THE RELATION BETWEEN THE DIGESTIBILITY AND THE RETENTION OF INGESTED PROTEINS.

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THE RELATIONS BETWEEN DIGESTION AND ASSIMILATION.[1]

The rate of the catabolism of a protein is determined principally by two factors, namely by the rate of its digestion and by that of its absorption. In the process of protein assimilation, according to the prevailing views, the controlling influence is exercised by the same two factors. Many writers assume that the reconstruction of body protein from the ingested material begins only after its complete deterioration. On the basis of these theories the value of a protein for the organism rises with the increase in its digestibility.

However, there are recorded facts which are not in full harmony with this assumption. Thus Falta first demonstrated that the degree of nitrogen retention is determined by the nature of the ingested proteins. This view was corroborated by Graffenberger and very recently by Voit. According to Falta the fragments of protein molecule which resist the destructive influence of the gastro-intestinal juices are those that are retained the longest in the organism, and are, perhaps, utilized for the purpose of protein assimilation. In a recent series of articles Levin, Manson and Levene, and Carrel, Meyer and Levene have reported experiments of an entirely different character which demonstrate the correctness of this view.

[1]The problem was suggested by Dr. P. A. Levene. Preliminary work was begun at Woods Hole in the summer of 1909 with Dr. Wm. M. Clark, at present of the U. S. Dept. of Agriculture, and C. B. Bennett of the University of Vermont.

It is known that the act of digestion and absorption is accomplished principally in the intestinal tract, while in the stomach the rate of protein absorption is low and the digestion does not pass the stage of proteoses.

On the basis of the generally accepted view of protein metabolism it seems reasonable to expect that the factors facilitating the transportation of a protein from the stomach to the intestinal tract should increase also the value of protein foodstuffs. The observations of Levene and his co-workers are contradictory to this expectation. They brought to light the fact that, after gastrectomy or gastro-enterostomy, when the food enters directly into the intestinal tract, the rate of nitrogen elimination is high, but the rate of retention is very significant; while the results are reversed after resection of most of the small intestine. On the basis of these experiments it seemed more reasonable to expect that factors causing a delay in transportation of protein foodstuff from the stomach to the intestinal tract would result in a higher rate of nitrogen retention.

The present investigation was originally undertaken with the purpose of establishing the food value of the proteins of the fish meats compared with each other and with that of lean beef. In course of the experiments it was found that the meats varied markedly, according to their source and mode of preparations, in the readiness with which they were digested. It was found possible, therefore, to test the influence of variations in the digestibility of proteins, shown by the curves of nitrogen elimination after their administration, upon the degree of nitrogen retention. The results are in harmony with the views expressed by Falta and by Levene and his co-workers.

THE NITROGEN EXCRETION CURVE AFTER PROTEIN FEEDING.

The curve for the excretion of nitrogen in the urine after protein feeding has been studied by a number of experimenters, beginning with Becher in 1855 and Voit in 1857. The literature is reviewed in recent papers by Stauber[1] and by Haas[2]. The results

[1] Stauber: *Biochem. Zeitschr.*, xxv, p. 187, 1910.
[2] Haas: *Ibid.*, xii, p. 203, 1908.

may be summarized briefly. In the case of a man or dog on a normal diet the greater part of the protein nitrogen consumed at a meal is rapidly transformed in the body and excreted in the urine. Consequently the hourly nitrogen excretion rises rapidly after a meal, and falls again to the original height, which may be termed for convenience the "fasting level," after digestion and absorption are concluded. The nature of the excretion curve is not markedly affected by bodily rest or activity (Haas), but is dependent upon the nature and amount of protein ingested, and, in man at least, to some extent upon the water intake. When larger amounts of protein are fed, more time is required for the curve to return to the fasting level. The nitrogen of certain proteins, as found by Falta[1], and Graffenberger[2] is metabolized more slowly than that of others.

In man, the excretion curve during digestion may be irregular. Within a short time after eating (half an hour, Veraguth[3]), a minor maximum usually occurs, due apparently to diuresis following the intake of water, which is quickly excreted, and washes out the nitrogenous waste products from the tissues (Haas). A second and third maximum usually occur 2 to 4 and 4 to 7 hours after a meal following, Tschlenoff[4] suggests, the periods of greatest resorptive activity in the stomach and intestine respectively. Stauber[5] found the highest excretion normally about 5 hours after feeding. When meat, predigested by pepsin was eaten, however, the chief maximum occurred within 1–2 hours, indicating that the products of protein digestion are absorbed and excreted soon after they are formed.

In experiments with dogs, in which the animals were fed meat equal to 2–4 per cent of the body weight and catheterized at intervals, the bladder being washed out thoroughly, the curve of nitrogen excretion following a meal was found to rise regularly to a maximum, which occurs after 4-8 hours, then to fall gradually to the fasting level, reached after about 20 hours.[6] Comparison with

[1] Falta: *Deutsch. Arch. f. klin. Med.*, lxxxvi.
[2] Graffenberger: *Zeitschr. f. Biol.*, xxviii, p. 337.
[3] Veraguth: *Journ. of Physiol.*, xxi, p. 112, 1897.
[4] Tschlenoff: *Correspondenz-Blatt f. Schweizer Aerzte*, 1896.
[5] Stauber: *loc. cit.*; *Centralbl. f. d. med. Wiss.*, 1896, p. 349.
[6] Feder: *Zeitschr f. Biol.*, xvii, p. 541.

the rate of absorption from the alimentary canal found by Schmidt-Mülheim[1] indicated that excretion lags behind absorption during the first 2 hours, nitrogen being retained in the body during this period. From then till the 12-14th hour absorption and excretion run almost parallel. Absorption is practically finished at this time, but excretion continues at a slow rate, gradually falling to the fasting level.

From the above it appears that under normal conditions the rate of excretion of nitrogen in a dog during the first 12 hours after feeding gives a fairly accurate picture of the course of digestion and absorption. Our own results indicate that, with an animal kept under uniform and normal conditions, the rate of nitrogen excretion is almost entirely dependent upon the food, and characteristic for any given diet. By combining the data concerning the rate of excretion with those from analysis of the feces, we aimed to obtain results showing both the rate and the completeness of the absorption of protein from the different foods employed.

METHODS.

A dog in approximate nitrogenous equilibrium was fed once in 24 hours, and the rate of nitrogen excretion in the urine followed by catheterizing at 3-hour intervals. The rate of excretion is taken as an index of the readiness with which the proteins are digested, absorbed from the alimentary canal, and metabolized in the body. The nitrogen of the feces is taken as an index of the relative completeness with which the proteins of the different foods are absorbed.

For all except the first experiments (Table I) the dog was fed the following diet.

Meat	to contain 3 gm. of nitrogen.
Starch	65 gm.
Fat (Lard + Meat fat)	26-27 gm.
Salt	5 gm.
Bone Ash	5 gm.

During alternate experiments charcoal was added to the food, in order to make possible a separation of the feces from the different diets. With the

[1] Schmidt-Mülheim: *Arch. f. Anat. u. Physiol., Physiol. Abt.*, 1879, p. 39.

addition of bone ash this proved satisfactory. The food was warmed to body temperature before feeding.

The meats were freed from visible fat, skin, etc., and ground as fine as possible in a machine. They were then boiled for 20-25 minutes, and thoroughly drained. The salt cod was shredded and soaked in fresh water over night before boiling. Immediately after taking samples for analysis the meats were frozen, and kept in that condition, as suggested by Gies.

FOOD ANALYSES. Kjeldahl determinations were performed upon 2-gram portions of the ground and drained meats. The samples were taken from different parts of the main portion; duplicates as a rule, agreed satisfactorily. The starch and lard were practically nitrogen free.

For fat determinations, 2 gm. of the meat, without preliminary drying, were ground up with anhydrous copper sulphate until the mixture became a dust-dry powder. This was then extracted 8 hours in a paper thimble with carbon tetrachloride. The method proved convenient and gave close duplicates throughout. The fat contents of the different meats were: periwinkle, 1.78 per cent; squeteague, 9.35 per cent; tautog, 2.03 per cent; eel, 6.79 per cent; boiled cod, 0.50 per cent; fried cod, 1.83 per cent.

ANALYSIS OF FECES. The feces as soon as gathered were placed in concentrated sulphuric acid, in which they formed a solution or homogenous suspension. This was diluted to a known volume at the conclusion of each food test, and aliquot portions used for nitrogen determinations.

URINE was collected chiefly by catheterization, the bladder being washed 4 times at each catheterization. Urine voided in the cage was washed into a bottle containing acid, and united with that obtained by catheterization at the end of the current period. The animal, a fox terrier bitch of 7.3 kilos weight, was catheterized every three hours after the daily feeding for 12 hours, then again at the end of the 24th hour.

RESULTS.

In order to test the effect of non-protein food on the digestibility of protein, the preliminary experiments tabulated in Tables I and II were performed. The animal was not yet in equilibrium. In the first 2 days of the series the dog received daily 60 grams of meat and 25 grams each of crackerdust and lard. During the next two days 60 grams of cornstarch was added to the diet. The effect of the added starch is markedly apparent in a retardation of absorption, as shown by the slower excretion of urinary nitrogen. While with diet 1 the height of excretion occurs in the first two periods, during which 0.47 and 0.69 gram of nitrogen were excreted, with diet 2 only 0.37 and 0.49 gram were voided during these periods, and thereafter the rate remained higher than with diet 1, indicating that a longer time was required for finishing the digestion and absorption when more carbohydrate was present. From these results it is apparent that not only the amount of protein fed, but also that of the other food constituents is a decided factor in the rate of absorption.

The nitrogen of the feces indicated little difference in the completeness of absorption, 84.8 per cent of the nitrogen in one case and 82.3 per cent in the other. The effect of the added starch in sparing protein and bringing about nitrogenous equilibrium is seen on the fourth day.

The remaining experiments were performed after the animal had attained equilibrium on the standard diet (p. 222). The tests were run in duplicate, except for the weakfish and periwinkle. The average results are summarized in Table II, the data from the different diets being arranged in a descending series, based on the relative rate at which the protein in each is digested and absorbed, as measured by the nitrogen excretion in the urine. The nitrogen excreted during the first 9 hours after feeding is taken as the index for comparison. The weight of the dog, remained without significant change during the experiments.

Table II shows a striking and unexpected relation between the relative rates at which the proteins were digested and metabolized, as shown by the nitrogen balances. The diets tabulated at the left, from which the nitrogen was absorbed and metabolized most rapidly, were least capable of maintaining equilibrium. The loss of nitrogen is in three of the four diets showing a negative balance, due at least partly to decreased absorption, as shown by the large nitrogen content of the feces. A possible explanation is that both the rapid digestion and the incomplete absorption were due to stimulation of peristalsis, which caused the alimentary contents to be digested and absorbed more rapidly, as the result of quicker mixing with the digestive fluids and more thorough contact with the absorbing surfaces of the digestive tract, but at the same time passed the contents through too rapidly for complete absorption. Another factor lies in the fact, shown by the recent work of Carrel, Meyer and Levene[1], that protein is more efficient in maintaining nitrogenous equilibrium when it is absorbed before cleavage has

[1]*Amer. Journ. of Physiol.*, 1909, 1910.

TABLE I.

Effect of Starch on Rate of Protein Digestion.

8, I, 1910			8, II, 1910				
HOURS	1	PER HOUR	2	PER HOUR		GRAMS	N
0–3	0.4885	0.1614	0.4540	0.1539	Weakfish-flesh	60.00	2.24
3–6	0.6834	0.2278	0.7060	0.2353	Cracker Dust	25.00	0.44
6–9	0.4150	0.1383	0.4450	0.1483	Lard	25.00	0.00
9–12	0.2941	0.0980	0.2900	0.0967	Salt	5.00	0.00
12–24	0.8360	0.0697	0.8266	0.0689			
Total	2.7170		2.722				
Feces	0.428		0.428				
N excretion	3.145		3.150				
Food N	2.680		2.680				
N Balance	–.465		–.470				
Digestion coefficient, 84.8 per cent.							

8, III, 1910			8, IV, 1910				
HOURS	1	PER HOUR	2	PER HOUR		GRAMS	N
0–3	0.3783	0.1261	0.3727	0.1242	Weakfish-flesh	60.00	2.24
3–6	0.5002	0.1667	0.4799	0.1600	Cracker Dust	25.00	0.44
6–9	0.4704	0.1568	0.4423	0.1474	Starch	60.00	0.00
9–12	0.3858	0.1283	0.2809	0.0936	Lard	25.00	0.00
12–24	1.029	0.0857	0.7078	0.0589	Salt	5.00	0.00
Total	2.764		2.283				
Feces	0.473		0.473				
N excretion	3.237		2.756				
Food N	2.680		2.680				
N Balance	–.557		–.076				
Digestion coefficient, 82.3 per cent.							

proceeded to the lowest stages. Whatever the cause, it is evident that ready digestibility of protein foods does not indicate complete availability, but that, within certain limits, the relations may be exactly the reverse. The relations between the fresh and salt cod diets form a marked illustration. The nitrogen from the fresh cod, boiled or fried, was digested and absorbed the most rapidly of that of any of the diets, but, absorbed and retained the least completely. The salt cod, which was fed in the interval between the two fresh cod diets, was absorbed and metabolized much more slowly, doubtless due to the physical effect of the preservation in salt, but the absorption and retention were proportionally more complete.

The above results are consistent with those recently obtained by E. Voit and Zisterer,[1] who find that casein is less capable of maintaining nitrogenous equilibrium when fed after artificial digestion with pepsin, than when fed without previous digestion; too early peptonization appeared to decrease the food value of the protein.

TABLE II.

Summary of Mean Results.

FOOD	BOILED COD	FRIED COD	BOILED BEEF	BOILED TAUTOG	BOILED EEL	BOILED WEAK-FISH	BOILED MUSSEL	BOILED SALT COD	BOILED PERI-WINKLE
N in urine during first 9 hours after feeding.	1.50	1.36	1.29	1.28	1.24	1.23	1.23	1.07	1.00
N absorbed in 24 hours......	1.98	1.80	2.58	2.55	1.91	2.53	2.40	2.58	2.57
N excreted in 24 hours	2.51	2.48	2.76	2.35	2.20	2.34	2.22	2.29	1.90
N retained......	−0.53	−0.68	−0.18	+0.20	−0.29	+0.19	+0.18	+0.29	+0.47

SUMMARY.

A dog was once fed in 24 hours and catheterized 3, 6, 9, 12 and 24 hours after each meal. The rate of nitrogen excretion is taken as an index of the rate of absorption from the alimentary canal.

[1] *Zeitschr. f. Biol.*, liii, p. 457.

Addition of starch to the diet decreased the rate of nitrogen metabolism, but had no significant effect on the completeness of absorption.

The diets containing boiled meats, other constituents being constant, rank as follows when arranged in order according to the relative *rates* at which their nitrogen was digested and absorbed, as indicated by the nitrogen excretion: fresh cod, beef, tautog, eel, weakfish, mussel, salt cod, periwinkle.

When ranked according to the amount of nitrogen *retained* from each, the order is practically reversed.

The failure to retain the nitrogen of the more quickly digested and metabolized proteins appears partly due, in the fresh cod and eel diets at least, to incomplete absorption. Another cause doubtless lies in the fact that a larger proportion of the more rapidly digested proteins is absorbed in the form of the lowest cleavage products, which appear, from recent work of Carrel, Levene, Meyer, and Manson, less capable than the higher cleavage products, of maintaining the nitrogenous equilibrium of the body. Apparently there is an *optimum rate of digestion* in the alimentary tract, which constitutes the condition for the formation and absorption of proteolytic products in a manner making possible their most complete assimilation by the body. This optimum rate of digestion may not only be fallen short of, but may be exceeded, as in some of the experiments above reported.

III.
Beef

HOURS	8, VII, 1910	8, VIII, 1910
0–3	0.3612	0.3182
3–6	0.5338	0.5038
6–9	0.4470	0.4439
9–12	0.4747	1.363
12–24	0.9870	
0–24	2.804	2.629
N of feces	0.423	0.423
	3.227	3.052
Balance	−0.227	−0.052

IV.
Weakfish (Cynoscion regalis.)

HOURS	8, IX, 1910
0–3	0.3400
3–6	0.5009
6–9	0.3972
9–12	0.3757
12–24	0.8740
0–24	2.488
N of feces	0.472
	2.960
Balance	+0.040

V

Tautog (Tautoga onitis)

HOURS	8, XII, 1910	8, XIII, 1910
0–3	0.3390	0.3150
3–6	0.6110	0.5150
6–9	0.3770	0.4052
9–12	0.3850	0.3161
12–24	0.7618	0.6940
0–24	2.474	2.245
N of feces	0.444	0.444
	2.918	2.689
Balance	+0.082	+0.311

VI

Periwinkle (Litorina litorea.)

HOURS	8, XIV, 1910	8, XV, 1910
0–3	0.2940	0.2880
3–6	0.4660	0.3780
6–9	0.3220	0.2549
9–12	0.2548	0.2507
12–24	0.6534	0.6457
0–24	1.990	1.817
N of feces	0.634	0.634
	2.624	2.451
Balance	+0.376	+0.549

VII

Eel (Anguilla chrysypa.)

HOURS	8, XVI, 1910
0–3	0.3657
3–6	0.5720
6–9	0.3010
9–12	0.3055
12–24	0.6515
0–24	2.196
N of feces	1.087
	3.283
Balance	–.283

VIII

Mussel (Mytilus edulis.)

HOURS	8, XVII, 1910	8, XVIII, 1910
0–3	0.3152	0.2720
3–6	0.4790	0.4480
6–9	0.4930	0.4580
9–12	0.3210	0.3402
12–24	0.6555	0.6570
0–24	2.264	2.175
N of feces	0.604	0.604
	2.868	2.779
Balance	+.132	+.221

IX

Boiled Cod (Gadus callarias)

HOURS	8, XIX, 1910	8, XX, 1910
0–3.......	0.2830	0.3580
3–6.......	0.6365	0.6560
6–9.......	0.5600	0.5095
9–12......	0.3360	0.3105
12–24.....	0.6370	0.7215
0–24......	2.452	2.555
N of feces	1.020	1.020
	3.472	3.575
Balance...	−.472	−.575

X

Salt Cod

HOURS	8, XXI, 1910	8, XXII, 1910
0–3.......	0.2645	0.2660
3–6.......	0.4550	0.4558
6–9.......	0.4610	0.3460
9–12......	0.2970	0.3725
12–24	0.7914	0.9430
0–24......	2.269	2.383
N of feces	0.422	0.422
	2.691	2.805
Balance...	+.309	+.195

XI

Fried Cod

HOURS	8, XXIII, 1910	8, XXIV, 1910
0–3.......	0.3623	0.2030
3–6.......	0.6840	0.5015
6–9.......	0.4985	0.4805
9–12......	0.2960	0.4170
12–24.....	0.7080	0.8050
0–24......	2.549	2.407
N of feces	1.197	1.197
	3.746	3.604
Balance...	−.746	−.604