The question then arose whether non-legumes could take up and utilise these nitrogen compounds directly, and to solve it pea and barley plants were grown together in sterile sand contained in three-necked Woulff bottles, the pea seeds being inoculated, and each kind of plant being made to grow out through a neck of the bottle. The nutrient solution used contained no nitrogen. The barley plants grew excellently, but in the control experiment, in which the pea seed was not inoculated, they failed to grow. It was also found that barley, as well as peas, grew exceedingly well right up to the flowering or fruiting stage when they were fed with an aqueous extract of a low moorland soil that contained no ammonia or nitric nitrogen; they also grew well when they were fed in sterile culture with peptone (Witte) as their sole source of nitrogen. It is therefore concluded that higher plants can take up and utilise directly organic nitrogen compounds present in soils before their nitrogen is mineralised by bacteria or other micro-organisms.

The nature of the organic nitrogenous compounds that are of value to plants, as well as their percentage utilisation, is now being investigated. Earlier experiments in which red and white clovers were grown under sterile conditions inside glass flasks showed that red clover responded well to hydrolysed casein and to aspartic acid, and less well to ammonium salts and nitrate, whereas with white clover the results were exactly opposite. In these experiments the hydrogen ion concentration of the nutrient solutions containing hydrolysed casein and aspartic acid remained constant, showing that the nutritional effects were due to the amino-acids themselves and not to ammonia that might be liberated by their decomposition. The later experiments, in which the plants were grown with their roots in sterilised nutrient solution or sand and their upper parts in the air, have shown that aspartic acid is a very good nutrient for legumes, but not for *Gramineæ*, and that asparagin is excellent for barley, but much less good for *Leguminosæ*.

The manner in which the plants utilise such organic compounds can at present only be conjectured. On one hand, it may be that enzymes like urease, asparaginase or aspartase liberate ammonia from these organic compounds when they are still in the root system, and that the plants utilise this ammonia to synthesise protein. On the other hand, it is also possible that the amino-acids may be utilised directly for the synthesis of protein.

Enzymes: A Discovery and its Consequences

By DR. E. F. ARMSTRONG, F.R.S.

IT is a hundred years since Payen and Persoz discovered diastase and recognised it as a ferment. To-day the ferments, or enzymes as we have preferred to call them, are in the forefront of interest as the factors concerned in all those chemical changes in the cell which in their totality are termed vital changes: they may indeed compose those invisible genes which make up the chromosomes. The discovery of diastase, apart from its broader consequences, has had a far-reaching effect also in introducing science into one of the oldest industries, one already established on the Nile in the days of the Egyptians, that of brewing: the determination of diastatic power is to-day one of the first exercises which is performed on the new season's crop of barley and malt. The studies on diastase made in the cause of brewing have in turn enriched chemical science, and there is a notable list of eminent brewers' chemists to inscribe on the roll of honour of enzyme pioneers.

The discovery of Payen and Persoz in 1833 followed an observation by Kirchhoff that germinating barley grains contained a principle capable of converting starch into sugar, a power which Leuchs discovered was also possessed by human saliva. Payen and Persoz found out how to extract ground germinating barley with water and, after filtration, precipitate a white flocculent material by means of alcohol, which when dried and redissolved turned starch into sugar. Their procedure was that which is followed to-day in extracting an enzyme, though our hands have gained not a little cunning in concentrating the enzyme and freeing it from this and that impurity, largely as the result of methods devised and elaborated by Richard Willstatter, who was Davy medallist of the Royal Society in 1932. The active material was named 'diastase' by its discoverers. It was found by them to be present in other cereals during germination, and during the 'seventies, numerous workers established that diastase is very generally present in vegetable cells so long as the latter are living. In the meantime, Miahle had made a diastase by adding alcohol to saliva, and other observers found it in the animal pancreas. Animal diastase is generally referred to as ptyalin in the literature and in many textbooks the term amylase is preferred to diastase; a proper sense of historical loyalty should, however, make us adhere to the older term.

In any mention of the diastase story, the work of Horace Brown and Morris in connexion with the translocation of starch comes immediately to mind. The formation of diastase in foliage leaves during darkness and its conspicuous diminution during bright sunshine, were discovered by them and used to explain the behaviour of starch, which varies in amount in the opposite sense during the twenty-four hours. Classical also are the studies of Cornelius O'Sullivan at Burton-on-Trent of the action of malt diastase on starch, published in 1876: they mark a beginning of the efforts to establish the kinetics of enzyme reactions and also to unravel the structure of starch itself.

We are still working at this problem—starch—nearly sixty years later, without any certainty that the goal is in sight, though the work of Haworth is definitely carrying us forward: it is successful perhaps because he is taking nothing for granted and is insisting on the need of relying only on the evidence furnished by quantitative laboratory work. The newer branches of chemistry, X-ray analysis and colloid conceptions, have been called in to help establish the size and form of the starch molecule, but the results obtained with their aid are definitely of a more speculative character.

Many have toiled, others may be said to have toyed, with the kinetics of enzyme action, but the chief result has been to introduce confusion into what at bottom is simple, once the disturbing factors have been eliminated. We would rather write of diastase in relation to starch from the point of view that the enzyme itself must be a composite material. The evidence is becoming more precise that the maltose units in starch are not all joined together in the same manner and that there are perhaps two different and separable diastases, which act differently upon starch, one producing the α-form of maltose, the other the β-form.

Starch is the largest item in our dietary, it is the most common reserve material of plants, in the industrial arts it has a thousand and one uses, it is the basis of beer, its study forms the most intriguing chapter of vital chemistry. Is it not remarkable that there is still more that is obscure than is definite in our knowledge of the structure of its molecule and its microscopic and macroscopic make-up?

Following the discovery of diastase, other enzymes attacking substances other than starch have been found in both plants and animals. Studying the behaviour of first one and then another of these, it has become possible to establish their general properties, in particular their high degree of specificity, the ease with which their activity is destroyed or modified by chemical or physical alteration. They have very many properties of the living unit, except the all-essential one—they are incapable of self-duplication. The enzyme particles are non-resolvable under the microscope. Jerome Alexander has emphasised their resemblance to the self-reproducing ultramicroscopic genes of which the chromosomes are thought to contain immense numbers, and there are adherents for the idea that the genes may be regarded as enzyme-like catalysts, capable of self-duplication, which dominate by their catalytic control of chemical changes what is to happen in their neighbourhood. They are capable both of self-duplication and of initiating and controlling each and every chemical reaction in the cell, the only proviso being that the necessary raw materials for the catalysed reactions are provided in the milieu and that the supply of the raw materials and the removal of the products of metabolism proceed at suitable rates of speed. Each gene may be supposed to catalyse one reaction only and its catalyst to be composed of a single molecule; in practice, there will always be large groups or strings of genes. Our methods of isolating and purifying or concentrating enzymes, after the breakdown and killing of the cell, aim at the selection of the catalyst derived from a single gene: it will be obvious how Utopian this quest must be.

The great majority of enzymes act, like diastase, as catalysts of hydrolysis; that is to say, the elements of water enter into the reaction, which indeed commonly takes place in the presence of an excess of water. The amount of water in the cell, its relative abundance in a free and active state, unattached to or uncombined with other substances, is an extremely important, if not the determining, factor in governing the direction and position of the final equilibrium, for it is in the highest degree probable that the same catalyst which favours hydrolysis can also, under other conditions, accelerate the reverse change, that of synthesis. The factors which control the activity of a cell are the catalyst, a supply of raw materials and the presence of more or less active water. When there is excess of water the reactions are those of hydrolysis; when there is a scarcity of water and the cell sap is concentrated or saturated, which is another way of expressing that the water molecules are all occupied, the reactions are those of synthesis.

There is now a consensus of opinion that enzymes consist largely of organic matter made up of at least two units, the carrier, forming a large colloid particle, and the other, the active substance, which is held or oriented in an efficient position and stabilised by the carrier. Without the carrier the catalyst cannot function, and consequently, when the process of purification leads to a disruption of the carrier, the catalyst loses its activity. Such a statement is to some extent incompatible with the possibility of isolating a definite crystalline enzyme, as Northrup claims to have done for pepsin and trypsin; more enlightenment is obviously required on this point before a final opinion can be pronounced.

The enzyme study began with Payen and Persoz; its first century of endeavour may be said to have ended with the anniversary address of Sir Frederick Gowland Hopkins to the Royal Society last November, wherein he summarises the present conception of an enzyme and emphasises the importance of the conviction which is now general amongst biologists that modern chemical methods, starting with isolated reactions made with tissue extracts and passing to studies of other tissue extracts in which the progress of a variety of reactions is studied, do in fact give a picture of the reactions which are taking place in intact or still living tissues or cells. In his words, we have escaped from the dilemma voiced in earlier dogma, that since chemical methods convert the living into the dead, they can do nothing to elucidate the dynamic events of life.

Such is the progress of the century. Since the discovery of the first enzyme we have travelled far to form a conception of an enzyme as an active spot of specific configuration at part of the surface of the colloid particle. Progress has been slow, largely because chemists and biologists have not been ready for such a conception and all that its application involves. Now at the beginning of the second century of enzyme study, things are different; the authoritative summary by the president of the Royal Society in closing the old period serves to open the new at a moment when the army of research workers is ready to apply it.

Obituary

Prof. C. Correns

THE death on February 15 of Prof. Carl Correns at the age of sixty-eight years leaves a gap in the ranks of those who were prominent in the Mendelian investigations from their inception in 1900. Much of his work was in the more obscure and difficult fields of plant genetics, such as self-sterility, variegation and the inheritance of sex. He brought a wide knowledge of plants and a broad biological outlook to bear on these and other problems, and will always be held in remembrance as one of the three co-discoverers of Mendel's principle of segregation in hybrids.

When Hugo de Vries published his paper "Sur la loi de disjunction des hybrides" in the *Comptes rendus* of the Paris Academy of Sciences in March 1900, announcing the re-discovery of Mendelism, based on the study of eleven different species, Correns was stimulated to write immediately, as he says, a paper for the *Berichte der deutschen botanischen Gesellschaft*, which was entitled "G. Mendel's Regel über das Verhalten der Nachkommenschaft der Rassenbastarde", in which he showed how he had independently arrived at the same conclusions as de Vries through experiments with maize and peas. He, like de Vries, at first thought his results and conclusions were new, and then found Mendel's paper of 1866 with similar results and the same explanation of them, namely, the principle of genetic segregation. In the previous year he had published an explanation of the phenomena of xenia in maize and in 1901 he wrote another extensive and classical paper on this subject.

Correns was thus a leading spirit in the rediscovery of Mendel's laws and in a clear statement of the mode of sex determinations in dicœcious and polygamous flowering plants. Important investigations on the latter subject were published in 1907, in which the Mendelian conceptions were applied to the inheritance and determination of sex in plants. This was the period when active discoveries of the sex chromosomes in insects were taking place, and in 1913 he published in collaboration with Goldschmidt a general work on what we would now call the genetics of sex. Several other investigations of sex in plants appeared during the next decade, *Melandrium*, *Silene* and *Rumex* being among the forms mainly studied.

Many of Correns's researches were fundamental and he preferred to labour in fields that were off the beaten track. The number of plant genera with which he worked at different periods was a surprisingly large one. In a series of papers on *Mirabilis* hybrids, beginning in 1902, he showed that when the yellow and white varieties are crossed, the F_1 is rose-coloured with red stripes while the F_2 produces no less than eleven colour types. This was important at a time when it seemed that Mendel's law of dominance might be as fundamental as his law of segregation. The influence on the offspring of the number of pollen grains placed on the stigma was also investigated in *Mirabilis*, and several interspecific hybrids of *Matthiola incana*, *M. glabra* and other species were analysed. Other genera the genetics of which he studied included *Urtica*, *Trinia*, *Hyoscyamus*, *Dimorphotheca*, *Lamium*, *Veronica* (long and short style), *Linum* and *Fagopyrum*.

Correns was interested in the rôle of the cytoplasm in inheritance and published an important paper on this subject in 1909. He studied variegation and its inheritance, finding cases where the development of the chloroplasts was under nuclear control and therefore Mendelian in inheritance, while in other cases the control was cytoplasmic. His paper at the Berlin Congress of Genetics in 1927, of which he was a vice-president, was a masterly summary of knowledge regarding non-Mendelian inheritance in plants and animals, but he was prevented by illness from giving it in person.

So early as 1889, Correns made a study of pollen germination and the pollen tubes of *Primula acaulis*, and much later he showed in *Melandrium*, by placing a few pollen grains on the stigma, that the upper ovules of the flower are generally fertilised first.

Darwin's experiments on self-sterility led him to the view that in such cases each plant, although self-sterile, can be fertilised by pollen from any other plant. This hypothesis of individual stuffs was held until the work of Correns on *Cardamine pratensis* in 1912, in which he found that the offspring of two crossed plants fell into four interfertile groups, and the inheritance of self-sterility could be explained on a Mendelian basis. This conception has since been confirmed and extended in other genera. In 1928, however, Correns found that in *Tolmiea Menziesii* the individuals from a cross were fully fertile with each other, as Darwin had supposed, and it is possible that in this species the determination of the stuffs inhibiting pollen tube growth is not genotypic but phenotypic.