ENZYMES OF ASPERGILLUS ORYZAE AND THE APPLICATION OF ITS AMYLOCLASTIC ENZYME TO THE FERMENTATION INDUSTRY

By Jokichi Takamine

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HISTORY

Aspergillus oryzae, an insignificant species of fungi, belonging to the genus *Aspergillacea*, plays an important role in the national economy of Japan, on account of the particular enzymes it generates during its growth. Other species of the same genus are also largely employed for production of various dietary articles in almost all countries of the Orient besides Japan. Nevertheless, its utilization in Occidental countries is singularly lacking. Calmette and Bodin's investigation on amylomyces with a view to utilizing it in the spirit industry is an isolated instance in Europe, and their process, known as the amylo-process, has been in operation in France since 1891.

In 1891, I made an arrangement with the Distilling & Cattle Feeding Co., of Peoria, Ill., and carried out on a practical scale the application of the *Aspergillus oryzae* to the American Distillery. My experiments, which ran for a couple of months on a 2000 bushel scale at the Manhattan Distillery, were partially successful, but unfortunately the process did not attain general recognition of its merit, because it still lacked means to overcome various impediments due to trade conditions and difficulties in adapting the process in the new field of application. I did not forsake the investigation after this first trial, but became even more enthusiastic about perfecting the method. Improvement after improvement was added and I now believe that I can soon demonstrate its usefulness.

For many centuries *Aspergillus oryzae* has been employed in Japan for varied purposes: Sake or rice beer, Soy and Miso are the products which are made by the use of this fungus. The fermentation of Sake for the fiscal year of 1912 contributed to the national treasury the goodly sum of $41,974,630 revenue. The tax on the production of Soy (Bean sauce) amounted to $2,048,141. The total cost of both and other articles produced by aid of the fungus in question is put at an aggregate of $200,000,000. It will thus be seen how important a role the fungus is playing in the national economy of Japan. If we add to the above statement the products and cost of the articles which are put out through the useful services of this fungus, or other species, rendered in all the countries of the Orient, the grand total is an enormous amount. Curiously enough this tiny and important hustler has scarcely attracted attention in the Occident, and this fact made me determine to work for its introduction to industrial use in the United States.

Scientifically, *Aspergillus oryzae* has attracted the attention of Occidental investigators as far back as 1875. Prof. Kozai, of Tokyo Imperial University, reviewed[1] the literature regarding the early investigations on the subject of *Aspergillus oryzae* and its industrial applications, and gives credit to Hoffmann and Korshelt as the first writers upon the subject. Korshelt[1] made an important contribution to the knowledge of the fungus in Europe. His report was upon Sake fermentation with special reference to an amyloclastic enzyme which occurs in the culture of the fungus on rice and which he named Eurotius. In his "Chemistry of Sake Brewing," Atkinson discusses the function of the enzyme just named. The fungus was then known as *Eurotium oryzae*, being first identified by Ahlburg in 1876 but Cohn later investigation led to renaming it *Aspergillus oryzae*.[2] It was re-examined by Bosgen, Schröter and later Wehmer who gave full morphological descriptions of it. O. Kellner and his pupils' investigations on invertase amylase and maltase are worthy of note. Controversies then existed with regard to the function of *Aspergillus oryzae* in Sake fermentation. It was thought, on the one hand, that the conidia (spores of the fungus) may transform, under certain favorable conditions, to peculiar yeast cells whose action induces the saccharified mash of rice to Sake, containing 10–15 per cent alcohol. Korshelt propounded this theory but was opposed by Atkinson. I, myself, confess that I shared the same view with Korshelt, but later was led to abandon it. Juhler, confirmed by Jorgenson[3] and Hansen,[4] also gave opinions agreeing with the transformation theory. Kozai and Yabe[5] showed that Aspergillus and Saccharomycetis have nothing in common. Klocker, Schioning, Seiter and Sorrel finally established the error of the transformation theory.

Acclimatization of Aspergillus Oryzae—For a few years I have been working on acclimatizing *Aspergillus oryzae* to various kinds of antiseptics. The art of acclimatizing fungi to antiseptics is not new, *e. g.*, the growth of yeast in a medium containing fluoric acid or other antiseptics has been tried and also put into practice. Effront's attempt to increase fermentation products in the distillery by means of his process is well known. It consists of acclimatizing yeast to hydrofluoric or some other inorganic acid and then employing it in a mash containing the acids so that yeast can multiply without being disturbed by the various bacteria which gradually infect the mash. So far as I know, nobody has yet tried to acclimatize *Aspergillus oryzae* for practical purposes. This is because the utilization of this interesting fungus is very little known, or is ignored in the U. S. and Europe, while the use of antiseptics is rather unnecessary in the fermentation industry of the Orient, since it prefers mixed culture to the pure, on account of the flavor or bouquet of products still thought to be imparted from a mixed culture.

Since my object in producing the culture of *Aspergillus oryzae* is chiefly concerned with the utilization of its amyloclastic property developed during its growth and multiplication, it matters little whether the culture medium employed contains antiseptics

[1] *Centralblatt für Bakteriologie*, **6** (1900), No. 12.

[1] *Mittheilungen deutschen Gesellschaft für Natur und Volkerkunde Ostasiens zr Tokyo*, Heft 16.

[2] "Wakreobericht der Schleisiochen Gessellschaft für Vaterl.," Kulter, Bd. LXI (1883), p. 226.

[3] *Centralblatt für Bakteriologie*, II, Bd I, pp. 16, 326.

[4] *Loc. cit.*, p. 65.

[5] *Centralblatt für Bakteriologie*, II Abt., Bd. I, p. 619.

or not, provided I can get the culture which is possessed of maximum enzymic function. To the growth of *Aspergillus oryzae* on wheat bran as a culture medium I gave the convenient name of Taka-Koji and have employed it for a number of years to distinguish it from that known in Japan as Koji which is a culture on steamed rice.

Taka-Koji is designed for a substitute for malt as an amyloclastic agent in varied fermentation and other allied industries. Its proposed use is encouraged by the fact that the cost of malt is subject to fluctuations according to the crop conditions of barley while bran is exempt from similar market conditions. Besides, the transformation of bran into Taka-Koji can be accomplished in 48 hours, while malting needs three or four times as long for completion of the process.

The making of Taka-Koji, as it was formerly practiced, was described by myself[1] some years ago, before the New York Section of the Society of Chemical Industry.[1] Later, several improvements were made and quite recently a radical departure was made in the mode of effecting the culture. I will, however, describe briefly the process as practiced some years ago, so as to facilitate my exposition of the subject matter.

The process consists of first moistening and then steaming wheat bran, so as to sterilize the material and at the same time to gelatinize the starch. After cooling the steamed mass down to 40° C. a small quantity of the spores of fungus are intimately mixed with it. It is now carried into a room where the floor is cemented, and spread in a thin layer of about $1^1/_2$ inches thick. It is still better if put into a number of trays with wood or metal frames, and provided with a false bottom of wire netting, fine enough to hold the particles of bran. The layer can be made in this case a little thicker since air supply can be obtained from both top and bottom. Such trays are placed upon a specially constructed rack to hold the trays one above the other and about 2 inches apart. The temperature of the room is kept at about 30° C. at the beginning by means of opening steam jets direct into the space. This also keeps the room moist and warm. Within 16 to 18 hours the fungus commences to multiply, which action is easily followed by the gradual rise in temperature in the room. From this time on steam is turned off gradually and at last entirely shut off. After 20 to 24 hours from the time of inoculation the growth and multiplication of the fungus becomes so vigorous that it is necessary to cool the room by the introduction of fresh, cool air. Carbon dioxide resulting from the vigorous growth of the fungus becomes very conspicuous and the room needs the renovation of air which is effected by the air draft introduced for cooling purposes. Care should be taken that the air is supersaturated with moisture, so that the culture medium can always be kept from drying. The temperature of the room often goes up as high as 40–42° C. As long as the growth of fungus is comparatively pure and the mass is kept moderately moist to furnish the necessary amount of water for the culture, such a high temperature does not interfere with the generation of amyloclastic enzyme in fungus cells and its secretion into the medium. The optimum temperature of the fungus growth lies, however, between 30–35° C. It is, of course, desirable to keep the temperature as nearly as possible between these limits. For 8 or 10 hours the multiplication of fungus is most vigorous and there high temperature prevails. Then a gradual decrease in temperature is noticed until the culture medium is replete with the mycelia of fungus within 48 hours from the time of inoculation. At this point vigorous and numerous conidiophores are already to be seen with yellow or greenish yellow conidia (spores). The diastatic strength of Taka-Koji has now reached its maximum and the Taka-Koji is ready to be taken out for use. When the vigorous growth of fungus has ceased the medium is liable to be easily infected with injurious bacteria which may destroy the diastase already generated. It is, therefore, necessary to conduct dry air into the room where Taka-Koji is manufactured, so that it shall be dried and made exempt from destructive infection. When the moisture of Taka-Koji is reduced to 10–15 per cent, it is immune from infection of bacteria and can be kept for several months. By use of antiseptics such as formaldehyde, benzoic or salicylic acid bacterial infection of Taka-Koji can be easily avoided.

While mould fungi in general can tolerate quite a quantity of antiseptics, nevertheless, if they are acclimated to same such toleration will attain to a conspicuous degree. One part of formaldehyde to 2500 parts of a culture medium does not considerably hinder the development of fungus. As to benzoic or salicylic acid, one part to 300 parts of a medium is almost immune to fungi. I had *Aspergillus oryzae* acclimated and the result of using it enabled me to obtain Taka-Koji 100 per cent stronger in amyloclastic power than the product obtained by the old process.

DRUM EXPERIMENTS

Encouraged by this result, I thought of growing the acclimated fungus on the culture medium placed in a cylinder similar to a pneumatic malt drum. This is not a new idea. I carried out a series of experiments nearly twenty years ago, but then without the use of the acclimatized variety of *Aspergillus oryzae*. My experiments were then a total failure, which made me think the fungus could not grow on the material in motion. If we consider the case of sprouting barley in a pneumatic drum, it is, of course, seen that the development comes from within, where the vitality of the seed resides; hence the slight impediment, due to frictions between the moving mass, is not sufficiently severe to totally inhibit the sprouting of the grains. The case is different when the fungus is grown on the culture medium in motion, since it must develop and multiply where frictions must always be injuring delicate mycelial cells of fungus. In my early experiments, it was noticed that the fungus shows an initial growth indicated by the rise of temperature and also observed under the microscope, but it gradually retrogrades. Meanwhile, bacterial infection sets in and totally destroys the fungus growth.

[1] *Journal of Applied Society of Chemical Industry*, London, Feb. 28, 1898.

The subject once almost forgotten, returned to my mind as I said above, and I decided to repeat experiments again. A small experimental cylinder revolving a few times per minute was made out of a Mason jar and clock mechanism. About 30 grams of steamed bran were put in it and mixed with the new variety of acclimated fungus and the whole was kept in an enclosure maintained at 30° C. Unexpectedly a fair result was obtained in which I noticed a very interesting fact, namely, one side of the bran particle is covered with the fungus. It is the inner side of the bran. This side is coarse in structure and is rich in starch and protein matter. If the bran is moistened and steamed, this particular side shows a tendency to curl inward and assumes the shape of pericarp previous to its severing from the kernel. When each particle of the bran has curled, the inner side is naturally protected from the friction between particles when they are put in cylinders and motion is imparted to the mass by revolution.

My success and observations encouraged me to construct larger apparatus for 100 grams, 1 kilo, 5 kilos, 20 kilos and finally 70 kilos capacity. I was very glad to confirm in every experiment my belief that the process of making Taka-Koji in a pneumatic drum is feasible. Therefore, I ordered a drum of about 4800 pounds capacity for Taka-Koji manufacture, which is the size for an 8-ton malt drum. In this drum many new devices were introduced, but to my grief, such elaborate devices became in fact stumbling blocks and the experiments were far from successful. The new devices were taken out one after another until at last the drum remained as simple as that of my own laboratory make. At length, after repetitions of more than fifty experiments, I was enabled to obtain a fairly good outcome and was able to show that the process could be accomplished successfully on a large scale. The first drum became so dilapidated from the alterations and changes that I had to order the second apparatus. It is now ready and I am soon to try it out. Its success is hardly to be doubted.

The drum consists of a huge, plain iron cylinder provided with inlet on one side and outlet on the other, through which air can be passed by means of a suction fan located on the outlet side. The apparatus is furnished with mechanism to turn the cylinder at the rate of once per minute. An iron pipe runs through the center of the cylinder independent from it. It branches out in several places along the whole length, each branch ending in a spray nozzle, through which water or steam can be turned upon the material. The Koji manufacture in this drum is carried out as follows: The wheat bran is dumped in, then the necessary quantity of water and finally the steam is added, the mass being put in motion by revolution of the drum. As soon as the mass is properly steamed, it is cooled to 45° C. cool moist air. Into this mass an aqueous solution of antiseptics and then spores of the fungus suspended in water are sprayed. The temperature of the mass is generally at 38–40° C. The drum is now brought to a standstill and left for about 12 hours, when the gradually decreased temperature again commences to go up. This means initial growth of the fungus. The drum is now put in motion and a slight air current is passed over the mass, care being taken that the temperature should not be lower than 30° C. At the eighteenth hour the temperature shows a tendency to rise quickly and at the twenty-fourth hour it reaches its maximum. The force of the air current is increased accordingly to keep the temperature at about 38° C. A suction pump of 1600 to 1800 cubic feet capacity per minute was put in full action yet the mass of 2400 pounds was barely within the limit of 38° C. According to my small experiment, a higher temperature of 42° C. did not impair the diastatic activity formed by the growth of the fungus; but if it is kept within 40° C. it is so much better for the process, that the air is previously cooled and saturated with moisture by drawing through a freely sprayed coke tower. In 48 hours the process is concluded.

The Taka-Koji manufactured in the drum is more compact and glossy than that grown otherwise. The inward side of the bran is covered with a felt-like growth of mycelia which do not branch out many conidiophores bearing spores; hence the color is whitish.

The labor needed for this process is reduced to one-sixth of that necessary in the old process. The saving of space is also considerable and the quality of Taka-Koji is excellent. Thus the process promises well for furnishing a substitute for malt in alcoholic fermentation and other industries, where amyloclastic enzyme is required.

Dr. Niels Ortved, Chief Chemist of Hiram Walker & Sons, of Walkerville, Ontario, Canada, kindly carried out a series of experiments in his distillery and published his results in his paper contributed to the last Congress of Applied Chemistry, held here in New York. To quote briefly, he said:

"On account of the numerous great variations in the price of barley malt (in two consecutive years the price varied 100 per cent), it would be of great value to the distilling industry if a converting medium of moderate and more uniform price could be employed instead of barley malt. Eliminating, therefore, the different grains as a source of converting medium, I turned to the diastase produced by a microörganism, the *Aspergillus oryzae*. Takamine was the first to introduce the Koji process in America. As far back as 1889 he advocated the use of Koji in the distilling industry. Instead of growing the fungus on rice, Takamine employed a material far cheaper for this country, namely, wheat bran. An extract of the wheat bran, on which the *Aspergillus oryzae* had been allowed to germinate, contained the diastase, produced by the Aspergillus, and this extract was mixed with the mashed grain, bringing about the conversion of the starchy materials. Lately, I understand, he has succeeded in adapting a modification of the Galland-Henning malt drum system to his process. This should be a great improvement over the old floor system, in so far as it makes it possible to work under absolutely sterile conditions. For my experiments I decided to use the Taka-Koji itself instead of the

diastatic extraction of same and add it to the mash in the same way as malt. Before beginning the practical experiments in the distillery, laboratory experiments were conducted on a small scale to ascertain the amount of Taka-Koji which was necessary to convert a certain amount of starch into sugar, and also the optimum temperature at which to conduct the conversion. It was found that 4 g. of Taka-Koji was sufficient to give a complete conversion in a mash made from 96 g. of corn and rye, the corn containing 15.0 per cent of moisture and the rye 14.0 per cent. Three experiments were made in the distillery. For the first experiment only a 14 gallon can was used and a portion of our ordinary mash from the mashtub was employed, the mash being taken from the main mash just before malt was going to be added for conversion. The second experiment was performed on a somewhat larger scale. Instead of using mash material from the mashtub, the mash was made separately. It consisted of 500 kg. altogether, of which 20 kg. were Taka-Koji. The third experiment was performed on a good-sized working scale. Two mashes, each consisting of 3,401.94 kg. (of which 131.5 kg. were Taka-Koji), were prepared. The two mashes were filled in Turn No. 25 of Friday, May 26, 1911. Turn No. 25 was distilled separately and the yield was 36 liters of 100 per cent alcohol per 100 kg. of mash material, just a trifle higher than the yield of the other mashes which were made the same day. In judging the adaptability of Taka-Koji for use in distilleries several questions must be asked and answered:

"Is Taka-Koji capable of giving a complete conversion of the starchy materials in the mash?

"Yes, 4 per cent of the air-dried Taka-Koji will in 15 to 20 minutes give a complete conversion of well prepared mash material.

"Is the fermentation a satisfactory one?

"While it is accompanied by a strong odor, which is prevalent in the fermenting room, the fermentation, however, is very rapid and complete, and on this account should give rise to the least amount of infection.

"Is the yield of spirit satisfactory?

"Yes, the yield obtained was a little higher than the yield gotten from the barley malt mashes, although the total fermentable extract available in the mash material was less. The yield of 36 liters of 100 per cent alcohol per 100 kg. of mash material is of course only a comparative yield. In distilleries which employ cookers and boil the corn under pressure, a higher yield would naturally result.

"Therefore, I should say as a final conclusion that in distilleries which make commercial or potable neutral spirit, the Taka-Koji process could be introduced to advantage. Aside from a probable higher yield in spirit, the saving in malt bill would be worth while in years with normal malt prices and very considerable in years when the malt prices become abnormal."

TAKA-DIASTASE

An aqueous extract from the Taka-Koji can be easily made by percolation and an enzyme can be precipitated by adding alcohol to such extent as to contain 70 per cent by volume of same in the mixture. The precipitate is dehydrated by means of strong alcohol, dried and powdered. It is a white or yellowish white powder of hygroscopic nature. It is marketed in this form for medical use under the name Taka-Diastase.

Though known as an amyloclastic agent, it contains various enzymes; nevertheless, amyloclastic and proteolytic enzymes predominate. O. Kellner and his pupils early reported[1] the presence of invertase maltase beside amylase. Newcombe[2] established the presence of cytase, while Aso reports, in a Bulletin of the Agricultural College of Japan, the presence of orydase in Koji extract. According to Brunstein, Koji contains an emulsin-like substance that decomposes helicin into salicylic aldehyde and glucose which former is later oxidized into salicylic acid; amygdalin is decomposed into cyanhydrin and glucose, which former is later oxidized to mandelic acid.

Investigations on peptase and ereptase carried out by S. H. Vines[3] are replete with interesting observations. He found that Taka-Diastase contains the above enzymes and effected the separation of one from the other. By treating Taka-Diastase with 50 per cent ethyl alcohol and leaving it over 48 hours ereptase went into solution while peptase remained behind. Equally valuable and interesting are researches of J. Wohlgemuth.[4] He states that Taka-Diastase contained an enormous amyloclastic function; the valuation of same according to his method showed to be $D^{38°}_{24\,h} = 62{,}500$ ("D" Diastatic power digested at 38° C. for 24 hours). Testing tryptic action of Taka-Diastase he found that one gram corresponds to nearly 100 cc. of the pancreatic juice of man as well as dog; hence he recommended the use of Taka-Diastase for therapeutics in cases of general debility. Wohlgemuth also proved the presence of maltase, chimosin, ereptase, and lipase in Taka-Diastase.

Quite recently, Kita[5] reports the discovery of a specific enzyme in Taka-Diastase or in a Koji extract, whose function is to transform starch direct into glucose. He doubts very much the opinions entertained by preceding investigators that Taka-Diastase first transforms starch to maltose and that the latter is converted to glucose by the action of the maltase present. His experimental data have not yet been confirmed by any other experimenter. Such an enzyme as Kita reports probably exists but confirmation by more exhaustive proofs than he has furnished must be had.

The resistance of amyloclastic enzyme of Taka-Diastase toward acid is reported by many investigators. This fact becomes very significant when applied to the practical conversion of grain mash. In a grain mash where 10 to 15 per cent sugar is to be finally produced an addition of mineral acid, say sulfuric acid, to an amount of 1 part to 2000 parts of mash accelerates the diastatic action at least 10 to 15 per cent. An equivalent of hydrochloric acid gives a

[1] *Zeitschrift für physiologische Chemie*, **14**, Part III.
[2] "Annals of Botany," **13** (1889), No. 49.
[3] *Ibid.*, **24** (1910), No. 93.
[4] *Biochemische Zeitschrift*, **39**, Parts 3 and 4.
[5] THIS JOURNAL, **5** (1913), 220.

similar result. Malt diastase is entirely inactive with such a high percentage of acid in mash.

Taka-Diastase possesses an important property as a medical agent; *i. e.*, it is more stable than the diastase of malt. The latter loses its activity gradually and within several months its activity dwindles, while Taka-Diastase remains almost unchanged for several years. How this stability is imparted to it is a subject full of interest for investigation. Taka-Diastase contains generally 10 to 15 per cent of ash; this can be reduced to 4 to 5 per cent by reprecipitations but the activity of the enzyme does not increase even by this apparent purifying process but on the contrary a loss of activity is occasioned in most cases.

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ALCOHOL IN THE MANUFACTURE OF PHOSPHORIC ACID AND PHOSPHATES

By PAUL J. FOX[1]

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The increasing tendency in the fertilizer trade in this country to produce a concentrated complete fertilizer makes the question of the production of cheap phosphoric acid of special interest. Whatever process of separation is used, it is clear that the original source can be only the natural tri-phosphates of lime—phosphorites, apatite, and the rock and pebble phosphate of the sedimentary beds.[2]

For the separation of the phosphoric acid, a large number of methods have been patented. It is not the writer's intention to review or even enumerate these methods. He desires merely to make a suggestion.

It is difficult to see, when the energy relations are considered, how anything cheaper can be found than sulfuric acid as the prime separator of phosphoric acid from calcium phosphate. When we consider that sulfuric acid is in part formed by the combustion of a substance very common in nature and very cheaply obtained, and moreover is a product which must be manufactured in some cases to avoid damage to vegetation, it is clear that with the progress of chemical technology, we may expect the present price of about four dollars and a half per ton to go down rather than up.

In view of these considerations, it appears that the cheapening of the production of relatively pure phosphoric acid is rather a question of extraction than of some other method of setting free the phosphoric acid from phosphate rock. The writer suggests the use of denatured alcohol for this purpose. The plan would be to treat the phosphate rock with the theoretical amount of sulfuric acid of about 50° Baumé, taking care to bring about thorough incorporation of the acid and consequent decomposition of the rock, by mechanical mixing. The mixed mass of phosphate rock and sulfuric acid is then leached with denatured alcohol, the residue being filter-pressed. This yields a pure alcoholic solution of phosphoric acid, the sulfates, undecomposed phosphates, etc., being left behind.[1]

The most favorable strength of alcohol, whether 95 per cent or more dilute, to be used in the process, would have to be determined under factory conditions, and would depend on the subsequent treatment adopted. The same remark applies to the sulfuric acid.

The method involves nothing new in theory, but so far as the writer knows has no application on an industrial scale. Besides the obvious advantage of yielding a phosphoric acid substantially free from calcium, the plan offers the following further features: If it is desired to evaporate the extract to produce pure phosphoric acid, the specific heat of alcohol is only 0.5452 to that of water as unity. The boiling point of alcohol is 78.4° as against 100° for water. The latent heat of vaporization of alcohol is 206.4° (at 78° C., Schall) as against 536 (at 100° C.) for water. The figures for specific heats and latent heat of vaporization refer, of course, to unit weight. When we consider unit volume, we see that the thermal capacity of a given volume of alcohol is (taking density of alcohol at 20° = 0.789) only 0.430 to water as unity, and the latent heat of vaporization similarly computed is 0.304 to water as unity.

Another phase of the question is of interest. In case it is desired not to separate the phosphoric acid, but to prepare immediately a concentrated fertilizer of saline character, many double decompositions and precipitations can take place in alcoholic solution which are impossible in aqueous solution except at a great concentration. For example, various potassium and ammonium phosphates may be more easily precipitated in alcoholic solution than in aqueous.

In particular various phosphates can be precipitated by merely adding aqueous ammonia, in *slight* excess, to an alcoholic solution of potassium chloride and phosphoric acid, or better by conducting in gaseous ammonia. The double decomposition depends on the solubility of potassium chloride in alcohol of various concentrations and the insolubility of potassium and other phosphates in that medium. Again, by merely digesting solid potassium chloride with alcoholic phosphoric acid, potassium phosphate results, though the progress of the double decomposition is limited by the hydrochloric acid set free. The progress of the double decomposition may be followed in a roughly quantitative manner with a microscope provided with nicol prisms, as the potassium chloride is isotropic and the potassium phosphate anisotropic. If the hydrochloric acid thus set free, however, is neutralized by aqueous or gaseous ammonia, ammonium phosphate and potassium phosphate are precipitated, certainly as a mixture of two or more salts and possibly in part as

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[2] It is not meant to imply that there is any such definite chemical compound as calcium tri-phosphate. The material of approximately this composition is probably a solid solution. See Cameron and Seidell, *Jour. Am. Chem. Soc.*, **27** (1905), 1503; and Cameron and Bell, *Ibid.*, p. 1512. Apatite always contains chlorine or fluorine. See Cameron and McCaughey, *Jour. Phys. Chem.*, [5] **15** (1911), 463.

[1] Dr. Cameron has suggested that a plan might be worked out whereby the phosphate rock would be treated in one process with a mixture of alcohol and sulfuric acid.