A COMPARATIVE STUDY OF THE METABOLISM OF PNEUMOCOCCUS, STREPTOCOCCUS, BACILLUS LACTIS ERYTHROGENES, AND BACILLUS ANTHRACOIDES.[1]

BY MARY LOUISE FOSTER.

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The most fascinating problem to the biochemist at present is the transition of the protein complex from its native state as albumin or globulin to its final state as amino acid. The study of this disintegration or cleavage is usually attacked from the sides of the nitrogenous excretion, but from Metchnikoff's investigations it is evident that the intestinal flora is largely concerned in this disintegration. These organisms are proteolytic and hence peptonize and reduce the protein material to its lowest terms. This paper represents some study on the proteolytic power of several organisms, the rate, the relation of different organisms to each other and the relation to each other of different strains of the same organism.

The method adopted was somewhat varied from Van Slyke's method as given in Abderhalden's "Lehrbuch der Biochemischen Arbeitsmethoden," Vol. V, 2, p. 1011. The native proteins were precipitated with hot trichloroacetic acid; the filtrate was precipitated with phosphotungstic acid, heated, dissolved and allowed to reprecipitate according to Van Slyke's method; the filtrate for the monoamino acids was also treated according to the same method. The total nitrogen and the nitrogen of the precipitation fractions were determined by Kjeldahl. Such a simplified method leaves much out of account, but my original plan was to open up a field which it was hoped might prove worthy of more intensive cultivation.

The first work was carried out on two strains, "T" and "H" of pneumococcus, grown on serum and given me by Dr. Augustus Wadsworth, of the College of Physicians and Surgeons, to whom I am deeply indebted. The serum used as the culture medium was one part serum and three parts water. The cultures were grown at two temperatures, 37° and 40°, at which points this organism is especially active in relation to the human system. Study of the table shows that the two strains "T" and "H" are very different in their power to split the protein molecule, strain "H" being by far the more active. Increase of temperature hastens the reaction and at 40° we find that the phosphotungstic acid fraction is more than doubled while the monoamino fraction is six times as great. Comparison with the control serum shows that proteolysis is progressive, the cleavage products apparently accumulating in the phosphotungstic acid fraction. This fraction contains no proteoses inasmuch as zinc sulfate

[1] Read by Dr. Alsberg at the meeting of the Biological Section, Milwaukee, March 28, 1913.

No.	Name of organism.	Length of incubation.	Medium.	Total N in grams in 100 cc.	Nitrogen by precipitation in 100 cc.				% of N in 100 cc.			Ratio of mono- to diamino acids.
					Native proteins.	Proteoses, peptones, diamino acids.	Mono-amino acids.	Total.	Native proteins.	Proteoses, peptones, diamino acids.	Mono-amino acids.	
1	.....	.....	serum	0.3162	0.3204	0.0056	0.0056	0.3316	96.10	1.67	1.67	1 : 1
2	Pnc. "T" @ 37°	.....	serum	0.3236	0.3152	0.0072	0.0040	0.3264	96.45	2.28	2.28	1 : 1.8
3	Pnc. "T" @ 40°	.....	serum	0.3206	0.3284	0.0100	0.0083	0.3367	94.66	2.92	3.03	1 : 1
4	Pnc. "H" @ 37°	.....	serum	0.2820	0.2612	0.0164	0.0052	0.2828	92.23	5.91	1.86	1 : 3
5	Pnc. "H" @ 40°	.....	serum	0.2864	0.2188	0.0432	0.0372	0.2980	73.45	14.06	12.44	1 : 1.1
6	.....	.....	milk	0.4386	0.3920	0.0164	0.0181	0.4265	91.9	3.8	4.2	1 : 0.91
7	Streptococcus	3 mos.	milk	0.4876	0.0510	0.2689	0.1821	0.5020	10.15	53.5	36.2	1 : 1.5
8	B. anthracoides	3 mos.	milk	0.4649	....	0.1904	0.0769	....	...	...	...	1 : 2.4
9	B. lactis erythrogenes	4 mos.	milk	0.5884	0.0511	0.3409	0.1990	0.5910	8.6	58.4	33.7	1 : 1.7
10	B. lactis erythrogenes	10 mos.	milk	0.5567	0.0299	0.1893	0.3188	0.5326	5.6	35.5	58.9	1 : 0.6
11	B. lactis erythrogenes	10 mos.	milk	0.5447	0	0.1991	0.3362	0.5352	...	37.5	62.5	1 : 0.6

and 7% tannic acid gave no precipitate. The relation of peptone to diamino acid was not determined. In the light of recent investigation into the toxic nature of peptone, this relation assumes new importance.

Similar experiments with other organisms show that this cleavage of the native protein is progressive, requiring some time, even months. Three organisms, *streptococcus*, *bacillus lactis erythrogenes* and *bacillus anthracoides* were studied. Milk was selected for the culture medium, sterilized five times, inoculated and grown for varying periods at 37° C. In another place[1] I have set forth the comparative results of *bacillus lactis erythrogenes* during varying periods of incubation. All of these flasks contained liquids which were drawn off by pipette for analysis after they had been proved to contain pure cultures. The liquids containing *streptococcus* (7) and *bacillus anthracoides* (8) gave alkaline reactions to litmus, were reddish and viscid and had a very strong glue-like odor. The liquid containing *bacillus lactis erythrogenes* was likewise alkaline to litmus, but was a clear, rich red with a slight granular precipitate on the bottom of the flask and it, too, gave the glue-like odor. The reaction of these organisms with milk is entirely different from that of *bacillus lactici acidi*, which sours milk by the production of lactic acid from lactose and thus precipitates insoluble casein. The enzyme in that instance seems of the nature of pepsin or rennet. In the case of Nos. 7, 8 and 9, no positive test for lactic acid could be obtained, indicating that the immediate attack was upon the native protein, which was broken down with the production of an alkaline reaction. Some coagulum was formed, but this rapidly disappeared, the rate apparently varying with the strain.

Study of Nos. 6, 7, 8, 9 and 10 shows how complete and how rapid is this disintegration. The ratio of the amino acids varies widely but in every instance it is very far from the normal as illustrated by the control milk, Number 6.

Number 11 is interesting because the result, obtained by an entirely different method, nevertheless corroborates the somewhat extraordinary figures obtained in Number 10. In the latter the usual method by immediate precipitation was used; in the former, the inoculated milk was heated with hydrochloric acid in a flask with a reflux condenser for twenty hours and then precipitated with phosphotungstic acid. By this method the native proteins are hydrolyzed and distributed between the diamino acids and the monoamino acids. It would seem from these results that after the breaking down of the native protein had reached a certain point, cleavage on the second fraction began, with rapid increase in the amount of monoamino acids.

The results show some qualitative discrepancies, but the difficulty of obtaining material and the length of time required for the reaction to be

[1] THIS JOURNAL, **35**, 597 (1913).

complete, make immediate duplication impossible. The results, however, are close enough to show that there probably exist certain chemical similarities in organisms which morphologically are widely different. It seems not impossible that study of bacterial action from the side of chemistry may lead to a different conception and a truer understanding of the nature of the virulence and the toxicity of microörganisms.

SMITH COLLEGE, NORTHAMPTON, MASS.

[FROM THE LABORATORY OF AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN.]

VOLATILITY OF LACTIC ACID.

BY E. B. HART AND J. J. WILLAMAN.

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In a recent article by Dox and Neidig[1] our methods for the determination of the volatil acidity of silage were criticized on three different grounds. First, because when distilling in steam at ordinary pressure a quantitative removal of the volatil acids is not possible, because even after eight liters of distillate are collected the distillate is not neutral. Second, because lactic acid keeps coming over in appreciable quantities, and is titrated as volatil acid. Third, because this lactic acid, when distilled according to Duclaux' method for estimating the volatil fatty acids, produces a curve very comparable to formic acid, and the figures for formic acid are thus always raised to the extent of the lactic acid that comes over. They reported experimental data to support their criticisms, of which the following are the most important:

"Lactic acid, equivalent to 178.75 cc. 0.1 *N* alkali, was distilled from a liter flask in a current of steam at ordinary pressure. Eight liters of distillate were collected and titrated separately.

Distillate.	Cc. 0.1 *N* $Ba(OH)_2$.
1st liter	4.6
2nd liter	3.6
3rd liter	3.6
4th liter	3.2
5th liter	3.2
6th liter	2.8
7th liter	2.8
8th liter	2.4

"The lactic acid that passes over then appears in the Duclaux calculations as formic acid. When 110 cc. of lactic acid equivalent to 39.4 cc. of 0.1 *N* barium hydroxide were subjected to the Duclaux fractionation, 5.8% of this acid passed over in the 100 cc. of distillate. From the titration figures we calculated the Duclaux constants for lactic acid,

[1] THIS JOURNAL, 35, 90 (1913).