

pears as a rule to be synonymous with increased ability to reduce nitrates and to ammonify peptone.

In the present paper we have dealt with the reactions produced by pure cultures of bacteria, it being necessary that these elementary reactions should be thoroughly understood before we attempt the study of mixed cultures, with the complicated series of secondary reactions which may occur between the reduction products of the different species. A practical application of the quantitative tests for the biochemical functions of various species combined with the numbers of bacteria of those species present in water and sewage has already been made, and the field is one which promises to bear fruit in the near future in a better understanding of the biological processes occurring in sewage treatment and in the more scientific control of the conditions which govern those processes.

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## ON THE SPECIFIC ROTATION OF SALTS OF CASEIN.

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IN THE literature there are a few references to the optical rotation of "casein" in solutions of weak alkalies, acids or neutral salts, but most of them refer to the old work of Hoppe-Seyler on products of doubtful purity,<sup>1</sup> or to later work by Béchamp with mixtures of somewhat uncertain composition.

Casein, as the term is now understood, is a nuclealbumin of marked acid character, which exists in milk in the form of a calcium salt mixed or combined with phosphate. This combination may be easily broken up by addition of weak acids, especially acetic, yielding the pure substance, which seems to be quite insoluble in water, but readily soluble in alkali solutions with formation of acid or neutral salts. Like many of the proteins, this casein holds certain groups which impart to it a basic character by reason of which it combines, to a certain extent, with weak acids, forming another class of salts, but in this short paper the alkali combinations only are considered. Some of these alkali combinations have some commercial importance; nutrose and plasmon are essentially impure sodium salts.

<sup>1</sup> For literature see "Optical Rotation of Organic Substances," Landolt, Long's translation, p. 728, and Hoppe-Seyler's *Handbuch der Phys. und Path. Chem. Anal.*, 6th ed., p. 259.

In the preparation of the casein employed in the present work about 5 liters of well skimmed milk were treated according to the general Hammarsten method. This milk had been run twice through the centrifugal separation process, the second run being made at high speed, especially for my work; but a minute trace of fat was left. To obtain the casein the milk was diluted with four or five volumes of water and treated with just enough dilute acetic acid to produce the maximum precipitation. The precipitate was allowed to settle and was then washed repeatedly by decantation with distilled water and finally dissolved in the requisite amount of weak sodium hydroxide, using phenolphthalein to show neutrality. This salt was again decomposed by weak acetic acid as before, and all the operations repeated several times. Finally the moist precipitate from the last acid treatment was drained on a Buchner funnel, washed with strong alcohol and with 2 liters of ether to remove the last traces of fat. The residue soon dried to a fine white, very light powder, which, after standing a week over strong sulphuric acid, was ready for use. It still held about 4 per cent. of water, which was determined by drying a small portion in the air-oven at  $105^{\circ}$ - $110^{\circ}$ . It contained about 0.6 per cent. of ash, as found by slow incineration at the lowest possible temperature.

To dissolve 5 grams (5.2 grams as used) of the powder 45 cc. of  $N/10$  alkali are required to produce a solution neutral to phenolphthalein, but the 5 grams will dissolve completely in just one-half this alkali, or 22.5 cc., yielding when diluted to 100 cc. a practically clear liquid, which may be easily polarized in a 100 mm. tube. There is, in all cases, a slight opalescence, which makes it necessary to employ a brighter field than is usually required. A good light must therefore be employed. It is also necessary to work with a rather large half-shadow angle, which diminishes somewhat the delicacy of the reading. The following individual determinations were made, all at  $20^{\circ}$ .

*Sodium Compound.*—Five grams of casein + 45 cc. of  $N/10$  sodium hydroxide made up with water to 100 cc. The observed angle of rotation,  $\alpha$ , in the 100 mm. tube was  $-5.175^{\circ}$ , from which we have, based on the casein weight,

$$(\alpha)_D = -103.5^{\circ}.$$

This is the product as made neutral to phenolphthalein. As mentioned above, a clear solution may be obtained with half

the alkali here used. For the salt formed with 5 grams of casein and 22.5 cc. of N/10 sodium hydroxide in 100 cc. I found  $\alpha = -4.76^\circ$  from which

$$(\alpha)_D = -95.2^\circ.$$

The effect of presence of increased amounts of alkali was found by making solutions with 67.5 of N/10 alkali and 45 cc. of N/5 alkali and water to 100 cc., for the first of these I found  $\alpha = -5.38^\circ$ , or

$$(\alpha)_D = -107.6^\circ,$$

and for the second  $\alpha = -5.59^\circ$ , or

$$(\alpha)_D = -111.8^\circ.$$

This alkali may all be present in salt form, as will be pointed out in a paper to follow in which observations in another direction will be recorded.

*Potassium Compound.*—One solution was examined. This contained 5 grams of casein and 45 cc. of N/10 alkali with water to 100 cc. for the angle of rotation I found  $\alpha = -5.22^\circ$ , or

$$(\alpha)_D = -104.4^\circ.$$

*Lithium Compound.*—To prepare this I made first pure lithium carbonate from the best commercial carbonate. This contained neither sulphate nor chloride. A few grams of the thoroughly washed preparation were suspended in water and converted into the more soluble bicarbonate by a stream of carbon dioxide. The normal carbonate precipitated from this by heat was made up to a solution approximately N/10 and used to dissolve casein as were the hydroxides above. The first salt was made by dissolving 5 grams of casein in the equivalent of 22.5 cc. of N/10 LiOH solution. For this I found  $\alpha = -4.74^\circ$ , with

$$(\alpha)_D = -94.8^\circ.$$

The solution was acid to phenolphthalein. A solution, neutral to the indicator, was made by using just twice as much lithium carbonate. For this I found  $\alpha = -5.04$ , or

$$(\alpha)_D = -100.8^\circ.$$

*Ammonium Compound.*—An ammonium salt was made with 45 cc. of N/10  $\text{NH}_4\text{OH}$ , 5 grams of casein and water to 100 cc. The polarization gave  $\alpha = -4.89^\circ$ , or

$$(\alpha)_D = -97.8.$$

In all these cases the specific rotation, based on the casein

present, is increased with increase of alkali. The values obtained are higher than the few given in the literature, but may be easily duplicated by other preparations from cows' milk. According to Béchamp<sup>1</sup> casein is slightly soluble in water; from such a solution he obtained a rotation  $(\alpha)_D = -117.7^\circ$ , which is equivalent to about  $(\alpha)_D = -105^\circ$ . This is practically the same as for the salts, as above described. The property is a constant, which may be utilized in the comparison of milks of different origin. Some experiments with human milk and the milk of the goat are now in progress.

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## THE EFFECT OF RUST ON THE STRAW AND GRAIN OF WHEAT.

BY FRANK T. SHUTT.

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THE prevalence of rust this season in certain districts of Manitoba led to many inquiries regarding the general effect of this fungus upon the wheat plant—both straw and grain—and more particularly as to how it might influence their feeding value. The importance of the subject made it desirable to obtain data on these points and accordingly samples of both rusted and rust-free wheat were obtained and analyzed.<sup>2</sup>

In order that the results should be strictly comparable, it was essential in procuring these samples that the clean and the affected wheat be of the same age and grown on the same soil. We were able to secure such specimens, both wheats being collected by hand on the same day in the same field on the farm of Sir Wm. Van Horne, at East Selkirk, Manitoba.

There was a marked difference in appearance between them, both in straw and grain. The rust-free wheat had a clear, bright yellow, a well-ripened straw, a normal ear, both as to size and color, and a plump, well-filled grain. On the other hand, the rusted wheat straw presented in general a dirty greenish brown appearance, and on closer inspection showed many spots or

<sup>1</sup> *Bull. Soc. chim.* [3], 4, 18; 11, p. 152. *Ber. d. chem. Ges.*, 1890, III, 741.

<sup>2</sup> Mention should be made that certain analyses of "rusted and frosted" wheat (grain) were made at the Minnesota Experiment Station in 1889, by Dr. Harper. From the results Dr. Harper concluded that the grain of such wheat was abnormal, both chemically and physically, and was about 2 per cent. richer in protein than "graded" wheat.