II.—THE NATURE OF AMYLASE. II.

By D. Narayanamurti.

Experiments on the purification of amylase by electro-dialysis and the resolution of the enzyme into two components by electro-osmosis were described in Part I (*J. Indian Inst. Sci.*, 1928, **11A**, 134). The enzyme subjected to electro-ultrafiltration has now been studied, and further evidence in favour of the two-enzyme theory follows also from ultrafiltration experiments.

ELECTRO-ULTRAFILTRATION.

This elegant and very useful process, devised by Bechhold (*Z. Elektrochem.*, 1925, **31**, 496), depends on the following principle. The collodial solution to be purified is placed on an ultrafilter from which the dissolved crystalloids are drained by suction; simultaneously an electric current removes ions and transfers water to one of the electrodes. It is thus a combination of electro-dialysis and ultrafiltration, and has the advantage of quickly removing all ionogenic and crystalloidal impurities from colloidal solutions. This is of fundamental importance when studying the action of salts on enzymes. In the case of oxidases it should be easy to settle the much discussed question whether manganese and iron, either as inorganic salts or part of the enzyme complex, are necessary for the action of these enzymes. Such a study is now being conducted by the author with tyrosinase. It is claimed that under favourable conditions the removal of electrolytes is about 180 times as rapid as by dialysis. Furthermore, bio-colloids liable to attack by micro-organisms can be quickly purified, and instead of dilution taking place concentration can be effected. Another advantage is that the process can be so conducted that the solution remains neutral or becomes acidic or alkaline; Bechhold has used it with great success for the purification of glue and gelatine, the ash after purification consisting entirely of silica and ferric oxide. The following experiments with amylase are the first of their kind with enzymes.

The Apparatus.—The electro-ultrafiltration vessels shown in Fig. I are made of special porous porcelain and resemble the ultrafiltration apparatus of Bechhold-König; they are covered with an ultrafilter membrane, and differ from ordinary ultrafiltration vessels in having an electrode of deposited platinum underneath. The deposit extends up the sides of the vessel in several places in order that electrical contact may be made by means of a tightly clamped collar.

Deposition of the membrane.—The dish is placed on a rubber washer in the funnel and 6 per cent. collodion poured to the brim, excess being poured back to the stock bottle by rotating the dish till no more drops fall. After drying in air for 5 minutes the dish is given two more similar coatings and then allowed to dry for 15 minutes before immersing in water for gelatinisation of the membrane. The flask is coated in similar manner by dipping in a beaker of collodion and applying suction for a minute.

Purification of the enzyme.—The enzyme solution is placed in the dish and distilled water in the ultrafilter flask, suction being applied on both sides. The electro-ultrafiltration was effected at 30 volts, tapped by means of a potentiometric arrangement, a milliammeter in series and a voltmeter in parallel being included in the circuit to follow the course of the electro-ultrafiltration. The ammeter reading became constant (almost zero) in the course of seven hours when the current was allowed to pass for an hour more to remove the last trace of electrolyte. Neutrality disturbance was avoided by having a more powerful suction at the anode than at the cathode, which being smaller is in the flask. In some experiments acidification was avoided by reversing the direction of the current every 2 or 3 minutes.

The results obtained with cholam malt diastase, partially dialysed according to Euler, are represented in Fig. II. The solid content of the cholam diastase solution was 4·8 times that of the electro-ultrafiltered solution. The activity of the enzyme being proportional to its concentration at low concentrations the electro-ultrafiltered solution might be considered to be 4·8 times as active as the dialysed preparation. It is evident from the figure that the curves are not similar, that representing the electro-ultrafiltered solution being autocatalytic in form. Whether this has anything to do with the two-enzyme theory of diastase only future experiments can decide. It must also be mentioned that the increase in activity obtained is not as great as in electro-dialysis. Experiments are in progress to study the effect of denser membranes and the most favourable conditions are being sought.

ULTRAFILTRATION.

In the earlier communication (*loc. cit.*) evidence was adduced in favour of the theory that diastase consists of two components, one responsible for liquefaction and the other for saccharification of starch; in one series of experiments a complete separation of the liquefier from the saccharifying component was effected. Ultrafiltration experiments described below give further support to this theory.

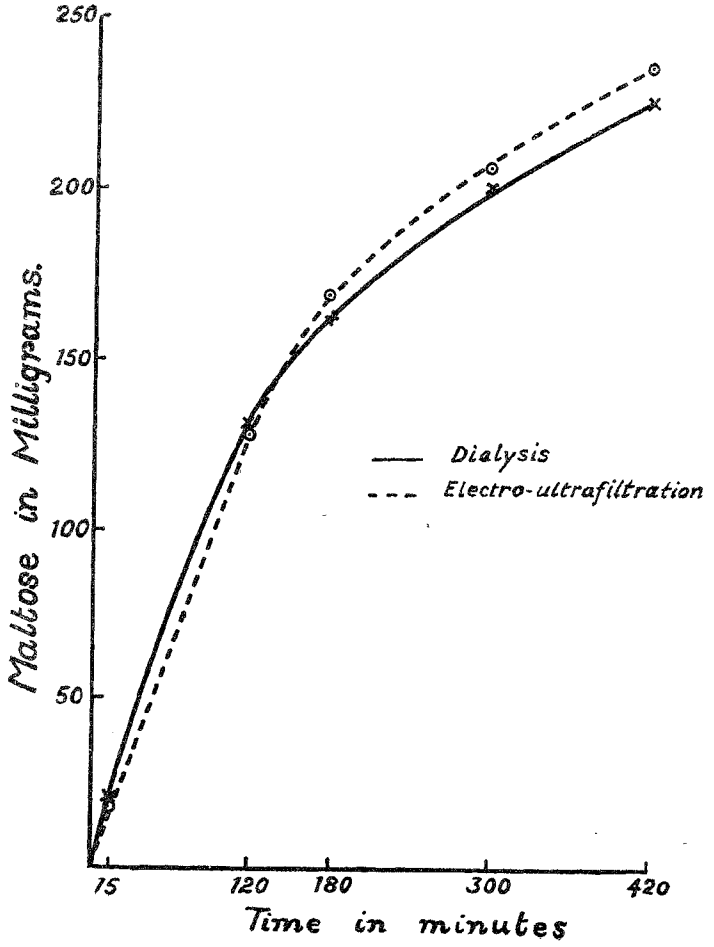
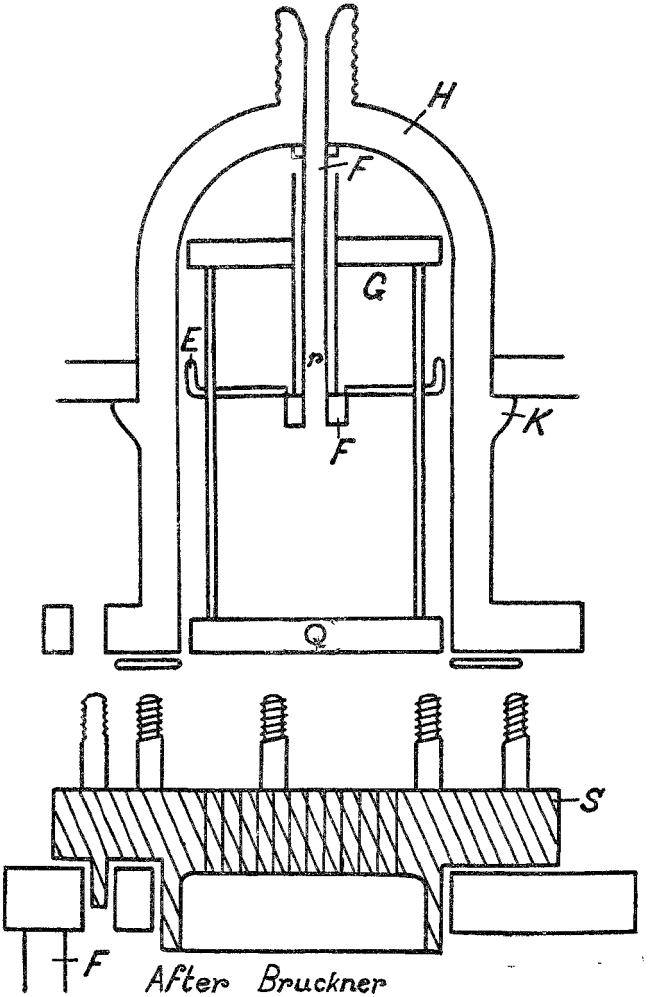

250
200
150
100
50
Maltose in Milligrams.
Dialysis
Electro-ultrafiltration
15
120
180
300
420
Time in minutes

Fig. II



After Bruckner

Fig. III

It was shown by Bechhold (*Z. physikal. Chem.*, 1908, **60**, 257) that colloids could be separated from their dispersion media by appropriate filters which he named ultrafilters, showing that by their use colloids of different dispersities could be separated from one another.

It was observed by Brückner (*Z. Ver. deut. Zucker-Ind.*, 1926, **76**, *Technischer Teil*, 837) that Zsigmondy's ultrafine filters in pressure filtration, especially at high pressures show some peculiarities. Filters which at a pressure of one atmosphere are permeable to protein become impermeable at 25 or 30 atmospheres. This could not be explained as due to a layer of protein on the filtering surface for the same phenomenon is observed when the filtration is accompanied by stirring. Brückner therefore concludes that at high pressures the pores become narrowed, the degree of change depending on the time during which the high pressure acts on the filter; further that the change is only partly reversible and that dense filters are more resistant to this change than more porous ones.

In ultrafiltration of colloidal solutions under pressure two possibilities arise. By choosing suitable filters particles of different dispersities could be separated from one another, or by using one filter, the filtrates collected at different pressures should contain the particles of different dispersities in different proportions. Moreover, in systems having an emulsoidal character, the particles which normally may be bigger than the pores of the filter might become elongated at high pressures and pass through. The results of some experiments on the ultrafiltration of diastase solutions with parchment as the filtering membrane at two different pressures are given below.

The apparatus.—The apparatus used was the one described by Brückner with slight modifications as indicated in Fig. III. It is made of brass, the parts which come into contact with the solution being tinned. It consists of the pressure dome H provided with a manometer, the sieve plate *S* about 14 mm. thick, the stirring arrangement *r* and a tripod on which the entire apparatus stands. The capacity of the dome is about 250 c.c., the upper surface of the sieve plate is smooth and three to six filter papers are placed thereon surmounted by the membrane. Nine screw bolts press the sieve plate and the dome firmly together. After filling the apparatus with compressed air, nitrogen, or carbon dioxide the valve of the manometer is closed.

The stirrer consists of a carrier *G*, attached to small tube *F* through a guide bush. It has a cross-piece *Q* moving a few millimetres above the surface of the filter. The armature *E* can be rotated by means of an electromagnet outside the dome.

Ultrafiltration of the enzyme.—B.D.H. diastase (1·5 g.) was dissolved in distilled water and made up to 250 c.c. of which 200 were

used for the ultrafiltration experiments reserving the rest for comparison. Compressed air was used and samples collected at 15–20 and 40–50 kgms. The two fractions were tested for their liquefying and saccharifying powers, results being given in Figs. IV, V, VI, and VII and showing that a partial separation of the two components has been effected. The liquefier is probably of a higher dispersity (cf. *J. Indian Inst. Sci.*, 1928, 11A, 134). Experiments are in progress to study the possibility of a complete separation of the two components by suitable choice of membranes and pressures.

SUMMARY.

Electro-ultrafiltration as a method for the purification of enzymes has been tried for the first time. Electro-ultrafiltered diastase is more active than the ordinary dialysed preparation.

The kinetics of starch-hydrolysis by the electro-ultrafiltered enzyme differs from that of the dialysed preparation. The possible significance of this to the two-enzyme theory has been indicated.

By ultrafiltration a partial separation of the two components of diastase has been effected.

Ultrafiltration experiments indicate that the liquefying component is probably of a higher dispersity than the saccharifier.

In conclusion the author desires to express his grateful thanks to Prof. R. V. Norris for the kind interest he has taken in this work.

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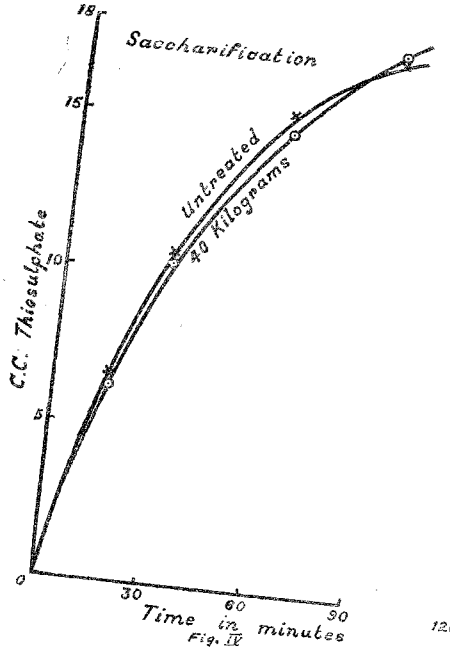
Saccharification
Untreated
40 Kilograms
C.C. Thiosulphate
18
15
10
5
0
30
60
90
120
Time in minutes

Fig. IV

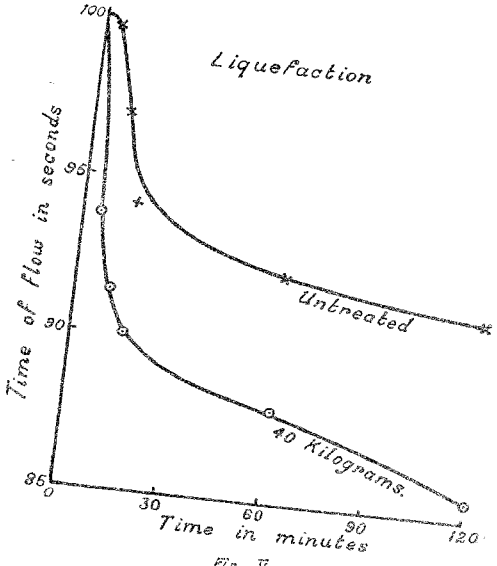
Liquefaction
Untreated
40 Kilograms.
Time of Flow in seconds
100
95
90
85
0
30
60
90
120
Time in minutes

Fig. V

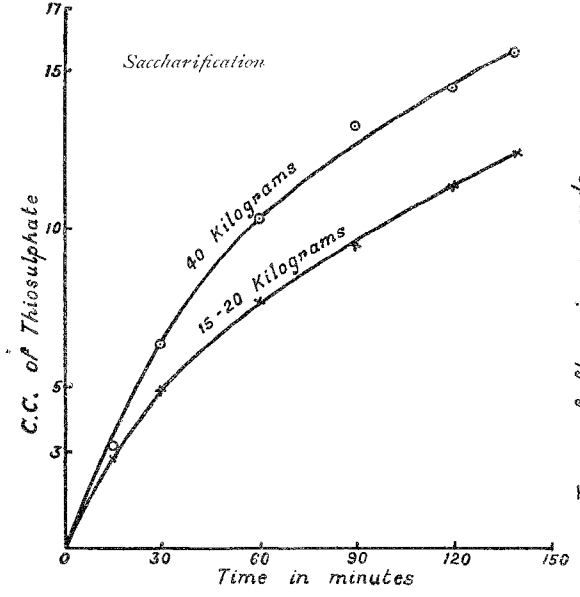

Fig VI

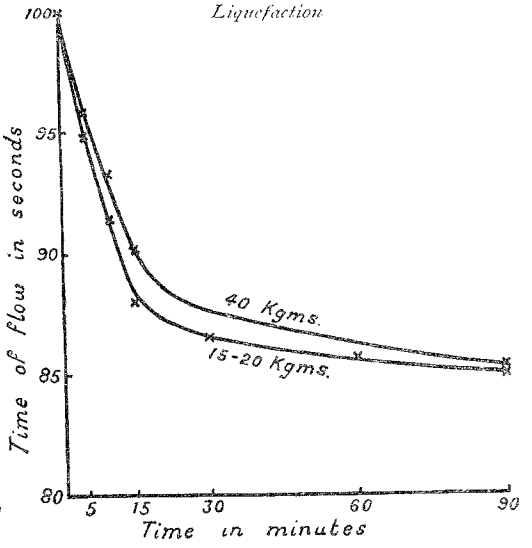

Fig. VII