XXI. THE INFLUENCE OF THE NATURE OF THE DIET ON THE RETENTION OF PROTEIN.

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The question of the capacity of the organism to store protein has always been one of considerable interest and although it is highly probable that such a storing is a normal process the critical conditions for its occurrence have not yet been fully elucidated, largely owing to the fact that the amount of information available from nitrogen equilibrium studies is small and its value difficult to assess. Still by the use of the method of superimposition results of importance can be obtained without recourse to such favouring conditions of storage as muscle work, growth or inanition, all of which conditions introduce secondary factors which render the interpretation of the data more difficult.

In the course of another investigation Tsuji [1915] found that the degree of retention of superimposed protein varied (1) with the nature of the standard diet, and (2) with the nature of the protein superimposed. As these experiments were, as regards the superimposition, only of one day's duration the present series of experiments was carried out to amplify the data obtained.

The animal employed was an Airedale bitch weighing 17.6 kilos. She was fed daily at 11 a.m. after catheterisation, the urine then obtained being added to the urine collected in the receiver of the metabolic cage.

The analytical methods employed were: total nitrogen, Kjeldahl; urea, Plimmer and Skelton's modification of the urease method; ammonia, Folin. The delimitation of the faeces was carried out by means of charcoal or carmine. The total faeces was collected for each period and a single analysis of the mixed specimens was made.

As regards the standard diets employed three different combinations of protein, fat and carbohydrate were used. They all contained the same amount

of protein and were of the same caloric value but varied markedly in their content of fat and carbohydrate. They were as follows:

I. Carbohydrate-rich, fat-poor diet.

Scott's oatflour	= 120 g.		
Dried skimmed milk	= 43 g.		
Margarine	= 34 g.	Nitrogen	7.2 g.
Caseinogen	= 19 g.	Carbohydrate	194 g.
Tapioca	= 93 g.	Fat	34 g.
Sodium chloride	= 2 g.	Caloric intake	1296

II. Intermediate diet.

Scott's oatflour	= 60 g.		
Dried skimmed milk	= 62 g.		
Margarine	= 77 g.	Nitrogen	7.2 g.
Caseinogen	= 19 g.	Carbohydrate	117 g.
Tapioca	= 47 g.	Fat	68 g.
Sodium chloride	= 2 g.	Caloric intake	1296

III. Fat-rich, carbohydrate-poor diet.

Scott's oatflour	= 30 g.		
Dried skimmed milk	= 40 g.		
Margarine	= 120 g.	Nitrogen	7.2 g.
Caseinogen	= 34 g.	Carbohydrate	42 g.
		Fat	101 g.
Sodium chloride	= 2 g.	Caloric intake	1296

The standard diet was continued for a pre-period of five to eight days until the nitrogen output was approximately constant, there was then added to the diet a definite amount of protein material, and this was continued for eight days when the diet again reverted to the original standard for a post-period of six or eight days. It was thought that in this way some definite information as regards retention would be obtained.

Caseinogen experiments.

EXPERIMENT I. *Retention of caseinogen nitrogen on the carbohydrate-rich, fat-poor diet.*

In this experiment the animal was fed on the carbohydrate-rich standard diet. After a pre-period of five days 30 g. of caseinogen containing 3.6 g. nitrogen were added daily to the standard diet during eight days. Then the diet was again changed, for a post-period of seven days, to the original standard diet.

It will be noted from the Table I A that although there was a progressive fall in the retention of nitrogen from + 0.4 g. on the first day to + 0.3 g. on the fourth day and even a slight negative balance on the fifth day the average daily retention was + 0.2 g. giving a total retention of 1.44 g. for the whole

period. During the chief period, I B, when caseinogen was being added to the diet there was a well-marked retention amounting to + 1.26 g. daily, with a total retention for the whole period of 10.0 g. nitrogen. As during this period 28.8 g. of nitrogen were superimposed there was therefore a retention

TABLE I.

A. *Pre-period.*

Day of experiment	Total amount of urine in cc.	Nitrogen intake in g.	Nitrogen output in g.				Daily nitrogen balance g.
			Urine			Faeces	
			Ammonia	Urea	Total nitrogen		
I	690	7.2	0.49	4.98	6.05	0.66	+0.49
II	540	7.2	0.48	5.02	6.10	0.66	+0.44
III	670	7.2	0.48	5.19	6.23	0.66	+0.31
IV	620	7.2	0.46	5.12	6.16	0.66	+0.38
V	560	7.2	0.51	5.60	6.72	0.66	-0.18
Average		7.2	0.48	5.18	6.25	0.66	+0.29

B. *Caseinogen period* (3.6 g. nitrogen in the form of caseinogen were added daily).

VI	700	10.8	0.64	7.13	8.36	0.79	+1.65
VII	700	10.8	0.66	7.24	8.56	0.79	+1.45
VIII	580	10.8	0.67	6.63	7.91	0.79	+2.10
IX	570	10.8	0.80	7.89	9.34	0.79	+0.67
X	730	10.8	0.76	7.73	9.07	0.79	+0.94
XI	730	10.8	0.69	7.22	8.49	0.79	+1.52
XII	770	10.8	0.70	7.61	8.98	0.79	+1.03
XIII	840	10.8	0.78	7.88	9.28	0.79	+0.73
Average		10.8	0.71	7.42	8.75	0.79	+1.26

C. *After period.*

XIV	790	7.2	0.51	6.03	7.26	0.66	-0.72
XV	900	7.2	0.49	5.83	6.93	0.66	-0.37
XVI	800	7.2	0.48	5.07	6.18	0.66	+0.38
XVII	800	7.2	0.55	5.75	6.90	0.66	-0.36
XVIII	760	7.2	0.47	5.27	6.34	0.66	+0.20
XIX	760	7.2	0.54	5.91	7.08	0.66	-0.54
XX	840	7.2	0.50	5.85	6.95	0.66	-0.41
Average		7.2	0.51	5.67	6.81	0.66	-0.26

of just over 35 %. In the post-period, I C, there was a quite definite negative balance, the average daily loss being - 0.26 g. or 1.84 g. for the total period. This loss, as it continues over the whole period, cannot in the light of Falta's [1906] careful experiments on the re-excretion of nitrogen be regarded solely as due to re-excretion. Falta found in his experiments with caseinogen that

the re-excretion ceased about the fourth day after the caseinogen was stopped. Moreover Tsuji's experiments demonstrate clearly that there is no prolonged excretion of excess nitrogen after the superimposition of caseinogen. This prolonged secondary loss is very difficult to explain except on the ground that there has been a stimulation of the total protein metabolism due to the increased intake.

EXPERIMENT II. *Retention of caseinogen nitrogen on the intermediate diet.*

TABLE II.

A. *Pre-period.*

Day of experiment	Total amount of urine in cc.	Nitrogen intake in g.	Nitrogen output in g.			Faeces	Daily nitrogen balance g.
			Urine				
			Ammonia	Urea	Total nitrogen		
I	590	7.2	0.42	5.94	6.99	0.56	-0.35
II	560	7.2	0.40	5.33	6.32	0.56	+0.32
III	530	7.2	0.46	5.65	6.74	0.56	-0.10
IV	620	7.2	0.45	5.64	6.76	0.56	-0.12
V	590	7.2	0.47	5.99	7.15	0.56	-0.51
Average		7.2	0.44	5.71	6.79	0.56	-0.15

B. *Caseinogen period* (3.6 g. nitrogen in the form of caseinogen were added daily).

VI	620	10.8	0.68	7.68	9.07	0.65	+1.08
VII	520	10.8	0.72	7.77	9.18	0.65	+0.97
VIII	610	10.8	0.67	7.28	8.69	0.65	+1.46
IX	530	10.8	0.69	7.54	8.85	0.65	+1.30
X	670	10.8	0.69	7.66	9.00	0.65	+1.15
XI	590	10.8	0.78	8.00	9.41	0.65	+0.74
XII	610	10.8	0.73	8.16	9.54	0.65	+0.61
XIII	460	10.8	0.71	7.85	9.23	0.65	+0.92
Average		10.8	0.71	7.74	9.12	0.65	+1.03

C. *After period.*

XIV	560	7.2	0.50	5.34	6.36	0.84	0
XV	580	7.2	0.47	5.80	6.90	0.84	-0.54
XVI	490	7.2	0.48	5.18	6.27	0.84	+0.09
XVII	610	7.2	0.47	5.04	6.07	0.84	+0.29
XVIII	540	7.2	0.43	5.11	6.09	0.84	+0.27
Average		7.2	0.47	5.29	6.34	0.84	+0.02

In this experiment the standard diet employed was the intermediate one and 30 g. of caseinogen were added during the chief period. During the after period there was a certain amount of difficulty as the faeces became semi-diarrhoeic and we may conclude that absorption from the intestine was

imperfect. During the pre-period the nitrogen balance was, with the exception of one day, slightly on the negative side giving a daily average of -0.15 g., i.e. 0.76 g. was lost during this period. When caseinogen was added the balance was positive throughout the period giving a daily average of $+1.03$ g. the total retention for the period being 8.23 g., i.e. 28.6% of the nitrogen added in the form of caseinogen was retained. In the post-period there was a slight positive balance.

EXPERIMENT III. *Retention of caseinogen nitrogen on the fat-rich, carbohydrate-poor diet.*

TABLE III.

A. *Pre-period.*

Day of experiment	Total amount of urine in cc.	Nitrogen intake in g.	Nitrogen output in g.			Faeces	Daily nitrogen balance g.
			Urine				
			Ammonia	Urea	Total nitrogen		
I	360	7.2	0.45	5.44	6.56	0.73	-0.09
II	330	7.2	0.50	5.30	6.52	0.73	-0.05
III	350	7.2	0.54	5.27	6.48	0.73	-0.01
IV	400	7.2	0.56	5.33	6.54	0.73	-0.07
V	610	7.2	0.58	5.67	6.79	0.73	-0.32
Average		7.2	0.53	5.40	6.58	0.73	-0.11

B. *Caseinogen period (3.6 g. nitrogen in the form of caseinogen were added daily).*

VI	610	10.8	0.81	8.01	9.44	0.67	+0.69
VII	440	10.8	0.84	7.79	9.32	0.67	+0.81
VIII	600	10.8	0.88	8.32	9.97	0.67	+0.16
IX	750	10.8	1.03	8.49	10.21	0.67	-0.08
X	690	10.8	0.88	8.50	10.08	0.67	+0.05
XI	680	10.8	0.90	8.33	9.86	0.67	+0.27
XII	690	10.8	0.89	8.36	9.97	0.67	+0.16
XIII	660	10.8	0.87	8.13	9.74	0.67	+0.39
Average		10.8	0.89	8.24	9.82	0.67	+0.31

C. *After period.*

XIV	852	7.2	0.66	5.14	6.45	0.76	-0.01
XV	620	7.2	0.66	5.35	6.65	0.76	-0.21
XVI	640	7.2	0.63	5.38	6.65	0.76	-0.21
XVII	620	7.2	0.59	5.24	6.43	0.76	+0.01
XVIII	850	7.2	0.58	4.85	6.18	0.76	+0.26
Average		7.2	0.62	5.19	6.47	0.76	-0.03

In this experiment the dog was kept throughout on the fat-rich diet, 30 g. of caseinogen being added daily during the chief period.

During the pre-period, as in the second experiment, there was a slight negative balance which amounted to a daily loss of -0.11 g. or of 0.54 g. nitrogen in all. In the chief period except for the first day when there was a small negative balance there was evidence of retention of nitrogen. The average balance for the eight days was $+0.31$ g. and the total amount retained was 2.45 g. or 8.5% of the total nitrogen ingested in the form of caseinogen. In the post-period during the first three days the balance was negative and on the two following days positive, giving however an average loss of -0.03 g. nitrogen per diem.

One fact emerges very clearly from the foregoing experiments namely that the nature of the standard diet employed plays a well-defined part in the degree of retention obtained. In each experiment the same amount of nitrogen in the form of caseinogen was added to the diet but the amount retained varied in each instance. Thus in the case of the carbohydrate-rich diet there was a retention of 10.09 g. corresponding to 35% of that superimposed; on the intermediate diet the retention amounted to 8.23 g. or 28.6% of the superimposed amount and on the carbohydrate-poor, fat-rich diet the retention amounted only to 2.45 g., equivalent to 8.5% of the total addition. The evidence, then, clearly points in favour of the view that carbohydrate plays some preponderant rôle in the retention of protein, that when a sufficiency of carbohydrate is not available there is a failure in the synthetic processes. This is borne out by the consideration, for example, of the marasmic condition associated with diabetes, where protein is given in large amount in the diet but without any gain on the part of the patient. It had been hoped that some light would have been obtained on the proportion of carbohydrate which was essential, *i.e.* on the ratio which must exist between carbohydrate and fat in a diet, but, owing to various difficulties, this point could not be fully investigated at present. From the results of Lang [1915] on acidosis it would seem probable however that the highest ratio of fat to carbohydrate which is possible is $1:2$.

Caseinogen is generally recognised as being a form of protein which is more readily retained than many other forms [Röhmman 1898; Caspari 1900], although this has been denied by some workers [Bloch 1900] for example. As the present experiments had given such a satisfactory result the question as to whether the superimposition of an imperfect protein like gelatin would yield an equally satisfactory result arose.

EXPERIMENT IV. *Retention of gelatin nitrogen on the carbohydrate-rich, fat-poor diet.*

TABLE IV.

A. *Pre-period.*

Day of experiment	Total amount of urine in cc.	Nitrogen intake in g.	Nitrogen output in g.			Faeces	Daily nitrogen balance g.
			Ammonia	Urea	Total nitrogen		
I	800	7.2	0.48	5.07	6.18	0.66	+0.38
II	800	7.2	0.55	5.75	6.90	0.66	-0.36
III	760	7.2	0.47	5.27	6.34	0.66	+0.20
IV	760	7.2	0.54	5.91	7.08	0.66	-0.54
V	840	7.2	0.50	5.85	6.95	0.66	-0.41
Average		7.2	0.51	5.57	6.69	0.66	-0.15

B. *Gelatin period* (4.1 g. nitrogen in the form of gelatin were added daily).

VI	840	11.3	0.60	8.87	10.16	0.78	+0.36
VII	840	11.3	0.64	9.04	10.33	0.78	+0.19
VIII	600	11.3	0.60	7.86	9.09	0.78	+1.43
IX	770	11.3	0.58	8.04	9.30	0.78	+1.22
X	880	11.3	0.63	8.69	10.03	0.78	+0.49
XI	860	11.3	0.60	8.37	9.63	0.78	+0.89
XII	760	11.3	0.64	8.39	9.77	0.78	+0.75
XIII	810	11.3	0.60	7.85	9.07	0.78	+1.45
Average		11.3	0.61	8.39	9.67	0.78	+0.85

C. *Gelatin and meat extract period* (4.1 g. nitrogen in the form of gelatin and 0.5 g. nitrogen in the form of meat extract were added daily).

I	760	11.8	0.59	8.77	10.24	0.96	+0.60
II	1020	11.8	0.68	9.55	11.08	0.96	-0.24
III	780	11.8	0.64	8.37	9.72	0.96	+1.12
Average		11.8	0.64	8.90	10.35	0.96	+0.49

D. *After period.*

I	980	7.2	0.58	6.34	7.50	0.97	-1.27
II	1040	7.2	0.56	5.88	7.02	0.97	-0.79
III	960	7.2	0.53	5.35	6.38	0.97	-0.15
Average		7.2	0.55	5.86	6.97	0.97	-0.74

As in the previous experiments the animal was put on the standard diet, in this case the carbohydrate-rich, fat-poor diet, for a pre-period of five days, then to this diet there was added daily during the chief period 30 g. of gelatin containing 4.1 g. nitrogen.

During the pre-period the balance was very variable, the daily average for the period being - 0.15 g. In the chief period the balance throughout

was positive, the average daily retention amounting to +0.85 g., the total retention for the whole period was 6.78 g. corresponding to 20.7 % of the nitrogen taken in, *i.e.* the retention in the case of gelatin is definitely lower than in that of caseinogen under exactly the same conditions. In view of Thompson's [1910] statement that the addition of meat extract to a diet brings about a more perfect retention it was thought that the present series of experiments offered a good opportunity of testing this matter. Accordingly the chief period was continued for a further three days during which time 5 g. of meat extract were added to the gelatin and superimposed on the standard diet. As the figures in IV C show this addition produced no increase in the amount retained, indeed the daily average was definitely lowered. Unfortunately, as the animal was tiring of the diet, the post-period could not be continued for the usual length of time. (Unless every precaution is taken to see that the animals, during these tests, maintain their appetites the experiments lose a very large part of their value.) During the post-period, in the present experiment, there was a very well-marked negative balance which may be due in large part to a re-excretion of the retained nitrogen. In this respect the apparent retention is even more unfavourable than the above calculation would suggest.

EXPERIMENT V. *Retention of gelatin nitrogen on a fat-rich, carbohydrate-poor diet.*

TABLE V.

A. *Pre-period.*

Day of experiment	Total amount of urine in cc.	Nitrogen intake in g.	Nitrogen output in g.			Faeces	Daily nitrogen balance g.
			Urine				
			Ammonia	Urea	Total nitrogen		
I	750	7.2	0.60	5.47	6.07	0.53	-0.03
II	680	7.2	0.62	5.40	6.02	0.53	+0.08
III	550	7.2	0.58	4.74	5.32	0.53	+0.78
IV	630	7.2	0.62	5.50	6.12	0.53	-0.12
V	640	7.2	0.63	5.84	6.47	0.53	-0.34
Average		7.2	0.61	5.39	6.00	0.53	+0.07

B. *Gelatin period* (4.1 g. nitrogen in the form of gelatin were added daily).

I	530	11.3	0.69	8.66	9.35	0.81	+0.48
II	580	11.3	0.73	8.81	9.54	0.81	+0.34
III	560	11.3	0.62	8.94	9.56	0.81	+0.28
IV	590	11.3	1.20	8.88	10.08	0.81	+0.50
Average		11.3	0.76	8.82	9.58	0.81	+0.40

In this experiment the standard diet was fat-rich, carbohydrate-poor. The amount of gelatin superimposed was the same as in experiment IV. This experiment is not complete as after the fifth day of the chief period the animal lost its appetite and could not be induced to finish its daily allowance of food. The data recorded are all, however, free from objection.

During the pre-period again the balance was variable but in this instance it was very slightly positive, the daily average retention being + 0.07 g. In the chief period the average daily retention amounted to + 0.4 g. or 1.6 g. for the four days corresponding to 9.76 % of the total nitrogen superimposed, a result slightly more favourable than that found for caseinogen under similar conditions for a period of eight days (8.5 %), but slightly less favourable if compared with the retention of the caseinogen nitrogen for the period of the first four days (10.97 %), which is more directly comparable with the gelatin figures. Here, therefore, as in the case of the caseinogen, although the retention is not quite so marked, the carbohydrate exercises a definite retaining influence on the superimposed nitrogen.

As regards the method by which the carbohydrate acts it is not proposed to deal with the problem in the present communication. It is hoped however to take up the consideration of this question in another paper.

CONCLUSIONS.

1. Nitrogen in the form of protein added to a carbohydrate diet is retained in greater amount than when added to a fat diet of equal caloric value.
2. Nitrogen given in the form of caseinogen is more completely retained than when given in the form of gelatin.
3. The addition of meat extract to gelatin does not increase the amount of nitrogen retained.

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REFERENCES.

- Bloch (1900). *Zeitsch. diätet. physik. Ther.*, 3, 482.
Caspari (1900). *Zeitsch. diätet. physik. Ther.*, 3, 393.
Falta (1906). *Deutsch. Arch. klin. Med.*, 86, 517.
Lang (1915). *Biochem. J.*, 9, 456.
Röhmman (1898). *Berl. klin. Woch.*, 35, 789.
Thompson (1910). *Brit. Assoc. Report*, 760.
Tsuji (1915). *Biochem. J.*, 9, 439.