solution concentrated on the steam bath and finally allowed to cool. Dimethyluracil separated in the form of prismatic crystals, which melted at 296° when heated rapidly. If the acid bath was heated slowly the substance melted at 292–294° with decomposition. A mixture of this compound with 4,5-dimethyluracil[1] melted at exactly the same temperature.

NEW HAVEN, CONN.

A PRELIMINARY STUDY OF THE BIOCHEMICAL ACTIVITY OF BACILLUS LACTIS ERYTHROGENES.

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Bacillus lactis erythrogenes belongs to the group of pigment-forming bacteria which diffuse coloring matter through the culture medium. This organism was first described by Grotenfelt and later by Hueppe as a "non-motile rod, 0.3–0.5 × 1–1.4. Sporeless. Colonies round, gray-yellow to pure yellow, slowly liquefying with rosy coloration the surrounding gelatin........ Milk is coagulated by lab. ferment and peptonized. Nauseating odor. The red pigment is insoluble."[2] This organism, found occasionally in dairies, is non-pathogenic to man. The object of this study was to determin the compositions of the fluid from time to time and to follow in this way the course of the catabolism; to find out the specific cause of this change; and to study the nature of the pigment.

A flask of steril milk, inoculated with this organism, shows, after a few days, a faint blush which gradually intensifies in hue until the entire liquid is a deep blood red. At the same time the milk coagulates. Later it liquefies, lumps of solid material appear on the surface while at the bottom of the flask is a thick viscous mass. This gradually diminishes in bulk and a granular precipitate settles to the bottom with a clear red supernatant liquid. There is a strong disagreeable odor of glue. These phenomena take place at room temperature—about 20°—and require from two to six months for completion.

The flask, before any chemical analysis was made, was examined bacterially and was found to contain a pure culture of the organism. The liquid was alkalin to litmus and gave a negative result for *lactic acid* by Uffelmann's test; a faint trace of *formic acid* with mercuric oxide; a strongly positive Molisch test indicating the presence of *carbohydrate*, which was identified as lactose by the phenylhydrazine test. At another time in another flask, some glucose was found to be present. While this second flask contained the *bacillus lactis erythrogenes*, it was a different

[1] Schlenker, Wheeler and Merriam, *Loc. cit.*

[2] Flügge, Vol. II, page 305.

strain and differed, in the amount of pigment produced, from the organism used earlier. It was an older, less active organism and apparently attacked the carbohydrate to some extent. However, it is evident that the *bacillus lactis erythrogenes* does not ferment the carbohydrate primarily. A rose-pink coloration with the biuret test indicated that the lactalbumin and globulin had been changed to proteoses and peptones; Millon's reagent gave a strongly positive test for tyrosine which was also obtained later as di-benzoyltyrosine; a strong xanthoproteic test showed the formation of nitro compounds; a negative Hopkins test showed absence of all tryptophane; a heavy precipitate with phosphotungstic acid gave abundant evidence of the presence of proteoses, peptones and diamino acids. These tests show that the milk is peptonized.

In order to determin the extent of the peptonization and the distribution of the cleavage products of the protein molecule, some quantitative experiments were undertaken. The Kjeldahl method was used to estimate the nitrogen. Inasmuch as the cleavage of the protein molecule is progressive, native protein, *i. e.*, albumin and globulin—changing gradually to proteoses, peptones, diamino and monoamino acids, the analysis of the metabolized material was to determin the *approximate* amount of each one of these present. The method employed is not strictly quantitative since a small amount of the phosphotungstic acid precipitate is soluble. A measured portion of the metabolized milk was completely precipitated with hot trichloracetic acid and filtered. The precipitate represents the native proteins present, the amount of which, as nitrogen, was determined. The filtrate was then treated with a solution of 40% phosphotungstic acid and 7% sulfuric acid until no more precipitate formed, filtered and the precipitate washed with acidulated water. The precipitate represents the proteoses, peptones and diamino acids, the amount of which, as nitrogen, was determined. The filtrate contains the monoamino acids. It was concentrated and the nitrogen determined here also. The following table gives the results of these analyses:

				Gr. of N in 100 cc.				Per cent. of N as			
	No.	Length of incubation.	Total N in gr. in 100 cc.	Native proteins.	Proteoses, peptones, diamino acids.	Monoamino acids.	Total.	Native proteins.	Proteoses, peptones, diamino acids.	Monoamino acids.	Ratio of mono to diamino acid.
	1 *a*	Milk	0.4386	0.3920	0.0164	0.0180	0.4265	91.9	3.8	4.3	1 : 0.9
R	2 *a*	3 mos.	....	0.1057	0.1907	0.2113	0.5077	20.8	37.6	41.6	1 : 0.9
R	*b*	4 mos.	0.5884	0.0511	0.3407	0.1990	0.5910	8.6	58.4	33.7	1 : 1.7
R	*c*	6 mos.	....	0.0408	0.3965	0.1842	0.6215	6.5	63.7	29.9	1 : 2.1
T	3 *a*	1 mo.	0.5577	0.3799	0.0852	0.0632	0.5283	71.9	16.0	12.0	1 : 1.3
T	*b*	10 mos.	0.5567	0.0299	0.1839	0.3188	0.5326	5.6	35.5	58.9	1 : 0.6
T	*c*	10 mos.	0.5447	0	0.199[illegible]	0.3362	0.5353	0	37.5	62.5	1 : 0.

Study of these tables leads to several conclusions. In the first place steril milk shows only a small proportion of changed protein, the amino acids amounting to 8.1%, about evenly distributed between the diamino and the monoamino acids. The cleavage of the native protein as illustrated by Nos. 2 and 3, is progressive and continuous; the longer the period of incubation the less the amount of native protein and the greater the amount of the other cleavage products. The ratio between the mono and diamino acids varies with the time and the strain of the organism. During short periods of six months or less, the amount of the phosphotungstic acid precipitate is in excess of the monoamino acid residue. Examination of 3*b* and 3*c* reveals a marked change. These two results were obtained from the same flask, *i. e.*, the same material, by different methods and may therefore act as checks to each other. In 3*b*, the cleavage products were obtained by immediate precipitation, while in 3*c* the material was hydrolyzed with 20% hydrochloric acid and boiled for several hours with a reflux condenser and then precipitated with phosphotungstic acid according to Van Slyke's method as given in *Biochemische Arbeits Methoden*, Vol. V, 2, page 1011. By this method, the native proteins are hydrolyzed and distributed between the diamino fraction and the monoamino fraction, largely to the benefit of the latter. In both analyses the ratio of the diamino fraction to the monoamino fraction is the reverse of the earlier experiments. This is unexpected and needs further investigation. In these analyses no attempt has been made to determin quantitatively the free or combined ammonia. These results are tentative and merely indicate the trend of the break down of the protein molecule through the agency of the metabolic processes of the organism or its enzyme. By the use of Van Slyke's method greater exactness in the ratio of the end cleavage products can be obtained but no knowledge about the native proteins. Perhaps a combination of the two can be effected.

I then undertook to find out whether an enzyme was present or not. A portion of the reddish milk was treated with alcohol and the yellowish white precipitate filtered off by suction. It was dried at room temperature. A few milligrams of this powder gave, in steril milk after three days in the incubator, a coagulation. The supernatant liquid was acid to litmus. Examination for organisms with methylene blue showed their absence. Several trials were made at different times, always with the same result. Even with the addition of hydrogen peroxide, coagulation took place and no evidence of the presence of any organism could be found. Chemical tests proved the presence of *acetic* and *formic* acids but no *lactic* acid. We have then associated with the organism a coagulative enzyme whose cleavage products, acetic and formic acids, are neutralized by the ammonia set free from the protein through the agency of the

organism. This would account for the difference in litmus test between the solution with the organism and the solution with the enzyme.

The experiments with strain "R" were made with an organism in excellent condition. It is well known that these chromogens form the pigment only under optimum conditions. This strain produced a red pigment which increased in depth of color as the milk became more translucent, from a slight reddish tinge to a deep blood-red. This change required about four months for completion and was accompanied by the disappearance of the coagulated or viscous mass and the presence of some insoluble granular material. This is the outward appearance of the tabulated results *2b* and *2c*, those which most closely corroborate each other although obtained from different flasks at different times. The same strain of *bacillus lactis erythrogenes*, however, was used in both. I succeeded in 1910 in extracting this pigment with amyl alcohol and in precipitating it in crystallin form with acetone. The precipitate was soluble in hot glacial acetic acid. The crystals were red needles arranged in clusters. A few colorless ones were found. They gave a strong alkaloidal odor but their chemical composition was not determined.

The work this spring was performed with a strain from an organism isolated from milk some four years ago in the Mount Prospect laboratory. It has nearly lost its pigment producing power, a common occurrence with chromogens. The milk inoculated February 26, 1912, shows only a dull reddish brown coloration. The nauseating glue-like odor has not been very noticeable. Is one the cause of the other? I have not yet the material at hand to determin. It is evident that rapid proteolytic activity and production of concentrated pigment accompany each other.

I have found that the action of *bacillus lactis erythrogenes* on milk is progressively catabolic; the native proteins are split with the ultimate formation of monoamino and diamino acids. This proteolytic change may be caused by an enzyme. A soluble ferment which was precipitated with alcohol split the carbohydrate with the production of formic and acetic acids. This would seem to indicate the presence of an intracellular enzyme which has been set free by the alcohol when it has destroyed the organism.

Accompanying these changes is the production of a pigment which causes a coloration varying from red to dull brown, according to the strain. This pigment can be extracted with amyl alcohol and is extracellular for it is contingent upon the life of the organism.

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