SCIENCE

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THE ISOLATION OF RENNET FROM BACTERIA CULTURES.

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EVER since the beginning of the study of micro-organisms it has been a debated question whether fermentations are to be regarded as biological or purely chemical phenomena. Beginning with the work of Schwann and others in the early part of the century, careful experimentation aided by microscopic study seemed to point to a biological explanation of nearly all forms of fermentation. The work of the third and fourth decades of the century proved beyond question that most fermentations were always intimately associated with the growth of micro-organisms, and the inference was a natural one that the micro-organisms themselves were the cause of the fermentations. Shortly after the valuable work of Schwann, however, appeared the brilliant investigations and discussions of Liebig upon fermentations in general. By Liebig all fermentations were regarded as purely chemical phenomena and the presence of micro-organisms was regarded only as a concomitant incident. According to him all albuminous matter was thought to tend spontaneously toward decomposition, and such decomposition was the basis of fermentation and decay. This, the chemical theory, was for twenty years the favorite theory. The biological and chemical theories were very rigidly opposed to each other and supposed to be contradictory. With the work of Pasteur and the great development of the study of micro-organisms thereby inaugurated, the biological theories of fermentations again came to the front, rapidly gained the ascendency, and soon displaced almost entirely the chemical theory as advanced by Liebig. For the next twenty years it was regarded as an almost settled fact that most natural fermentations were biological phenomena, and the theory of Liebig was at last practically abandoned.

Within still more recent times there has been a partial swinging back of the pendulum toward a chemical explanation of many forms of fermentation at least. This has not, however, been in the direction of Liebig's theory, but rather toward a theory of the action which unites together a chemical and biological explanation. It has been recognized for more than half a century that there are forms of ferments, such as pepsin, trypsin, etc., which do act in a purely chemical manner. These ferments, it is true, are produced originally by living organisms, but when once produced they are not living themselves, at least in any proper sense, and their action is not dependent upon growth or multiplication, for they are not organisms. So far as can be determined their action is purely chemical. On the other hand, a large number of fermentations, such as the alcoholic fermentations, the souring of milk, etc., have been traced with certainty, not only to living organisms in the form of bacteria and yeasts, but to the actual growth and multiplication of these organisms. These fermentations occur only when micro-organisms are present and only when these micro-organisms grow and multiply. The amount of growth of the organism is a measure of the amount of fermentation. Undoubtedly these biological fermentations are of a different nature from the other class. We are, however, learning now to look upon some of the biological fermentations as chemical in their immediate nature.

In the first place, the bacteriologist has been learning that germ diseases, which are caused primarily by the growth of micro-organisms in the body, are caused immediately by certain poisonous bodies which these organisms produce. He has called these bodies ptomaines, and at present biologists are very rapidly becoming convinced that it is the direct action of these poisonous materials which produces the symptoms and disturbances associated with most germ diseases. It is not the simple growth of bacteria which produces disease, but the poisonous products of their growth; and thus a chemical explanation is added to the biological.

Not only in germ diseases but in other forms of fermentations, not associated with disease, bacteriologists are learning of the production of chemical ferments by the micro-organisms. Many organisms have been found to produce dyastase, sucrase, glucase, etc. Within the last year or two it has been demonstrated that many bacteria produce a chemical ferment very similar in its character to trypsin. The general class of bacteria which liquefy gelatin have long been known to have an action quite similar to that of pancreatic juice. Indeed, it is this peptonizing action which is the cause of the liquefaction of the gelatin. Recently Brunton and Macfadyen and especially Fremi have succeeded in actually isolating from bacteria cultures a chemical ferment which has this power even when acting in sterilized media.

Some recent work in the bacteriological laboratory at Wesleyan University has isolated another chemical ferment from bacteria cultures. A large class of bacteria have the following actions on milk: They first curdle the milk rendering it slightly alkaline; subsequently the curd is slowly dissolved into a more or less watery liquid. Chemical study shows that this last action is a simple digestion and peptonization of the curd and that it is due to the trypsin-like ferment above mentioned. The curdling, which precedes the digestion, however, must be due to a different action, inasmuch as trypsin produces no curdling of the milk. It has been suspected for some time that this curdling is really due to a "rennet-like" ferment which is produced by the bacteria. It has been my good fortune recently to demonstrate the truth of this supposition. My method of work has been as follows :—

The bacteria in question are cultivated in milk for several days, in some cases for two weeks. By this time the curd is precipitated and at least partially dissolved, and the result is a somewhat thick liquid containing, of course, immense numbers of bacteria. This liquid is filtered through a porcelain filter to remove organisms, and a clear, usually amber-colored, filtrate is thus obtained. The filtrate, of course, contains in solution all of the soluble chemical ferments which may have been formed by the bacteria. This filtrate is now acidified with H_2SO_4 and then common salt is added to a state of super-saturation. When this condition is reached there appears on the surface of the liquid a considerable quantity of snow-white scum. This scum is removed from the liquid, purified if necessary by reprecipitation, and then dried. It produces a snow-white powder, which upon experiment is found to be active in its curdling action upon milk and to have all of the essential characters of rennet. The ferment which is thus obtained is not chemically pure, containing, besides the rennet ferment, a varying amount of the tryptic ferment formed at the same time. But the rennet ferment is most abundant and is very active. This ferment can be kept indefinitely, is killed by heat, acts best at a temperature of 30°–35° C., and curdles sterilized milk under proper conditions in half an hour. Experiment shows that no organisms are present in the curdled milk, and there is thus no doubt left that we are dealing with a chemical ferment similar to rennet, and which is produced by the growth of these micro-organisms in milk. The ferment does not appear to be exactly identical with rennet, some of its chemical tests being different. This may be due to the impurities which are present or to an actual difference in the ferment.

A large number of bacteria possess this power of producing rennet, though not more than nine or ten have thus far been experimented upon. Those studied differ much in the amount of the ferment produced, some giving large quantities, and others only traces. Thus far it seems that all species of bacteria which liquefy gelatin produce this rennet ferment, although some of them only in small amount.

This general line of work is thus leading bacteriologists to a better understanding of the fermentations, although we are as yet doubtless far from any real knowledge of their nature.

IS THE SÂO FRANCISCO DO SUL (SANTA CATHARINA) IRON A METEORITE?

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THE possibility of a terrestrial origin for masses of native iron being established by the well-known occurrence at Ovifak, Greenland, doubt may be cast on the mode of origin of such so-called meteorites as in their chemical and physical characters depart widely from the ordinary type of meteoric iron. From its size and prominence in meteoric literature the most important example of such a doubtful iron is that found in 1875 near the city of Sâo Francisco do Sul, in the State of Santa Catharina, Brazil, and generally known as the Santa Catharina iron or meteorite. Professors Daubree and Stanislas Meunier regard it as undoubtedly meteoric; while Dr. A. Brezina, whose opinion is equally entitled to respect, considers it as probably terrestrial, placing it alongside of the Ovifak iron in his catalogue of the Vienna collection.

So far as known to the writer, no such minute study as Brezina, Cohen, and others have given to various undoubted meteorites has as yet been made of the Ovifak iron, so that at present we are in the dark as to whether or not it presents definite criteria by which a terrestrial iron can be positively separated from a meteoric one. So many of the characteristics once presumed to be purely meteoric have already been noticed, either in the Ovifak or artificial irons, that it may be doubted if any such definite criteria exist. The published analyses of Ovifak show that neither the nickel-iron alloys nor the association with a monosulphide (troilite) carbon free or combined and phosphorus can be taken as distinctive of meteorites. It is not clear, however, if the carbon presents the same form as in meteorites, that is to say, free in the form of amorphous carbon, graphite, cliftonite, diamond (?), etc., and combined as cohenite, nor if the phosphorus is combined with iron and nickel as in the meteoric minerals schreibersite and rhabdite. On the other hand, the compact (*Dichte Eisen*) group of Brezina containing at least nine undoubted meteorites, one of which, Nedagolla, was seen to fall, proves that the absence of a certain crystalline structure, indicated by the so-called Widmanstätten and Neumann figures, is not necessarily proof of non-meteoric origin. On the contrary, evidence is accumulating that a very similar if not absolutely identical crystalline structure may, under favorable circumstances, appear in artificial irons. Huntington has illustrated figures very like those of Widmanstätten in spiegel-eisen; Linck has described a crystal from a furnace slag, with cube faces and a polysynthetic twinning structure, which he identifies with the Neumann figures, and a perfect octahedral crystal with a similar twinning structure has recently come into the possession of the writer from a Brazilian blast-furnace.

It may be presumed that it is mainly on the absence of the characteristic meteoric figures that Brezina depends in placing the Sâo Francisco do Sul with the Ovifak iron, as in no other respect are they markedly similar. The Brazilian iron does, however, show, at least in places, a very fine rectilinear cross-hatching indicative of polsynthetic twinning, but according to some law different from that giving the Neumann lines. Another point of agreement is in the abundant occurrence of the magnetic oxide of iron which is either absent from most meteorites or has been overlooked in their description. Both irons are brittle, being readily broken to fragments with a hammer, in which respect they differ from the tenaceous malleable metal of most meteorites. Both also crumble to fragments in the atmosphere of museums, though apparently not from the same cause, the crumbling of Sâo Francisco do Sul being due to alteration of the sulphide, which presumably is not the case with Ovifak.

Lawrence Smith and Becherel found the magnetism of the Brazilian metal abnormally weak but that it became normal on heating, from which they concluded that the mass could not have been subjected to great heat. There seems, however, to be a variation in this respect in different parts, since Daubree notes that many fragments exhibit polar magnetism while others do not. Any argument drawn from the magnetic properties would apparently tell as much against a terrestrial as a cosmic origin, since the only conceivable mode of terrestrial origin is in the midst of an igneous magma made fluid by heat.

In other respects, however, a parallel for the characteristic features of the Sâo Francisco do Sul iron is to be looked for in the group of meteoric irons rather than in those, so far as published, of the terrestrial iron of Ovifak. It is particularly characterized by the high percentage of nickel and the extraordinary abundance of sulphide. In the first respect its nearest ally is the Oktibbeha meteorite with nearly double the proportion of nickel, and not the Ovifak iron in which that metal is below the meteoric average. Unlike any other known iron, meteoric or terrestrial, the sulphide forms a gangue inclosing the metallic parts, but it is interesting to notice that it also presents itself in pencil-like inclusions in the metal, surrounded by carbon and other accessories, as in the Bendegó meteorite. The meteoric phosphurets, schreibersite and rhabdite, not yet described from, though perhaps present in, the Ovifak iron, are abundant accessories. In the carbonaceous residue, soft, friable granules, with a crystalline form suggestive of the cliftonite of the Youngedin, Magura, and Bendegó meteorites, have been noticed.

Thus, so far as at present known, the chemical and physical characteristics of the Sâo Francisco do Sul iron do not point very markedly to an association with that of Ovifak. What is known of the geological conditions of the place of discovery, although too incomplete to be decisive, points rather to a meteoric origin.

The geological information regarding the place of discovery is derived from verbal communications by the late Professor Ch. Fred. Hartt, a paper in the *Revista do Observatorio* de Rio de Janeiro for 1888 by Dr. Luiz F. Gonzaga de Campos, and a recent article in the *Jornal do Commercio* of Rio, May 29, 1892, by Dr. J. P. Calogeras. All these accounts agree in representing the island of Sâo Francisco do Sul as composed essentially of granitoid gneiss cut by dykes of tourmaline granite and diabase and covered by a heavily-wooded soil-cap due to the decay of the underlying rocks. That is to say, it is a typical locality of the coast-belt from Cape Frio to Montevideo, at any point of which, so far as geological indications go, native iron might be looked for with as great probability as at this particular locality. The rocks, granite, gneiss, and diabase, are well exposed about the shores of the island. The latter, which approaches most nearly in character the Ovifak rock, being apparently no more abundant than in any other similar locality. In the interior of the island the soil-cap and forest growth make geological observations difficult. Dr. Campos, in his examination of the place of discovery of the iron, opened numerous paths and pits, and on my recommendation paid particular attention to the question of the possible occurrence of basic rocks in immediate connection with the iron. He says, "Although the rock on this hillside is much altered, giving an argillaceous soil of a red color, here more, there less deep in tint, it shows perfectly in some points the mineral composition of granite. At the top of the hill near the point *c* [one of the points where masses of iron were found] there are large blocks of granite, at times tourmaliniferous. In the vicinity, in all the directions that I followed, all the soil is granitic. In the bed of the stream, which I ascended in various sections, the material was always that of the disaggregation of granitoid gneiss. Finally, I did not find at this place a single exposure of basic rock." The numerous specimens that have come to hand showing foreign material cemented to the iron by the limonite crust formed by its alteration are in accord with this description, indi-