arrived at by analysis of the residual urine for N, S and creatinine and then by a calculation based on the constancy of the creatinine excretion in these experiments.

Diet

Period I. Meal 1 P+F 8.10 a.m. Meal 2 P+F 1.15 p.m. Meal 3 C+F 4.45 p.m. Meal 4 C+F 10.30 p.m.

Period II, as above, but 12.5 g. cane sugar added to meals 1 and 2 and removed from 3 and 4.

Period III, similar to I.

Period IV, as for I, but 25 g. cane sugar added to meal 2 and removed equally from 3 and 4.

Period V, similar to Period I.

Period VI, as for I, but 100 g. cane sugar added to meal 2 and removed equally from 3 and 4.

Period VII. Days 18 and 19 as for Period VI, but half cane sugar replaced by starch. In addition 100 g. carbohydrate were removed from meals 3 and 4 and taken at meal 1 as cane sugar. Meals 3 and 4 contained fat and equal amounts of starch and sugar. Day 20, as for preceding 2 days, but half sugar at breakfast replaced by starch.

Period IV, but on this occasion the depression was more transitory than in Period II.

In Period V a single day return to the dietary of Period I was made. Then in Period VI, 100 g. carbohydrate (as cane sugar for the first 2 days) were transferred from the evening meals to the protein meal at 1.15 p.m.; the resulting fall in N and S excretions averaged 0.87 g. and 0.084 g. respectively below the mean of Period III. On the last day of the period starch was substituted for half of the cane sugar, but this did not appreciably affect the N and S excretions.

During the last 3 days of the experiment further slight changes were made in the distribution of the diet, as indicated in Table VI. In the end, loss of body-weight was stopped and N equilibrium was reached at a level 2.02 g. lower than the original level of Period I, and 1.80 g. lower than the intermediate value of Period III. The S excretion also decreased during the final period; its unsteady nature, however, prohibited calculation of the exact amount of the fall. In this experiment the main changes in N and S were confined to the day urines.

II. *Partial separation of protein from carbohydrate*

Exp. 6. The possibility that separation of a part only of the protein from the dietary carbohydrate might also result in a loss of N from the body is considered in this experiment. The subject H. N. M. (Table I) was put on a diet containing some 2880 cal. and 116 g. protein, arranged (Table VII) so that he partook in Period I of mixed meals (each containing 2.32 g. N) at 9.30 a.m. and 8 p.m.; along with these meals a protein-free jelly of arrowroot and sugar (480 cal. per meal) was consumed. In the intervening hours of the day, 200 g. lean beef were taken at 12.45 p.m. and at 4.30 p.m. respectively. Thus during this period, some 75 % (14.2 g.) of the dietary N was unassociated with carbohydrate; the average daily urinary excretion was 16.57 g. N, representing a slight storage with the intake of 18.8 g. N, if an allowance of 1.3 g. N for faecal and cutaneous losses is made (Table VIII).

Table VII. *Dietary of subject H. N. M., Exp. 6*

	Period I				Period II			
	9.30 a.m.	12.45 p.m.	4.30 p.m.	8 p.m.	9.30 a.m.	12.45 p.m.	4.30 p.m.	8 p.m.
	g.	g.	g.	g.	g.	g.	g.	g.
White bread	81	—	—	81	81	—	—	81
Brown bread	42.5	—	—	42.5	42.5	—	—	42.5
Cheese	3.75	—	—	3.75	3.75	—	—	3.75
Bran	3.75	—	—	3.75	3.75	—	—	3.75
Jam	25	—	—	25	25	—	—	25
Butter	30	—	—	30	30	—	—	30
Apple	50	—	—	50	50	—	—	50
Lean beef	—	200	200	—	—	200	200	—
Arrowroot	45	—	—	45	—	45	45	—
Sugar	75	—	—	75	—	75	75	—
Total N	2.32	7.10	7.10	2.32	2.32	7.10	7.10	2.32
Carbohydrate	195	—	—	195	75	120	120	75

Table VIII. *Exp. 6, subject H. N. M. Partial separation of protein from carbohydrate*

Day of exp.	Diet	Body wt. kg.	Total urinary N output (g.)
2	Period I	61.35	16.39
3	(association of 25% of the protein	61.15	16.54
4	with carbohydrate)	61.26	17.19
5		61.20	16.19
6		61.25	16.21
7		61.10	16.88
	Mean	61.05	16.57
8	Period II	60.80	16.65
9	(association of 100% of the protein	60.90	16.20
10	with carbohydrate)	60.95	16.47
11		60.85	16.34
	Mean	60.90	16.42

In Period II the jelly of arrowroot was transferred to the meat meals at 12.45 p.m. and 4.30 p.m. The average daily output during this period was 16.42 g. N. Thus it may be said that, within the limits of our experiment, variation in the quantity of protein unassociated with carbohydrate in a diet does not affect the N balance, amounts as low as 4.64 g. N being sufficient, when ingested along with carbohydrate, to maintain N equilibrium.

III. Separation of surfeit carbohydrate from dietary protein

Exp. 7. This portion of the work was undertaken to investigate the function of carbohydrate eaten in excess of energy requirements under conditions similar

Table IX. *Dietary of subject H. N. M., Exps. 7 and 8*

	9.30 a.m.	12.45 p.m.	4.30 p.m.	7.15 p.m.
	g.	g.	g.	g.
White bread	50	150	100	25
Brown bread	25	60	60	25
Cheese	—	15	—	—
Bran	—	—	—	15
Apples	—	100	—	100
Butter	—	—	125 g.	—
Jam	—	—	100 g.	—
Milk	—	—	250 ml.	250 ml.

Fig. 5. *Exp. 7.* Subject H.N.M. Effect of varying the proximity of a carbohydrate surfeit to the rest of the diet.

to those in which the total dietary carbohydrate was ingested in the foregoing experiments, i.e. along with and also separate from the dietary protein. It will be remembered that the somewhat similar experiments of Larson & Chaikoff [1937]

were only one-day superimpositions of carbohydrate, and we wished to obtain evidence based on more prolonged surfeit feeding. The subject H. N. M. (Table I) was given a mixed diet (Table IX) containing 76 g. protein and some 2930 cal. In Period I urinary N equilibrium was established at a level of 10.32 g. with an intake of 12.10 g. (Fig. 5).

In Period II 150 g. sucrose were added daily at 11.30 p.m. ($4\frac{1}{2}$ hr. after the last meal). For 2 days this surfeit failed to induce any appreciable alteration in N output, but on the 3rd day of surfeit a sharp fall was registered and a new level of 9.14 g. N established. After an interval of one day, on which surfeit was discontinued, Period III was entered on by superimposing the 150 g. sucrose along with the 12.45 p.m. and 4.30 p.m. meals (the surfeit being divided between each) for 2 days. This maintained the N output at an average level of 9.04 g. per day.

An examination of the day (9.30 a.m.–9.30 p.m.) and night (9.30 p.m.–9.30 a.m.) urines shows that both in Period II and in Period III the major portion of the fall in N was attributable to the day fraction, no matter whether the carbohydrate were superimposed in the late evening or in the middle of the day. The fall in N was mainly due to a proportional reduction in urea excretion. In general, total S excretion paralleled total N, any changes noted in the level, however, being confined to the day fraction.

The creatinine output remained relatively constant throughout the course of the experiment. Body-weights were recorded and it will be observed that the giving of surfeit carbohydrate was accompanied by a rise in weight. This rise in weight in no way accounts for the marked and sustained reduction in urinary volume persisting throughout the periods of surfeit; probably this is to be explained by an increase in insensible perspiration, representing an attempt by increased energy metabolism to deal with the surfeit.

IV. *N balance in relation to the number of meals*

Exp. 8. Since in Exps. 1 and 2 the number of protein-containing meals was reduced from 4 to 2 in the second period of each, it was considered advisable to eliminate this change as a possible cause of the phenomena observed in these experiments (this objection does not apply to Exps. 3 and 4).

Table X. *Exp. 8, subject H. N. M., N balance in relation to the number of meals*

Day of exp.	Diet	Body-wt. kg.	Urine		Faeces	
			Volume ml.	N-output g.	N g.	Fat g.
4	Period I (8 meals per day)	64.05	1895	10.53	Total 3.64	Total 17.08
4		63.50	2238	10.89		
5		63.95	1838	10.83		
7		64.05	1915	10.72		
	Mean	63.89	—	10.74	0.91	4.27
8	Period II (2 meals per day)	63.60	2075	10.74	Total 3.54	Total 17.73
9		63.50	1650	11.83		
10		64.15	1650	11.07		
11		64.00	2155	11.00		
	Mean	63.81	—	11.16	0.89	4.43
12	Period III (8 meals per day)	63.90	2048	10.20	—	—
13		63.95	1960	10.45	—	—
14		—	1990	10.75	—	—

The subject H. N. M. (Table I) was placed on a mixed diet similar to that of Exp. 7 (Table IX), but divided in this case into 8 equal meals separated by intervals of 2 hr. from one another. This gave a very constant N output averaging daily 10.74 g. in the urine and 0.91 g. in the faeces (intake 12.10 g. N). In Period II this diet was compressed into 2 equal meals, taken at 11 a.m. and 5 p.m. respectively. The effect on the N output was an evanescent rise (probably due to the alteration in the times of protein ingestion), the mean for all 4 days of this regime being only 11.16 g. N per day. The faecal fat and faecal N were unaltered by this change. In Period III the 8 meals were restored; this produced a temporary depression of N, followed by a return to the average output in Period I (Table X).

It may accordingly be said that since a change from 8 to 2 meals induces only a slight alteration in daily N output, the reduction of the protein-containing meals from 4 to 2 in Exps. 1 and 2 is at most only a minor factor, if indeed one at all, in the production of the marked losses of N observed therein.

DISCUSSION

The salient features of our experiments may be grouped under the divisions suggested in the introductory remarks.

I. Exps. 1-4 indicate that the output of N and S from the body exceeds the intake when the adult human subject is fed on a diet abnormal only in one respect, viz. that the ingestion of the protein moiety is dissociated in time from that of the carbohydrate. This loss of N and S from the body occurs immediately on separating protein from carbohydrate and persists throughout the period of separation. In assessing the extent of this loss we have taken the mean daily level of urinary N output during the period of mixed meals as representing N equilibrium and have regarded as N lost from the body any excess output over and above this mean level. On such a basis, during the periods of separation of protein from carbohydrate the mean daily output of urinary N in our 4 experimental subjects was 1.93 g. greater than the N output when on mixed meals, i.e. there was a daily average of 1.93 g. N lost from the body. The individual data for these 4 subjects are appended in Table XI.

Table XI

Exp.	Subject	Material lost daily			Body-wt. kg.	Loss per kg. wt. g. N	Loss per sq. metre surface area g. N
		N g.	S g.	N:S ratio			
1	A. H. S.	1.77	—	—	64	0.025	0.94
2	H. B. C.	2.02	—	—	80	0.028	1.04
3	N. N. M.	2.13	0.129†	15.5:1*	61.5	0.035	1.29
4	D. P. C.	1.81	0.118	15.3:1	83.7	0.022	0.89

* Urea-N: preformed sulphate-S.

† Preformed sulphate-S.

It will be seen that the losses of N vary between 1.77 and 2.13 g. daily for the different subjects. Age may be a factor in determining the extent of the loss; in the present series of experiments there is an inverse relationship (see Table I). However, the variations in individual loss are found to be reduced if calculated per kg. body weight, or per square metre body surface. The relative constancy from individual to individual suggests that the loss is conditioned by the level of the endogenous metabolism; this possibility is substantiated by an examination of the more extensive data given for the endogenous N output, such as those

of Deuel *et al.* [1928], in which the value of 0.0241 g. per kg. body-weight was the daily excretion of urinary N after the subject had lived for more than a month on a protein-free diet—a figure which strikingly resembles the figures given in Table XI.

Is it by mere accident that there is a similarity between the daily amount of endogenous N excretion and the daily loss of N which occurs in these separation experiments, or does it really imply that the separate ingestion of carbohydrate and protein results in an inability of the organism to make good the endogenous loss of tissue protein? It must be remembered that only in Exp. 5 was the separation of carbohydrate and protein maintained over a prolonged period, but in this experiment the evidence is not clear-cut, for the separation was intermittent. We cannot therefore exclude the possibility that this loss of N and S might diminish in the course of prolonged separation, perhaps owing to the development of some compensatory mechanism. Exp. 5 does however clearly indicate that the N loss is accompanied by a decline in body-weight (Table VI). The significance of the other results of this experiment are discussed below.

The possibility of an endogenous source for the N lost is also supported by an examination of the separation of the 24 hr. urines into day and night fractions (Exps. 3 and 4). It is seen that the loss of N during the separation of the two food-stuffs is equally distributed between each fraction. This would apparently indicate a source of N which is unaffected by digestive and absorptive processes, i.e. an endogenous source. If this is so one might anticipate that the output of S would follow the same course, but examination shows that S loss is mainly confined to the day urine (Figs. 3 and 4). It is common knowledge that the metabolism of the S moiety of protein generally occurs more rapidly than that of the N fraction.

As regards the nature of the material lost during separation, Table XI shows that the N:S ratios of this material resemble closely the N:S ratio of muscle-protein (15.5:1), but it must be admitted that the quantities of N and S involved are small. (In Exp. 3, the S estimated was preformed sulphate-S, and accordingly the appropriate ratio is urea-N:preformed sulphate-S.) This finding of an N:S ratio similar to that of muscle is not however proof that the loss was from such a source; the material involved may well have been derived from a storage protein of similar ratio, utilized at the time of separation to replenish the endogenous requirements. It is significant that the creatinine excretion was relatively unaffected during the course of these separation procedures.

II. Exp. 6 indicates that for the normal metabolism of the dietary protein only a small fraction of it requires to be ingested simultaneously with the carbohydrate. In this experiment protein corresponding to only 4.64 g. N out of the 18.8 g. N in the diet was taken with the dietary carbohydrate, and yet the N output was the same as when all the protein (18.8 g. N) was taken with the carbohydrate (Table VIII).

The corollary does not appear to hold, viz. that the association of a small amount of carbohydrate with the protein of the day will necessarily maintain normal protein metabolism and prevent a loss of N (Exp. 5, Table VI).

III. By way of contrast to these N-sparing effects of carbohydrate in an adequate diet, the ingestion of carbohydrate in excess of energy requirements produces an actual storage of N. This is shown mainly in the day fraction of the 24 hr. urine, suggesting that it is the immediate products of digestion which are affected by the surfeit. The excretion of S is similarly depressed, the fall being again confined to the day output (Fig. 5).

It was observed by Larson & Chaikoff [1937], in their one-day superimposition experiments on dogs, that surfeit lost its N-storing effect when applied more than 4 hr. before or after the protein meal. With this we are in agreement, but we would also add that over longer periods than a single day, surfeit carbohydrate ingested separately from protein does eventually exercise a saving effect similar to that of surfeit carbohydrate taken simultaneously with the protein. The distribution of the saved N between the day and night fractions of the 24 hr. urines is identical under both conditions, viz. when the carbohydrate is taken apart from the protein (in the late evening) or along with the protein (in the afternoon) (Fig. 5).

IV. The possibility that the phenomena observed in Exps. 1 and 2 might be due to the change in the number of the protein-containing meals in the course of these experiments is discounted by Exps. 3, 4 and 5, to which this objection does not apply (Figs. 3, 4 and Table VI). It is also negated by Exp. 8, in which the number of meals of a fixed diet were varied from 8 to 2 without seriously affecting the level of N output (Table X). This is consistent with the results of other workers, as reviewed by Cathcart [1921]. It may be further noted that this change in the number of meals in Exp. 8 did not alter the utilization of the protein and fat, since both faecal N and faecal fat were found to be unaltered in amount by this procedure. It would indeed appear that no appreciable economy in food is effected by eating more frequently than twice a day.

Mechanism of N-sparing and N-saving

In the foregoing discussion we have presented two separate aspects of carbohydrate in its relation to protein metabolism. These two may be termed N-sparing in the case of the adequate diet (Exps. 1-5), and N-saving in the case of the surfeit diet (Exp. 7). They differ in certain respects.

The separate ingestion of the carbohydrate and the protein of the adequate diet results in a loss of N from the body, and this loss may be spared if the carbohydrate and the protein are ingested together. To produce this sparing effect requires the close approximation of the protein to the carbohydrate (Figs. 1, 2, 3, 4 and Table VI). It occurs at once on ingesting the carbohydrate along with the protein; moreover, the sparing is equally distributed over the day and night excretions of N (Figs. 3 and 4). The N spared is not necessarily proportional to the amount of carbohydrate present along with the protein (Table VI).

The N-saving effect of carbohydrate present in excess of energy requirements differs from the N-sparing effect in that surfeit carbohydrate ingested at any period of the day is capable in the long run of causing this storage of N in the body (Fig. 5), in that it takes several days of surfeit to exert its full effect [Cuthbertson & Munro, 1937] and in that, in contrast to N-sparing, N-saving is mainly effected on the day excretion of N, even if the carbohydrate be taken at night (Fig. 5). The N thus stored in the body is roughly proportional to the amount of surfeit carbohydrate taken.

In seeking to explain the loss of N from the body which results from the separate ingestion of the protein and the carbohydrate in an adequate diet, four hypotheses may be considered. All four assume that the underlying factor is an increased immediate disposal of the food protein, leading to an insufficiency of protein remaining to replace the endogenous N loss.

(a) If an insufficient quantity of energy were present in the protein-fat meals of the separation period, it might make necessary an excessive utilization of food protein for immediate energy purposes; hence, less protein would be left over for tissue needs.

(b) The absence of carbohydrate from the protein-fat meals might have to be made good by production of carbohydrate from food protein, thus lessening the amount of protein available for endogenous requirements.

(c) In the absence of carbohydrate from the protein-fat meals, a more rapid combustion of protein by its own specific dynamic action might occur.

(d) A specific inhibitory action of carbohydrate on the deaminases of the body might exist, an inhibition which would not come into play unless both carbohydrate and protein were simultaneously ingested. This last theory can be made to embrace both N-sparing and N-saving effects.

The first of these hypotheses may be disposed of by reference to Tables II-V, in which it is shown that there is no deficiency of energy in the protein-fat meals of the periods in which carbohydrate and protein were separately ingested. One cannot therefore ascribe the N loss in these experiments to insufficiency at any particular period of the day, unless it be assumed that fat is not metabolized at a sufficiently rapid rate to cover the energy needs immediately after the protein-fat meals of the day.

The second argument seeks to explain the N loss as an attempt to make available the non-N residues of protein metabolism for carbohydrate synthesis. It is true that these residues would tend to compensate for the absence of carbohydrate in the protein-fat meals of the separated diet, but they would also be productive of an excessive deamination of the protein of the diet and a consequent protein-starvation of the tissues. This suggestion would seem unlikely in the face of the adequate carbohydrate available at other periods of the day (Tables II-V). It might of course be further contended that for the complete oxidation of the fat present in the protein-fat meals, newly formed carbohydrate might be required, thereby increasing the demand on the non-N protein moiety. If such be the case, the large variations in the quantity of fat present in the protein meals of our diets might be expected to cause proportionately varying losses of N. Such was not the case, the losses of N being on the contrary reasonably uniform (Table XI).

The third consideration is that, in the absence of freshly ingested carbohydrate, the specific dynamic action of the dietary protein must be met at the expense of the protein itself. That this is unlikely is suggested by a study of the dietaries of Exps. 1-4 (Tables II-V). In the period of separation of protein from carbohydrate in Exps. 1 and 2, the 2 meals of protein were separated from those containing carbohydrate by some 4 hr., whereas in Exps. 3 and 4, the periods of separation of the first protein meal from the first carbohydrate meal were 7 hr. and 8½ hr. respectively. Since specific dynamic action after a protein meal is a prolonged effect [Williams *et al.* 1912], one would expect a commensurately greater loss of N in the second pair of experiments, to say nothing of variations dependent on protein intake, if such losses were actuated by specific dynamic action. Such a view is not tenable in the light of the relative uniformity of our results (Table XI).

We prefer to believe that both N-sparing and N-saving have one underlying mechanism, suggested by the observation of Krebs [1935] that deamination by liver slices is inhibited in the presence of easily oxidizable substances such as lactate, pyruvate, succinic and α -keto acids (but curiously enough, not glucose). In regard to the N-sparing of the ordinary adequate diet, the presence of carbohydrate (perhaps yielding a more active form of glucose within the body) along with the protein is sufficient to secure that some of the amino-acids escape deamination and go to replace the effete products of endogenous metabolism; N balance is thus attained. However, if all the dietary protein be ingested

separately from the carbohydrate, the deaminases are given a free hand, this sparing effect of carbohydrate no longer obtains and deamination is then complete. Further, to this waste of food N is added the unreplaced endogenous quota, so that there is produced an output of N which exceeds intake by an amount equal to the daily endogenous N.

The N-saving effect of surfeit carbohydrate may possibly be explained by the same mechanism; here, even more amino-acids are spared deamination by the inhibiting action of the greater concentrations of carbohydrate resultant on the surfeit intake. Thus in the blood a plethora of amino-acids is produced, and by the law of mass action the storage depots are further charged with protein. This would account for the finding of Larson & Chaikoff [1937] that, in one-day superimposition experiments, carbohydrate was only effective when ingested within 4 hr. before or after the protein, i.e. when its absorption fell within the main phase of protein metabolism. In more prolonged surfeit feeding (Exp. 7) storage of the carbohydrate would also seem to play a part, for surfeit taken outside the 4 hr. limits does have a N-saving effect in the course of a few days (Fig. 5), which probably represents the (variable) time taken to fill up the carbohydrate stores. The eventual result of charging these stores would be that the freshly ingested carbohydrate of the ordinary daily meals is unable to pass out of circulation with its wonted rapidity; it thus produces a higher concentration of carbohydrate within the organism than it normally would and simulates thereby the effect of giving a large draught of surfeit carbohydrate along with the meal. This would of necessity imply that owing to the prolonged period of surfeit feeding the Staub-Traugott phenomenon would be less marked, indicating a less effective disposal of freshly absorbed carbohydrate. Whatever may be the mechanism involved, carbohydrate ingested apart from protein seems to produce an inhibition of deamination of exactly similar extent and time-distribution to that of surfeit carbohydrate taken along with the protein.

That this egress of the carbohydrate from currency and that the availability of stores for this purpose play an integral part in determining the effect of dietary carbohydrate on protein metabolism seems more certain from the results obtained in Exp. 6. Here 2 meals of an adequate (but not surfeit) diet were loaded with all the dietary carbohydrate (some 390 g.) but yet a similar N balance was obtained as when there was a distribution of the same carbohydrate over all 4 meals of the day. That is, if there is excessive ingestion of carbohydrate at one particular meal at the expense of carbohydrate at other meals of that day, the excessive ingestion is at once counteracted by a legacy (from the carbohydrate-poor meals) of stores depleted in carbohydrate. The fluctuations in concentration of current carbohydrate at the sites of deamination would thus be more or less similar after each meal, even if the dietary carbohydrate were disposed unevenly between the different meals. We are of the belief that such a mechanism is the basis whereby N balance is made possible in the organism on the ordinary adequate diet, subjected as it is to vagaries of time and tide of carbohydrate intake.

This capacity for storage of carbohydrate probably also explains the curious results of Exp. 5 (Table VI), in which varying amounts of carbohydrate were given along with the dietary protein, in an attempt to reduce the loss of N which occurs on separation of carbohydrate from protein in an adequate diet. It was found that 100 g. carbohydrate so given were no more effective than 25 g., the N loss being equally reduced, but not abolished. Only when a larger proportion of the total dietary carbohydrate was transferred to the protein meals did N loss eventually cease to occur. This would seem to indicate that the carbohydrate stores at the times of the protein meals were not replete, but rather depleted, and

hence that 25 g. sugar and 100 g. sugar were equally well disposed of, the larger amounts being however less rapidly dealt with by the carbohydrate stores.

Further evidence of a carbohydrate mechanism in protein metabolism may be adduced from the experiments of Silver [1937] which indicate that the amount of carbohydrate in a diet is a factor in the establishment of N equilibrium on that diet. It was found that, by keeping the protein and energy content similar, but varying the carbohydrate-fat ratios of a series of diets, the N balance was favourably influenced by larger proportions of carbohydrate in the diet. The metabolism of the Esquimaux, and of other races whose dietaries contain very little carbohydrate, must presumably be such that the non-N moieties of the large quantities of protein in these diets are deviated to the filling up of the carbohydrate stores and thus successfully take the place of carbohydrate. In this connexion it is recalled that Voit [1881] showed that N and C equilibrium could be eventually established in dogs fed on a high meat diet in which virtually no carbohydrate was present.

SUMMARY

1. It has been demonstrated on 4 human subjects that when the protein and the carbohydrate moieties of an adequate diet are separately ingested over short periods of time, there is a negative N balance of some 2 g. daily (mainly urea).

2. This loss is equally distributed between the day and night portions of the 24-hourly urines. There is a corresponding loss of S which, however, is confined to the day urines. Creatinine excretion is relatively unaffected by this procedure.

3. N metabolism is undisturbed by dissociating a part only of the dietary protein from the carbohydrate; this is in contradistinction to the N loss resultant on complete separation of protein and carbohydrate. The association of a small fraction of the dietary protein with the carbohydrate is sufficient to maintain N equilibrium.

4. Carbohydrate, if ingested over a sufficiently long period in excess of the energy requirements, causes storage of N and S no matter whether the carbohydrate be taken along with, or apart from the dietary protein.

5. The variation of the number of meals in a fixed diet from 8 to 2 causes a transitory loss of N due to the disturbance in time relationships. The digestibility and absorbability of the protein and fat of the diet are apparently unaffected by this procedure.

6. The changes in protein metabolism involved in these experiments and the possible mechanisms of their production are discussed.

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