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

I. THE EFFECT OF SUPERIMPOSING RAW AND BOILED MILKS ON A DIET ADEQUATE FOR MAINTENANCE

II. THE SUPERIMPOSITION, ON A DIET ADEQUATE FOR MAINTENANCE, OF BEEF (OR SOYA FLOUR) + LACTOSE + BUTTER, EQUIVALENT IN PROTEIN, CARBOHYDRATE AND FAT CONTENT TO A LITRE OF MILK

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I. THE EFFECT OF SUPERIMPOSING RAW AND BOILED MILKS ON A DIET ADEQUATE FOR MAINTENANCE

THE main series of experiments which are described in this paper really comprise an investigation of the effects of moderate overfeeding on the protein metabolism of man, although they owe their origin to the untoward results which were obtained during a re-examination of certain observations which had come to our knowledge and which had indicated that a difference existed between the nutritive values for the adult of the proteins of raw and boiled milks. The dietary method which was adopted in these experiments was of the superimposition type, and as the work proceeded it became obvious that retention phenomena of an unexpected nature were occurring and were of such an order as to disturb seriously our interpretation of this type of experiment in dietary studies. Thus the original design of the investigation was submerged in an attempt to elucidate these remarkable phenomena and in the process some fresh light has been shed on an old and largely forgotten chapter of physiology in which the observations of Voit [1869], v. Noorden's pupils [Milroy, 1907], Rubner, [1903], Grafe and co-workers [1910; 1911; 1923; Eckstein & Grafe, 1919] and Neumann [1919] had already indicated that N retention may result from overfeeding.

EXPERIMENTAL

The subjects selected for these experiments were students and members of the teaching staff performing routine laboratory duties. They were all of good physique. During the experimental period they took no special exercise apart from short walks of a relatively uniform character.

Urine was collected in 24-hour specimens, made up to constant volume and preserved with thymol in chloroform. The various periods during which faeces were collected were marked off with carmine, taken in the form of capsules. The faeces were acidified with H_2SO_4 and dried on a steam-bath and in an air-oven before being stored in sealed bottles. The analytical methods adopted were similar to those already employed by one of us [Cuthbertson, 1929].

First series. One-day superimposition experiments

Seven healthy male subjects of good physique ranging in age from 19–35 years were placed on self-selected constant basal diets comprised of white and brown breads, 100 g. fresh butter, cheese, 500 ml. raw milk (Grade A, T.T.), apple jelly and raw apples. The water intake was kept constant. Subjects 5, 6 and 7 received lettuce and tomato in addition. The diet was consumed in four meals per day. The calorie and protein intakes are given in Table I. The variations in protein intake among the members of the group are mainly due to differences in the consumption of cheese.

Table I

Subject	Age	Initial body wt. kg.	Final body wt. kg.	Cal./kg.	Prot./kg.	% first class protein
(1) Gi.	19	66.75	66.70	45	1.09	51
(2) Gr.	20	74.3	74.70	37	0.82	41
(3) Or.	19	56.6	56.90	51	1.21	54
(4) St.	21	74.9	74.80	39	1.01	52
(5) Cu.	35	87.0	86.70	34	0.92	33
(6) Mu.	20	60.2	60.25	45	1.16	30
(7) McC.	29	56.1	57.0	52	0.87	25

A fairly steady urinary excretion was observed on the basal diets. Although no determinations of the faecal N excretion were made in this series the individuals were probably in N equilibrium on these diets. This is supported by the fact that the body weights tended to remain constant during the experimental periods which lasted 3–5 weeks. Subject 7 was the only person to show a definite increase in weight during the period (0.9 kg. in 4 weeks). This was due to the higher calorie diet selected.

Having established equilibrium Subjects 1–7 received on one day an extra litre of milk, the extra intake of water in the milk being compensated by a withdrawal of an equivalent volume of drinking water. As soon as N equilibrium was re-established the addition of extra milk was repeated. The order of administration is indicated in Table II, the extra milk being consumed in two lots of 500 ml. each, at 1 p.m. and 5 p.m.

It may be noted here that even when boiled milk was superimposed on the basal diet the latter contained its usual quota of raw milk. A constant basal intake of raw milk was maintained so that if any differences were subsequently apparent in the fate of the N of the milk, these could definitely be ascribed to a change in the protein caused by boiling, and not to some unknown deficiency in the diet which might be presumed to be due to the absence of raw milk.

During the month of May 1935 when Subjects 1–4 were on diet the average N content of a litre of Grade A, T.T. milk was 5.58 g. and during July when Subjects 5–7 were on diet, 5.63 g. In boiling the milk a cast aluminium pan was used, the milk being stirred constantly till the boiling point was just reached. It was then cooled. The skin and residue which stuck to the sides and bottom of the pan were consumed.

Table II

Subject	Calories	Basal N (urine) g. prior to each milk supplement				Extra milk N per litre g. av.	Type of milk	Rise of N over basal (urine) g.			Rise of S over basal (urine) g. 1st day
		1	2	3	4			1st day	2nd day	Total	
1	2932	11.02	11.27	11.69	—	5.58	Raw	1.85	1.52	3.37	0.205
							Boiled	2.03	1.50	3.53	0.202
							Raw	1.32	1.45	2.77	0.155
2	2674	9.18	9.06	9.33	—	5.58	Raw	1.74	0.26	2.00	0.137
							Boiled	1.54	0.17	1.71	0.140
							Raw	1.75	1.22	2.97	0.166
3	2909	10.45	—	10.70	11.18	5.58	Boiled	2.05	0.47	2.52	0.151
							Raw	—	—	—	0.181
							Boiled	2.45	0.34	2.79	0.200
							Raw	1.82	0.36	2.18	0.169
4	2976	11.77	11.54	11.73	—	5.58	Boiled	2.16	0.61	2.77	0.138
							Raw	1.67	1.38	3.05	0.161
							Boiled	2.14	1.87	4.01	0.179
5	2959	13.39	13.03	12.85	12.94	5.63	Raw	1.51	0.00	1.51	—
							Boiled	2.61	0.66	3.27	—
							Raw	1.53	1.26	2.79	—
							Boiled	1.81	0.46	2.27	—
6	2698	11.29	11.29	—	—	5.63	Raw	1.96	0.54	2.50	—
							Boiled	1.03	0.19	1.22	—
7	2907	9.28	8.61	8.80	—	5.63	Raw	1.84	—	—	—
							Raw	2.21	—	—	—
							Raw	1.25	—	—	—

In Table II are recorded the amounts of extra N excreted by the different individuals on the day of ingestion of the extra milk and the day following. The differences between raw and boiled milk are not statistically significant (Table III). The data for Subject 7 are excluded from the analysis.

Table III. *Statistical summary of data on Table II*

	N						S	
	1st day		2nd day		Total 2 days		1st day	
	Raw	Boiled	Raw	Boiled	Raw	Boiled	Raw	Boiled
Mean	1.683	1.980	0.889	0.697	2.571	2.677	0.168	0.168
S.D.	0.1923	0.4494	—	—	0.5495	0.8228	—	—

Urea paralleled the total N excretion whereas ammonia exhibited a steady rate of elimination. Total sulphates paralleled the total N and urea-N output but the excess output appeared to be all excreted on the first day, indicating a more rapid elimination of S, a fact in harmony with most work on N and S metabolism. The mean of the excess S excretions over basal was 0.168 g. S for both raw and boiled milk. This emphasizes the fact that no significant difference in the quality of raw and boiled milk proteins could be detected under these experimental conditions. The variability in the excess N excretions in these experiments is due to the fact that the individual's reaction to a superimposed protein is never quite the same each time. This was confirmed by noting the reaction of Subject 7 to the ingestion of 1 litre raw milk on three separate occasions, N equilibrium having been established in the intervals. The excess N outputs were 1.84, 2.21 and 1.25 g.

The mean percentage retention of the extra milk N was 54, that of S, 53. The material retained during these raw and boiled milk experiments had apparently an S : N ratio of 1 : 13.4 and 1 : 13.9 respectively. Since the extra milk ingested had an S : N ratio of 1 : 15.5 this would indicate that the material stored was richer in S than that ingested. The S : N ratio is close to that found in the urine during the later days of a starvation period.

To test if the capacity of the organism to retain milk N was equal to or greater than its capacity to store the N of an equivalent amount of sodium caseinogenate, Subject 6 ingested a litre of sodium caseinogenate solution in place of a litre of milk. Only 33% of the caseinogenate N was retained as compared with retentions of 56% and 69% noted with raw and boiled milks. It appeared as if the non-protein moiety of the milk contributed to the storage of the N. The question naturally arose as to whether this extraordinary retention would still occur when the period of milk ingestion was prolonged. For this purpose a second series of experiments was designed.

Second series. Eight-day superimposition experiments

Subjects 5 and 6 took part in this series. The basal diets were identical in quality with, and only slightly different in quantity from, those selected in the first

Table IV

Diet	Subject	Initial body wt.	Total Cal.	Cal./kg.	Total protein g.	Protein/kg. g.	% first class protein
Basal	5	86.2	3300	38	86	1.00	31
	6	61.5	2850	46	71	1.15	30
Basal + 1000 ml milk	5	86.2	3980	46	121	1.40	51
	6	61.5	3530	57	106	1.72	53

Fig. 1.

Fig. 2.

Figs. 1 and 2. The effect of superimposing a litre of raw or boiled milk on a diet adequate for maintenance.

series and were consumed in the winter of 1935-36. The ingestion of the extra litre of milk increased the total caloric value and protein content of the diets by some 680 Cal. and by 35 g. protein (first class). It was continued for a period of 8 days in each instance.

Subject 5 consumed the extra raw milk in two equal lots at 1 p.m. and at 4.30 p.m. Subject 6 drank the litre of milk at 9.15 a.m.

Following the first experimental period which lasted about three weeks there was an interval of a fortnight on normal diet before the procedure was repeated with boiled milk of the same quality as the raw.

It will be noted that the urinary N excretion exhibits the same general trend in the same subject irrespective of the treatment of the milk (Figs. 1 and 2, Tables V and VI). The two subjects behaved rather differently to this extra milk N.

Table V. *Subject 5. Eight days' superimposition of raw and boiled milks*

Diet	Body weight kg.	Daily N intake g.	Urine vol. ml.	N output daily, g.		
				Urine	Faeces	Total
Basal 3300 Cal.	—	14.85	2260	13.15		
	86.25		2185	12.57		
	86.20		1880	12.47		
		Mean		12.73	1.967	14.70
Basal + 1 litre raw milk 3980 Cal.	86.60	20.63	1476	14.23	1.963	
	86.80		1605	14.81		
	—		1548	15.42		
	86.90		1365	15.18		
	86.90		1760	15.92		
	—		2100	16.45		
	87.15		1755	16.46		
	87.10		2065	16.15		
		Total 8 days		124.62	15.70	140.32
Basal 3300 Cal.	86.60	14.85	1955	13.36		
	86.00		1480	13.47		
	86.50		1510	13.05		
		Total 11 days		164.50		
Basal 3300 Cal.	85.90	14.97	2015	13.55	1.967	
	—		1755	12.66		
	86.60		1860	12.59		
	—		1930	13.33		
	86.40		2070	12.48		
		Mean		12.92		
Basal + 1 litre boiled milk 3980 Cal.	86.50	20.78	1375	14.37	2.396	
	—		2095	15.43		
	—		1870	15.19		
	—		1830	15.07		
	87.05		1705	16.17		
	—		1900	16.15		
	87.0		2140	15.66		
	87.05		1705	15.95		
		Total 8 days		123.99	19.17	143.16
Basal 3300 Cal.	—	14.97	1870	13.64		
	86.35		1775	13.84		
	—		1700	13.22		
		Total 11 days		164.69		

Table VI. *Subject 6. Eight days' superimposition of raw and boiled milks*

Diet	Body weight kg.	Daily N intake g.	Urine vol. ml.	N output daily, g.		
				Urine	Faeces	Total
Basal 2850 Cal.	—	12.02	1520	9.20		
	61.85		1465	9.64		
	61.90		2023	10.21		
	61.85		1982	9.75		
	61.60		1652	10.38		
	61.70		1996	9.79		
	61.65		2216	9.41		
	61.50		1997	10.38		
		Mean		9.85	1.21	11.06
Basal + 1 litre raw milk 3530 Cal.	61.40	17.80	1384	12.64	1.591	
	61.60		1397	13.19		
	61.60		1667	13.24		
	61.75		1450	13.62		
	62.05		1875	13.55		
	61.90		1748	13.51		
	61.40		990	12.79		
	61.75		1810	12.68		
		Total 8 days		105.22	12.73	117.95
Basal 2850 Cal.	61.95	12.02	1955	10.96		
	62.00		1725	9.68		
			1410	9.76		
		Total 11 days		135.62		
Basal + 2850 Cal.	60.50	11.98	1740	9.36		
	60.55		1770	10.89		
	60.25		1568	10.54		
	60.30		1550	10.56		
	60.60		2262	9.94		
	60.40		1725	10.30		
		Mean		10.30	1.21	11.51
Basal + 1 litre raw milk 3530 Cal.	60.50	17.79	1677	11.73	1.560	
	60.70		1628	12.92		
	60.60		1731	12.95		
	60.75		1445	13.17		
	60.90		1844	13.36		
	61.15		2012	14.04		
	60.55		1728	13.47		
	61.15		1610	13.00		
		Total 8 days		104.64	12.48	117.12
Basal 2850 Cal.	61.25	11.98	1940	10.89		
	61.15		1840	10.27		
	61.15		1548	10.38		
		Total 11 days		136.18		

Table VII

Subject	Period	Output, g.			Intake g.	Retention g.
		Urine	Faeces	Total		
5	Pre-raw	12.73	1.97*	14.70	14.85	+0.15
	Pre-boiled	12.92	1.97	14.89	14.97	+0.09
	Pre-meat (1)	12.26	1.51	13.77	14.97	+1.20
	Pre-meat (2)	12.46	1.765	14.225	15.23	+1.005
6	Pre-raw	9.85	1.21*	11.06	12.02	+0.96
	Pre-boiled	10.30	1.21	11.51	11.98	+0.47
	Pre-meat	9.67	1.18	10.85	12.11	+1.26
	Pre-soya	11.09	1.12	12.21	12.11	-0.10

* The basal faecal N was not determined during the pre-raw period. The value obtained during the pre-boiled period is adopted.

Table VII contains the N balance data for the basal periods of these and subsequent experiments. The apparent retention of +0.15 and +0.09 g. N per day noted in the pre-raw and pre-boiled milk periods of Subject 5 point to slight negative balances since the cutaneous N losses must have exceeded these values. In the case of Subject 6 true balances probably existed, for the second milk period was carried through during a colder spell of weather than the first and hence there would be less loss of N by the sweat. Taking the sweat N loss as 0.5–1.0 g. per day N intake practically equalled N output in the case of Subject 6 when on basal diet.

It is apparent that a very definite retention of milk N occurred during these experiments and that the retained N appeared to remain stored within the organism on return to basal diet. The evidence on this latter point was somewhat equivocal.

In computing the percentage retention of the additional food N the following calculation was used:

$$\frac{\left(\begin{array}{c} \text{Excess N intake} \\ \text{over 8 days} \end{array} - \begin{array}{c} \text{Excess N output (urine + faeces) over 8 days} \\ + 3 \text{ days of post-milk period} \end{array} \right)}{\text{Excess N intake over 8 days}} 100$$

Table VIII. *Subjects 5 and 6. N balance during 8 days' extra milk intake and during first 3 days of subsequent basal period*

Milk	Sub- ject	Basal excretion	Output g.			Reten- tion g.	Extra intake g.	% extra N retained
			Urine	Faeces	Total			
Raw	5	160.48	164.50	21.60	186.10	20.62	46.24	44.6
	6	121.66	135.62	16.36	151.98	15.92	46.24	34.4
Boiled	5	163.76	164.69	26.36	191.05	19.19	46.48	41.3
	6	126.61	136.18	16.11	152.29	20.80	46.48	44.8

Of the extra raw and boiled milk N respectively 44.6 and 41.3 % were retained by Subject 5, and by Subject 6, 34.4 and 44.8 %.

These retentions are of approximately the same order and confirm the marked storage of superimposed milk N witnessed in the earlier series and substantiate the fact that no real difference exists between the nutritive values of the proteins of the two milks as determined by this method in the adult or adolescent subject.

These extraordinary N retentions were coupled with increases in body weight. Utilizing Rubner's value of 1 g. N as being equivalent to 33 g. body substance (mainly muscle) we have calculated the expected weight increase from the N retentions and compared it with the actual (Table IX).

Table IX. *Body weight changes (kg.)*

Sub- ject	Raw milk				Boiled milk			
	Basal weight	Weight after extra raw	Weight increase	Calcu- lated weight increase	Basal weight	Weight after extra boiled	Weight increase	Calcu- lated weight increase
5	86.40	87.12	0.720	0.680	86.45	87.0	0.550	0.640
6	61.45	61.85	0.400	0.530	60.45	61.20	0.700	0.690
Meat exp. over equivalent period								
6	61.58	62.35	0.770	0.460				

It is apparent that the calculated data fit the actual with a reasonable degree of accuracy considering the many factors which may influence body weight.

The increments in weight of Subject 5 were not maintained on return to basal, there being an immediate loss of weight. No significant difference could be detected in the alteration in body weight which occurred through feeding raw and boiled milks in this case. The fact that the rise in body weight was not maintained on return to basal suggests that the bulk of the material stored during the period of surfeit feeding was apparently not required by the organism to improve its "nutritive state".

The assumption has been made in all these experiments that the additional milk protein is all absorbed. In view of the fact that there was a slight increment in the faecal N during the periods of extra milk intake this assumption may perhaps appear unjustified. It is uncertain, however, how much of this additional faecal N may have arisen during the passage of the increased bulk of mineral matter down the alimentary canal. Even if the whole of this additional faecal N represents unabsorbed milk N, the loss by this route would not have exceeded 8% of the additional N intake.

In the case of Subject 6 the weight changes were rather peculiar in that the steady gain in weight on both types of milk was broken on the seventh day of additional milk. This loss in weight was transitory, being made good the day following. On return to basal conditions the body weight did not fall but was maintained during the two to three days of the post-milk period. This is in striking contrast to the marked fall in body weight of Subject 5 on the first day of the post-milk period. This fall was not coupled with a diuresis and so it is not accounted for by a loss of water.

In a subsequent experiment Subject 6 consumed daily, beef+lactose+ butter, equivalent in protein content and caloric value to a litre of milk. No fall in body weight took place for some time during the period of observation following the resumption of the basal dietary. The presumption is that we are dealing here with the reaction of the adolescent in contrast to the adult as 5 was aged 36 years and 6, 20 years.

The metabolism of calcium following the superimposition of raw and boiled milks on a diet adequate for maintenance

In view of the marked retentions of milk N it was considered of interest to determine if the same phenomenon occurred with the Ca of the milk. For this purpose the Ca balance was investigated during the periods of high milk intake. The data for Subject 5 are given in the following table:

Table X

	Ca g.	
	Raw milk	Boiled milk
Urine (8 days)	1.596	1.323
Faeces (8 days)	17.254	17.520
Total excretion	18.850	18.843
Basal excretion	9.666	9.495
Extra Ca excreted	9.184	9.348
Extra Ca intake	9.797	9.912
Milk Ca retained	0.613	0.564
% retained	6.26	5.69

It is obvious that compared with the N retentions the retentions of Ca are slight, and indicate no real difference between raw and boiled milks.

It is not in the province of this paper to enter into a discussion of the relative merits of raw and boiled milk. It will be convenient however to consider

briefly (1) the nature of the changes, if any, produced in the protein moiety of the milk by boiling, and (2) the effect of boiling on the retention of milk N.

Boiling milk causes only one significant change in the proteins and that is a coagulation of the albumin and globulin fraction [Kieferle & Gloetzel, 1930].

Metabolism experiments on infants are few in number and Morris & Graham's [1933; 1936] are the most satisfactory. Their results indicate that boiling milk does not interfere with its usefulness as a source of dietary protein. According to Symington [1936] the rate of digestion of the proteins of milk in the stomach is unaltered by boiling the milk. We have not detected any significant difference in the specific dynamic actions exerted by a litre of raw or boiled milks.

The remarkable fact about the experiments which we have described is the unexpected and considerable retention of N and S which occurred when a litre of milk was superimposed on a diet adequate for maintenance of the pre-experimental body weight, a retention unaffected by boiling the milk.

II. THE SUPERIMPOSITION, ON A DIET ADEQUATE FOR MAINTENANCE, OF BEEF (OR SOYA FLOUR) + LACTOSE + BUTTER, EQUIVALENT IN PROTEIN, CARBOHYDRATE AND FAT CONTENT TO A LITRE OF MILK

In the first part of this work attention has been directed to the remarkable retention of N and S which may occur in the adult human organism during the superimposition of milk on an already adequate diet. The question naturally arose as to whether this marked storage of N would occur when some other protein of animal origin was superimposed. To determine this point the experiments were repeated superimposing on the same basal diet, lean beef with supplements of lactose and butter so that the final quantities of protein, fat and carbohydrate ingested daily were equivalent to these principles in the extra litre of milk. For this 160 g. lean topside beef (av. T.N.=5.68 g.), 46 g. lactose and 35 g. butter were required. Water equal in volume to the water content of the milk was drunk at times corresponding to the extra ingestion of milk.

Over the first 8 days of the superimposition period Subject 6 exhibited a retention of N representing 31.4% of the additional intake. On a similar basis the retentions observed with raw and boiled milk were for this subject, 36.3 and 46.1% respectively.

Fig. 3. The effect of superimposing beef + lactose + butter daily equivalent in protein, carbohydrate and fat to a litre of milk. Subject 6.

In order to determine the effect of this continued superimposition, the additional food was continued for a total period of 15 days (Table XI, Fig. 3).

Table XI. *Subject 6. Superimposition of beef + lactose + butter.*

Day of exp.	Daily diet	Body weight kg.	Daily N intake g.	N output daily, g.		
				Urine vol. ml.	Urine	Faeces
1	Basal (2850 Cal.)	62.05	12.10	1520	10.06	
2		62.20		1660	9.53	
3		61.70		2190	10.30	
4		61.30		1930	9.15	
5		61.65		1240	9.39	
6		61.65		1571	9.60	
7		61.45		1565	9.63	
8		61.25		1589	9.77	
Average (1-8 days)			12.10	9.68	1.18	10.86
9	Basal + 160 g. beef + 46 g. lactose + 35 g. butter (3550 Cal.)	61.20	17.925	1794	12.93	
10		61.20		2052	13.10	
11		61.40		1583	13.34	
12		61.60		1570	13.57	
13	Lactose replaced with butter	61.90		1620	13.95	
14		62.25		1577	13.69	
15		62.35		1835	13.77	
16		62.20		1847	14.12	
Total (9-16 days)			143.40	108.47	10.40	118.87
17	Lactose restored	62.45	17.925	1630	13.16	
18		62.45		1790	14.38	
19		62.50		1907	13.54	
20		62.60		1490	14.42	
21		62.60		1725	14.23	
22		62.50		1425	14.25	
23		62.50		1895	14.84	
Total (9-23 days)				268.88	207.29	19.50
24	Basal (2850 Cal.)	62.30	12.10	2052	11.00	
25		62.40		1757	10.48	
26		62.45		1847	10.49	
27		62.75		1680	11.03	
28		—		1720	10.63	
29		62.45		1740	10.54	
30		62.30		1907	10.30	
31		61.65		1925	10.81	
32		61.35		2195	10.41	

Table XII. *Subject 6. N balance during 15 days' extra beef intake and during first 3 days of subsequent basal period.*

Basal excretion	Output, g.			Retention g.	Extra intake g.	% extra N retained	Body wt. increase
	Urine g.	Faeces g.	Total g.				
195.48	239.26	23.04	262.30	18.55 (618 g. flesh*)	85.37	21.7	ca. 1000 g.

* On basis of 1 kg. body substance $\equiv$ 30 g. N (mainly muscle).

The urinary N excretion slowly rose reaching a maximum value on the fifteenth day of the experiment—a value still short of that to be expected if N equivalent in amount to the daily additional intake of N were excreted. N equilibrium was therefore still unattained although the degree of retention was steadily becoming less. On reverting to the basal intake again the curve of

urinary N fell to a new more or less constant level in the course of two days. A slow elimination of the retained N appeared to take place, so slow that it scarcely affected the body weight, for coupled with the retention of N there had occurred a slow increase in body weight which was maintained during the subsequent first 8 days of the basal period. The body weight fell on the 9th day of basal diet and by the following day had reached its original level. In this respect the experiment corresponds to the previous experiments on Subject 6. The actual body weight increase was about 1 kg. whereas that calculated from the N retention gave a value of 618 g. assuming that the material stored had a N content comparable to muscle.

Exchanging an isocaloric equivalent of butter for the lactose did not appear to affect the trend of the N retention apart from causing an initial lag in N excretion.

The retention of N in this experiment was 21.7% over the period of 15 days (31.4% for the first 8 days), account being taken of the first 3 days of return to basal diet.

In the light of our observations we have analysed the data accumulated by Wilson [1930; 1931] in the course of his study of the fate of the N and S of meat supplements. Wilson assumed that when a relatively constant excretion of N and S was attained these outputs represented the total N and S absorbed from the alimentary canal. This involved assuming a coefficient of absorption of only 75%. Recalculation of his data shows that in his case also an extraordinary retention of N and S took place when the basal ration was supplemented with 250 g. beef daily.

Soya bean experiment

To determine the effect of a supplement of good vegetable protein the foregoing experiment was repeated using in place of beef, 81 g. "Soyolk" (T.N. = 5.7 g.) with the necessary supplements of butter and lactose to make the addition comparable to a litre of milk. As difficulty was experienced in consuming this preparation the experiment was closed after 5 days' superimposition. For these 5 days and the first 2 days of the subsequent basal period the % retention of the extra protein was 34. The increase in weight corresponded fairly closely with that calculated from the N retention on the basis that the material retained was comparable in N content to muscle. There is no justification for the assumption that the material stored was transformed into muscle either in this or previous experiments in this series.

DISCUSSION

These experiments have demonstrated that in the adolescent and even adult human subject the superimposition of a litre of milk for 1 day on a basal diet, adequate for the maintenance of weight and N equilibrium and already containing 500 ml. raw milk, caused retentions of N and S equivalent to 54 and 53% of the N and S added to the diet. No statistical difference could be detected between the retentions observed with raw and boiled milks. It is of interest that superimposed sodium caseinogenate equivalent in N content to a litre of milk was only half as well retained, indicating that probably the other constituents in the milk were playing a role in effecting this retention.

This unexpected retention of N persisted even when the supplementing of the diet with extra milk was continued for 8 days. As was to be expected, again no real difference was detected between the retentions effected by raw and boiled milks. The retention of Ca in contrast to that of N was relatively insignificant with both types of milk.

In calculating the retention of the additional N it has been assumed that all

the extra N was absorbed. It is unlikely that the amount of the additional milk N escaping absorption would exceed 8%.

That this retention phenomenon was not a peculiar property of the super-added milk was demonstrated by adding lean beef+lactose+ butter equivalent in protein, carbohydrate and fat content to a litre of milk, a comparable retention of N being observed. The daily retention gradually diminished but even after 15 days was still in evidence. Wilson's data [1930; 1931] indicate that quite considerable although steadily diminishing retentions may be found even when beef is the sole supplement.

Soya flour+lactose+ butter equivalent to the protein, carbohydrate and fat in a litre of milk also led to a marked retention of N when superimposed on an adequate basal diet. The writers feel that too much emphasis must not be laid on the differences in retention values which have been observed over comparatively short periods of time. Extraneous factors, particularly those which affect the faecal N excretion, may introduce variations in the day to day N exchange. The main fact of importance is that here again evidence of a considerable N retention is forthcoming. We cannot define which protein moiety of these mixed diets is contributing mainly to the N retention. For example in our last experiment we are not justified in considering the N retained to be of "Soyolk" origin. It may have come from the protein of the milk in the basal dietary. The superimposition type of experiment must therefore be used with great caution for the determination of the nutritive value of a foodstuff.

We may conclude by stating that when a dietary which is adequate for maintenance is supplemented by protein, whether of animal or vegetable origin, and particularly if the energy content of the diet is still further increased by additions of carbohydrate and fat, a considerable retention of N and S may result, the degree of retention bearing a relationship to the total increment in the energy value of the diet. This retention of nitrogenous material diminished with the passage of time but may still be considerable even after fifteen days of surfeit. The nature and site of the retained material will be discussed in a subsequent paper.

SUMMARY

1. The superimposition of a litre of raw or boiled milk on a diet adequate for maintenance of body weight and N equilibrium in the adult human subject caused an increase in body weight and a marked retention of N and S but not of Ca. The addition of sodium caseinogenate equivalent in N content to a litre of milk to the diet of one subject resulted in a definite but much less marked storage of N.

2. Comparison of the N retentions effected by the addition of milk whether administered for 1 day or 8 consecutive days revealed no real difference between the metabolic fates of the proteins of raw and boiled milk.

3. The superimposition of beef+lactose+ butter equivalent in protein, carbohydrate and fat content to a litre of milk also effected a definite saving of food N which was still in evidence even after 15 days of surfeit. Soya flour+lactose+ butter achieved a similar effect.

4. On resumption of basal conditions the retained N was apparently eliminated at a slow even rate.

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