plants, and that the great variations, recorded in this paper, were partly due to the peculiar soil conditions prevailing on the Station farm.

This investigation has been continued since 1901, and is still being continued.

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[CONTRIBUTION FROM THE NEW YORK AGRICULTURAL EXPERIMENT STATION.]

RENNET ENZYME AS A CAUSE OF CHEMICAL CHANGES IN THE PROTEIDS OF MILK AND CHEESE.

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INTRODUCTION.

THE object of the work described in this article was, primarily, to ascertain to what extent the proteolytic phenomena, observed in cheese-ripening, are due to the action of an enzyme contained in the rennet extract used in cheese-making. It was also our purpose to learn how the proteolytic action of rennet compares with that of commercial pepsin.

It has been quite generally believed that the rennet extracts used in the manufacture of cheese contain not less than two enzymes or ferments, called rennin and pepsin, one ferment coagulating milk-casein and the other converting milk-casein and paracasein, under favorable conditions, into soluble forms of nitrogen compounds. The present tendency, however, is in the direction of the belief that both kinds of action are due to the presence of only one enzyme. The presence of a proteolytic ferment in rennet extract is readily understood, when we consider its source, which is the stomach of a suckling calf.

For years the weight of opinion was against the belief that rennet has any other function in cheese-making than simply to coagulate milk-casein. In some work[1] done by one of us in 1892, it was shown that cheese, made with larger amounts of rennet, furnished greater quantities of soluble nitrogen com-

[1] New York Agr. Expt. Sta. Bull. No. 54, p. 267 (1893).

pounds than did cheese made with smaller amounts of rennet. In 1899, some additional work[1] was done, confirming the results previously obtained. Babcock, Russell and Vivian[2] have made a very thorough investigation of this subject, showing that, in the case of normal cheese, increased use of rennet resulted in a more rapid increase of soluble nitrogen compounds, especially of those nitrogen compounds grouped under the names of caseoses and peptones. They also made cheese from milk to which purified commercial pepsin had been added and found similar chemical changes taking place in the cheese thus made. They concluded, from these experiments with normal cheese, that rennet exerts a digestive influence on casein, due to the presence of peptic enzymes contained in rennet extracts, the action of which is intensified by the development of acid in the cheese-curd. Jensen,[3] working independently and along quite different lines, reached the same conclusions at the same time.

In the case of the work previously done here and elsewhere, the effect of rennet ferment has not been studied apart from the action of other factors that are present in normal cheese-ripening. So far as our present knowledge goes, the different agencies taking part in the normal process of cheese-ripening are the following: (1) Some acid, usually lactic; (2) enzymes present in the milk before it is made into cheese; (3) an enzyme contained in the rennet extract added to milk in the cheese-making process; and (4) micro-organisms, chiefly bacteria. In previous studies of the effect of rennet ferment on cheese-ripening some or all of these factors have been present, so that the specific action of rennet has had to be inferred rather than been clearly proved. It has been the special aim of our work to study the action of the rennet ferment as far as possible apart from the other agencies of cheese-ripening. Under these conditions, we have studied the action of rennet extracts in cheese-ripening: (1) Without acid, (2) in the presence of acid, (3) without salt, and (4) with salt. In addition, we have studied the action of rennet extracts of different ages upon the casein of milk, and also the proteolytic action of commercial pepsin on milk-casein and in the process of cheese-ripening. We have also studied the action of rennet enzyme and pepsin on paracasein dilactate.

[1] New York Agr. Expt. Sta. Bull., No. 236, p. 150 (1903).
[2] Annual Report, Wis. Expt. Sta., 17, 102 (1900).
[3] *Landw. Jahrb. d. Schweiz.*, 14, 197 (1900).

DESCRIPTION OF EXPERIMENTAL WORK.

DIFFICULTIES INVOLVED IN THE WORK.

In order to destroy all enzymes present in milk, our general plan of procedure has been to heat the milk to a temperature varying in different cases from 85° C. to 98° C. (185° F. to 208° F.). Then, in order to prevent possible contamination by the entrance of enzyme-producing organisms, the milk, after being heated and cooled, has been treated with 3 to 5 per cent. of chloroform by volume, previous to being made into cheese. The heating of milk to the temperature stated diminishes the readiness and completeness with which it is coagulated by rennet extract, but the power of prompt coagulation by rennet can be restored by the addition of calcium chloride or carbon dioxide or any ordinary acid. In thus eliminating other factors of cheese-ripening than rennet enzyme, we necessarily produce conditions that do not exist in normal cheese-making, such as (1) heated milk, (2) absence of milk-enzymes, (3) the use of calcium chloride or carbon dioxide, and (4) absence of enzyme-forming and acid-forming organisms. In a study carried on under such conditions, we cannot expect our results to be entirely comparable with results obtained under normal conditions; but we can secure data that enable us to determine the ability of the rennet enzyme to cause proteolytic changes under the conditions of experiment employed. Later we will inquire as to whether the introduction of such unusual conditions seriously affected the value of the results obtained, in their application to the process of normal cheese-ripening.

EFFECT OF ACID UPON THE PROTEOLYTIC ACTION OF RENNET ENZYME.

Table I contains the averages of results given by four sets of experiments, in which the effect of the presence and absence of acid upon the action of rennet enzyme in cheese-ripening was studied.

TABLE I.—EFFECT OF PRESENCE AND ABSENCE OF ACID UPON THE ACTION OF RENNET ENZYMES.

		Nitrogen, expressed as percentage of nitrogen in cheese, in form of			
Acid.	Age of cheese when analyzed.	Water-soluble nitrogen compounds. Per cent.	Paracasein monolactate. Per cent.	Paranuclein caseoses and peptones. Per cent.	Amides. Per cent.
Present	Fresh	4.55	26.80	3.78	0.77
"	12 mos.	25.85	11.59	20.87	4.98
Absent	Fresh	3.13	1.88	2.73	0.80
"	12 mos.	4.23	1.68	2.26	3.96

A comparison of the data embodied in this table shows a relatively large formation of water-soluble nitrogen compounds in twelve months in the presence of acid, while in the absence of acid practically no proteolysis had occurred. Paracasein monolactate was present in only minute quantities, if at all, in the absence of acid, while a considerable amount was formed in the presence of acid. The increase of soluble nitrogen compounds was confined largely to the paranuclein, caseoses and peptones, the amount of amides remaining small. In normal cheese-ripening, we find these relations reversed, that is, the amides form a considerably larger part of the soluble nitrogen compounds than do the higher groups.

The results embodied in Table I may properly be interpreted as showing that the proteolytic action of the rennet enzyme, in cheese-ripening, is dependent upon the presence of acid.

EFFECT OF RENNET ENZYMES IN CHEESE CONTAINING ACID-FORMING AND SOME PROTEOLYTIC ORGANISMS.

For the purpose of comparison, it was desired to have some cheeses made from milk pasteurized at 85° C. (185° F.). As factors active in causing proteolytic changes, we had in the cheeses made in these experiments (1) acid, (2) rennet enzyme, and (3) such micro-organisms as happened to be introduced with the "starter" and from the air of the room. As compared with a normal cheese, there were no milk enzymes present and the biological factor would be expected to be considerably less marked. In comparison with the cheeses referred to in Table I, we had in these no chloroform, a difference that meant absence of a biological factor in the former case. In these cheeses, the acid was furnished by a sour-milk "starter." In Table II, we give the results of chemical analysis made when the cheese was fresh from the press and when nine months old.

TABLE II.—SHOWING COMPOSITION OF CHEESE MADE FROM PASTEURIZED MILK.

Age of cheese when analyzed.	Nitrogen, expressed in percentage of nitrogen in cheese, in form of				
	Water-soluble nitrogen compounds. Per cent.	Para-casein mono-lactate. Per cent.	Paranuclein, caseoses and peptones. Per cent.	Amides. Per cent.	Ammonia. Per cent.
Fresh	3.13	11.36	3.13	0	0
9 months	25.54	5.14	12.79	12.75	1.31

In studying these results, we notice that there was an increase in all the different classes of water-soluble compounds during the period of experiment. The amount of amido compounds was considerably in excess of that noticed in Table I in the case of cheeses made and kept in the presence of chloroform. Ammonia was formed, while none was present in Table I. The increased amounts of amido compounds and the presence of ammonia, observed in these experiments, as compared with the results of the experiments given in Table I, must be ascribed to the presence in the former of a biological factor and not to rennet enzyme.

COMPARISON OF THE EFFECT OF COMMERCIAL PEPSIN WITH THAT OF RENNET ENZYME IN CHEESE-RIPENING.

In the following experiments, the cheeses were made in the normal way, without chloroform, except that the milk was pasteurized at 85° C. (185° F.) and hydrochloric acid was used in the place of lactic acid or a "starter." In 55, rennet extract alone was used at the usual rate of 2.5 ounces for 1000 pounds of milk. In 56, in addition to rennet extract, we added 1 gram of Parke, Davis & Co.'s aseptic scale pepsin dissolved in water, and in 57, we used 15 grams of the pepsin and the usual amount of rennet extract.

TABLE III.—SHOWING EFFECT OF COMMERCIAL PEPSIN IN CHEESE-RIPENING.

No. of experiment.	Age of cheese when analyzed.	Enzymes added.	Nitrogen, expressed as percentage of nitrogen in cheese, in form of: Water-soluble nitrogen compounds. Per cent.	Paracasein monolactate. Per cent.	Paranuclein, caseoses and peptones. Per cent.	Amides. Per cent.	Ammonia. Per cent.
55	Fresh	Rennet extract	4.76	65.45	2.41	2.36	0.0
55	6 mos.	Rennet extract	28.37	17.14	15.87	6.35	2.00
56	Fresh	Rennet and 1 gram pepsin	6.97	36.76	4.11	2.86	0.0
56	6 mos.	Rennet and 1 gram pepsin	29.80	17.04	16.47	7.10	1.91
57	Fresh	Rennet and 15 grams pepsin	25.00	59.53	22.80	2.20	0.0
57	3 mos.	Rennet and 15 grams pepsin	46.67	11.61	41.00	5.68	0.49

In studying the results contained in Table III, we notice:

(1) The use of 1 gram of commercial pepsin in addition to rennet extract slightly increased the proteolytic results in the cheese. This cheese contained considerably less moisture than 55 or 57.

(2) The use of 15 grams of commercial pepsin along with rennet extract produced very marked results. This is strikingly evident in the fresh cheese, where we have 25 per cent. of the nitrogen in the cheese present in the form of water-soluble compounds, while in the case of Experiment 55, in which rennet extract only was used, the amount of soluble nitrogen compounds is less than 5 per cent. At the end of three months, we still have much more of the soluble nitrogen compounds in 57, the pepsin cheese, than we have in 55, the rennet extract cheese, at the end of six months.

(3) In comparing the proteolytic factors in Experiments 55 and 57, the conditions of work were such that the chief essential difference was the presence of pepsin in the latter, though 57 contained more moisture than 55. The observed difference in the chemical results could, therefore, be due only to pepsin, and this would be particularly true of the results obtained in the fresh cheese.

COMPARISON OF THE EFFECT OF RENNET ENZYME AND OF COMMERCIAL PEPSIN IN MILK, WITH AND WITHOUT ACID.

We made a comparative study of the effect of rennet enzyme and of commercial pepsin upon milk-casein and upon casein monolactate. These experiments were carried out in the following manner: We heated 8.6 liters of milk for fifteen minutes at 85° C. (185° F.), and after cooling added 2 per cent. of chloroform by volume. Of this milk, we placed in each of several bottles 100 cc. In one case, we added, to the neutral milk, 0.22 cc. of Hansen's fresh rennet extract, and in another the same amount of old rennet extract. In other bottles, we added, in addition to the rennet extract, 0.5 cc. of pure concentrated lactic acid, which was sufficient to convert the milk-casein into the monolactate. For comparison, we placed in other bottles, with and without acid, the same amount of milk and 0.06 gram of Parke, Davis & Co.'s aseptic scale pepsin for each 7 grams of proteid contained in the milk. Duplicates were used in all cases. The

contents of these bottles were kept at 15.5° C. (60° F.) and were examined at intervals, both chemically and bacteriologically. With the exception of a single determination, in the case of one bottle, the germ content was below 50 per cubic centimeter, which undoubtedly represented spore forms. The old rennet extract was used for the purpose of answering the question as to whether the proteolytic changes, observed in Table I, were due to rennet enzyme alone, or whether the rennet may not have contained some proteolytic bacterial enzymes produced in the rennet extract previous to its use.

The results of chemical analysis are given in the subjoined table. The determinations of nitrogen in the form of amides were made by the use of phosphotungstic acid, since it has been shown[1] that, in the case of peptic digestion, phosphotungstic acid is a more satisfactory reagent than tannic acid, especially in solutions having an acid reaction. The amount of nitrogen originally in the milk was 0.561 per cent.

TABLE IV.—SHOWING THE EFFECT OF RENNET ENZYME AND COMMERCIAL PEPSIN UPON MILK-CASEIN AND CASEIN MONOLACTATE.

Kind of enzyme used.	With or without lactic acid.	Age of milk when analyzed.	Nitrogen, expressed as percentage of nitrogen in milk, in form of		
			Soluble nitrogen compounds. Per cent.	Caseoses and peptones. Per cent.	Amides. Per cent.
.....	.....	Fresh	9.98	....	....
Fresh rennet	Without	1 mo.	11.35	6.00	5.35
" "	With	1 "	29.80	22.22	7.58
Old rennet	Without	1 "	12.57	4.99	7.58
" "	With	1 "	25.23	18.55	6.68
Commercial pepsin	Without	1 "	8.91	2.22	6.69
" "	With	1 "	33.51	25.93	7.58
Fresh rennet	Without	9 mos.	18.98	13.63	5.35
" "	With	9 "	53.57	45.64	7.93
Old rennet	Without	9 "	17.03	12.13	4.90
" "	With	9 "	47.96	39.67	8.29
Commercial pepsin	Without	9 "	10.08	6.51	3.57
" "	With	9 "	56.96	48.05	8.91

The data embodied in Table IV appear to be quite definite in respect to the following points:

(1) The increased activity of rennet extract as well as of pepsin in the presence of acid is very marked. Expressed in another

[1] New York Agr. Expt. Sta. Bull. No. 215, pp. 90 and 98 (1900).

way, these enzymes act upon casein monolactate much more extensively than upon milk-casein.

(2) If we compare the results secured by the use of the purest commercial pepsin with those given by the rennet extracts, we find that, in the presence of acid, there are formed soluble nitrogen compounds quite close in amount to those formed by rennet extract. The amount of soluble nitrogen compounds formed in neutral solution was fairly stationary during the nine months, while, in the case of the rennet extracts, there was a slow increase. The amount of amido compounds was surprisingly uniform in the case of the pepsin and the rennet extracts, in both neutral and acid reaction.

(3) At any given time, the fresh rennet extract had, in most cases, formed a larger amount of soluble nitrogen compounds than had the old extract. This was particularly true in acid solution. This result does not indicate that we had bacterial enzymes in the old rennet in addition to rennet enzyme. The difference in action of the two rennet extracts is not marked in the class of amido compounds. If the old extract contained bacterial enzymes, we should expect it to produce larger amounts of amido compounds. These results fail to show that the old rennet extract contained any proteolytic bacterial enzymes, as compared with the fresh extract. Moreover, the results given by pepsin suggest that the pepsin was able to account for all the changes observed in the case of the rennet extracts in the presence of acid.

COMPARISON OF THE EFFECT OF RENNET ENZYME AND OF COMMERCIAL PEPSIN ON PARACASEIN DILACTATE.

Paracasein monolactate was extracted from several pounds of cheese by a 10 per cent. solution of sodium chloride and this was treated with acid, precipitating paracasein dilactate. Of this compound washed free from salt, we placed 25 grams, suspended in water, in each of several flasks and sterilized by heat. We then sterilized some solution of pepsin and rennet extract by treating with 0.5 per cent. of formalin, containing 0.2 per cent. of formaldehyde. According to Bliss and Novy,[1] pepsin is not affected by a 1 per cent. solution of formaldehyde nor rennet by a 4 per cent. solution. In one set of flasks, we added to each

[1] *Jour. Expt. Med.*, **4**, No. 1 (1899).

0.06 gram of the sterilized pepsin, and in each of the other set of flasks 0.5 cc. of the sterilized rennet extract. Duplicates were used in all cases. These were examined bacteriologically and chemically, at intervals for three months. The formalin was very effective in destroying bacterial forms. In some cases a few molds were found, but not in sufficient number to affect the work. The nitrogen in the material was 4.35 per cent.

TABLE V.—SHOWING EFFECT OF RENNET ENZYME AND COMMERCIAL PEPSIN ON PARACASEIN DILACTATE.

		Nitrogen, expressed as percentage of nitrogen in mixture, in form of				
Enzymes used.	Age when analyzed.	Water-soluble nitrogen compounds Per cent.	Para-casein mono-lactate. Per cent.	Para-nuclein caseoses and peptones. Per cent.	Amides. Per cent.	Am-monia. Per cent.
Pepsin	2 weeks	33.68	2.30	....	....	0
Rennet	"	34.95	2.30	....	....	0
Pepsin	1 mo.	41.61	...	37.87	3.74	0
Rennet	"	43.68	...	40.00	3.68	0
Pepsin	3 "	55.75	...	46.55	9.20	0
Rennet	"	57.25	...	49.53	7.72	0

From the data contained in Table V, we can see that the results of our work indicate that:

(1) Both pepsin and rennet enzyme exerted a marked proteolytic effect upon the paracasein dilactate, digesting about one-third of it in two weeks and considerably over one-half in three months. While the rennet enzyme appears somewhat more active in forming water-soluble nitrogen compounds, the actual difference is small.

(2) Both enzymes formed amides in small quantities, but neither produced any ammonia.

(3) If we compare the results in Table V with those in Table III, we find that more proteolysis occurred in this experiment than in the presence of chloroform. This is true of both enzymes. This suggests that the chloroform may exert a retarding influence upon the action of pepsin and rennet. Malfitano[1] makes the statement that the action of pepsin is considerably diminished by chloroform. The difference noted in our work may be due to the greater amount of acid present in the experiment in Table V. However, both sets of experiments practically agree in showing small formation of amides and entire absence of ammonia.

[1] *Ann. Inst. Pasteur*, **16**, 853 (1902).

EFFECT OF COMMON SALT ON THE ACTION OF RENNET ENZYME IN CHEESE-RIPENING.

Previous work[1] shows that salt exerts a marked repressing influence upon the proteolytic action of those enzymes that are present in milk when made into cheese. We have also found that in normal cheese the addition of increased quantities of salt decreases the rapidity of proteolysis. We planned several of our experiments with a view to study the action of salt on cheese-ripening, when rennet enzyme is the only proteolytic factor present. The results were not at all conclusive, but seem to indicate, as far as they go, that, in cheese-ripening, salt, in the proportions commonly used, has little or no influence upon the action of rennet enzyme. It may be mentioned, in this connection, that Chittenden and Allen[2] have shown that the action of pepsin, in digesting blood-fibrin, is diminished by the presence of common salt.

EFFECT OF ABNORMAL CONDITIONS PRESENT.

We have already called attention to the difference of conditions present in the experiments described in this bulletin and those found in normal cheese. We will now consider these in more detail. These abnormal conditions, found in our experiments, but not present in normal cheese, are the following: (1) Milk heated to 85° C. to 98° C. (185° F. to 208° F.) to destroy all enzymes originally existing in milk; (2) the use of calcium chloride or carbon dioxide gas to restore the coagulating property of milk-casein by rennet extract; and (3) the use of chloroform to suppress all activity of organisms. The question naturally arises as to whether the introduction of these unusual conditions seriously affected the results obtained and, if so, in what manner and to what extent.

If the conditions mentioned showed any influence upon the action of rennet enzyme, the tendency was an unfavorable one for the action of this enzyme.

SUMMARY AND DISCUSSION OF RESULTS.

In the work described in the preceding pages, we have studied the proteolytic action of rennet enzyme under the following conditions:

[1] New York Agr. Expt. Sta. Bull. No. 203, p. 241 (1901).
[2] "Studies in Physiological Chemistry," Yale Univ., I, 92 (1884-'85).

(*1*) *In Cheese Containing Rennet Enzyme as the Only Proteolytic Agent, with and without Acid, and also with and without Salt.*—In these experiments, all milk-enzymes were destroyed by heating at 95° C. to 98° C. (205° F. to 208° F.); the coagulable property of the milk-casein was restored by the addition of either calcium chloride or carbon dioxide gas, and all organisms were rendered inactive by chloroform. Acid, when present, was furnished by addition of pure lactic acid.

(*2*) *In Cheese Containing Rennet Enzyme together with Acid-forming and Some Proteolytic Organisms.*—In these experiments, the milk-enzymes were destroyed by heating, acid was furnished by a lactic acid "starter," but no chloroform was used. We thus had, as our only proteolytic agents, rennet enzyme in the presence of acid and some liquefying organisms that were introduced in the "starter" or that got into the milk or curd during the operation of cheese-making.

(*3*) *In Cheese Containing Commercial Pepsin in Addition to Rennet Enzyme, together with Hydrochloric Acid and Such Organisms as Were Introduced during the Process of Making Cheese.*—In these experiments, the milk enzymes were destroyed by heat and commercial pepsin added in different amounts.

(*4*) *In Comparison with Commercial Pepsin on Casein in Milk, with and without Acid.*—In these experiments, the milk enzymes were destroyed by heat and all organisms were rendered inactive by chloroform.

(*5*) *In Comparison with Commercial Pepsin on Paracasein Dilactate.*—In these experiments, rennet enzyme and commercial pepsin, sterilized by formaldehyde, were allowed to act upon sterile paracasein dilactate.

The results of these experiments appear to us to justify the following statements:

(1) In the case of every experiment made, whether with cheese or milk, there was little or no proteolytic action of either rennet enzyme or commercial pepsin in the absence of acid, while there was marked action, though in varying degrees, in the presence of acid.

(2) In the absence of acid in cheese, no paracasein lactate is formed and little or no proteolysis occurs; in the presence of acid in cheese, or more strictly in the milk and curd, paracasein mono-

lactate is formed and proteolysis takes place, with the rennet ferment as the active agent. The ability of rennet enzyme to convert paracasein into soluble nitrogen compounds appears to depend upon the presence of paracasein lactate. In cheese-making, therefore, the primary function of acid appears to be the formation of a chemical compound with paracasein, commonly paracasein monolactate but, in excess of acid, paracasein dilactate. The conversion of paracasein monolactate by rennet enzyme into soluble nitrogen compounds is strongly suggested by the fact that, when the soluble nitrogen compounds increase, the paracasein monolactate decreases.

(3) In comparing rennet enzyme and commercial pepsin in the case of cheese, milk and paracasein dilactate, the experiments that were strictly parallel have shown about the same extent of proteolytic action.

(4) In the case of both rennet enzyme and commercial pepsin, the chemical work performed by the ferments is confined mainly to the formation of paranuclein, caseoses and peptones, while only small amounts of amides are formed, and no ammonia.

(5) Rennet enzyme is a peptic ferment, as shown by the following characteristics: (*a*) Neither rennet enzyme nor pepsin causes much, if any, proteolytic change, except with the help of acid; (*b*) the quantitative results of proteolysis furnished by rennet enzyme and pepsin agree closely when working on the same material under comparable conditions; (*c*) the classes of soluble nitrogen compounds formed by the two enzymes are the same both qualitatively and quantitatively; (*d*) neither enzyme forms any considerable amount of amido compounds, and neither produces any ammonia; (*e*) the soluble nitrogen compounds formed by either enzyme are chiefly confined to the groups of compounds known as paranuclein, caseoses and peptones.

(6) The experiments made to determine the influence of salt on the proteolytic action of rennet enzyme, while not conclusive, suggest that salt has little or no effect upon the action of rennet enzyme in cheese-ripening.

(7) In obtaining our results relating to the study of the function of rennet enzyme in cheese-ripening, we were necessarily compelled to work under conditions more or less abnormal as compared with the conditions commonly present in cheese-

making. The effect of such unusual conditions would tend, if they had influence at all, to diminish the proteolytic action of rennet enzyme. We are, therefore, justified in believing that our results represent the minimum effect of rennet enzyme in cheese-ripening and that, under normal conditions, it takes, if anything, a larger part than that indicated by our experiments.

(8) In some experiments, we eliminated all milk enzymes and all active forms of organisms contained in the milk before making it into cheese. In some cases, we had rennet enzyme in the presence of acid as the only proteolytic agent in the cheese; in others, we had the same conditions and, in addition, such proteolytic organisms as chanced to get into the milk and curd during the process of cheese-making. In the latter case larger amounts of amides were formed, and some ammonia, while, in the presence of rennet enzymes alone, no ammonia was formed and only small amounts of amido compounds. When we compare normal cheese with cheese containing only rennet-enzyme, we find the same difference, except that it is more pronounced, as we should expect. Hence, the special work done by the rennet enzyme as a factor in cheese-ripening is that of a peptic digestion, forming groups of water-soluble nitrogen compounds, intermediate in complexity of structure between paracasein and the amido compounds, *viz.*, paranuclein, caseoses and peptones.

In normal cheese, we find an accumulation of amides and ammonia, as the cheese grows older and a corresponding diminution of the compounds previously formed. The formation of all the ammonia and of a large proportion of the amides found in ripened cheese must be due to some agency other than rennet enzyme, and the only other agents present, besides milk enzymes, that can do this work appear to be organisms or their enzymes. The first stage in normal cheese-ripening is essentially a peptic digestion of paracasein monolactate. Gradually amides are formed and later ammonia. It is probable that the first chemical work done in normal cheese-ripening is the conversion of paracasein monolactate by rennet enzyme into paranuclein, caseoses and peptones. The question naturally arises as to whether these compounds must be formed before other agents can take part in the work and carry it along farther, producing amides and ammonia. We are at present engaged in studying this phase of the problem.

(9) When rennet enzyme was the only digesting agent in cheese, we were unable, in any case, to find the slightest traces of cheese flavor. Apparently, we must look to other sources for this important product of cheese-ripening.

NEW YORK AGRICULTURAL EXPERIMENT STATION, GENEVA N. Y.

A METHOD OF GRADING SOAPS AS TO THEIR DETERGENT POWER.

BY H. W. HILLYER.

Received October 7, 1903.

AS FAR as known to the writer, no method for directly determining the detergent value of a soap is now known. It is assumed, and with much justice, that the value of a soap is a function of the amount of combined fatty acids present, and the valuation of soaps is now based on the determination of the fatty acids. Besides this, the determination of the free alkali and of water and various filling materials is required. These determinations give evidence on which the valuation of the soap may be based and, to some extent, for ascertaining the materials from which it is made, but do not give any direct measure of the value as a cleansing agent and, further, require a complex judgment to interpret them.

The method now proposed is based on the study of sodium soaps, previously publishd in this Journal, **25**, 511 and 524. In the articles indicated, it was found that when a solution of a soap is made to form drops beneath the surface of an oil, the number of drops formed from a given volume of the solution is dependent on the amount of soap present in the solution. Further, the conclusion was reached that the number of drops formed was a measure of the emulsifying power of the given soap solution, and that the emulsifying power was so large a part of the cleansing power that it might stand as a measure of it, especially, since two of the other probable factors in cleansing, namely, penetrating or wetting power, and lubricating power are dependent on the same physical properties as the emulsifying power.