(8) Many desirable and no undesirable effects were obtained by the use of water with meals, and in general, the more water taken the more pronounced were the benefits.

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STUDIES OF THE TRYPTIC DIGESTION OF SILK.[1]

FIRST PAPER.

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Introduction.

Städeler[2] in 1859 first demonstrated that silk was composed of two parts, fibroin and silk glue or sericin. The dissociation products of these parts were, however, first studied by Cramer,[3] who obtained serine from silk glue, and subsequently by Weyl,[4] Vignon,[5] Wetzel[6] and E. Fischer.[7]

Silk fibroin is insoluble in superheated water, dilute acids or alkalies. It has been shown by E. Fischer that fibroin may be heated with water for hours at 117–120°, if the reaction be exactly neutral; however, in the presence of acids or alkalies considerable change takes place under these conditions. According to Weyl it is not attacked by either pepsin or trypsin, a statement disproved with respect to the latter enzyme, by my investigations.

Fibroin contains more than 50 per cent. glycine and alanine and 10 per cent. tyrosine, leucine being present in only small amounts, while

[1] This investigation was undertaken at the suggestion of the late Professor W. F. Koelker, at the University of Wisconsin, and was pursued in its early stages under his direction. The author also wishes to acknowledge his indebtedness to Professor Richard Fischer and Louis Kahlenberg for their interest in the work.

[2] *Ann.*, **111**, 12.

[3] *J. prakt. Chem.*, **96**, 76 (1865).

[4] *Ber.*, **21**, 1407 (1888).

[5] *Compt. rend.*, **115**, 613 (1892).

[6] *Z. physiol. Chem.*, **26**, 535 (1899).

[7] *Ibid.*, **33**, 171 (1902).

glutaminic and aspartic acids are absent, thus differing materially from other albumins.

The silk glue[1] resembles ordinary gelatin in its solubility, but does not gelatinize so readily and is precipitated by acids. In its chemical composition, however, it differs greatly from gelatin in that glycine is present only in small amounts, if at all, while tyrosine and serine are present in considerable quantity. The following table, based on the results of Fischer and Skita,[2] and Fischer,[3] shows the partial composition of silk fibroin and silk gelatin.

	Glycine.	Alanine.	Valine.	Leucine.	Isoleucine.	Phenylalanine.	Tyrosine.	Serine.	Cystine.	Proline.	Oxyproline.	Aspartic acid.	Glutaminic acid.	Tryptophan.	Arginine.	Lysine.	Histidine.	Total.
Silk fibroin	36.0	21.0	0	1.5	—	1.5	10.5	1.6	..	+	..	+	0	..	1.0	+	+	73.1
Silk gelatin	0.2	5.0		...	..	...	5.0	6.6	..	..	..	..	..	..	+	..	4	..

According to Wetzel,[4] silk gelatin upon hydrolysis yields 1.87 per cent. ammonia and shows a positive reaction for tryptophan.

Since over two-thirds of silk fibroin is composed of only three amino acids, *viz.*, glycine, alanine and tyrosine, it is in this respect the simplest known protein.

Experimental.

The silk used in this investigation was the product technically known as "Winder's Waste," which was purified by repeated digestion with boiling alcohol and final thorough washing with water. Four hundred and eighty grams of this purified material were divided into two equal parts, each of which was placed in a bottle of several liters capacity. Five liters of a 0.2 per cent. sodium carbonate solution and half the extract from three hogs' pancreases were added to each bottle, the solutions being kept aseptic by supersaturating with chloroform. One portion was digested for two months, the other for seven months, a constant temperature of 40° being maintained, the bottles thoroughly shaken several times daily and one gram of Merck's trypsin added to each bottle every two weeks.

At the end of the above-stated periods the filtrate in each case responded to Millon's as well as to the Hopkins-Cole tests. Tyrosine and tryptophan were identified according to the method of Hopkins and Cole.[5] However, it was found impossible to entirely prevent pigmentation or to obtain

[1] *Z. physiol. Chem.*, **34**, 481.

[2] *Ibid.*, **33**, 151; **35**, 221.

[3] *Ibid.*, **37**, 508.

[4] *Ibid.*, **29**, 386.

[5] *J. Physiol.*, **27**, 418.

an entirely crystallin product, although the dry residue contained numerous crystals throughout an amorphous magma. This residue gave the glyoxylic acid test very positively and quickly. Those products which had been most highly purified by reprecipitation did not give any test for tyrosine. They were moderately soluble in cold water, freely so in boiling water, and on subjecting to dry heat emitted the characteristic skatole-like odors. There can therefore be no doubt that the product contained tryptophan or some compound of tryptophan.

The washings from the first mercuric sulfate precipitate were heated to boiling and hydrogen sulfide passed in until all the mercury was precipitated. The sulfuric acid was removed with barium hydroxide, the excess of barium precipitated with ammonium carbonate, and the solution concentrated to a small bulk, when a precipitate formed. This precipitate on repeated crystallization gave well defined, needle-like crystals, which, however, were still contaminated with a reddish, gummy substance from which it could not be freed even after boiling with charcoal a number of times. That most highly purified product so obtained gave Millon's test very positively, but it, as well as the impure product, gave a negative test for tryptophan.

The filtrate from which the tryptophan and tyrosine had been removed was freed from hydrogen sulfide by passing a current of air through it for several days, and the peptones precipitated out according to the method of Siegfried[1] as follows: The solution was acidulated to the extent of five per cent. with sulfuric acid, saturated with ammonium sulfate, powdered ferric ammonium alum added and the mixture allowed to stand two days. In this precipitation with ferric ammonium alum it was found that the precipitation was much facilitated by keeping the liquid at about 40°. The peptones are contained in the "Eisen niederschlag,"[2] which is dissolved readily and completely in water, the iron and sulfate removed by adding a saturated solution of barium hydroxide and the excess of the latter removed with ammonium carbonate solution. The colorless, neutral solution thus obtained was concentrated to about 10 cc. from a water-bath under diminished pressure at 42°. Absolute alcohol was added to the golden yellow liquid thus obtained, a white gelatinous precipitate resulting, which it was impossible to filter off on account of its tendency to run through the filter. However, upon adding more alcohol small granules began to form in the magma and upon shaking a few times the whole mass became finely granular. This was recrystallized three times in the same way, the total yield of the purified product being less than one gram. It gave a brown ring with the Hopkins-Cole reagent, to Millon's reagent it responded only very slightly, and it gave

[1] *Z. physiol. Chem.*, 35, 164.

[2] *Ibid.*, 38, 289; 35, 164.

no biuret reaction. This peptone shows a rotation in aqueous solution; $[\alpha]^{D}_{20} = +25.0$. A nitrogen determination by the Kjeldahl method gave 14.71 per cent. of nitrogen compared with 14.94 and 14.96 per cent. for the original silk and 14.88 and 14.89 per cent. for the residual silk after digestion.

Results and Conclusions.

1. It is possible to hydrolyze silk with trypsin.
2. Tyrosine is one of the products of this hydrolysis.
3. Tryptophan or some of its products were isolated from silk for the first time.
4. Tryptic peptones with a dextrorotation were obtained.

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THE ORIGIN OF CREATININE IN SOILS.[1]

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Creatinine has been found in soils. Its presence was demonstrated first by Dr. E. C. Shorey,[2] of this laboratory, who extracted it from various soils by means of dilute alkali. Subsequently, as shown in the present paper, it was found in the water and glycerol extracts of planted soils. The creatinine of the soils might have its origin (1) as a result of metabolic activity of microörganisms; (2) from stable manure introduced into the soil; (3) from the disintegration of plant debris and the direct passage from the living plant.

The particular phase of the question of the origin of creatinine in soils, which is considered here, is its presence in plants and consequently in plant debris and the passage of the creatinine into the soil either by the disintegration of the plant debris or as a result of cell sloughing or direct excretion from the living plant.

Creatinine, $C_4H_7ON_3$, is the anhydride of creatine, $C_4H_9O_2N_3$. Creatine is an ever present constituent of muscle and is found in addition in blood and urin. Creatinine is likewise found in muscle, blood and urin, particularly in the urin. The origin of these substances and their physiological relationship within the animal organism have long been under study.

Whatever may be the relationship between creatine and creatinine within the animal, it is certain that one can be converted into the other by appropriate means in the laboratory. Creatine, for example, goes to creatinine on boiling with acids, or, as shown by Folin and Denis,[3]

[1] Published by permission of the Secretary of Agriculture.

[2] *Sci.*, **33**, 340 (1910).

[3] *J. Biol. Chem.*, **8**, 399 (1910).