creatine are left in water and soil where they exercize an effect on subsequent plant growth. Creatinine seems to persist for a considerable time in soils and may indeed increase in the soil by accumulation. Since both creatine and creatinine have a favorable effect on plant growth, their presence in plants and in the medium in which plants grow has considerable bearing on soil fertility.

WASHINGTON, D. C.

[FROM THE WOODS HOLE LABORATORIES OF THE U. S. BUREAU OF FISHERIES.]

COMPARATIVE PROTEOLYSIS EXPERIMENTS WITH TRYPSIN.

BY GEORGE F. WHITE AND WILLIAM CROZIER.

Received October 20, 1911.

Considerable work has been done on the artificial digestion of various proteins in an attempt to establish their relative values as food. The proteins are digested with either pepsin or trypsin and the rate at which solution takes place is determined. No attempt will be made to review the large amount of literature on this subject, as the present investigation is entirely independent of previous results. These former experiments are of somewhat limited value, as no idea is obtained of the extent to which the soluble products have been hydrolyzed, and the degree of cleavage of the proteins is of great importance, naturally, in a study of comparative digestive properties.

In an article entitled "A Method for Quantitative Determination of Aliphatic Amino Groups," D. D. Van Slyke[1] has described a convenient and generally accurate process by which the amino nitrogen in proteins and their cleavage products can be readily determined. An aqueous solution of the protein is treated with sodium nitrite and glacial acetic acid and the gases evolved passed into alkaline permanganate solution, in which solution nitric oxide is completely absorbed. There is left free nitrogen, which corresponds to the amino nitrogen in the original protein, and the volume of which is measured by passing the gas into a suitable buret. Van Slyke has suggested his method for studying the rate and course of hydrolysis of various proteins by either acids, alkalies, or enzymes, and it is the object of this article to apply it to a study of the comparative digestibility of beef, the edible fish, cod (*Gadus callarias*), and the unutilized dogfish (*Mustelus canis*).

Trypsin was chosen instead of pepsin as the proteolytic enzyme acting on the meats, as the hydrolysis proceeds much farther and a greater insight into the complexity of the end-products of digestion can be obtained.

The meats were boiled with a little water for about fifteen minutes, allowed to drain, and kept ice-cold for analysis and the digestion ex-

[1] *J. Biol. Chem.*, 9, 185 (1911).

periments. The following table gives results of analyses showing percentage of moisture, and of nitrogen in the dry substance.

TABLE I.

		Cod.	Dogfish.	Beef.
Moisture	(1)	23.10	26.20	42.51
	(2)	23.02	26.10	42.65
Nitrogen	(1)	4.60	6.36	10.54
	(2)	4.62	6.38	10.65

For the proteolysis experiments, enough protein to contain 0.717 gram of nitrogen was weighed out and ground up thoroughly with a little water in a mortar. The sample, together with 0.4 gram trypsin (Merck) and 0.4 gram sodium carbonate, was poured into a 100 cc. measuring flask and made up to the mark with water. After the addition of a little chloroform to prevent putrefaction, the flask was placed in a thermostat kept at a temperature of 36–38.5°. Duplicate analyses were made, and separate mixtures used for the time periods of $^1/_2$, 1, 2, 4, and 8 hours respectively. For analysis, the solutions were rapidly filtered through folded filters, and 10 cc. portions used for total soluble nitrogen by the Kjeldahl process, for the amino nitrogen according to the method of Van Slyke, and for the amino nitrogen after total hydrolysis. The last was carried out by digesting with concentrated hydrochloric acid and removal of this acid after hydrolysis by evaporation on the water bath. The residue was then taken up with a little water, made up to 25 cc. and 10 cc. portions used for the amino determination.

The buret for collecting the nitrogen gas in the amino determination was carefully calibrated and corrections made. Also blank experiments were performed in which no protein was used, but the same quantities of trypsin and other reagents as were used in the proteolysis experiments. Corrections were thus obtained for the total soluble nitrogen and for the amino nitrogen before and after total hydrolysis.

The following tables contain data showing the time of digestion, the soluble and insoluble nitrogen, and the amino nitrogen before and after complete hydrolysis with hydrochloric acid. The figures are the average of duplicate analyses, which showed satisfactory agreement.

In a study of the digestion of protein in the alimentary tract of the dogfish, Van Slyke and White[1] found that there was a large quantity of urea in the intestinal contents, and its source was traced to the gall-bladder. 1.7 per cent. of the bile was urea, or 72.3 per cent. of the total nitrogen was in the urea form. The presence of this urea was mentioned as a possible source of error in the amino determinations of the intestinal matter. These facts suggested testing the dogfish flesh used for the

[1] *J. Biol. Chem.*, **9**, 211 (1911).

TABLE II.—PROTEOLYSIS OF BEEF.

Time in hours.	Sol. N.	Insol. N.	Am. N.	Am. N. after hyd.	Av. size of peptids.	$100 \times \frac{\text{Sol. N.}}{\text{Tot. N.}}$	$100 \times \frac{\text{Am. N.}}{\text{Sol. N.}}$
1/2	0.391	0.326	0.093	0.196	2.11	54.54	23.79
1	0.481	0.236	0.130	0.268	2.06	67.08	27.03[1]
2	0.637	0.080	0.147	0.291	1.98	88.84	23.08[1]
4	0.659	0.058	0.191	0.330	1.73	91.91	28.98
8	0.704	0.013	0.230	0.311	1.54	98.19	32.67

TABLE III.—PROTEOLYSIS OF COD.

1/2	0.613	0.104	0.121	0.293	2.43	85.51	19.73
1	0.683	0.034	0.166	0.319	1.92	95.26	24.31
2	0.715	0.002	0.185	0.303	1.64	99.72	25.87
4	0.716	0.001	0.229	...	..	99.86	31.99
8	0.707	0.010	0.285	0.341	1.20	98.60	40.31

TABLE IV.—PROTEOLYSIS OF DOGFISH.

1/2	0.506	0.211	0.069	0.175	2.54	70.58	14.11
1	0.588	0.129	0.091	0.240	2.05	82.02	15.47
2	0.641	0.076	0.121	0.252	2.08	89.41	18.88
4	0.697	0.020	0.152	0.259	1.71	97.21	21.81
8	0.716	0.001	0.186	0.262	1.42	99.86	25.98

present experiments, for urea. The method of Levene and Meyer[2] for the determination of urea was utilized, and duplicate analyses gave the following: 0.84 per cent. and 0.83 per cent. urea in the boiled dogfish flesh, or 8.27 per cent. of the total nitrogen was in the urea form. This percentage is quite low and should cause little trouble in the amino determination. Any error involved would not affect our later conclusions, which are based on the *low* amino content of the proteolyzed dogfish, while the presence of urea would raise this.

In the seventh and eighth columns of the tables are given the per cent. soluble nitrogen in the total nitrogen, and the per cent. amino nitrogen in the total soluble nitrogen respectively. These results are shown graphically in Figs. 1 and 2. From Fig. 1 it may be noted that conversion of protein to the soluble form takes place very rapidly, and almost entirely in the first 2 hours of digestion, in the case of all the meats. The most rapid hydrolysis, shown by the steepness of the curves, is during the first half hour, while after about 2–4 hours the increase in soluble nitrogen is slow up to 8 hours, when the digestion is practically complete. Of course the trypsin used may not have been a very pure enzyme, but all that was desired in these experiments were relative values, and it may be seen that there are definit differences in behavior of the three proteins. The cod is made soluble the most rapidly of all, while the

[1] Obviously in error.

[2] THIS JOURNAL, **31**, 717 (1909).

dogfish falls intermediate between the cod and the beef. König and Splittgerber[1] found that pepsin digested some boiled fish more readily than boiled beef, which fact agrees with the results above obtained with trypsin. For further literature relating to artificial digestion experiments with fish see Chittenden and Cummins[2] and Popoff.[3]

In Fig. 2, curves are given which show the relationship of the amino nitrogen as determined by Van Slyke's method, and the total soluble nitrogen. This ratio appears to increase slightly with time of digestion, the increase being most apparent with the codfish. This fact will be

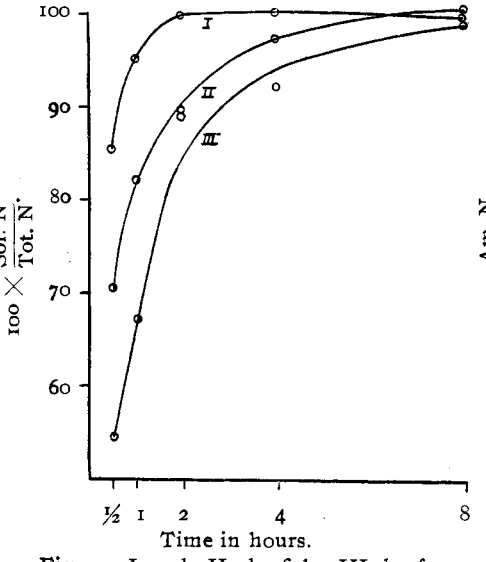

Fig. 1.—I, cod; II, dogfish; III, beef.

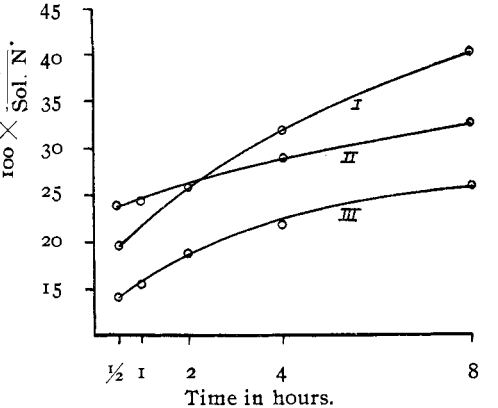

Fig. 2.—I, cod; II, beef; III, dogfish.

referred to later. It may be noted that the ratio is lowest with the dogfish during the whole period of digestion, while the cod, during the first 2 hours having a ratio a little lower than that of the beef, has the highest ratio of all during the remainder of the period.

It is evident then, that while the codfish in tryptic digestion is most readily converted into the soluble form, at the same time the soluble proteins are more easily hydrolyzed than either the beef or the dogfish after an hour or two of digestion, as they furnish more amino nitrogen. The peptides, in other words, are broken down most readily with the cod. Although the beef is least easily made soluble, the hydrolysis of its soluble proteins lies between that of the cod and the dogfish. In columns 6 of the tables of data are given the ratio of the amino nitrogen, obtained by

[1] *Z. wiss. Landwirts.*, **38**, 144 (1909).

[2] *Am. Chem. J.*, 65,

[3] *Z. physiol. Chem.*, **14**, 524 (1890).

complete hydrolysis of the soluble proteins, to the amino nitrogen before hydrolysis. This gives approximately the average size of the peptides present in the solution,[1] and the results are in fairly good agreement with the observations regarding the relations between the amino and soluble nitrogen. At the end of the first half hour, the complexity of the peptides in the dogfish solution is 2.54, in the cod 2.43, and in the beef 2.11. With the longest digestion experiments, the average complexity of the peptides decreases to 1.20 in the cod, while the hydrolysis proceeds to 1.54 and 1.42 with the beef and dogfish respectively. This agrees therefore with the figures, for the percentage amino nitrogen, which are in just the reverse order of magnitude. These facts are peculiarly interesting when considered in connection with the following.

It has been suggested recently by various lines of evidence, that the value of a protein to an organism does not depend necessarily on its ready digestibility, but exactly the reverse may be true; that is, that the assimilation of protein in the digestive tract, its conversion into body material and corresponding retention are most complete when the proteolysis has not been carried too far. Van Slyke and White[2] have briefly reviewed the literature concerning the relation between the digestibility and the retention of ingested proteins, at the same time carrying out some experiments which seem to completely substantiate the above views. In a series of metabolism experiments, they fed dogs with boiled beef and various fish meats with a view to establish the relative food value of the latter. The *rate* of digestion was followed during 24 hours by measuring the nitrogen content of the urin drawn at regular intervals, while the amount of nitrogen retained in the organism was measured by the difference between the nitrogen of the ingested protein and that excreted in the urin plus that in the feces.

It was found that the boiled meats varied considerably in their power to maintain nitrogenous equilibrium, and in the rapidity with which they were digested. The important fact was brought out that those proteins that were most readily metabolized caused a negative nitrogen balance; that nitrogen was lost from the organism while they were fed. Those proteins digested slowly brought about a positive retention of nitrogen. Van Slyke and White suggest as causes for loss of nitrogen with rapid digestion, incomplete absorption as shown by the high nitrogen content of the feces; further, as mentioned above, that the organism in absorbing the low cleavage products brought about by rapid and complete hydrolysis, is less capable of building them up into body protein than higher cleavage products. They suggest an optimum rate of digestion for proteins,

[1] *J. Biol. Chem.*, **9**, 203 (1911).
[2] *Ibid.*, **9**, 219 (1911).

which shall maintain nitrogenous equilibrium, although this rate may be exceeded as shown by their experiments.

In the above metabolism experiments, of nine meats studied, boiled cod was digested the fastest and was the least capable of maintaining nitrogenous equilibrium. Beef fell approximately in the middle of the series, a little nitrogen being lost during the feeding of it. The following table taken from the above work[1] makes these relations clear, the nitrogen in the urin during the first 9 hours after feeding being a measure of the rate of digestion.

Food.	Boiled cod.	Fried cod.	Boiled beef.	Boiled tautog.	Boiled eel.	Boiled weak-fish.	Boiled mussel.	Boiled salt cod.	Boiled peri-winkle.
N in urin 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

The present artificial digestion experiments, it is evident, are in harmony with the metabolism experiments. Boiled cod was found to be digested the fastest of the three meats, at the same time during the greater part of the time of digestion the proportion of low cleavage products was much higher than the other two as shown by the high amino ratio. The dogfish, having only a little greater rate of digestion than the beef, is not broken down as fast as either the beef or the cod. In the light of the metabolism work above quoted and other experiments tending to indicate that too early proteolysis lowers the value of a protein to an organism, it seems safe to conclude that boiled dogfish has a higher food value than cod, and at least as great value as beef. As regards the palatability of dogfish and the economic significance of the above statement, reference may be made to the work of Field.[2]

Summary.

1. The rate and course of hydrolysis by trypsin of the proteins, boiled beef, cod, and dogfish, were studied according to Van Slyke's method. The meats were made soluble at a decreasing rate in the following order: cod, dogfish and beef. The proteolyzed cod solutions gave the greatest amount of amino nitrogen, while the dogfish solutions contained the least. The complexity of the peptides is shown by the ratio of the amino nitrogen after hydrolysis with hydrochloric acid to that present before.

2. The results with the artificial digestion agree with those from metabolism experiments performed on dogs with beef and cod, and indicate

[1] *J. Biol. Chem.*, **9**, 226 (1911).

[2] U. S. Bureau of Fisheries Document, No. 622, 44 (1907).

that dogfish would be digested slowly in the organism, and would be capable of maintaining nitrogenous equilibrium—more capable than either beef or cod.

3. There is 0.84 per cent. urea in dogfish flesh, its presence in the blood and bile having been proved by other experimenters.

4. The dogfish, at present considered an unutilized fish, is suggested as a valuable food product.

The authors wish to express their gratitude to Dr. D. D. Van Slyke, of the Rockefeller Institute for Medical Research, for valuable suggestions in the use of his method.

RICHMOND COLLEGE,
RICHMOND, VA.

[CONTRIBUTION FROM THE NEVADA AGRICULTURAL EXPERIMENT STATION.]

MYRISTONE OBTAINED FROM ALFALFA.

BY C. A. JACOBSON.

Received September 6, 1911.

Since alfalfa (or lucern) is now generally recognized as the best, all-round forage crop, being richer in nitrogen, fats and proteins than the ordinary forage crops under cultivation, it seemed of no small consequence to undertake an exhaustive study of its chemical constituents.

The variety of alfalfa employed for the present investigation was the *Medicago sativa* L. which was grown from carefully selected seed and cut in the early blooming period. After curing in the field, the hay was further dried by spreading it out on a granary floor and left for several weeks. When fully air-dried it was ground to a fine meal by passing through a feed mill used only for this purpose.

The alfalfa meal was then put into an extractor of my own construction, described elsewhere in this issue, and extracted with hot 95 per cent. alcohol for about seven hours, or until the alcohol came through colorless. The alcoholic extract was then removed from the extractor, heated to boiling on the water bath, and filtered. The filtrate was set aside to cool and then left in the ice box over night, after which the dark green precipitate, which had separated out, was filtered off and dried in the air. The resulting material, which represents 6.8 per cent. of the weight of the dry alfalfa meal, was then extracted with ether in a Soxhlet apparatus until the ether siphoned over clear, which required from 16 to 20 hours. The green ethereal extract from the Soxhlet was poured into a beaker and allowed to evaporate at the room temperature to about one-third of its original volume, when a greenish precipitate had separated out. This precipitate was then filtered and washed several times with ether on a Hirsch funnel connected with the filter pump. The