urines at 18° and at 37°, their content of alkaline and acid sodium phosphate and uric acid, and the amount of uric acid precipitated on cooling, in order that the condition in urine be made still clearer, and that we may be able to predict quantitatively from the chemical composition and acidity of the urine, the effect of a certain amount of alkali on a urine and the amount of uric acid which will precipitate spontaneously on cooling. We can then, perhaps, do something for those suffering from uric acid calculi and gravel. Later we hope to study the blood and tissue fluids by physico-chemical methods. This rich and unexplored field must certainly offer a good harvest to the physiological chemist. The results will, perhaps, throw light not only on gout but on many other of those diseases which are classed as disorders of metabolism.

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THE GERMINATION OF BARLEY.

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ACCORDING to the prevalent theory, the germination of the barley is caused largely by the action of enzymes in the presence of oxygen, moisture, and a suitable temperature.

Of the enzymes contained in the barley, the diastase and the peptase have been most thoroughly investigated. The diastase changes the insoluble starch into soluble sugar, and the peptase is supposed to change the insoluble albumen into soluble, coagulable albumen, albumoses, peptones, and amido-bodies.

Late researches, however, have made the existence of enzymes in the barley rather doubtful, and more than one investigator has failed to detect enzymes in appreciable quantities, even after the steeping of the barley. It is only after the growth is well on the way that the enzymes make their appearance in quantity, and they, therefore, seem to be rather the consequence than the cause of the growth.

I do not thereby deny that the enzymes are indispensable to the germination, but claim that they are not the primary cause of the growth, and that some other agent must be looked for

which releases the energy stored in the barley by the mother plant in the form of insoluble albumen and starch.

This agent which starts the series of chemical changes that constitute the germination is, in my opinion, the lactic acid-producing bacteria, which are always present in the barley grain under normal conditions.

When the barley is steeped, these bacteria are carried by the steeping water under the hull of the grain. They then develop, feeding upon the ready formed sugar of the grain, and splitting it up into lactic acid. The acid thus produced dissolves the insoluble albumen, and evidently in some way or another sets free the enzymes, which then act upon the albumen brought into solution by the acid, changing it from the colloidal state into diffusible amido-bodies, and at the same time changing the insoluble starch into sugar, thereby supplying the germ with digestible food.

That the insoluble albumen of the barley is not brought into solution by the proteolytic enzyme of the barley, that is by the peptase, has been proved by W. Loé, and I have found that the increase of soluble albumen goes hand in hand with the increase of acidity in the barley. I have, however, as yet no experimental proof of the supposition that the acid produces the enzyme from the insoluble albumen molecule, though many indications point towards this conclusion.

It is a well-known fact that the enzymes act only in slightly acid solutions, at least such enzymes as the diastase and peptase of the barley. If ground malt, for instance, is treated with a 0.01 normal solution of caustic soda, in the proportion of about 1 part of malt and 10 parts of solution, the resulting mash shows a very slight alkalinity. But this alkalinity is enough to render the conversion of starch very incomplete, and also to lessen the total amount of albumen, or, in other words, enough to destroy almost all enzymatic action.

If we then take a ten times stronger solution, or 0.1 normal solution of caustic soda, and steep some barley in this solution, it is safe to say that, even if the enzymes are present in small quantities in the barley, they will be put out of action by such a strong alkalinity, and that, consequently, if the enzymes were the starting factors in the growth of the barley, no growth would follow.

The barley becomes dark brown after twenty-four hours' steeping in such 0.1 normal caustic soda, but when it is placed in the growing-box it gradually begins to pale in spots; after a day or two the rootlets appear, and, as the growth proceeds, the barley regains its normal color.

Even a 0.15 normal caustic soda (0.6 per cent. NaOH) will not entirely prevent growth, but higher concentrations destroy the structure of the barley grain, and a solution of normal caustic soda turns it into a jelly in twenty-four hours.

The bacteria, on the other hand, are not affected by solutions of caustic soda of such strength as 0.15 normal. They develop, produce the acid which not only neutralizes the alkalinity, but renders the contents of the barley of a decidedly acid reaction, and makes growth possible.

The addition of a substance detrimental to the enzymes does then not hinder the growth of the barley, as long as this detrimental influence can be neutralized by the action of the bacteria, and since the action of the bacteria under such abnormal conditions precedes the growth, it is very likely to do so under normal circumstances also.

On the other hand, if we interfere with the development of the bacteria without injuring the enzymes, the result is very different.

As a general rule, the living cell is more sensitive to antiseptics than the enzymes produced by the cell. Utilizing this fact, I steeped barley in water saturated with toluene. Water takes up only a small quantity of toluene, and the solutions used always had a surplus of the oil floating on the top.

A steeping of the barley for twenty-four hours in such toluene water effectually stops the growth, and repeated washings of the steeped barley with pure water does not remedy it.

That the toluene has but a slight action upon the enzymes is seen from the fact that solutions of enzymes can be preserved with toluene for several days without losing their strength, and if malt is mashed with toluene water, the conversion of starch to sugar is as complete as when pure water is employed. The total albumen in such wort containing toluene is slightly less than that of a pure water mash, because the lactic acid bacteria are active in the mash with pure water up to a temperature of 50° C.,

producing lactic acid, and, as a consequence, more soluble albumen, whereas all bacterial action is suppressed in the mash with toluene water.

Destruction of the bacteria of the barley grain inhibits, then, the germination, even if the enzymes are left intact.

It is, however, only the start of germination that is checked by toluene. If barley is steeped in water and allowed to grow until the blade begins to show, then the growing barley may be sprinkled with toluene water repeatedly, without any apparent injury to the plant, showing how little the enzymes, once formed, are affected by the toluene.

Since the alkalinity of a 0.1 normal solution of caustic soda does not prevent the germination of the barley, it might be expected that an equivalent quantity of ammonium hydroxide would also be overcome by the acid produced by the lactic acid bacteria. But such is not the case.

If barley is steeped in $^1/_{20}$ normal and even in $^1/_{25}$ normal ammonia for twenty-four hours, the grain acquires the dark color of the alkaline reaction, but when placed in the growing-box it does not pale, nor does it germinate. The lactic acid bacteria are evidently weakened by the ammonia to such an extent that they have not the energy to decompose the sugar of the grain. They may, nevertheless, be coaxed into action, if offered a more suitable medium. When 2 per cent. of cane-sugar is added to the $^1/_{20}$ normal ammonia, in which the barley is steeped, and the steeping is carried on at a slightly higher temperature, for instance, 25° to 28° C., then after a couple of days lactic acid fermentation sets in, and the browned barley turns yellow again. If placed in the growing-box, a few kernels may sprout, but most of them are weakened too much by the long steeping. Though the steeping of the barley in dilute ammonia prevents germination, it does not stop the development of bacteria. If the barley after a twenty-four hours' steep in $^1/_{20}$ normal ammonia is placed in a sterilized flask, closed with a cotton plug, putrefaction of the barley rapidly follows.

There exists, then, in the barley grain more than one species of bacteria, which, of course, might be expected.

In order to gain a little more definite knowledge concerning these bacteria, a few wort-gelatine plates were prepared in the following manner:

Some barley was washed by repeatedly shaking it with distilled sterilized water, two kernels were then rapidly ground in a sterilized mortar with some sterilized water and the mixture made up to 100 cc. After thoroughly mixing, 0.1 cc. of this mixture was run into 10 cc. of liquefied wort-gelatine, and a plate made in a Petri dish. The same process was carried out with 2 kernels of the barley after it had been steeped for twenty-four hours in sterilized water, in a sterilized flask, closed with cotton, and also with 2 kernels after twenty-four hours' growth in the same sterilized flask.

The first plate showed a few colonies, the second a considerably larger number, the third had almost too many to be counted, showing the gradual development and increase of bacteria during steeping and growth. The colonies were of two distinctly different kinds. One kind was formed by bacteria which, under the microscope, appeared as short non-motile rods, and these colonies remained elevated on top of the gelatine plate; the other kind of colonies were formed by bacteria of longer rods with a lively motion, and these colonies sank into the plate, liquefying the gelatine, and formed hollows filled with liquid. This liquid colored red litmus paper blue, and evidently contained ammonia.

The fact that barley steeped in dilute ammonia will putrefy but not grow, would then be explained by the presence of these ammonia-producing bacteria. Such bacteria would naturally not be as sensitive to a small amount of ammonia as the lactic acid-producing bacteria; the former would increase, producing ammonia enough to counteract the acid produced by the latter species of bacteria, thus keeping the barley alkaline and preventing the formation of enzymes.

The germ of death lies then in the barley grain side by side with the germ of life, and outside circumstances decide whether the grain will live or die. Should the barley, for instance, be surrounded in the ground by too much of decaying substances, the ammonia produced would cause the barley to rot instead of grow.

It seems then that the higher plant is still dependent upon that simpler form of life from which it has evolved, and that it rises up from and again falls back into that current of bacterial life

which flows forever, and is self-contained to the same extent as it is original.

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THE DETERMINATION OF TOTAL CARBON IN COAL AND SOIL.[1]

BY S. W. PARR.

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WHERE many determinations of carbon are to be made, the ordinary combustion process becomes a very heavy task. The method here offered was developed in the first instance for technical purposes, more especially in the study of fuel economy, but in practical use it has exhibited a degree of accuracy and a range of service which may give it wider application.

The material employed is the residue from the determination of heat values by means of the calorimeter, recently described by the writer.[2] The combustion of organic material is effected by means of sodium peroxide, the charge being contained in a closed bomb or cartridge surrounded by water. The resulting product, sodium carbonate, together with the excess of sodium peroxide is dissolved in a minimum amount of water and boiled for about five minutes to decompose the peroxide and remove all free oxygen. The carbon dioxide in the residue is determined by volume. To meet the conditions as to solution, volume, etc., the following apparatus has been devised. It has been found of much greater accuracy than various forms of alkalimeters, which have been tried for comparison, and its ease of manipulation makes it preferable to the absorption method, especially for technical work.

The holder A is of 200 cc. capacity and contains sulphuric acid of about 1.4 specific gravity. The flask B is of 125 to 135 cc. capacity. The receptacle C is for the solution of the carbonate. P is a jacketed gas burette with three-way cock at O. To operate, the acid is run in from A, filling B completely, and to the zero mark of the burette at O. Exactly 100 cc. of air at ordinary pressure is measured in the burette and this is forced over into the flask B,

[1] Read at the St. Louis meeting of the American Chemical Society.

[2] This Journal, **22**, 646.