

THE MORE NOTABLE EVENTS IN THE PROGRESS IN AGRICULTURAL CHEMISTRY, SINCE 1870.

In Germany, where the greater part of the recent progress in agricultural chemistry has been made, a wide field is included in this branch of chemical science. The table of contents of the *Jahresbericht ueber die Fortschritte auf dem Gebiete der Agrikulturchemie* includes these several subdivisions of the subject:—Soil, water, atmosphere, the chemical composition of the plant, the vegetation of the plant, diseases of the plant, culture and manures, methods of agricultural investigation, animal production, and accessory manufactures; this last item including dairy products, wine, beer, spirits, fermentation, disinfection, preservation of foods, cane sugar, starch, dextrose, glucose.

Within the limits of this paper I must confine myself to a narrower field.

Concerning *methods of chemical analysis*, *Kjeldahl's* publication in 1883 of his now well known method for the determination of nitrogen in organic substances, was an event of the highest importance for agricultural chemists; it may be safely said that it has been of far greater service to them, than in any other branch of the science; and since it has been made applicable also to the determination of nitrogen in nitrates as well as in organic matter, by *Scovell's* simple modification of adding zinc dust and salicylic acid or zinc sulphide, in the treatment with sulphuric acid, we do not now need any other method for our determinations of this constituent of soils, fertilizers, plant products, or animal products. At the time that this method was published, the Association of Official Agricultural Chemists of this country had come to the conclusion that the absolute method was the only one that was reliable for all their purposes. Only when we compare the cumbersome nature of this method, even with the advantage of the excellent forms of apparatus devised by them, but all depending on the collection of the nitrogen over mercury, with the simplicity and inexpensiveness of the *Kjeldahl* method, and, withal, without any

sacrifice of accuracy, and remembering that no determination has to be made so often by the agricultural chemist as that of nitrogen, the great value of this new method to him can be fully appreciated.

Of *agricultural experimentation*, a most important part consists of experiments with plants in general, or with the different species of plants of agricultural importance, under different conditions, especially as to the supply of food, the only factor over which the farmer has any essential control. Before the date of the beginning of my history, 1870, the very valuable method of *water culture*, by which plants were grown with their roots in water only, and where the conditions as to supply or exclusion of any nutrient is under almost perfect control, had been so fully developed as to leave little room for improvement since. Of *experimental culture in soils* there are three methods prominently in use, that of pot culture on carriages that can be run under shelter to protect the plants from injury by unfavorable atmospheric disturbances; second, the system of box culture in boxes as large as possible without being unmanageable, usually from two to three feet square and deep, sunk in the ground till their upper edges are even with the surface; with this method it is plain that the natural conditions of plant growth are much more like those in the field, and that the results obtained may be applied to actual field culture with less liability to error than with the first; third, actual field culture on plots of known area, with one or more unfertilized plots, or plots manured with stable manure, or both, as standards by which to measure the effect produced by the particular nutrient experimented with. Concerning this last method, the one most generally followed, there has been much discussion as to the best size of the plot, or rather the smallest size compatible with reliability of results, but without any general agreement, areas being, advocated ranging from one square meter up to a quarter of a hectare the proper size depending, as some would have it, on the number of plants growing on a given area, a smaller plot answering for barley or wheat than for potatoes or corn. While improvement has been made in this method of experimentation as the result of all this discussion, much remains yet to be done: and for want of

a better understanding of the conditions required for success, a great amount of time and means are wasted at our agricultural experiment stations. The last and best stage of improvement in box and plot culture is well represented at a new station in Dresden, Germany, established for the special purpose of experimentation on the soils of that region. A part of the station farm was stripped of its soil to the depth of three feet and in plots four by twenty-five meters; these pits were then filled each one with soil brought from a plot of the same size on some farm in the neighborhood. And for the box experiment, boxes of cement and lined with plate glass are used, one meter square and deep; the cement sides retain their shape permanently, and the glass lining will be as little corroded by the water of the soil as any material that it would be practicable to use for the purpose; these boxes are sunk in the ground as usual in box culture, in rows with excavated passage-ways between, and with provision for drawing off or supplying water from below. A system like this of box and plot culture at the station, combined as is the intention, with plot culture with the same soils *in situ* at the farms from which the samples were imported, is far ahead of anything attempted elsewhere, and is far more promising of really useful results.

Concerning the *ash ingredients of plants*, of those always present, potassium, sodium, calcium, magnesium, iron, chlorine, sulphur, phosphorus and silicon, the necessity of none but chlorine and silicon was questioned in 1870; as to the functions of any of those in the life of the plant nothing was known, except that sulphur is one of the constituents of the protein. In 1871 *Nobbe* and others observed that without *potassium* no starch was formed in the chlorophyll grains, and that, unless the potassium was supplied as chloride, there was some difficulty in the transportation of the starch grains from the leaf where they are first formed, and a consequent congestion of the starch in those organs ensued. Experiments were made in 1871 apparently conclusive in their results, at least so far as concerns the plants experimented with, buckwheat and rye, that sodium cannot take the place of potassium in whatever function is performed by the latter. As to this function *Lupke*,

in 1887 showed that if this element is excluded from the food of the plant experimented with, *Phaseolus vulgaris*, all the parts are developed, even to the reproductive organs; and even if the cotyledons are removed, these containing a large part of the potassium stored up in the seed, the only indication of want of this element was a very much reduced development, as if on a scale proportional to the much diminished amount of it at command. Unlike others, he concludes that potassium is not required on account of any special function fulfilled by it; but, like nitrogen, phosphorus, sulphur and other elements it is only essential to the building up of every cell.

As to *calcium*, *Böhm* appeared to have shown that its coöperation was in some way necessary in completing the transportation of the reserve stores of carbohydrates from the cells of the seed to those of the seedling; but in 1883 *Raumer* showed that the only specialization that could be proved in respect to its function was that of taking part in the production of the building material for the cell walls; and that *magnesium*, for which no function had then been found, assisted in the transportation of the starch out of the chlorophyll grain.

In 1883 *Kreuzhage & Wolff* proved that *silica*, up to that time considered as unessential even in the cereals, where it was supposed to be necessary to the strength of the slender reed-like stalk of which it forms so large a part, is at least requisite for the fullest development of the cereal plant; that it brings about in some way a better utilization of the other nutrients, and a better production of seed, by effecting a more rapid flow of sap to the seed parts at the time of flowering, and a consequent earlier and more uniform ripening.

Concerning the necessity of *chlorine*, earlier results have been conflicting. *Aschoff* takes up the subject in 1890 and shows that it is essential at least in the case of the bean and maize; the cotyledons of the bean were removed, it having been first proved that this operation did not affect the growth in a solution containing with chlorides all the other essential nutrients, in any other way than to diminish the size of the plant; by this removal the quantity of seed chloride was considerably lessened. In all cases where

no chlorides were supplied in the food, and whether the cotyledons had been removed or not, the terminal bud soon died, and further growth ceased; the congestion of starch grains in the leaf was observed, as in earlier investigations with potassium when not supplied as chloride. Nevertheless, Aschoff concluded that no specialized function could be made out for this element, but that, like nitrogen, sulphur, phosphorus and potassium, probably a certain quantity of it is essential to the building up of every cell.

Thus, while some addition has been made to our knowledge of the necessity of certain ash ingredients, the most that has been gained as to the very interesting question of their function in the life of the plant has been in the direction of generalization rather than of specialization of function.

Not long since *boron* was found in the ash of some California wines, and the inference was naturally drawn that a borate or boracic acid had been added as a preservative, but *Crampton*, in 1890, found it in the ash of thirty-four out of thirty-six wines, of such a character as to preclude the probability that it had been added to them; later, *Hotton* found it in watermelons, and confirmed many results reported by others indicating its wide but sparing diffusion; he found it in most fruits. All this means simply that this element is uncommonly widely diffused in the soil. When boron is supplied in the food of the plant it is harmful except when present only in traces.

Sestini calls attention to the interesting fact that *all the elements essential to plant life* are in the first four series of *Medelejeef's* classification; that no element with a higher atomic weight than 56 is used by the plant; he thinks it might be reasonably inferred that only such elements as are light, having low atomic weights, would have the necessary mobility, or would possess the power of storing up so much energy as to enable them to take part in the atomic movements continually going on in the plant.

The feeding of the plant. The *carbon dioxide* of the atmosphere is the source of all the carbon of the plant. Prior to 1870 it was, I think, everywhere stated that the atmospheric air contained .04% of carbon dioxide. *F. Schulze* was the first to show, in 1870, as the result of a large number of determinations, that the correct

percentage is nearer .03, the average as found by him being .029. *Fittbogen*, in 1876, obtained the higher average of .033 on 347 determinations, and accounted for *Schulze's* lower result by his proximity to the sea ; *Henneberg* also obtained an average of .033.

It may be said to have been for a time the prevailing opinion among agricultural chemists that plants get their *nitrogen altogether from nitrates*. Experiments made since 1870 appear to prove that this view is untenable. In 1875 *J. Lehmann*, found on repeated trials that while buckwheat produced only a sickly growth if confined to ammonium salts instead of nitrates, maize and tobacco required ammonium salts in the first part of the season for normal growth and nitrates in the latter part ; this preference was so marked that if a sickly maize plant, making the best it could of its unsuitable nitrate food during the first half of the season, was transferred to a solution containing ammonium salts, it quickly changed its appearance and began to thrive ; or if a similar change of a thrifty ammonium salt plant to nitrate food was made during the same part of the season, the change in the other direction was no less marked ; corresponding changes in the latter half of the season gave corresponding results. *Pitsch* in 1887 showed that cereals would grow normally, producing a large amount of organic substance and protein, without a trace of nitrate in the food ; but for a certain period after germination these plants stood still, making no growth above ground ; then, as if they had in some way adapted themselves to the unwonted state of affairs, they began growing again and continued to grow vigorously. Other results have been published to the same effect, or results indicating a preference for ammonium salts ; but still the opinion is predominant that the nitrogen presented as nitrate is the more acceptable food ; it would appear to be the case that this is the ordinary nitrogen food of crops, when the ever prevailing tendency is taken into account of ammonia, in any of its compounds in the soil, to pass on into nitrates by nitrification.

Concerning the important question as to whether the *composition of the fertilizer affects the composition of the crop*, small gain has been made on the little knowledge that we had prior to 1870. Some confirmation has been given to older results showing that

increase of nitrogen in the food of the plant increases the protein in the product. It has been shown that any treatment favoring the luxuriant growth of the tobacco plant raises the production of nicotine in it ; and it may after all be possible that this is the only ground for the increased production of protein, that the plants were in a more vigorous condition. The extent of our recently acquired knowledge on this interesting and important question is very limited.

The manner of the construction of the organic substance of the plant out of inorganic materials, is a subject of great interest to agricultural chemists. Starch is the first plainly distinguishable organic substance produced from carbon dioxide and water, with the evolution of oxygen ; if this is really so, then oxygen should be evolved only when carbon dioxide is present. But *Mayer* showed in 1875 that the green parts of plants, in which the first organic substance is undoubtedly produced from inorganic materials, can give off oxygen in the absence of carbon dioxide, while at the same time organic acids disappear ; it is thus suggested that these acids, oxalic, tartaric, citric and malic may be stepping stones in the otherwise long leap from carbonic dioxide plus water to starch plus oxygen ; but further than this no advance has been made on this line of research. No more do we know about the formation of the albuminoids, products of still further and larger deoxidation. *Emmertling* showed in 1874 that nitric acid is set free by oxalic acid in the plant, and states that it probably becomes reduced by the same powerful agency that reduces the carbon dioxide, and that its nascent nitrogen then becomes available for the construction of the albuminoid molecule ; three years later he attempts to show that the proteids are formed in the protoplasm at the cost of amides ; and that the preliminary production of amido-acids constitutes an important part of the total assimilative processes of the plant.

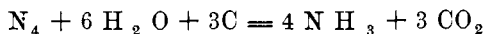
The absorptive power of soils for some of the most important of the plant nutrients, preserving them from loss by leaching, is one of their most important and interesting properties. This property is allowed by all agricultural chemists to be due, so far as the bases are concerned, to hydrated double silicates or zeolites, to a large

extent, these zeolites exchanging their lime or magnesia or soda for the ammonia or potash in solutions of salts of these bases coming in contact with them. In 1875 *Eichhorn* published one of the most interesting confirmations of this explanation of this remarkable property of soils; on leaving powdered chabazite, a hydrated aluminum and calcium silicate, in contact with a solution of an ammonium salt, a new chabazite was produced containing nearly seven per cent. of ammonium in the place of an equivalent amount of calcium which had passed into solution. *Knop* gave further support to this explanation of the absorptive power, by showing that in the large majority of cases it is approximately proportional to the amount of silicate present, decomposable by dilute acid; of forty-six soils examined only one possessed high absorptive power that did not also show a large per cent. of decomposable silicate or zeolite. *Armsby* in 1878, observing as others had that this exchange of bases between the silicate and the solution of potassium or ammonium salt is never complete, stated this limitation as being the consequence of a constant striving to re-form the original compounds, the condition of equilibrium being attained only when this striving balances the opposite tendency to exchange bases.

Van Bemmelen published two important papers on this subject, one in 1878, the other in 1888; in the first one taking the position that if the soil is freed from its basic silicates by treatment with acid, absorption of potassium from its ordinary salts except the carbonate ceases, but that it will still absorb this element from this last salt, no base being set free, while silica still takes some unexplained part. In the last paper he sums up his results and views thus; that the new compounds formed as the result of this absorption are bodies of variable molecular composition, and that they must be distinguished from chemical compounds, although they can often be converted into them; that colloidal substances form such absorptive compounds, as he calls them, with water and with bases, acids and salts, when brought into contact with their solutions; all the phenomena peculiar to this absorption can be exhibited by the behavior of these colloids to solutions of salts. The soil contains them—colloidal silicates, ferric oxide, silicic

acid and humus substances ; it is mostly the colloidal silicates that do the absorptive work in the soil. Thus this latest and most carefully developed explanation of this property of soils does not differ from the first one, except in applying a different name and different constitution to the chief agent concerned, and in assigning one common physical condition to that and the minor agents, all of which have for a long time been recognized as taking part in it.

The reasons of the universally recognized value of the products of the partial decay of the vegetable and animal remains in the soil, known under the collective name of *humus*, have received much attention ; no subject has been more discussed than the relation of the humus, and with it the clay of the soil, to the free nitrogen of the atmosphere. *Simon*, in 1875, affirmed as the result of numerous experiments that humic acid, one of the constituents of humus, absorbs free nitrogen, and in connection therewith evolves carbon dioxide, the carbon of the acid, and moisture, coöperating thus :



Armsby, in the same year, reaffirmed an older statement of *Deherain's*, that in the presence of a caustic alkali free nitrogen is absorbed, with the formation of some organic compound. *Truchot*, in the following year published results showing a nearly uniform relation between the proportion of nitrogen absorbed and the proportion of carbon in the soil ; and since the proportion of ulmin, one of the humus compounds, is closely related to the proportion of carbon, he infers that this is the absorbent agent of the nitrogen. In the same year *Berthelot* published his investigation, proving the absorption and fixation of free nitrogen by many organic compounds, notably some carbohydrates, as gum arabic and the cellulose of filter paper, under the influence of a feeble electric tension, and showing that such a result could be produced under the tension existing between the earth and the atmosphere.

In 1887 *Berthelot* published the results of three years' observation, indicating an absorption of free nitrogen by clay and sandy soils, poor in nitrogen and rich in potash, and that this is accomplished with the coöperation of microbes. *Tacke*, in 1889, confirmed this

statement by results of his own, adding that the presence of vegetation on the soil is not necessary, that there is no nitrification accompanying the fixation of nitrogen, and that it goes on best during the warm summer months, when vegetation is growing most actively; the last statement accords with the view that microbes, also in general flourishing most actively under such conditions, are engaged in the work. In the same year *Deherain* brings support to these views by results in field culture. The soil first had its nitrogen reduced, by cropping, from 2 grams per kilogram to about 1.5 grams; and from this time on no manure was added, while crops were removed each year; yet the soil gained in nitrogen, on two fields, at the rate of 354 and 275 kilograms respectively per hectare. *Deherain* calls attention to the necessity of the reduction of the nitrogen already present to a certain low point, before the work of the microbes begins, in accordance with *Berthelot's* statement of the requirements in the case.

This question of the absorption of free nitrogen by the soil, or by some living organisms in the soil, is naturally of very great agricultural importance, and *Berthelot's* views have been the subject of much discussion, pro and con; his special opponents have been *Th. Schloessing*, father and son, who have insisted that no such results were obtainable by them, and *Lawes and Gilbert* of England, known to all agricultural chemists for the high character of all their work and for their conservatism, have doubted the reliability of *Deherain's* work, particularly on account of the difficulty of getting thoroughly satisfactory determinations of nitrogen in the soils of fields in grass. But *Kreusler*, editor of one of the best agricultural chemical journals in Germany, in a recent criticism of the dispute between *Berthelot* and the *Schloessings*, inclines to the support of the former. In view of still more recent discoveries as to the work of microbes in the soil, the contention of *Berthelot* as it stands does not seem so impossible or unreasonable as it would be, were not such coöperation of living organisms allowed by him.

In consideration of the most important relations of the soil to the crops as their chief feeder, the subject of the *analysis of soils* has received a large share of the attention of agricultural chemists from the outset, but without much satisfactory progress even in

recent years. In 1872 *Biedermann* made so much account of the determination of the absorptive power of the soil, as a means of deciding upon its relative value, as to consider other determinations of small importance; he urged especially, therefore, the determination of what he called the "aufgeschlossene silicat Basen," or practically the zeolite oxides, and also the absorptive power; he considered that one would then have done the best that he could towards obtaining chemical data for a relative valuation or "Bonitirung" of a soil. *Knop* followed in the same line. Other discussions of the subject in long papers in the German journals, by *Fesca*, *Orth* and others, appear to have resulted in little unification of opinion even as to salient points in the analysis, or those that might be made specially prominent, to the exclusion of others to be regarded at least for the present as of comparatively small importance. *Hilgard* of California has I think made more important contributions to the discussion of the question along this line than any one else; but even he would make a complete analysis of the silicate part, a determination of the phosphoric pentoxide, of the silica set free by acid digestion, of the humus by *Grandean's* method, and of the nitrogen, besides a mechanical determination of the proportion of finest material by silt analysis; in connection with this he makes many valuable suggestions as to the interpretation of results, based on his wide experience and observation.

Both the Association of German Experiment Stations, and our own Association of Official Agricultural Chemists, have at last taken this important matter seriously in hand, and I think we may reasonably look for important scientific and practical results within the next decade.

A very large amount of valuable work has been done by *Wollny*, all of it within the last twenty years, on *soil physics*, especially the relations of the soil to heat and to water, of which I should at least make mention, besides work on the same lines in this country by *Whitney*, *King* and others; but, as it is not germane to my subject, and as there is not time for a fair digest of these researches, this mere allusion to them must suffice.

The matter of the *production of sugar* in the Northern States has received a very great amount of investigation in this country,

mostly under the direction of agricultural chemists, chiefly *Collier* first and *Wiley* afterwards, in the department of agriculture. So many of us are undoubtedly acquainted with the history of this work that has been done in our midst, that it will suffice if I present here a brief summary of the views of the latter, who is without question the best judge of the prospects of this industry, whose success may mean so much to the farmers of the North.

Concerning the manufacture of sugar from beets he says that in "certain parts of California, Oregon and Washington, all of them coast areas, certain localities in Minnesota, Iowa, Wisconsin and Michigan, and certain parts of Northern Illinois, Indiana, Ohio and New York, good beets for sugar production are yielded; but it remains yet to be demonstrated how far profitable sugar manufactures can be carried on, on this basis."

Concerning sorghum as a source of sugar for manufacture he says that the "possibility has been fully assured of developing from existing varieties a permanently improved plant, capable of cultivation for manufacturing purposes. But to develop this plant we must have a climate thoroughly adapted to it, scientifically tested seed of the most approved varieties, and much more careful attention than is given to ordinary maize." Another chemist who has given much attention to the matter, *Neale*, affirms that there is a margin of profit on cane averaging 9 per cent. of sugar; but the margin is too small to justify the hazard of any considerable amount of capital. In the last season in Delaware, all the seed sown was guaranteed to have been taken from lots of cane testing as high in some cases as 15 per cent. of sugar, while the per cent. in the cane harvested from that seed ranged from 8 to 11 per cent." The season was unfavorable; but just in this respect one of the most serious risks seems to be incurred—the sensitiveness of the plant to the season as concerns its content of sugar. As to the suitable climate referred to by Mr. Wiley, I believe it is generally allowed that reasonable assurance of it, such as would attract capital, is to be found only in certain limited areas in the Northern States.

An enormous amount of work has been done in Germany, and much, also, in this country, on the *relation of the crops pro-*

duced by the farmer to the feeding of his stock. Within the limits of my history the importance of the occurrence of the different forms of nitrogenous matter in foddering materials has been fully shown, and the nitrogenous constituents have received by far the largest share of the attention of agricultural chemists. That part of the nitrogenous substance of agricultural crops used for stock feeding, which is not genuine albuminoids, is made up chiefly of amide substances. Presuming that they cannot have the same nutritive value as the genuine proteids, their quantitative estimation is of great importance. *Kern* and *Schulze* attempted their direct estimation in 1879, but failed of success, and the latter proposed to remove the real protein, which I will designate by this name, protein, and then determine the nitrogen in what is left. *Kellner* claimed sufficient accuracy for his process of extracting the non-proteid substance with alcohol, acidified with acetic acid, and determination of the nitrogen in the residue. In 1880 *Armsby* claimed that all the non-protein in hay could be extracted simply by boiling water; but he does not affirm that this method can be applied to all fodders. In the same year, *E. Schulze* and *Barbieri* proposed to distinguish three classes of nitrogen compounds in the fodder: (1) the protein, precipitable by cupric hydroxide, (2) the pepton-like substances, alkaloids, ammonium salts, etc., precipitated by phospho-tungstic acids, and finally (3) what is not precipitated by either of these reagents, or the amide substances. But no proposal has met with such general acceptance as that of *Stutzer*, in the same year, of precipitating the protein by cupric hydroxide, determination of the nitrogen in this precipitate, and, by subtraction of this from the total nitrogen, getting the non-proteid nitrogen. This method is everywhere used now, and essentially as he first described it. In the examination of a large number of fodders, the amide nitrogen has been found to range from nothing in a few cases up to sixty per cent. of the total nitrogen; even in wheat and other grains, in which, at first, the nitrogenous substance was supposed to be pretty much all protein, there may be from thirteen to forty-six per cent.

Before leaving this part of the subject mention should be made of the valuable work done by *Osborne*, of the Connecticut Ex-

periment Station, on the *proteids of the oat kernel*, and a new proteid extracted at 65° C. by ten per cent. solution of sodium chloride, and separating from the solution on cooling, in spheroids; and the work of *Chittenden* and *Osborne* on the proteids of the maize kernel, in which three globulins, one or more albumins, and zein, a new proteid, soluble in alcohol, were isolated; the zein contains an unusually high per cent. of carbon.

The question of the *value of the amide substances of fodder* is of the highest agricultural significance, because of the supreme importance of the protein itself for purposes in the animal economy that no non-nitrogenous constituent can serve. This question has therefore received very careful attention on the part of agricultural chemists. Asparagin is taken as a type of at least a large part of these compounds in fodders.

In 1887 *Weiske* experimented with it on hens, guinea pigs and sheep, and concluded from the results obtained that it saves protein from waste or loss, or, in other words, in some way leads to the conversion of a larger proportion of the digested protein of the fodder into animal substance than would take place without its presence. Similar results were obtained in other experiments, with goats, sheep and geese. Hence these amide substances may become particularly useful in connection with the feeding of a ration poor in protein. In the case of milch cows *Weiske* found that half of the protein of the ration could be replaced by asparagin, without loss in body weight, or in the flow of milk; if carbohydrates were substituted for the asparagin, the animal lost weight and the milk yield was diminished. This result with the asparagin is remarkable, because no one supposes that it can be converted into proteids in the animal. In the case of carnivorous or omnivorous animals, asparagin produces no such effect. But not all amide substances work in the same way; in the cooking of fodder the protein is converted partly into peptones and partly into amide bodies, some of which may cause greater waste of digested protein, instead of saving it: such bodies are tyrosin and leucin. *Weiske*, in 1890, published this result, and gave, also, an interesting illustration of the effect of differences in the chemical structure of some of these

bodies. Amido-succinic acid, and amido-succinamic acid, work in opposite directions on the utilization of digested protein: one has the amide group in the radical, and the other in the carboxyl group. In 1890 *Gabriel* made public the results of investigations on the steaming of lupine, the temperature being kept so low as to avoid the production of injurious amide bodies; he had found that this production was a matter of temperature; in these results the value of the useful amide bodies was strikingly shown. On a daily ration of raw lupine containing 12.44 grams. of digestible protein and 0.7 grams. of non-proteid nitrogen, 2.39 grams. of nitrogen were fixed as animal substance in the body; on a daily ration of steamed lupine, the temperature being kept below 135° C. in the cooking, containing 8.25 grams. of digestible protein and 1.88 grams. of non-albuminoid nitrogen, 2.75 grams. of nitrogen were fixed in the body. These results indicate that a mixture of protein with amide bodies may have a higher nutritive value than protein alone.

In the process of preserving fodder in closely packed masses in pits or wooden inclosures, known as ensilage of fodder, it is proved beyond doubt that, in the fermentation taking place, a notable proportion of the protein is converted into amide substances; and yet it is no less indisputable that this fermented fodder will cause at least as good a flow of good milk as will an equal weight of dry substance in the same kind of fodder, cured in the usual manner. This experience, hundreds of times repeated, as it has been, serves to confirm the conclusions of *Weiske* and others as to the usefulness of these bodies.

And yet *König*, a high authority on this subject of fodders and feeding, affirms in one of the most recent numbers of a German journal that the latest investigations on this subject have given results which lead him to believe that the question must still be considered as an open one, and that we may yet be forced to conclude that amide substances do not act in the manner above indicated.

In 1889 a paper was published by *W. E. Stone* on a new class of carbohydrates called *pentagluco*ses derived from plants, now more often called *pentoses*, the number of atoms of carbon in the sim-

plest formula being five, instead of six as in all other carbohydrates. They are derived from a peculiar class of gum-like bodies, occurring in common fodder plants in proportions ranging from 1 to 12 per cent. They were found also in large quantities in the manure of animals fed on two quite distinct rations. Since, as in digestion experiments, the digestibility of any constituent of the fodder, as the carbohydrates, always reckoned together in the analysis under the name of the nitrogen-free extract, is taken as the difference between the nitrogen-free extract in the ration consumed and the nitrogen-free extract in the solid excrements, and since these pentoses, or their corresponding gums, would be included in the nitrogen-free extract as usually determined, their existence stands in an important relation to these determinations of the digestibility of fodders. Further experiments with them have shown that they are to a marked degree less digestible than starch or sugar; consequently they accumulate in the solid excrements, giving to the nitrogen-free extract of this matter a different composition from that of the ration fed, while we have been in the habit of assuming that the composition was essentially the same. Thus all the laborious digestion work will need to be done over again to make it accurate.

These pentose-yielding gums are found to be widely diffused in nature, in condensed molecules, as is the case with the hexoses, as the regular carbohydrates may be called; wheat straw or ears is one of the best substances from which to prepare them, by simply heating with acid, producing hydrolysis. When heated in certain proportions with hydrochloric acid furfural is produced, instead of laevulinic acid, as with the hexoses; and this production of furfural is made use of in their quantitative estimation. Arabinose and xylose are the two pentoses thus far isolated. Much of our present knowledge of these bodies is due to *Tollens*, with *Stone*.

One of the proximate constituents always determined in the analysis of fodder is the so-called *crude fibre*; a part of this is digestible, as of the protein, fat, and nitrogen-free extract; this digested part was pronounced by *Schulze* and *Maercker* in 1875 to be identical with pure cellulose; and it was for a long time regarded as equally valuable for fuel, in the animal economy, with starch and

sugar. But in 1884 *Tappeiner* published researches indicating that all this crude fibre of the ration that did not appear in the solid excrements, and was therefore supposed to be digested, was never digested at all, or not taken into the circulation; but that it was simply fermented in the intestines, with the production of intestinal gases; this conclusion was accepted by *Weiske* and others eminent in this line of work; it must have been accepted unwillingly since it made of no account a large part of their own previous investigations.

In 1889 *F. Lehmann* made this matter the subject of a long investigation; and he appears to prove conclusively that *Tappeiner* was wrong; his results showed that the protein-saving effect of sugar is to that of digested crude fibre, or cellulose, as 100 to 74.7, and that the protein-saving effect of starch is to that of digested cellulose as 100 to 61. This constituent of the fodder seems, therefore, to be fully relegated to its former place.

In 1880 appeared *Stutzer's* first statement of his method of *determining the digestibility of the protein of the fodder by treatment of it with artificial gastric juice*, instead of passing the fodder through the animal and getting digested protein by protein in the fodder eaten minus protein in solid excrements; the method was improved, later, by following the treatment with gastric juice by that with pancreatic juice; its greater simplicity commended it at once; and it avoids the error in all results by the natural method, due to the fact that a part of the nitrogenous matter in the intestines belongs to secretions which have passed in from some organs of the body, and is not therefore really undigested crude protein of the fodder. The natural inference that since these substances were produced in the animal out of digested matters, they would be extracted from the solid excrements by treatment with digestive agents, as pepsin, was tested; and it was found that when digested protein by the natural method was considered to be crude protein of the fodder, minus crude protein of the dung calculated from the nitrogen left *after this extraction by pepsin*, the results agreed more closely with those obtained by the artificial method—that in fact the agreement, in general reasonably fair in most cases without this correction, was with the correction very good. If equally

satisfactory methods could be developed for the determination of the digestibility of the other proximate constituents of the fodder, it is not at all unlikely that the cumbersome and tedious natural method would be relinquished. But all attempts in this direction having failed of success, the fodder whose digestibility is to be tested must still be passed through the animal; and it makes less work to include the protein in this set of operations than to determine it by the artificial method. Some chemists, as *Pfeiffer* in 1890, express themselves as not yet sure of the reliability of the artificial method, even though it would seem to be unquestionable that a laboratory experiment must be less exposed to error than a barn experiment with a living animal, the materials and reagents used in both cases being practically the same, and the conditions the same as to temperature, duration of treatment and so on. And the conclusion would seem to be sound, that, if there is disagreement between the results by the two methods, the natural method should be regarded as the faulty one.

A very large number of analyses of fodders and feeding stuffs has been made within the past twenty years, in Europe and in this country, in the laboratories of the Agricultural Experiment Stations. Furthermore, many of the German Experiment Stations and some of our own, notably those of Maine, New York (Geneva), Pennsylvania and Wisconsin, are fitted out with the necessary appliances for digestion work by the natural method; they are continually adding to our knowledge of the subject and working on new practical problems that are constantly presenting themselves. In 1874 *Dietrich* and *Koenig* issued a work of 84 pages on the Composition and Digestibility of Fodders, giving in the form of Tables the results of the work that had been done up to that time. In 1891 the second edition of this work was published in two volumes of 1,500 pages in all, and its compilation was the result of ten years' work. No more striking presentment could be given of the amount of work done on these lines of investigation, but it would be useless to attempt to summarize the results of this work, in the brief space of a few minutes; and none of them are notably prominent, although all are valuable as a part of the whole.

In respect to the composition of milk some new and interesting

results have been obtained within these twenty years. The question of *the existence of an enveloping membrane of albuminous matter*, inclosing the fat of the globule, has been conclusively settled by the investigations of *Soxhlet* in 1874, and especially of *Babcock* in 1885. The latter prepared an emulsion of oil by trituration with sugar and water, which was then diluted with water, as it could be to a large extent without destroying the emulsion. Such a diluted emulsion was identical with milk in all its properties except that there was more variation in the size of the fat globules; the fat gradually rose to the surface in the same manner, the cream so obtained could be churned to a substance resembling butter in consistency; there was the same difficulty in extracting the fat from the emulsion, by shaking up with ether, that there is with milk, which was one of the strongest arguments used in support of the existence of a membranous sack inclosing the fat. Another no less conclusive proof of the non-existence of the envelope was found in the increase in the number of the fat globules that can be brought about by simply stirring milk vigorously with an egg beater, while kept at a temperature of about 40° C. or above the melting point of the fats; the number being 159 in 0.0001 millimeter cube of a sample of fresh milk, it was raised to 174 by the first stirring, then by another stirring to 231 and by a third to 283; in another experiment the number of globules above a certain size was found to be greater before stirring than after, demonstrating by observation what must necessarily be the case if the globules are divided by this operation, that the number of smaller globules is relatively increased.

Two new *normal constituents of milk* have been discovered. In 1888 *Babcock* announced the discovery of a fibrin in milk, to which he gave the name of lacto-fibrin. Soon after milk is drawn the fat globules, before uniformly distributed, are more or less grouped together, which is supposed by *Babcock* to be due to their entanglement in the clots of coagulated fibrin; solution of potassium hydroxide prevents coagulation of the fibrin; and if the milk is drawn directly into such a solution, no grouping of the fat globules is observed. Fibrin, unlike other proteids, decomposes hydrogen peroxide; milk possesses this property, because of its fibrin, and a

method of determining the relative amount of fibrin in different samples of milk was based on this reaction.

In 1888 *Haeckel* announced the discovery of citric acid as another normal constituent of milk. Soxhlet had concluded that there must be an organic acid in milk, whose neutral lime salt is soluble, because the quantity of lime in solution is incompatible with the quality of dissolved phosphorus pentoxide. There is no lactate in fresh milk; later, citric acid was found and unquestionably identified.

Experiments made in 1891 showed that it does not come from citric acid in the fodder, for its quantity is not increased if this acid is added to the ration, nor is it produced by fermentation in the large intestines; it appears to be one of the normal products of the special metamorphosis of which the milk as a whole is the product. The quantity of it varies from 1.7 to 2 grams. per liter, while it was calculated that there should be 2 grams. to take up all the lime that could not otherwise be satisfactorily disposed of.

No question has been more discussed or investigated by agricultural chemists than that of the *nitrogen supply of plants*, and some of the finest researches in chemical science have been devoted to this subject. It has been already referred to in this paper in the account of some studies on the relation of the soil to the free nitrogen of the atmosphere; and mention was made of the possible coöperation of a third agency in the accomplishment of some of the results there claimed to have been obtained, namely microbes. No student of that side of chemistry which relates more particularly to the conditions of our own existence and comfort needs to be told that, within less than the limits of the period of my history, the existence has been established of such intimate and important relations between higher vegetable life and animal life on the one hand, and microbial life on the other, that the chemist who would be an investigator in vegetable and animal chemistry must either be a bacteriologist himself, or else must often call in the aid of the bacteriologist, before he can solve some of the most important questions that confront him.

Since some of the most interesting contributions toward the solution of vitally important agricultural chemical questions have

been made through this coöperative study of the chemist and the bacteriologist, and since these researches are published in agricultural chemical journals as if properly belonging there, I certainly shall not be accused of going outside of proper bounds, if I devote the closing part of my paper to an account of some of the most striking results that the bacteriological chemist or the chemical bacteriologist has given us.

The process of nitrification as a natural process, or the natural tendency of nitrogen in certain widely diffused compounds to pass on into the form of nitrates, has long been known. The discovery of ozone as a constituent of the atmosphere, with its great oxidizing power at common temperatures, led naturally to the belief that nitrification even of free nitrogen took place through its aid.

Schönbein affirmed that even in connection with the evaporation of water, nitrites and nitrates are formed, as well as in connection with the combustion of organic and inorganic substances—ozone being first produced in both cases. Schönbein's fundamental experiment was simply this: to collect the vapor from water dropping slowly into a hot crucible, on the walls of a large beaker; this water usually gave after acidification a blue color to iodized starch paper. *Baumann*, in 1888, went over all of Schönbein's experiments, and, with the aid of the much more delicate tests for nitrous and nitric acid that we have now than any that were known in Schönbein's day, proved that these acids are always formed in connection with any combustion, but especially the burning of illuminating gas, and that this was the source of the nitrogen acids in Schönbein's fundamental experiment above described. *Baumann* also proved that calcium carbonate and all oxides, basic bodies or hydroxides prepared and dried in the ordinary manner, either by exposure to air or in chambers heated by gas, contain these acids, taken from the air while being dried, if not in some previous stage of their preparation. These experiments by *Baumann*, and others along the same line, appear to have deprived the contention of all support, that manganic hydroxide in conjunction with alkaline carbonates possess the property of producing nitric acid from water and free nitrogen, or that free nitrogen or the nitrogen of

ammonia is oxidized by the oxygen of the air, by the simple co-operation of alkaline carbonates or calcium carbonate.

The production of nitrite and nitrate from the nitrogen of dead vegetable or animal substances, or of ammonium salts, in porous soils, by simple exposure to warm, moist air, was supposed, prior to 1877, to be due to simple oxidation. In that year *Schloessing* and *Muntz* proved that the coöperation of microbes was required, since this nitrification would not go on in sterilized materials, and is altogether stopped by vapors, as of chloroform, poisonous to microbes. As soon as the discovery was made, it was seen that all the conditions of nitrification, as to temperature, moisture, free access of oxygen, are such as are required for the active life of aerobic bacteria. The only mycologist of any note who at first refused to accept this explanation of nitrification was *Frank*, of Berlin, who maintained that nitrification could take place in a porous soil as in platinum sponge. In answer to him *Pluth*, working under Landolt, in 1887, went over the whole ground with great care testing all the usual constituents of soils separately, and proved that in none of them would nitrification of nitrogen in any form take place if they were previously sterilized.

Much importance was attached by Frank to the failure of all attempts to isolate the microbe by pure culture. But *Winogradsky*, in Zurich, and the *Franklands* and *Warrington* in England, found, after much patient investigation, and announced at about the same time, in 1890, that the nitrifying bacterium would not grow in Koch's gelatine plate. *Winogradsky*, by successive cultures in a solution containing no organic matter whatever, but only ammonium sulphate and potassium phosphate, with a small quantity of basic magnesium carbonate, after many inoculations of solution after solution, succeeded in obtaining the nitrifying organism in a zooglœa, or jelly-like mass, coating the deposit of magnesium carbonate in the bottom of the vessel; here it was, however, invariably accompanied by another microbe, which, as was found, could grow slowly in the gelatine plate; to separate the two, therefore, all that was necessary was to distribute the zooglœa in a gelatine plate, then after several days carefully cut out those parts of the plate that were *not* occupied by any colonies, and with these

inoculate new solutions. The single species of bacterium thus isolated was proved to possess the power of nitrifying ammonia, and to it the name *nitromonas* was given.

The very interesting features of the life of *nitromonas* are these : that like other plants it gets its carbon from carbon dioxide, obtained from the carbonate around which it grows, and accumulated in these cultures in the form of a jelly-like substance ; but unlike other plants which get their carbon from carbon dioxide, this one works without light and without chlorophyll. Moreover, while other microbes decompose organic matter, and get their carbon from that source, this microbe produces organic matter out of materials entirely inorganic. The most interesting feature remains yet to be mentioned. *The function of the vegetable kingdom in relation to the animal is the storage of energy for the use of the animal ; the ordinary plant, producing organic substance with the aid of chlorophyll and the sun, gets its energy from the sun ; the parasite, or the microbe that decomposes the organic matter on which it lives, gets its energy from the organic matter so decomposed ; where does nitromonas get its energy ? The answer is simple : from the oxidation or combustion of the nitrogen of ammonia which accompanies its growth and multiplication, and for which the oxygen of the air is used—not simply the oxygen of the carbon dioxide decomposed, for that would be no gain.*

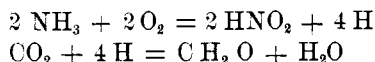
In the same year the *Franklands* published their results in a paper read before the Royal Society of England ; they also failed entirely with gelatine plate culture, but did succeed with simple sterilized distilled water culture ; and in their cultures also, another microbe accompanied the nitrifier ; or, rather, starting with another portion of the same solution, and proceeding in precisely the same manner, the final product was a microbe which did not nitrify, and did grow in the gelatine plate.

In 1891, *Warrington*, of the Rothamstead laboratory, published the final results of his work on this subject, which had been going on since 1880. Like the other investigators he failed in all attempts to cultivate the microbe in organic media, but succeeded in solutions containing ammonium chloride, calcium carbonate and a phosphate ; he isolated an organism that would oxidize ni-

trite to nitrate, but would not oxidize the nitrogen of ammonia, and another that would oxidize only the nitrogen of ammonia; both lived only in solutions of inorganic matter; the nitrite microbe was isolated from the nitrate microbe by successive cultures in solutions containing the nitrogen in the form of ammonium carbonate, while the nitrate microbe was isolated from the other by successive cultures in solutions containing nitrogen as nitrite, and sodium bicarbonate to supply the carbon.

Winogradsky also announced, in 1891, that he had isolated the nitrate organism, after much difficulty, and that his *Nitromonas* oxidized only nitrogen of ammonia to nitrite.

As to the mode of production of organic substance by *Nitromonas*, it was at first suggested that, as in plants containing chlorophyll, carbohydrates were at once produced; but *Oscar Loew* regards it as more probable that the action of the nascent hydrogen of the ammonia should come into play, forming formaldehyde with carbon dioxide.



This formaldehyde by simple molecular condensation may yield carbohydrates, or it may also serve at once in the synthesis of proteids. This is purely theoretical, but nevertheless interesting in this connection.

The next interesting step in this matter of nature's methods of providing nitrogenous food for the plant is the announcement by *Müntz* in 1890, of the discovery of microbial action in the production of ammonia from the nitrogen of organic matter; if the soil in which production of ammonia is going on is sterilized by heating it to 120° C., the production is arrested, and is started again when a little unsterilized soil is added in which ammonia production is going on.

Before leaving *Nitromonas* and his companions who are engaged in this most important work of converting the nitrogen of soils and manures into the most valuable form of nitrogen food, I must notice one other operation of no less importance in which they take part indirectly. Since *Nitromonas* can produce organic

matter wherever there is ammonium carbonates together with phosphate and potash, and since the first mentioned salt is always present in the atmosphere and the others are widely even if sparingly diffused in the rocks, we need not be surprised by the discovery announced by *Müntz*, in 1890, of these organisms engaged in the production of organic substance on bare rocks and mountain peaks; in the Alps and the Pyrenees he found the disintegrated particles of those rocks always covered with organic matter, and the nitrifying microbes always present; he has even found them "alive and ready to resume activity after a sleep of ages under the enduring ice of glaciers;" on the walls of the smallest crevices of the rock; on fragments of rock in or on the soil; and he affirms that by this production of the powerful nitric acid within these masses and fragments of rock, they become indirect agents in the conversion of rock into soil. As a striking illustration of this he cites the case of the Faulhorn in Switzerland, literally translated Rotten Peak. This rock consists of calcareous schist, friable and disintegrating; and he found it to be invaded through its whole mass by the nitric ferment.

So far in the consideration of this microbial work, we have dealt only with nitrogen already in combination; but the work of microbes in the farmer's behalf by no means stops there; in a mysterious and as yet unexplained way they seize hold of and put at his service the free nitrogen of the air. The classical researches of Boussingault, and of Lawes, Gilbert and Pugh proved conclusively that, at any rate, cereal plants, and in all probability all other agricultural plants, are entirely unable to assimilate the free nitrogen of the atmosphere; but nevertheless it was impossible to explain away the universal opinion firmly held by farmers, and supported also by numerous experiments, that leguminous plants, clover, lucerne, and the like, were not only comparatively indifferent to the supply of nitrogenous food in the soil, but would, while demanding much nitrogen for their own growth, leave the soil even in a better condition for crops which must have a liberal supply of assimilable nitrogen in the soil.

In 1858 *Lachman* described, in a Vienna scientific journal of narrow circulation, the existence of small swellings or tubercles

on the roots of some leguminous plants, recognized the bacterial nature of the contents of these swellings, and said it might be supposed that they stand in some relation to the claim of the farmer that these plants gain nitrogen; but that probably they only help the plant to make better use of the ammonia and nitrate in the soil. There the matter rested for nearly twenty years, when *Wilfarth* and *Hellriegel* published the remarkable results of a series of experiments with a small number of leguminous and non-leguminous plants. The seeds of these plants were planted in sand containing all required food except nitrogen compounds: the growth of the seedlings soon stopped in all cases: if, however, there was added to the sand of each pot about a cubic centimeter of a cold aqueous extract of arable soil, containing at the most not over 0.7 mgm. of nitrogen, every one of the leguminous plants began to grow again after a short time, and continued growing luxuriantly; but no change took place in the condition of the non-leguminous plants. These experiments were repeated with 178 pots, and in all cases with the same results; as a variation in the method some of the sand was put in the bottom of a carboy, a pea, buckwheat and oat seed were planted, the soil extract was added, a supply of carbon dioxide was provided, and the carboy was sealed up: only the pea grew beyond the first, seedling, stage and the whole plant contained 137 mgms. of nitrogen, for less than 0.7 mgms. added. Here are the yields of nitrogen in four pots of lupine in the first mentioned set of experiments, without soil extract added: 14.6, 13, 13.6, 13.3 milligrams; with soil extract added, 1099, 1194, 1337, 1156 milligrams. The sand in each pot weighed four kilograms; the soil of an acre a foot deep weighs about 3,500,000 lbs.; allowing that the sand in each pot weighed 10 lbs. instead of about 8.8 lbs., and supposing that each lupine plant would have done at least as well in the field with 10 lbs. of surface soil plus indefinite depth below the level of one foot, which is not unreasonable, since in these experiments the roots were confined altogether to the few inches in depth of the pot, this gain in nitrogen would represent over 900 lbs. per acre; an extremely liberal dressing of sodium nitrate, 400 lbs. per acre, would not contain over 65 lbs. of nitrogen.

Hellriegel and Wilfarth suggest that the tubercles on the roots of the legumes had something to do with this assimilation of free nitrogen of the air, for such it was ; the nitrogen could have come from no other source. They do not mention Lachmann's work, and must have been ignorant of it; they are investigators of high rank, and would have given due credit to him, had they known of the existence of his paper, which was brought to light only two or three months ago.

Of course such an intensely interesting discovery was at once taken up by other agricultural chemists and by bacteriologists; the results above described were confirmed and endorsed by such investigators as Lawes, Gilbert and Warrington, of England; Schloessing's son and Laurent, Berthelot, Deherain, in France; Nobbe and Beyerinck, in Germany; Atwater and Woods in this country; and at the last meeting of the chemists of the German Experiment Stations the work of Hellriegel and Wilfarth was endorsed by a special and unanimous vote.

Nobbe observed that the organisms that do this work are almost universally diffused in arable soils ; that the extracts of soil on which leguminous trees or shrubs are growing are very powerful as inoculating material; that when inoculation is made with a pure culture product it is most efficient on that species of plant which was occupying the soil from which the culture was started.

Schlössing (son) and *Laurent* proved not only the gain in combined nitrogen made by the plant, but also the loss in free nitrogen of the atmosphere, in a confined space in which the plant grew.

As to the explanation of the phenomenon different views are held by different bacteriologists. All are agreed that there is here a case of symbiosis—a partnership or association between two plants for their mutual benefit ; the fungus is benefited, because it finds in the roots of these plants conditions favorable or necessary for its growth; the tubercles produced on the roots of leguminous plants, and apparently only after infection with the fungus, are its home in which it carries on at least a part of its development; the growth of these tubercles does not harm the host plant; free nitrogen is taken up and worked into organic matter; and eventually the

fungus dies and this nitrogenous organic matter is used by the host plant for its own growth. Whether the organism is a bacterium, or more nearly allied to the yeast fungi in its mode of growth, is the special point in regard to which the results of researches differ.

It may seem that since Boussingault, and Lawes, Gilbert and Pugh, included legumes in the plants which they experimented with, these latest results are incompatible with theirs. But it is not at all so. They used sterilized soils; that is, the soils had been ignited to expel all nitrogen compounds, but they did not call it sterilizing in those days; and their plants were as a general thing carefully kept from contact with any other atmospheric air than such as had been carefully washed by passage through strong sulphuric acid, to free it from ammonia; and there was no possibility of that inoculation of soil or plant roots with extracts of arable soil, which was in nearly all cases essential to carry the growth of the leguminous plants, in their latest experiments, beyond the hunger period.

In its relations to the operations of the dairy, the study of bacteriology has only just begun; but already, in so far as the work of bacteria has been looked for where it might be expected, it has been found. It has for a long time been known that milk is a most efficient means of transporting infectious diseases; as we now know, this is because its chemical composition adapts it so well for the nourishment, while in transitu, of the microbes which are the actual carriers of the diseases. But their work is not all of this baneful character. Two illustrations must suffice: cream must undergo a certain change, called its ripening, before butter of the best flavor can be produced from it. The microbe that causes this change has been, at least to a large extent, separated from many others in the milk, and it is claimed that the pure culture of it can be practised on a large scale in the dairy; the directions for this operation were first given by *Weigmann*, in 1891, with seed to be obtained from some bacteriological institute, and the use of this pure culture product is specially recommended at certain seasons of the year when it is particularly difficult to make good butter.

Again, in the ripening of cheese, we should naturally look for the action of microbes. Several investigators have taken this matter up, but by far the most careful research was made by *Adametz*, and published in 1889. He identified twenty-eight species of bacteria in ripening cheese, and a very large number, 850,000, in a gram of a hard cheese (*Emmenthal*), and 5,600,000 in a gram of one of the soft cheeses. Two distinct kinds of change take place in the ripening of the cheese: the conversion of some of the insoluble casein of the fresh curd into a soluble and more digestible form; and, second, the development of the flavor, by which one kind of cheese is distinguishable from another. If the curd is sterilized at the time when it is ready to be put away in the ripening room, and is there protected from infection, neither of these changes takes place; the dependence on microbial coöperation is thus established. Adametz was not able to identify any one species of bacterium as that one which exclusively produced the flavor of the one kind of cheese or of the other. The most he could establish was that the solubilizing of the curd was effected by one or more kinds, and the production of the flavor by one or more other kinds. A great amount of work remains to be done in this special field alone.