

rad., root of facial nerve; *a.o.t.*, acustico-olivary tract; *viii B. nucl.*, Bechterew's nucleus of auditory nerve; *s.p.c.*, superior cerebellar peduncle.

Plate II.—*a.p.*, anterior pyramid; *f.*, fillet; *c.t.*, corpus trapezoideum; *B.t.*, Bechterew's tract; *sup. olive*, superior olive; *vii nucl.*, nucleus of facial nerve; *v.Asc.*, ascending root of fifth; *c.r.*, corpus restiforme.

Enzyme Action in Lower Organisms. By G. E. Cartwright Wood, M.D., B.Sc.

(Read December 16, 1889.)

The soluble ferments or enzymes have always aroused the deepest interest, partly from the mystery which enshrouded their mode of action, partly from the importance of the processes with which they are associated. The peculiar power which each possesses of decomposing apparently unlimited quantities of a specific medium, without itself being used up in the process, has occasioned the confusion of enzyme action with processes truly vital in their nature. Although this action had only been demonstrated as subserving an alimentary function, its aid was invoked to explain many of the more obscure phenomena of biology. The series of decompositions which carbohydrates may undergo, known as the alcoholic, lactic, and butyric fermentations, were long ascribed to it. Even when Pasteur had proved that these processes were always correlated with a vital fact—the growth and multiplication of living cells—Traube,* Hoppe-Seyler, and Liebig † still contended that these might act only indirectly by the formation of soluble ferments. The analogy between fermentation and the infectious processes is so striking that the latter have long been grouped together under the term zymotic diseases, and these we are every day coming to recognise more and more as parasitic diseases conditioned by micro-organisms. Here again, however, many tend to regard the microbe as not acting directly, but through the production of soluble ferments. The consideration of enzyme function in lower organisms has accordingly another interest than that which attaches to it, as throwing light upon the

* *Theorie der Fermentwirkungen*, Berlin, 1858.

† *Ueber Gährung, Quelle der Muskelkraft und Ernährung*, Leipsic, 1870.

processes of digestion in the higher animals. Upon the view which we take as to its origin and meaning will depend the standpoint from which we regard many important physiological and pathological questions.

Although Duclaux and Hueppe, in their investigations on milk, had suggested the probable existence of soluble ferments, H. Bitter in 1887 first furnished rigorous proof that bacteria produce enzymes separable from the organisms which form them. He managed to kill the organisms by sterilisation at 60° C. without materially destroying their products, and in this way demonstrated that two organisms, when grown on gelatine, produced enzymes which were able, apart from the organism, to liquefy gelatine and peptonise albumen. Sirotinin, in an investigation carried out in Flüge's laboratory, noted incidentally, that culture fluids which had been freed from organisms by means of Chamberland's porcelain filter, still retained the power of liquefying the gelatine. Brunton and Macfadyen have, more recently, communicated the same fact for another organism, and have stated in addition that when grown on starch a diastatic ferment was there produced. My own experiments were undertaken, not for the purpose of demonstrating the existence of such enzymes, but if possible to investigate more minutely their properties. For this purpose the four organisms which form what is known as the cholera group—Koch's cholera bacillus, Deneke's cheese bacillus, Finkler's cholera nostras bacillus, and Miller's bacillus—were selected. These organisms exhibit in all directions a most striking similarity, and the point which I wished to investigate was whether their enzymes could be shown to differ in any way. The reaction in which they would continue to act offered itself as the most convenient test. The method of experimentation pursued was as follows:—A tube containing 20 c.c. of sterilised milk was taken, and to it a certain quantity of acetic acid was added. The fluid was shaken, and then delivered, by means of a sterilised pipette, in equal quantities, into four test tubes. To each of these 1 c.c. of the *sterilised* products of one of the organisms was added, and the result noted. By making a series containing varying quantities of acid the effect on the enzyme action was ascertained. The cholera enzyme was found to be most sensitive towards acid reaction, even very small quantities inhibiting its activity. Deneke came next, while

Finkler and Miller bacilli continued to act in distinctly acid solutions. But the two enzymes which each of these organisms possess seemed not to be equally affected by the acidity. The precipitation of the casein occurred in reactions where the peptonisation was apparently completely inhibited. We must therefore conclude that the enzymes of these organisms are, in each case, distinct, and that the enzymes, even in the same organism, are not alike as regards their capacity of acting under different conditions.

Gelatine and blood serum cultures of the four organisms were found, in no case, to exert any diastatic action on starch. A medium containing starch was then inoculated with cholera bacillus, which after being allowed to grow for some time was sterilised at 60° C. The amount of sugar present was then estimated in a small quantity. By means of a pipette, 25 c.c. of the sterilised culture was then introduced into a starch solution, and incubated for six days, when the amount of sugar was again titrated. The quantity found was not, however, greater than what had been introduced with the sterilised culture fluid, so that no evidence of ferment action was exhibited. Although the experiment was repeated twice, a negative result had, on both occasions, to be recorded. It is possible that the ferment may have been destroyed, or that some condition was present which prevented its action. It appears to me more probable, however, that the power of converting the starch into sugar may here exist only in the protoplasm, it not having yet become developed into an enzyme separable from the organism. It is not necessary to consider the primary processes of digestion as dependent on soluble ferments. The enzyme function should, as Hueppe * has insisted, be regarded as a *secondarily* derived function of the protoplasm. The process of digestion, as observed in its simplest form, is an intracellular digestion, and is not, as Krukenberg† has conclusively shown, dependent on enzymes, but is inherent in the protoplasm itself. The enzymes are merely further differentiations of this primitive power of the protoplasm in the same way as muscle and nerve are to be regarded as represented in the contractility and irritability of a structureless mass of undifferentiated protoplasm. We are accordingly prepared

* *Die Methoden der Bakterien-Forschung*, 1888; "Abschnitte ueber Biologie," *Die Hygienische Beurtheilung des Trinkwasser*, 1887.

† *Vergleichend-physiologische Vortrge*, Heidelberg.

to find very different degrees of development in different organisms, and even in the same organism, as regards the different substances to be digested. Thus cholera appears to digest its proteids by means of enzymes, but the faculty of digesting the carbohydrates appears still to reside in the protoplasm. How far the process is carried out in each case by means of enzymes, and how far, directly, by the protoplasm, must be decided by experiment. Even in the alimentary canal the process is not carried out by ferments alone. Starch is there converted into equal quantities of maltose and dextrine, and the latter is unaffected by the further action of the enzymes; yet as neither of these bodies is absorbed as such, the portal vein containing the carbohydrates only in the form of dextrose, these must undergo their further changes at the hands of the cells. In a similar way lactose and cane sugar are split up by an inversive ferment into equal quantities of dextrose and levulose, and the further transformation of the latter is evidently to be referred directly to the protoplasm.* The greater part of the albumen is undoubtedly peptonised before being absorbed, yet, as Voit and Bauer † have shown, a part may undergo direct absorption. More recent experiments, in cases of artificial anus, where the influence of the ferments from the upper straits of the canal was completely excluded, have shown that egg albumen undergoes direct absorption; and since egg albumen is excreted by the kidneys as a foreign body, the disintegration and rebuilding up which is necessary for its assimilation must be attributed purely to cellular action. In all probability the cells lining the alimentary tract perform a much larger part of the preliminary processes of digestion than is at present conceded to them.

An enzyme is accordingly to be looked upon as a function which has undergone a high degree of differentiation, indeed, as a property which is able to exist and act apart from the protoplasm. As each organism is adapted to special conditions, we should expect the enzymes also to act best under these conditions. It has already been stated that the enzymes of cholera—Deneke, Miller, and Finkler—

* Although the enzyme obtained from yeast can convert only the half of the cane sugar into dextrose, Payen has shown that the living yeast-cells convert the levulose also into dextrose.

† *Zeit. für Biol.*, Bd. v. 562.

exhibit a varying susceptibility to acid reaction precisely as the organisms themselves do. This does not indicate that the organisms are more susceptible to acidity, according as their enzymes are more sensitive to its presence, but that the protoplasm as a whole, and with it the enzymes, is adapted to a certain set of conditions—its usual environment.

This correspondence between the conditions under which the organism exists and those under which the enzymes act, is exhibited even more strikingly by a consideration of their temperature relations. Every enzyme has an optimum temperature at which it works most effectively, and a lower and a higher limit at which it ceases to act. In the case of the cold-blooded animals this optimum temperature is much lower than in the warm-blooded animals. Fick * and Murisier† found that the gastric juice of the frog, pike, and trout dissolved albumen at 0° C. Hoppe-Seyler‡ has confirmed this result, and states in addition that at higher temperatures they did not act so effectively as at moderate temperatures. The gastric juice of a mammal, on the other hand, peptonises best at the temperature of the body, 39° C., and becoming less active at lower temperatures, is finally inoperative at 10° C. Again, while the diastatic ferment of the pancreas and saliva acts best between 37° and 40° C., that of germinating barley, where the temperature is usually much higher, has its optimum at from 54° to 63° C. Micro-organisms exhibit even greater differences as regards the temperature to which they are adapted; some grow and liquefy the gelatine at 0° C., and as is seen in the case of the micrococcus, which causes the phosphorescence at sea, can at this temperature exhibit their characteristic action; § others do not grow at a temperature under 50° C., and at 70° C. continue to vegetate actively.|| In accordance with the adaptation of the organism as regards temperature, the liquefaction of the gelatine, occasioned by a microbe, may vary greatly, even to the point of cessation, with the temperature; but how far this is due to lessened production of the enzyme, and how far to its action being interfered with,

* *Arbeiten Physiol. Lab. Würzburg*, ii. p. 181.

† *Verhandlung. d. phys. med. Ges. in Würzburg*, Bd. iv. p. 120, 1873.

‡ *Pflüger's Archiv*, Bd. xiv. p. 395, 1877.

§ Forster, *Central. f. Bakt.*, 1887, vol. ii. No. 12; Fischer, *Central. f. Bakt.*, 1888, vol. iv. No. 3.

|| Globig, *Zeit. für Hygiene*, vol. iii. p. 295.

has not yet in the case of these organisms been definitely ascertained.

We have just seen that each enzyme has its own most appropriate temperature, and as the organisms vary as regards their natural food, we should expect their enzymes to exhibit great variety as regards their capacity of acting on different media. This is very strikingly indicated by their action on the different sugars. The yeasts as a group are able to attack dextrose, giving rise to what is known as the alcoholic fermentation, and they are also able to act on cane sugar, which is, however, first converted by an inversive ferment into dextrose and levulose. Yet of all the yeasts only three are known which are able to ferment milk sugar, the power of converting it into dextrose not being possessed by the *saccharomyces* as a group, although it is a property very widely distributed among bacteria. Of still greater interest is the varying manner in which the same organism conducts itself towards different albumenoids. Thus, as a very general rule, those organisms which liquefy gelatine are able to coagulate milk, and then peptonise casein which has been separated, but some organisms which peptonise gelatine are without action on milk. Again, we should expect that those organisms which exhibit no enzyme action on gelatine would be inoperative on milk, and although this is usually the case there are exceptions to this rule. It has been already noted that organisms vary greatly as regards the reaction in which they peptonise the milk, and it would appear that they even peptonise it in different ways. Although the vast majority first coagulate the casein and then dissolve it, certain microbes appear to peptonise the casein directly; this would indicate that the process in the two cases is not identical.* Very striking is the way in which the same organism conducts itself to the different albumenoids, gelatin, fibrin, blood serum, and egg albumen. One organism is unable to liquefy gelatine, but peptonises fibrin; another liquefies the gelatine, but cannot peptonise the egg albumen. They exhibit, in this respect, the utmost diversity, each having its own special idiosyncrasies. We have thus seen, in the course of our investigation, that the enzymes vary in every direction as regards the temperature and reaction in which they act, and the nature of the medium on which they act. They must, accord-

* Caneva, *Ueber die Schweineseuchen*.

ingly, be regarded as specific in their nature, depending on the specific nature of the protoplasm of which they are merely further differentiations.

The question now arises, Are the products originating from the action of different enzymes the same? We know, already, that the sugars and peptones, in certain cases, are different, and more exact investigation, coupled with more delicate methods, will, without doubt, in the future, add greatly to the number of these. We should not, then, regard the peptonisation as merely a breaking down of the complex proteid molecule into smaller, more diffusible molecules, which are in this way more easily absorbed. It is also a disintegration into simpler bodies of probably a quite definite composition, which the protoplasm can either convert into colloid catalysable material to be stored up in the organism, or build up into its own proper substance. The protoplasm of bacteria has been shown to exhibit the greatest possible difference towards bodies all but indistinguishable from a chemical point of view. We possess indeed in these organisms one of the most delicate reagents at our disposal in differentiating organic bodies of allied constitution. Thus, to take one example out of many, it has been found that the natural and artificially prepared inulins are treated as distinct bodies by a microbe, the first being assimilated, the latter being left absolutely untouched. It is probable, then, that the process of peptonisation, in which the proteid molecule is, as it were, taken to pieces, is not always the same, but varies according to the specific nature of the protoplasm, in which it may have, afterwards, to undergo a process of building up to form an integral part.

The enzymes have not yet been isolated as chemically pure bodies; indeed, there is good reason to believe that, in most cases, these would be found to consist of several bodies corresponding to different stages of their action. This has been indicated most clearly in the case of the diastatic ferment obtained from malt.* The starch, after undergoing a first change into a soluble form, is converted into a molecule of maltose and dextrine; the latter then undergoes successive hydrations, until 52 per cent. of the maltose which the starch could have yielded, has been formed, at which stage the action of the ferment ceases. If, however, the ferment had been

* *Annales de l'Institut Pasteur*, 1887.

previously heated up to 60° C., the starch is converted completely into dextrine as rapidly as previously, but only 28·4 per cent. of the possible maltose producible from the starch is formed, and this quantity is not increased by the addition of more of the weakened ferment. It would appear, from these and other experiments, that the successive stages in the conversion of the starch into maltose depend on different ferments, which are destroyed, perhaps through coagulation, at different temperatures. Little is known of the products which intervene between albumen and peptone, and nothing of the action which the various secretions exert upon them. Yet a complete knowledge of these, and of the action upon them of the various secretions which are poured into the alimentary canal, is essential to a proper understanding of the process. At present the action of a ferment is always tested on the unchanged albumen, usually egg albumen or fibrin, although, as is probably the case with the intestinal secretion, it may operate only in the later stages in the splitting up of the albumen. This may perhaps account for the want of action of this secretion upon the chief articles of food which so many observers have recorded. The products resulting from the action of the secretions on the different forms of albumen in raw and in cooked condition,—the nature and relative amount of the peptone and bye-products formed in each case,—still require more exact investigation. By a consideration of the relative amount of peptone yielded by each we should be able to form a much more accurate conception of its dietetic value. The nature and amount of the bye-products, and the effect, in this relation, of prolonged action of a secretion, as may frequently occur in gastric digestion, might be expected to throw much light on the effect of diet in such disorders of assimilation as gout.

The enzyme function is, as we have seen, to be regarded as a higher development of a primordial function of the protoplasm, and we have now to consider the question as to whether the degree of development in each organism is a fixed invariable quantity or one subject to variation. It follows, from what has been already stated, that such external conditions as temperature, nature, and reaction of the medium affect its action; we have still to mention here that Flügge* has observed that organisms when grown without the

* *Die Mikro-Organismen*, page 470, 1886.

presence of oxygen—under conditions of *anaërobiosis*—appear then to lose their enzyme function, inasmuch as they then cease to liquefy the gelatine. As great inconvenience results in many investigations from the liquefying colonies destroying the gelatine plates before the other, more slowly-growing organisms, have had time to develop, Chantemesse and Widal * have attempted, by the addition of some substance to the medium, to hold the peptonising power temporarily in check. To facilitate the discovery of typhoid fever bacilli in the presence of other organisms, as in suspected earth and water, they recommended the addition of carbolic acid to the gelatine. Control experiments carried out by me at the request of Professor Hueppe,† indicated that the quantity required to effect this in different species of bacteria varied from 0·01 per cent. to 0·1 per cent. carbolic acid, and that the higher concentrations necessary for the more refractory, entirely prevented the development of more sensitive organisms. Other substances were accordingly investigated for the purpose of selecting one which, while retarding the liquefaction of the gelatine, would still exert no injurious action on the microbes. Of those experimented with, glycerine seemed most suitable for general use, although the influence which it exerted upon this function varied greatly in different organisms. This is explained by the fact, that its action appears to consist not in operating directly upon the enzymes, but rather in offering the microbe a substance as pabulum, which it selects in preference to the gelatine. It has been already mentioned that Brunton and Macfadyen found that an organism produced a peptonising ferment when grown on albumen, but a diastatic ferment on starch, so that these would appear to be produced according to the special temporary requirements of the organism. I had previously observed that all the members of the cholera group, when inoculated on blood serum, produced sulphuretted hydrogen, and at the same time liquefied the medium. If, however, glycerine is added to the blood serum the liquefaction and production of sulphuretted hydrogen appear much later, which is, I think, to be attributed to the organism obtaining the energy which it required, first from the more easily decomposed carbohydrate, and, only when this had

* *Gazette Hebdomadaire*, 1887, page 146.

† *Die Methoden der Bakterien-Forschung*, page 299, 1888.

become exhausted, resorting to the albumenoids present. The degree of retardation in the liquefaction of the gelatine will in each case depend on the protoplasmic idiosyncrasies of the organism as regards the form of nourishment best adapted to its metabolism. It is not improbable that an analogous selective action of the protoplasm may come into play when organisms are grown *anaërobically* on gelatine containing grape sugar, and that the absence of liquefaction in some of these cases is to be ascribed to this.

It is not, however, such temporary abeyances of function that I wish to consider now, but more permanent changes in the organisms themselves as regards this function. The first occasion on which I observed the complete loss of the power of liquefying the gelatine was in the case of an old gelatine culture of Koch's cholera bacillus. The colonies originating from it presented the usual irregular margin and granulated-glass appearance, and, under the microscope, cover-glass preparations gave the characteristic comma form, but it grew in gelatine without any signs of liquefaction, and in milk no separation of the casein took place. On potatoes, which are naturally of acid reaction, the growth was either much impaired or even completely inhibited, although other samples of cholera grew luxuriantly on slices of the same potato. This sensitiveness of the organism to acid reaction, when it has lost its enzyme function, will be dealt with later. The variety of cholera which had lost its enzyme faculty, preserved its new properties for many months when cultivated on agar-agar. When grown under other conditions, it rapidly regained its power of liquefying the gelatine. Thus, if frequently re-inoculated on fresh bouillon, and retained at a temperature of 25° C., it recovered its former characters in about three weeks. Since noting this loss of function in the case of cholera, I have had occasion repeatedly to observe the same fact in old cultures of many other organisms. Gelatine plates from such tubes presented non-liquefying colonies often intermingled with the characteristic liquefying ones, which had alone been expected. At first sight it would appear that the former must be impurities, a view which, by preventing further investigation, has no doubt misled many who must at some time have observed the same appearances. I was able, in such cases, by cultivation under appropriate conditions, to cause such aberrant forms to recover their original characters.

The question now arises, What are the factors which come into operation in old cholera cultures, and cause this loss of function? It has been already stated, that when oxygen is excluded, the organism exists without the aid of enzymes. Now, if a culture is left, for long, undisturbed, a membrane forms on the surface which effectually prevents the entrance of oxygen to organisms which have fallen to the bottom of the tube, and the habit of taking its food otherwise than by enzymes may perhaps persist for some time after it is again grown under ordinary conditions. The most important factor, as evidenced by the fact that the age of the culture is essential, is undoubtedly the action of the metabolic products of the microbe, which accumulate under such circumstances. These products may act either as general depressants devitalisingly upon the protoplasm as a whole, or specifically upon one or other function. We must in this case attribute the action to the presence of some special substance, which acts upon this function, as organisms which were unaffected by their own products suffered this loss when cultivated in sterilised cholera cultures. As the phenols had been found to act powerfully in restraining the action of the soluble ferments, attention was directed to indol, which Brieger and Salkowski had shown to be formed by cholera. The great difficulty and expense involved in the preparation of pure indol prevented the experiment from being carried out with it, so recourse was had to phenol. The method of procedure was as follows:—A series of tubes each containing 10 c.c. of sterilised bouillon, received quantities, varying from 1 to 10 drops, of 5 per cent. carbolic acid solution. The organism was then seeded in these tubes, and the effect of the varying concentrations investigated. The microbes which were experimented upon in this way were Koch's *Cholera bacillus*, *Micrococcus indicus*, *Micrococcus prodigiosus*, and *Bacillus pyocyaneus*. These varied greatly as regards the quantity required to effect the loss of their enzyme function, 1 to 2 drops being sufficient for cholera, while *Prodigiosus* and *Indicus* required the highest concentrations. *Bacillus pyocyaneus* being still more intractable, showing itself unaffected, even by 10 drops of carbolic acid, it was finally omitted from the series. The state of the enzyme function in the other organisms was tested from time to time by inoculation on milk and gelatine. By this means, in the space of about six weeks, varieties of cholera, *Prodigiosus*

and *Indicus*, were produced which grew on gelatine without any signs of liquefaction. The condition was not, however, a very permanent one in cholera, as after a certain time a slow liquefaction of the gelatine began to show itself.

In the case of all the organisms, the longer they were subjected to the influence of carbolic acid the more fixed was the new condition. On the other hand, the strongest solutions in which the organism could be got to grow were not those best suited for the production of more permanent species. No doubt, the enzymes were lost more quickly by the use of the higher concentrations, but on recultivation on other media they almost immediately returned. It would appear that the carbolic acid, by acting devitalisingly on the protoplasm as a whole, restricted the exercise of that lower function, which by replacing its enzyme action, prevented the tendency to relapse reasserting itself, when cultivated again under normal conditions. At any rate, it was the use of medium concentrations over more prolonged periods which were found most effective, and indications were not wanting that a previous growth of the microbe in the more dilute solutions would have produced even better results. The enzyme which acted on the gelatine, and that which occasioned the rennet-like separation of the casein in milk, were not affected alike in these experiments. In all three organisms the power of coagulating the milk appeared to be lost before the power of liquefying the gelatine. If this were so, one might consider the rennet-like ferment as being a later evolved function, and so more easily lost when subjected to degrading influences. For, as the precipitation of the casein is only a splitting, preliminary to the peptonisation which is to follow, such an enzyme would be of no use to the organism unless the peptonising power were also present. The absolute proof that it is first lost, is however wanting, as another factor must here be taken into consideration. These organisms become more sensitive to acid reaction in proportion to the loss of their enzyme action, as is evidenced by their less vigorous growth on potato. Now, as they all produce acid from the sugar in milk, their further enzyme production may be inhibited by the acid they themselves have formed before such ferment is present in sufficient quantity to cause the precipitation of the casein.

It may be mentioned here that *Prodigiosus* and *Indicus*, which

had lost the power of liquefying the gelatine had also lost the faculty of forming their characteristic pigments.* This function was, however, much more easily lost than the enzyme function, for perfectly colourless cultures could be obtained, which nevertheless acted on the gelatine and milk, and it was evident that by properly graduating the agent brought to bear on the organism, the one might be lost without the other being appreciably affected.

It has already been stated that Flüggé found that micro-organisms when grown in the absence of oxygen, appeared to obtain their food without the aid of enzymes, as they then ceased to liquefy the gelatine. The most natural supposition is, that oxygen being necessary for the formation of the enzymes, when it is excluded, the protoplasm reverts more or less to the primary form of digestion—that without enzymes. This may, in some cases, be the explanation, but there are a number of facts which tend to show that in some cases under *anaërobiosis* the products of the organism are different from those which occur under *aërobiosis*. This has led Hueppe and Brieger to suggest that the metabolism of the organism under *aërobiotic* and *anaërobiotic* states may be different, these two being entirely different adaptations of the protoplasm. On this view, under *anaërobiosis*, they do not liquefy the gelatine, because they then take their food in a different way from what they do under *aërobiosis*. If this conception should, at first sight, appear rather fantastic, I must refer to certain facts which indicate that such organisms may have an adaptation to even so impalpable an agent as light. Prove † found that *Streptococcus ochroleukus* produced its yellow pigment only when exposed to diffuse daylight, while Scholl states that *Bacterium mycoides roseum* formed its red pigment in darkness only. There is no escape, here, from the conclusion that these two organisms have developed their pigment function under different conditions, which alone permit of its exercise, a result in perfect harmony with the beautiful researches of Engel-

* *Staphylococcus aureus*, when obtained from an abscess, must frequently be cultivated for some time before the pigment faculty returns; other organisms when exposed to the action of the tissues exhibit a temporary loss of the enzyme function. In both cases this may be attributed, in part to an adaptation to the conditions present in the animal organism, and in part to the attenuating influence exerted by the cells.

† Cohn's *Beiträge zur Biol. d. Pflanzen*, Bd. iv., 1887.

mann,* on the influence of light on the *Purpurin bacteria*. We may, accordingly, regard the protoplasm as having several adaptations, latent memories ready to spring into action on the application of the appropriate stimulus.

As we have already seen, the enzyme faculty is to be regarded as a differentiation of a primitive power of the protoplasm, and we have now to consider the probable cause of its origin. The resolution of the complex into the simpler, more diffusible molecules, is accompanied by the setting free of a certain amount of energy which is lost to the organism when this occurs by means of enzymes in the external medium. What compensating advantage could its development entail on the organism? From experiments on *anaërobiosis*, I was led to suggest some time ago, that, under this condition, where the enzyme action appears cut off, the organism was more sensitive to acid reaction. Further investigation has indicated so clearly a relation between the loss of this function and a corresponding increase in sensitiveness of the organism to injurious agencies, that this view becomes more than a mere hypothesis. The development of enzymes, by which the complex indiffusible compounds which supply it with nourishment are rendered diffusible outside the organism, would allow of the protoplasm being invested by a firmer, resisting, bounding membrane; and as this function was gradually evolved, each stage of development would be associated with a corresponding condition of the cell membrane. In a similar way each loss of this function would be accompanied by a change in the membrane, rendering it more sensitive to external injurious agencies. We must now consider how far this accords with a series of facts which have lately been observed by Flügge.

Flügge † has come to the conclusion that Pasteur's anthrax and fowl-cholera vaccines are really degenerated cultures, in which the protoplasm as a whole has suffered by the devitalising conditions to which they have been exposed during their production. He founds this view on a series of very careful experiments, which indicated that the attenuated cultures grew less rapidly than the virulent, and were also much more sensitive towards antiseptic agents. In addition, they grew more slowly and were more sensitive

* *Archiv f. d. ges. Physiologie*, Bd. xlii., 1888.

† *Zeitschrift für Hygiene*, Bd. iv., 1888.

to external injurious agencies in proportion to the degree of attenuation they had undergone. The incapacity of the vaccines to grow and cause disease in the bodies of susceptible animals he ascribed to their lessened reproductive power and greater sensitiveness, which gave the victory to the animal in the struggle for mastery. He saw no reason to suppose that the action of these microbes depended in any degree on the production of specific toxins. As his experiments were carried out in greatest detail on anthrax, I will limit myself at present to its consideration. On comparing the action of the virulent organism and its vaccines on Koch's gelatine, the vaccines were seen to liquefy the gelatine much more slowly, and had in fact lost in great part their enzyme function. This effect was much more clearly shown in milk. The normal anthrax coagulated the milk on the second day, while Pasteur's 1st vaccine only began to show signs of this after, at earliest, two weeks. My suggestion that the phenomena were in great part to be accounted for by interference with enzyme function was confirmed by this result. The method by which Flügge compared the resistance of the organisms to acids was, shortly, as follows:—A series of test tubes, each containing the same quantity of gelatine, was prepared, and to each set a given number of drops of the acid to be tested was added. The organisms were then inoculated by means of a platinum wire in a set of tubes containing the same quantity of acid, and their growth compared. I was able, however, to register more exactly the effect of the acidity by the following modification. The acid gelatine was rendered fluid, and after being inoculated was, as it cooled down, distributed uniformly on the walls of the tube. By this means the organism was enveloped on all sides by the medium whose influence was to be tested. In addition, as the organisms became separated from one another in the liquefied gelatine, the colonies which appeared later would each originate approximately from one organism, and thus the disturbing influence arising from the varying quantity introduced in each case was eliminated. I was, by this method, able to confirm Flügge's statement, that the vaccines were much more easily inhibited in their growth than the unaffected organism. A new tube was now added to each set, and inoculated with virulent anthrax which had been grown for some time *anaërobiotically*. The *anaërobe* organism was found to be more sensitive than the vaccines,

so that we must conclude that the loss of virulence and susceptibility to antiseptic agents have no necessary connection. The susceptibility was in all probability to be referred to the change in the cell membrane induced by the loss of the enzyme function. The pigment microbes which had lost their power of liquefying the gelatine were also compared with the unaffected organisms, and found to have become much less resistant to acidity.

It has been already stated that if the application of the degrading agency is properly graduated, *Prodigiosus* and *Indicus* can lose their pigment faculty, although their power of liquefying the gelatine is not appreciably lessened. It is probable that in a similar way anthrax can lose its toxine faculty without its other functions being seriously affected. In support of this it may be mentioned, that Gamaleia states that his *vaccines*, prepared by means of bichromate of potash, did not exhibit the great loss of vitality which Flügge observed in Pasteur's *vaccines*. Pasteur's method of attenuation by the use of high temperatures would appear to be so coarse a method that not merely the pathogenic function, but the vitality of the organism as a whole is affected. But this general loss of function and lessened vitality, depending as it does on the method employed, can in no sense be regarded as characteristic of the *vaccines*.

We have had occasion, frequently, in the course of this paper to refer changes in the power of resistance of an organism to a probable change in the condition of the cell membrane, and it may be not uninteresting here to briefly mention certain morphological variations in the bacillus, some of which may, perhaps, be regarded from this point of view. I have repeatedly observed that when virulent anthrax has been grown for some time in bouillon, *anaërobically*, the shape of the organism undergoes a very remarkable change. The rods and filaments are no longer to be seen, and in their place we have a series of larger spherical or oval bodies, apparently multiplying by fission to form chains and clusters. This torula-form is best investigated in its natural state, as it does not stain readily and appears to shrivel up when dried. When examined in this way the appearance of these spherical bodies is so different from what one finds normally in anthrax, that one feels inclined to put down its presence to the entrance of some chance impurity. Gelatine plates laid down from such cultures proved however that the cultivations

consisted of pure anthrax, and inoculation of mice indicated that their pathogenicity was unimpaired. It is but right to state here that Pasteur * has observed precisely similar phenomena in the case of certain moulds and mucors. These fungi, when grown with exclusion of the air, tend to produce, instead of the usual *mycelium* and *hyphæ*, cells much larger than normal and more globular in form, so much so that Pasteur would refer in part to this, the erroneous statements so many botanists have made concerning the transformation of these into yeast. The *anaërobie* cells of these fungi suggest by their appearance a tendency more or less pronounced to revert to the amoeboid state. Another appearance which may be noted in anthrax cultures grown *anaërobiotically* also indicates a change in the cell membrane. Under normal conditions this organism never forms on its cultivation fluids anything approaching to a surface membrane, at most only a narrow ring of growth appearing round the walls of the tube, which falls to the bottom as soon as it has attained any size; but when the air is excluded the superficial growth remains attached until almost the whole surface is covered. Now a surface membrane or zooglœa results, as does the matrix of cartilage, from the changes which the cell membranes undergo in swelling up and yet remaining in continuity, and a change in this respect is what we might expect when grown in this way. The *vaccines* under certain conditions exhibit an appearance, probably of a somewhat similar character, which points in the same direction. When virulent anthrax is grown *aërobiotically* in bouillon, the fluid remains clear and limpid, the growth collecting at the bottom as a whitish fluffy mass. The *vaccines*, if allowed to grow slowly, present a precisely similar appearance; but if placed at a higher temperature, where they grow more rapidly, the tubes of bouillon, especially in the case of the most attenuated, present a uniformly turbid aspect. The apparent change in the specific gravity of the organism, which permits it to remain in suspension in the fluid, is evidently to be ascribed to the condition of the membrane in the *vaccine* being still further accentuated by the rapid division of the cells at the higher temperature. The *vaccines* are described as morphologically indistinguishable from the virulent organism; Gamaleia,† however, asserts,

* *Études sur la bière*, 1876.

† *Annales de l'Institut Pasteur*, 1888.

in opposition to other observers, that the bacilli of the *vaccines* are smaller, this corresponding to the degree of attenuation they have undergone. On comparing the bacilli of Pasteur's *vaccines* with the virulent forms when grown on gelatine and bouillon, only a very slight difference in their size was usually to be detected; when, however, they originated from a potato culture, the difference became very marked, the 1st *vaccine* being only about one-half as broad as the virulent organism. This appears to be mainly due to the acid reaction of the medium affecting the *vaccines* more than the virulent organism, the size of the bacillus apparently varying with the more or less favourable conditions to which it is for the moment exposed. Huber * has observed that anthrax bacilli vary in size as they occur in the tissues of different species of animals, being much larger in the more sensitive than in the less susceptible animals. This difference in size of the organism in different animals, or even in different tissues of the same animal, should not be regarded as indicating a more or less favourable medium in a physical sense, but rather as the vital expression on the part of the microbe of the influences to which it has been there exposed, resulting from the reactive powers of the tissues in that special animal or even tissue. This point will be dealt with more fully presently, since it is important as evidencing an action exerted by the cells on the microbe which lies outside them.

There is still a relation of the enzyme function to the organism which has not yet been discussed—the action, or rather want of action, which the ferments manifest on the living protoplasm. The organisms are constantly bathed in their own enzymes, and may even be exposed to those of other microbes without their substance being attacked by these. The explanation of Pavy,† of why self-digestion of the stomach does not occur in the living animal, even if it were sufficient in that special case, would not apply to those ferments which act in alkaline reaction. The “living principle” of John Hunter,‡ as inhibiting the action of the enzymes, seems still the only plausible explanation which applies to all cases. This question has assumed importance from a bacteriological point of

* *Deutsche med. Wochenschrift*, 1881.

† *Philosoph. Transactions*, page 161, 1863.

‡ *Philosoph. Transactions*, June 18, 1772.

view, since Metschnikoff has ascribed the death of microbes in the animal body to the mesoderm cells which are able to take up the organisms as foreign bodies and *digest* them. Now, as the organisms appear to be unaffected by enzymes,* by general consent the antiseptic action of the gastric juice being ascribed entirely to its acidity, even assuming that they actually undergo their death in the cells, we have no reason to believe that this is due to a process of digestion, but rather to some influence exerted by the living protoplasm with which it must there come into such close relations. It is probable that the struggle which may occur between two microbes in our test tube cultivations does not differ essentially in *nature* but only in degree from that which takes place in our body between the tissues and the disease germ. In both cases the ultimate result of the action and reaction of the cells upon each other may turn on the sum of the "conditions of existence" to which they are exposed, happening to favour one more than the other. Thus when two organisms are sown together in a culture fluid, which will "overgrow" the other may depend on the relative quantity of the two primarily introduced, the nature and reaction of the medium, and the temperature at which they are held : in a precisely analogous fashion, the growth of a disease germ introduced into a susceptible animal and the disease which follows is dependent on the quantity introduced (Davaine, Watson-Cheyne), the tissue inoculated (Koch, Watson-Cheyne), and it may be the temperature at which the animal is kept (Gibier, Metschnikoff, Pasteur). It was for long supposed that the products of one organism might be highly injurious to another, and that thus the sudden disappearance of an organism introduced into a mixture might be explained, but more recent experiments tend to ascribe much less influence to the products as direct poisons. Kitasato has

* Organic matter in nature is, in the process known as putrefaction, resolved by microbes into its simpler constituents, which in the protoplasm of plants are again built up into complex organic bodies. But a part of the organic matter undergoing this process is built up into the substance of the microbes. These, when dead, appear to undergo a process of disintegration, to be ascribed in all probability to the action of their own enzymes, the cellulose ferment which Vignal has found to be secreted by "potato-bacilli" exerting its action on the cell wall, and the peptonising enzyme upon the albuminoid constituents. In this way the constant circulation of organic matter between the animal and vegetable kingdoms may proceed without intermission.

recently reported that he found that the organisms which, when together, destroyed cholera, did not exert nearly the same effect when seeded as pure cultures alone with cholera, a result which he seemed to regard as very anomalous. This, however, becomes easily understood if we consider the course of events in putrefaction as it occurs normally in nature. If we examine from time to time a fluid undergoing this change, we find that at different periods of the process different organisms predominate, the others, for the time, sinking into the back-ground or even disappearing; yet, as Hauser has shown, several of these organisms can, when grown alone as pure cultures, carry out, although much more slowly, the greater part of the process. In nature we have, however, a physiological division of labour, each organism becoming adapted to a special stage of the process, during which by its more active growth and the molecular changes it sets up in the medium, it seems to exert a certain inhibitive action on the other microbes present. This is more clearly shown by the remarkable way in which yeast * remains essentially pure when grown under favourable conditions, although exposed to every chance impurity. If the wort is held at a suitable temperature, and sufficient yeast is at the first added, so that it may increase sufficiently rapidly to get at an early period "within striking distance" of any chance intruders, no other organism appears able to obtain a footing, unless when the process is allowed to proceed so far that the products of the yeast begin to act depressingly on its own cells. The inhibitory action which one microbe appears to exert upon another during the vigorous exercise of its vital functions, is strictly comparable to that which the tissues may exert upon such organisms. The cells of the human body have undergone great differentiation morphologically and physiologically, fitting them to carry out the various stages in the *dissociations* and *oxidations* necessary in the combustion of albumen to urea, carbonic acid, and water. A microbe on entering the tissues finds itself in conflict with cells specially adapted to the conditions present there, and more or less vigorously carrying out their stages in the vital processes, so that it has small chance of invading the organism, or even holding its ground, unless it exerts a depressing influence by means of its products—unless it is pathogenic. That we are not here ascribing too

* Naegeli, *Theorie der Gährung*, München, 1879.

important a rôle to the *toxines* is indicated by the varying grades of virulence of the organism which at present may be said almost certainly to depend on the production of varying quantities of the poison, and by the fact that when a *tolerance* of the specific poison has been acquired either by a passing attack or by the previous introduction of the toxine, the disease germ loses its power of invading that animal. The microbe under such circumstances need not immediately perish; but may, and probably usually does, undergo a gradual process of attenuation from the increased reactive powers of the tissues, and then falls a victim to the "*phagocytes*" as does an ordinary saprophyte, or even a foreign body. This has been shown by Hueppe and myself * to be the case when animals have been rendered only partially immune to anthrax, the course of the disease is then much prolonged, and when death does occur the microbe is found to have become attenuated.† An Italian observer ‡ has rendered it probable that in a precisely similar way anthrax, when grown with another organism, whose products exerted no injurious action on it, undergoes a slow attenuation, an effect which we must ascribe apparently to the direct action of the one cell on the other. There is accordingly, as already asserted, no reason to assume that any other forces come into play in the destruction of disease germs in the animal body than those we see operating in our cultures.

From a review of what has been said on the subject of enzymes, it is evident that very much less value attaches to the power of liquefying the gelatine, as indicating a fundamental distinction between two organisms, than that which is usually accorded to it. It is a property which is liable to great fluctuations in the degree of its development, each organism in this respect exhibiting specific idiosyncrasies; and although the normal degree of development usually tends to return under cultivation, yet, as in the case of the anthrax vaccines, the lower grade may be retained with great

* "Saprophytismus und Parasitismus," *Berliner klin. Woch.*, No. 13, 1889.

† Since then, it has been shown by Woodhead and myself (*Comptes Rendus*, Dec. 23, 1889) that, if the toxine is antagonised, the tissues are able to come into action, and we have either complete recovery, or the disease is mitigated or prolonged in its course; the microbes on the death of the animal being then found to have undergone a process of attenuation by the action of the cells.

‡ "Sur la concurrence vitale des bacilles la fièvre Typhoïde et du bacille Charbon," *Giorn. Internaz.*, ix., 1887.

tenacity. Hueppe, from the mere fact of its being a *secondarily* derived function, considers it of little value for classification, as all characters for this purpose should depend on primary powers of the protoplasm, which as such, are unchangeable. At the same time, the extreme convenience and value of the gelatine methods as a means of diagnosis cannot be overrated, although the result arrived at in this way should in every case be further tested by the appearance of the growth on potato and in milk.

The varying power of resistance of the same microbe, which we have had so frequently to refer to, has a great practical interest in its relation to the most important application of bacteriology—the antiseptic system. Koch proposed anthrax as a standard by which we might compare the relative efficiency of different disinfecting agents. Great confusion has, however, arisen from this, as not only anthrax, but other organisms also, exhibit varying powers of resistance. In determining the absolute value of an antiseptic agent, those conditions which cause the powers of resistance of the organism to vary must be most carefully considered. This is still more necessary at present in consequence of the search after *specific* * disinfectants. It has been found that each organism exhibits idiosyncrasies in relation to antiseptic agents, being exceptionally susceptible to one, and more than usually resistant to another. The object being to find that substance, or combination of substances, which acts most vigorously on the disease germ, and yet injures least of all the tissues, the confusion occasioned by the organism itself not being constant in its properties, can be readily understood. In such experiments even a difference in the age of the culture may cause a serious discrepancy in the results. Thus, if the organisms experimented with are a brood of very young cholera cells obtained by incubation for 18 to 24 hours at the temperature of the body, the action of the antiseptic is much more marked than on an older culture. This is probably to be ascribed to the organisms in this stage of development possessing a more permeable membrane. The nature of the medium appears also to exert a certain influence, as anthrax which has been grown on potato is slightly less resistant than that from other strata, as agar-agar. We have here again to consider the influence which the acidity of the medium exerts on

* Hueppe, *Berliner klin. Woch.*, Nos. 46 and 47, 1889.

the state of development of the enzyme function, a factor which must be constantly borne in mind in all such investigations. In each organism the range of variability, as regards its enzyme action and varying susceptibility, is a specific quantity depending on the nature of the protoplasm, and can only be determined by direct experiment.

The greater sensitiveness of organisms when grown with exclusion of the air may be of more general interest, as perhaps throwing light on a question which has long remained obscure—the manner in which certain diseases are propagated. In cholera, typhoid, and yellow fever, the intestinal symptoms predominate, and it is through the evacuations that the poison is disseminated. Yet although the organisms are present in large numbers, in the case at least of the first two diseases, direct infection from the fresh stools is by almost general consent considered the exception rather than the rule. Pettenkofer has emphasised and attempted to explain this view, especially in the case of cholera, by assuming that the organisms do not leave the intestine in a condition capable of infecting others, but that they must first undergo a process of “ripening” in the soil. It has been suggested by others that the organisms must first grow outside the body to form spores, and that these may be the only means of infection. This is, however, completely negatived by direct bacteriological investigation. Now, the mode of existence of organisms in the intestine must be from the first practically an *anaërobic* one. The small quantities of oxygen which are swallowed with the food are rapidly absorbed by the walls of the stomach, or converted into carbonic acid by the organisms always found there, so that, in the upper straits of the small intestine, at most, only traces of oxygen can be present. Experiments carried out by Hueppe and myself, have shown that cholera can under precisely these conditions produce its poison in great quantity. But if the organism lives *anaërobically* in the intestine, it will leave it in a peculiarly sensitive condition, especially as regards acids. This greater susceptibility to acids would raise a barrier to its passage through the stomach, through which infection must occur. But, if allowed to grow in contact with the air, as on soiled linen, they would acquire their normal power of resistance, and with it that fearful infectiveness which has been, under such cir-

cumstances, so often remarked. The need for that stage of growth outside the body and the influence of the "time" and "place" disposition as affecting this becomes thus more readily understood. It is not denied that many other factors, such as personal predisposition, and all conditions local or seasonal which favour a state of intestinal irritation, come also into play ; but to discuss these at present would be obviously out of place. It is sufficient to have noted that we have here a factor in this question which has not as yet received consideration.

The whole question of enzyme action is of profound interest, from a general physiological point of view. Burdon Sanderson has recently advised us to study function, not in its simplest, but in its most specialised form. Contractility is not to be investigated in a mass of undifferentiated protoplasm, but in striped muscle. We have in the case of the enzymes a function of the organism, separable from the protoplasm, whose mode of action we can investigate at our leisure. It is a catabolic function of the organism, and by a complete knowledge of its mode of action we should learn how the process of combustion in the animal body proceeds at so low a temperature. The action of enzymes is generally recognised as consisting in a splitting up of complex molecules into simpler, accompanied by hydration. The same result is attained by the chemical action of acids and alkalies or even by a much higher temperature alone. Thus hydrochloric acid can convert fibrin into peptone and starch into sugar. But this occurs only at 100° C., whereas with enzymes the change takes place at a very much lower temperature. The power possessed by a comparatively small quantity of an enzyme of decomposing relatively very large quantities of a special medium is spoken of as due to *catalytic* action—an action bordering on the chemical and the physical. The dependence of its optimum action on a definite temperature suggests that it may consist in the transmission of a certain molecular motion, which enables the molecule to be decomposed at a much lower temperature than if the force were less accurately adjusted to the work to be performed,—just as certain chemical substances explode most readily when sound waves of a certain length and rapidity impinge on them, or as certain rays of light decompose or cause to combine certain chemical bodies. The energy which is present in the shape of temperature, instead of acting

as it usually does for the most part in merely increasing the inter-molecular spaces by increasing the mean free path of the molecule by means of the enzyme, is presented in such a form that it is taken up by the molecule or rather by a part of the molecule until its integrity is wrecked and *dissociation* occurs. Now we have already seen that the enzyme is merely a property separable from the protoplasm, but not differing otherwise from other functions. Each enzyme has its optimum temperature, and may not the different *catalysing* powers of the protoplasm of an organism have different optimum temperatures? A series of facts which have been accumulating for some time must, I think, be explained in this way. We find that the pigment bacilli have each an optimum temperature at which they produce most readily their pigment, although this does not necessarily coincide with that at which they grow most rapidly. Thus *Prodigiosus* grown at the usual temperature of the room, is of a striking red colour, but, when cultivated at the temperature of the animal body, it is absolutely colourless.* I had recently occasion to

* This need not be entirely attributed to the direct influence of the temperature on the catalysing processes; the protoplasm, as we have already seen, exhibits a selective action as regards the different substances offered it as food, and it may be that when one process is partially interfered with, a similar power may come into play, and this would exaggerate the direct effect of the temperature. Correlated with these perhaps only quantitative changes which may be catabolic or anabolic in character, the metabolism as a whole may become more or less altered, so that the centre of gravity of the organism becomes as it were shifted. It is to this, probably, that we must refer the fact noted by Schottelius that, when *Prodigiosus* is grown for a certain time at the higher temperature, it loses the faculty of producing its pigment even when grown at a lower temperature. In a more recent communication he states that, when grown sufficiently long at the lower temperature, the property returned, so that we have here an example of that form of "reversion" which Romanes has recently so ably discussed. Dallinger has found that Infusoria can be gradually accustomed to withstand very high temperatures, and this adaptation may be associated with a similar change in the metabolism. The "tolerance" which Kossiakoff (*Annales de l'Institut Pasteur*, No. 10, 1887) has shown that microbes can acquire towards antiseptic agents when previously cultivated in more dilute solutions, is to be referred chiefly to the organisms becoming gradually accustomed to exist without those "*associations*" and "*dissociations*" which the chemical substance tends to inhibit, and to a corresponding development of others to take their place. The permanence of this new habit of the organism, when grown again under the old conditions, will depend on the more or less stable nature of the new combination or complex functions which has been evolved. This modification of the organism as a whole, which may result from a change in one direction, and is dependent on

investigate the growth of a series of organisms on media coloured with litmus, and found, to my great perplexity, that a certain number reddened it, but only at a definite temperature which varied with the organism and the medium under consideration. Relatively larger quantities of acid than ammonia (?) appeared to be formed only within a certain range of temperature. Warrington * found, with quite a series of organisms, that when cultivated in milk, at higher temperatures they produced relatively more ammonia, at lower relatively more peptone. It has been recently communicated that certain yeasts produce at lower temperatures relatively more alcohol, at higher temperatures relatively more glycerine. The one *dissociation* appears to be favoured at a higher, the other at a lower temperature. I conclude that the temperature acts more or less directly on those *catalysing* processes inherent in the protoplasm itself, in a way similar to that in which it acts on the enzymes which are separable from the protoplasm. It has already been noted, that in the organisms of the cholera group the rennet-like ferment appeared able to act in acid reaction in which the pepsin-like ferment was inhibited. We should not then be surprised to find that a chemical substance is able to inhibit one *catalysing* process in the protoplasm without materially affecting the others. There are already many facts in Bacteriology which speak for this, but a systematic examination of the influence which such bodies exert on the functions of microbes is much to be desired, as throwing light upon the way in which a drug affects the reactions of the living protoplasm, and as furnishing us with a basis on which a cellular therapeutics may perhaps in the future be founded. Interesting as this subject is, it cannot now be further entered upon in a paper whose proper theme is the enzyme function in its relation to the general physiology of the cell.

In conclusion, I wish to acknowledge my great indebtedness to Professor Ferdinand Hueppe, not merely for invaluable assistance in the experimental work, but also for the general biological outlook, I have here adopted. My thanks are also due to Dr Woodhead, for

what we may term the solidarity of the organism, has never yet received the attention which it deserves as a factor which may determine the degree of fixity of a new adaptation.

* *Journal of the Chemical Society*, 1888.

much kind assistance in the work carried out in the Laboratory of the Royal College of Physicians.

A New Synthesis of Dibasic Carbon Acids.

By Prof. Crum Brown.

(Read February 17, 1890.)

(*Abstract.*)

The electrolysis of potassium salts of the form $\text{K}-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R}$ in strong aqueous solution has been shown by Kolbe* to lead to the

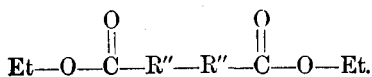
formation at the anode of R_2 . $\text{K}-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R}$ decomposing into

the ions K and $-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R}$; the former giving at the cathode caustic potash and hydrogen, and the latter giving at the anode carbonic acid gas and R_2 . It occurred to me that if in dibasic acids, containing two carboxyls, one carboxyl could be temporarily shut off from taking part in the electrolysis, an interesting synthesis might be effected. Guthrie's observation† that $\text{K}-\text{O}-\text{SO}_2-\text{O}-\text{Et}$, when subjected to electrolysis with an anode of amalgamated zinc, gives caustic potash and hydrogen at the cathode, and zinc ethyl sulphate at the anode, gave a hint how such temporary eclipse of one carboxyl might be effected. I therefore determined to try the

electrolysis of such a salt as $\text{K}-\overset{\text{O}}{\parallel}{\text{C}}-\text{R}''-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-\text{Et}$, in the

hope that it would give at the cathode caustic potash and hydro-

gen, and at the anode carbonic acid and $(\text{R}''-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-\text{Et})_2$, that is



* *Annalen*, lxi. 257.

† *Chem. Soc. Quart. Jour.*, ix. 131.