STUDIES IN ELECTRO-CULTURE.

Part II. Influence of Electrical Treatment on the Germination of Barley and the Diastatic Activity of Malt.

By K. Venkata Giri and V. Subrahmanyan.

Butterfield, Barclay and other earlier Australian workers conducted systematic work on the stimulation of seeds by electricity (Mackinnon, *Sci. and Ind.*, 1920, 2, 24). They observed that barley soaked in a solution of copper sulphate could be electrified with an ordinary medical coil in 3–5 minutes, and that plants raised from the seeds thus treated, gave about 40 per cent. more yield than the controls. Fry developed a system of electrical treatment which was subsequently patented as the 'Wolfryn' process (*U. S. Pat.*, 1,106,039) and which excited considerable attention in both England and Australia. Reporting on the results of the treatment, Dunn (*Elec. Rev.*, 1919, 84, 89) mentions that quickened germination, prolific tillering, increased yields of richer grain, sturdier straw and resistance to a variety of diseases may be expected by the use of the Wolfryn-treated seeds. Others were not so hopeful. Thus, Brenchley could observe no difference between plants raised from treated seeds and those from untreated. Gerrard reported that trials carried out with electrified seeds at Wye resulted in failure. The investigations of the British Board of Trade suggested that the beneficial effects of the treatment might have been largely due to the preliminary soaking of the seeds in the electrolyte (*Elec. Rev.*, 1919, 85, 377). Various other systems of seed treatment have lately been developed, among which mention may be made of the method of Bennett (*Brit. Pat.*, 268, 291) as the result of which highly satisfactory yields of grain and straw are claimed to have been obtained.

The precise nature of the action of electricity on seeds is still not understood, though various theories based on the ionisation of the electrolyte, permeability of the seed, osmotic pressure of the solution, and sterilisation of the seed-coat have been advanced by different workers. Very little is known also regarding the physiological response of the seed to electrical treatment. It is possible that the passage of current through the seed brings about some physical or chemical changes which influence certain phases of living activity such as germination, development of the seedling, response to plant nutrients, assimilation and translocation. Systematic study of such physiological effects is required before the significance of the various field observations cited above can be properly understood.

In the present investigation, some aspects of the changes occurring during the germination of electrically treated barley have been studied. Barley was chosen because (*a*) consistently favourable reports regarding the beneficial effects of so treating it have been received, no less than 30–40 per cent. increase in yield being claimed by many observers, and (*b*) the changes attending the germination of barley are of considerable economic importance and have already been studied, in great detail, by different workers from other points of view. It was therefore thought that the significance of the various changes attending the germination of electrically treated seed can be better understood in the case of barley than in that of any other grain.

The most conspicuous changes attending the germination of barley are the activities of the different enzymes resulting in the transformation of starch into dextrins and sugars and of protein into proteoses and amino-acids; and the movement of the various products into the seedling, which is thus provided with its early nutrition. The effects of the treatment were therefore sought from (*a*) the rate of germination, (*b*) the diastatic and the proteolytic activities of the germinating seed at various stages and under different conditions of electro-culture, and (*c*) the development of the seedling.

In view of the almost fanciful prices paid for 'quality' in malting barley, considerable attention has recently been paid by various workers to that subject. To the maltster, the value of barley depends largely on the amount of extract in the wort prepared from it. The investigations carried out under the auspices of the Institute of Brewing Research Scheme (Rothamsted Conference on Malting Barley, 1928; Bishop, *J. Inst. Brewing*, 1930, **36**, 352) have shown that the yield of extract is, generally, inversely related to the nitrogen content of the malting barley. Field experiments carried out during the past ten years at Rothamsted and elsewhere (Russell, Agricultural Research in 1929, 1930, 144–145), have shown that where the soil cannot supply all the nitrogen required by the plant, the yield of barley, without impairing the quality, can be increased by judicious application of nitrogenous fertilisers. It is not, however, generally possible to control the nitrogen uptake of the grain because this is determined, not by the absolute quantities added to the soil, but by the amounts rendered available under the existing soil and climatic conditions. It is also influenced by the available amounts of the other plant nutrients, such as phosphate and potash present in the soil. Moreover, there are several tracts of land, like the Fen districts of England, where the soil is so rich in nitrogen that even without the addition of nitrogenous manure, the amount of nitrogen passing into the grain is so high that its quality is seriously impaired. The malt prepared in the usual way from such barley contains, invariably, a high percentage of nitrogen and yields a wort of poor quality.

The observations of earlier workers regarding the quicker and the more efficient germination of electrically treated seed, together with those of Punnet (*Biochem. Bull.*, 1913, **2**, 555) and Lob and Sato (*Biochem. Z.*, 1915, **69**, 1) regarding the favourable influence of ionised air on enzyme action suggested that, during the germination of the treated barley, there may be quicker transport of nitrogen into the seedling than in the case of the untreated. It therefore seemed probable that if the activity of the protease in the electrically treated seed is greater than that in the untreated, the malt prepared from the former will contain less nitrogen and give a larger yield of wort of the desired gravity than the latter. In other words, it appeared probable that the quality of malting barley may be improved by electro-culture.

With a view to testing the above hypothesis, the nitrogen content of malts prepared from treated barley and the gravity of their worts were determined at various stages.

EXPERIMENTAL.

Method of treatment.—Malting barley of the Plumage Archer variety, obtained from the United Breweries of Kaiti in the Nilgiris, was used for the present series of trials. Before treatment, the seeds were soaked first in water for 24 hours and then in sodium nitrate solution of known concentration for a definite length of time. They were then packed between chemically pure carbon electrodes in glass cells and electric current (A.C., 25 cycles, 210 V.) of varying densities passed through them for different periods. A stream of salt solution was kept gently circulating through the cells to preclude the otherwise inevitable rise of temperature, and to maintain the current strength constant throughout each experiment. At the end of the specified period, the seeds were removed, washed carefully from the adhering salt and germinated at a constant range of temperature (24–26°). Representative specimens of the germinating grains were removed at intervals of 24 hours and dried by stages, at temperatures rising from 50° to 95°. The diastatic activities of the different preparations of malt thus obtained were compared with those prepared under the same conditions from similar specimens of barley after merely soaking them in water and salt solution respectively. The diastatic powers were determined by Ling's volumetric method as modified by Hind, Threadgold and Arnold (*J. Inst. Brewing*, 1926, **32**, 26).

General effects of the electrical treatment.—Barley was first steeped in water for 24 hours and then in 1 per cent. solution of nitrate for

2 hours, followed by passage of current ($7{\cdot}1 \times 10^{-3}$ amp. and $8{\cdot}6 \times 0^{-3}$ amp.) for 2 hours. The diastatic powers of the malts obtained from the treated specimens were compared with those prepared after being steeped in water and salt solution respectively.

In the course of preliminary trials it was observed that the passage of comparatively heavy current caused the temperature of the electrolyte to rise rapidly. The barley thus treated germinated in 12-24 hours, chiefly as the result of the high temperature of the electrolyte; intermittent passage of current was next tried, but did not prevent the rise in temperature. Passing a continuous stream of electrolyte through the cells was found useful in maintaining a uniform temperature and thus helping to bring about a normal germination. The malts prepared from the different specimens dried at about the same rates, but it was noted that those electrically treated developed a sweeter flavour.

A study of the diastatic power of the different preparations (Fig. I) shows that the treated specimens developed activity more readily than the others. The latter, however, made up for the difference within 48 hours, so that after that period there was no significant difference between the two sets of preparations. Later trials showed that the current densities used in the above experiment were too high. It was also found advantageous to reduce the concentration of the electrolyte.

Effect of current density on diastatic activity.—The seeds were soaked in a dilute solution (0·1 per cent.) of the electrolyte and treated with current of different densities, for two hours in each case, the electrolyte being kept circulating as before. The results (Fig. II) show that a comparatively weak current ($1{\cdot}7 \times 10^{-3}$ amp.) is almost without effect on the diastatic activity. Slightly stronger currents ($2{\cdot}86 \times 10^{-3}$ amp. and 430×10^{-3} amp. respectively) evoke very much betterresponse and lead to considerably enhanced diastatic activity, but further increase in current density to $7{\cdot}1 \times 10^{-3}$ amp. is without any apparent effect.

It is difficult to explain, at this stage, the significance of the disproportionate effects of the different current strengths. It is possible that a very weak current does not supply sufficient energy to activate the enzyme. The distinct rise in activity noticed at the end of 24 hours in the case of seeds treated with $7{\cdot}1 \times 10^{-3}$ amp. shows that the enzyme is, to some extent, stimulated by the treatment: there is, however, no marked rise observed after that period while in the case of seeds treated with $2{\cdot}86 \times 10^{-3}$ amp. and $4{\cdot}3 \times 10^{-3}$ amp. respectively, the favourable effect is most prominently seen between 48 and 96 hours. The results suggest that after producing the initial effect, the denser current leads to a reaction which hinders further rise in activity.

It may be mentioned, in this connection, that the different observations recorded above are in keeping with those of many other biological phenomena which respond suitably only under such physical conditions as are their specific optima.

Period of treatment as affecting diastatic activity.—Seeds soaked in a 0·1 per cent. solution of sodium nitrate were treated with currents of $1{\cdot}7 \times 10^{-3}$ amp. and $4{\cdot}3 \times 10^{-3}$ amp., which were interrupted after 1, 2, and 3 hours, respectively. They were then washed, spread out to germinate and the diastatic activities determined at different subsequent intervals.

The results (Fig. III) show that up to the end of 48 hours there was no significant difference between the effects of the various treatments. After that period, the specimens treated with $4{\cdot}3 \times 10^{-3}$ amp. showed very much greater activity than the others and continued to do so almost to the end. Treatment with $4{\cdot}3 \times 10^{-3}$ amp. for 1 hour was less effective than that for 2 hours: in the former case, the activity showed a distinct decrease at the end of 96 hours, while in the latter, it continued to rise up to 120 hours. Passage of the current for 3 hours was not, however, so beneficial as that for 2 hours: in the former case, the activity was somewhat depressed in the earlier stages, and although it showed a distinct rise in the later ones, it was at no time so high as in the latter. It may, therefore, be inferred that the maximum increase in diastatic activity can be obtained by treatment for 2 hours at $4{\cdot}3 \times 10^{-3}$ amp.

Observations on seeds treated for 1 and 3 hours respectively with $1{\cdot}7 \times 10^{-3}$ amp. show that there was no significant response in either case, the diastatic activities of their malts being at no time appreciably different from those of the untreated ones. Even prolonged treatment with a current strength below the optimum does not, therefore, appear to be beneficial.

The mechanism of stimulation of diastatic power.—The increased activity resulting from the passage of an electric current under optimum conditions may have been due to one or more of the following causes:—(*a*) Fundamental change in the composition of the substrate, (*b*) stimulation of the enzyme present in the germinating seed, and (*c*) larger amounts of the enzyme being developed by the treated seed.

A study of the progress of hydrolysis shows that the electrical treatment could not in any way have affected the composition of

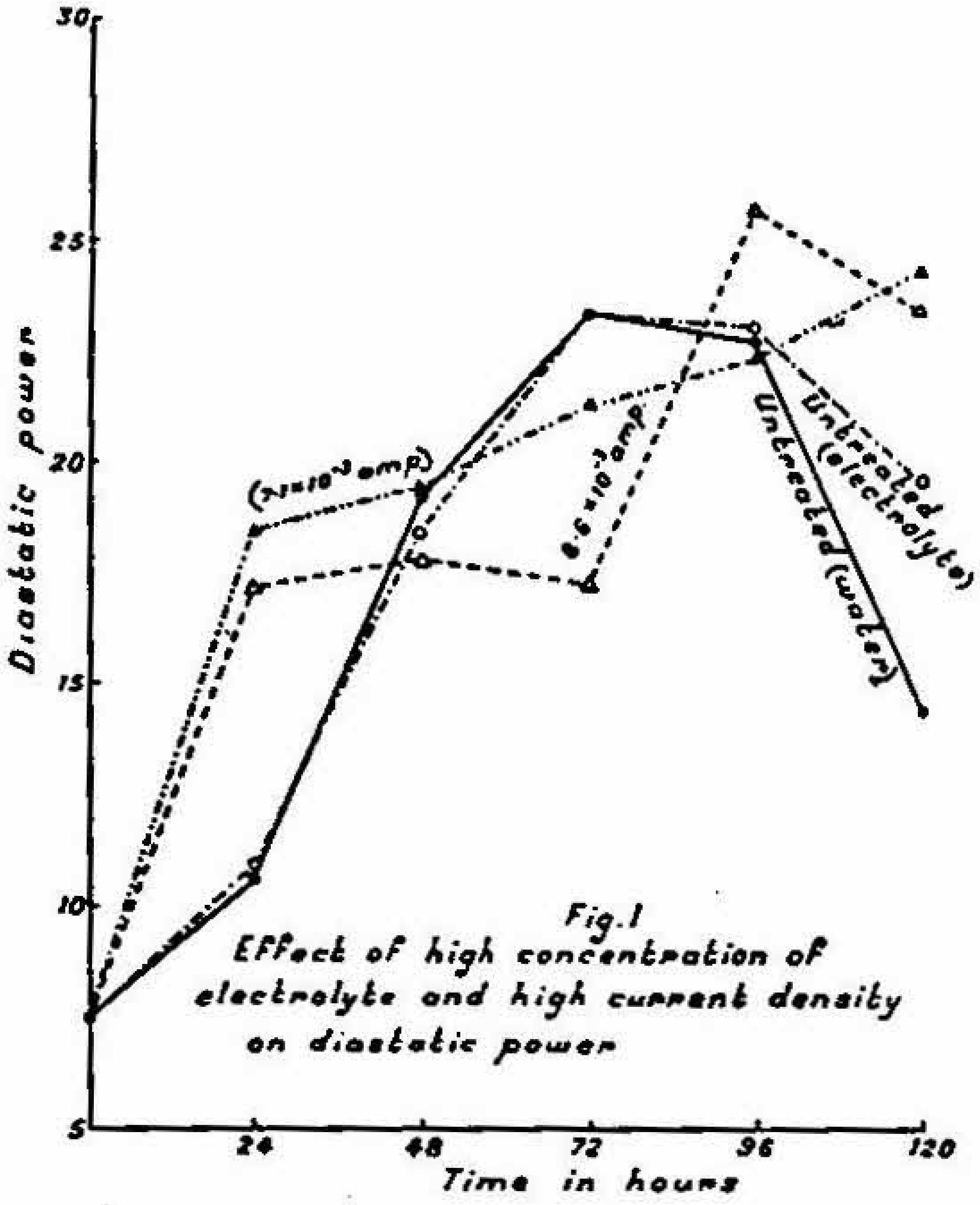

Fig. I
Effect of high concentration of electrolyte and high current density on diastatic power

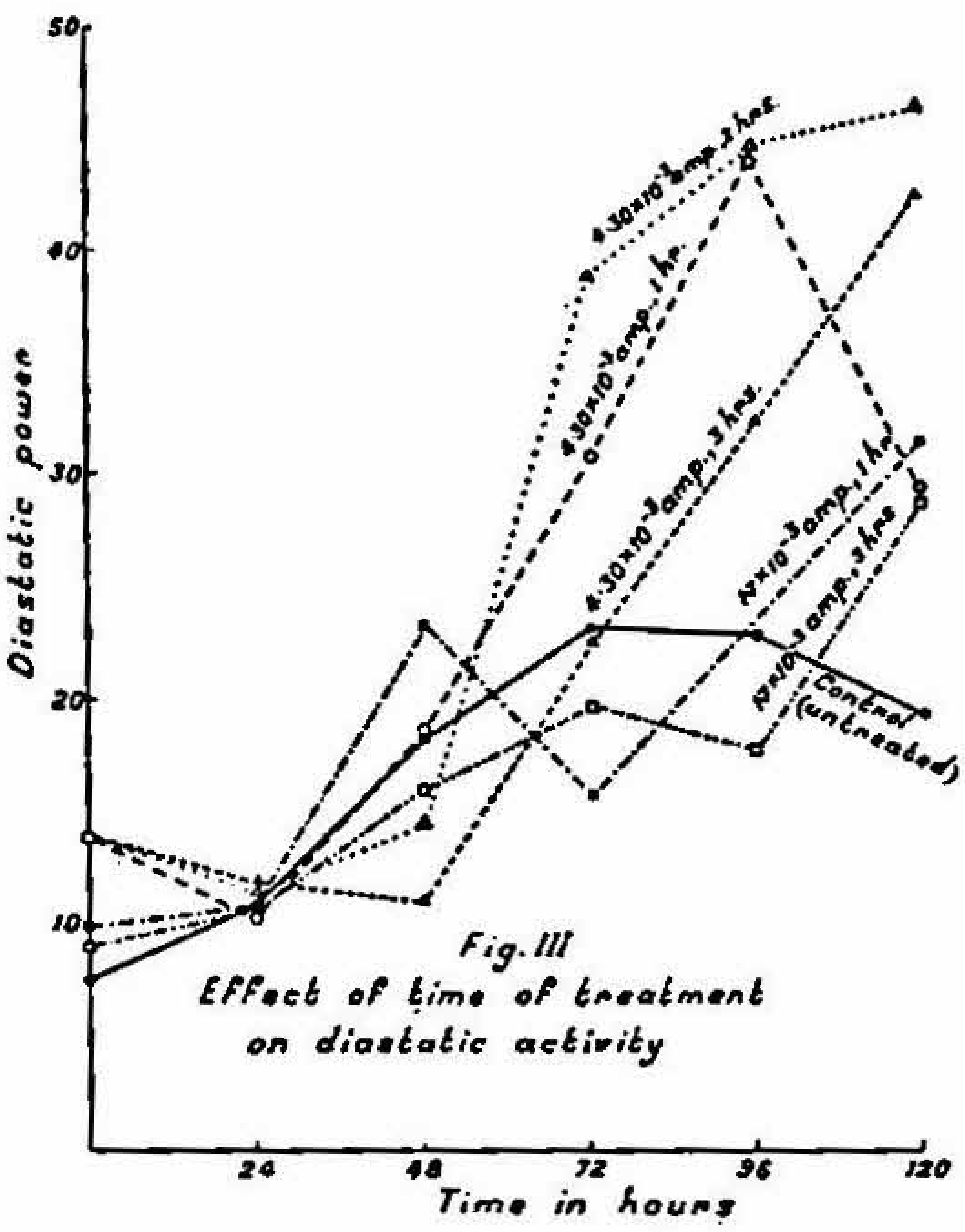

Fig. III
Effect of time of treatment on diastatic activity

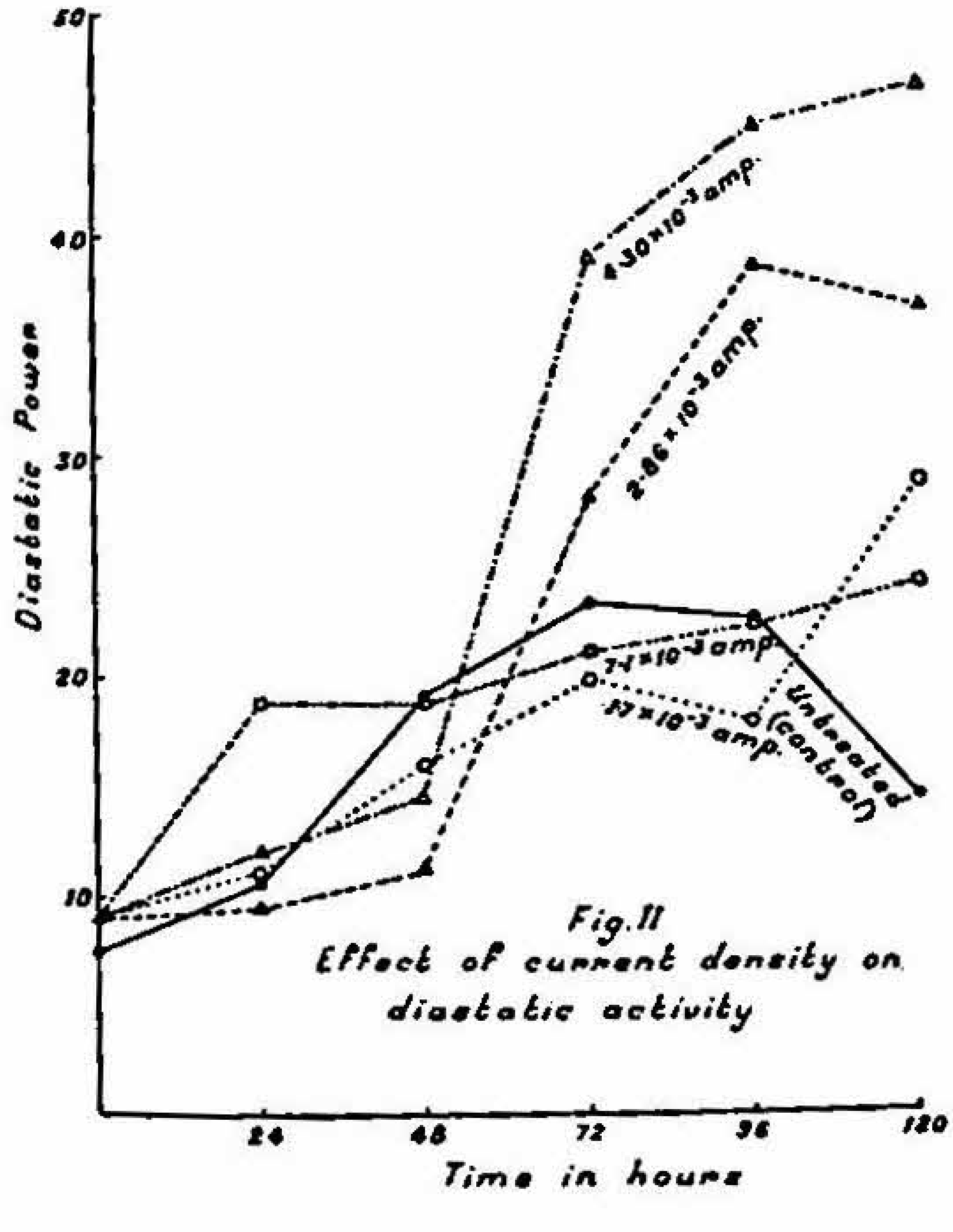

Fig. II
Effect of current density on diastatic activity

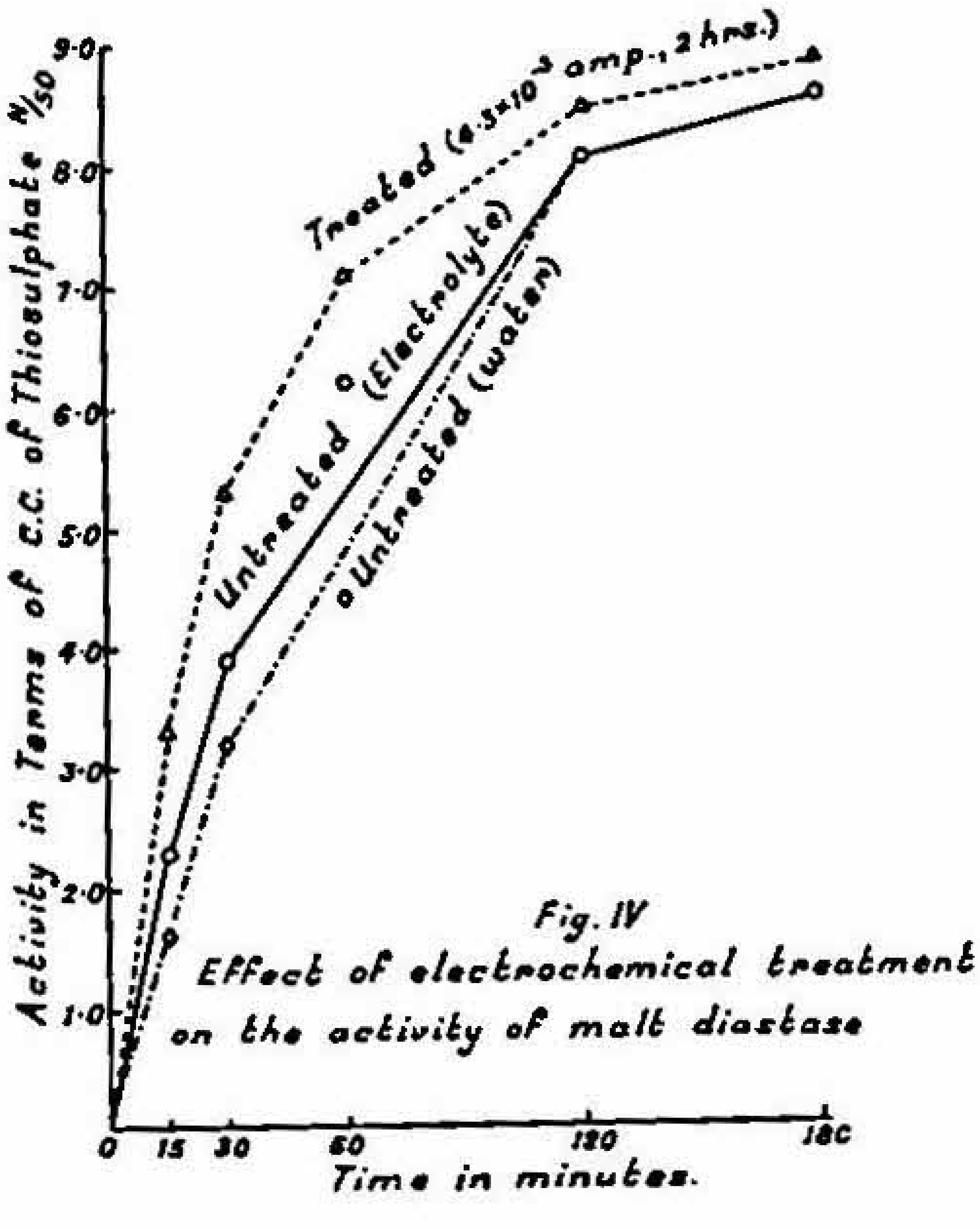

Fig. IV
Effect of electrochemical treatment on the activity of malt diastase

barley starch, since in that case the diastatic activity of the treated malt should have been higher than that of the untreated, throughout the experiment. The fact that the diastatic powers were almost the same in all the cases for the first 48 hours suggests, on the other hand, that only the enzyme was affected.

To determine whether the electrical treatment led to increased activity of the enzyme unit, the following experiment was made :—Specimens (60 g.) of treated as well as untreated malts were shaken with distilled water (180 c.c.) for 6–8 hours and the mixtures filtered first through cloth and then through paper pulp. The filtrates were centrifuged and the solid contents of the clear extracts determined. After dilution with water sufficient to render the solid contents the same in all the cases, the diastatic activities of the different extracts were determined. To 50 c.c. lots of 1 per cent. starch solution, 10 c.c. of Walpole's acetate buffer of P_H 4·6 and 2 c.c. of enzyme extract were added and the mixtures maintained at 35° in a thermostat. Aliquot parts of the mixtures were removed at stated intervals and their sugar contents estimated by the method of Shaffer and Hartmann (*J. Biol. Chem.*, 1920–21, **45**, 365).

The results (Fig. IV) show that although the dialysed enzyme from treated malt is slightly more active than that from the untreated, the difference is not so considerable as to account for the observations of the earlier experiments. Since it has already been shown that the extract from a unit weight of the treated malt brings about a larger transformation of starch in a given time than that from the untreated, it should be inferred that the former contains a higher concentration of either the enzyme or the necessary activators, or both. In view of the fact that only the crystalloids in the extracts were removed by dialysis, the activators probably present in larger amounts in the electrically treated malt might have belonged to that class.

Some doubt has, however, been recently cast on the existence of any definite group of substances capable of activating vegetable amylases. Sherman and Thomas (*J. Amer. Chem. Soc.*, 1915, **37**, 623) and Sherman and Walker (*J. Amer. Chem. Soc.*, 1919, **41**, 1867 ; 1921, **43**, 2461) found that addition of neutral salts and amino-acids led to increased rates of starch hydrolysis by amylases of vegetable as well as animal origin. On the other hand, Koga (*Biochem. Z.*, 1923, **142**, 159) observed that neutral salts were without any effect on vegetable amylases, while Fricke and Kaja (*Ber.*, 1924, **57**, 310, 313) noted that they actually inactivated electro-dialysed diastase. Patwardhan and Karmarkar (*J. Indian Inst. Sci.*, 1930, **13A**, 159) recorded that amino-acids were without any effect on wheat amylase and, in a later unpublished investigation showed that these had no influence on the activity of most vegetable amylases examined by them. In view of the above and the fact that the different preparations of malt examined in the present investigation were obtained from the same specimen of barley, it appeared to be hardly probable that any particular activator developed in the electrically treated seed was responsible for the increased diastatic power.

The results of the investigations of the British Board of Trade (*loc. cit.*) suggest that at least a part of the beneficial effect attributed to electrical treatment may be traced to preliminary soaking in the electrolyte. Although the control experiments of the present series showed that mere soaking in the electrolyte led to no response, it was still considered possible that, on passing the current, active ions might have penetrated the seed-coat and brought about, either directly or indirectly, the increased diastatic activity.

To determine whether traces of the nitrate were present in the malt of treated barley, the following experiment was made :—Specimens of dry malt were powdered and extracted with large excess of distilled water. The extract was passed through a coarse filter and the filtrate treated with cupric hydroxide (Van Slyke, *J. Biol. Chem.*, 1917, **32**, 455) to remove sugars and other organic substances. The clear and colourless extract was concentrated to small bulk and then tested for the presence of nitrates with phenoldisulphonic acid and diphenylamine, respectively. The tests were negative, thereby indicating that any nitrate originally adhering to the barley was either removed by the thorough washing following electrical treatment, or transported to the plumule and the radicle during germination.

The aqueous extract of treated malt was found, after dialysis, to contain 71·8 mg. of solid per 100 c.c. as compared with 45·8 and 52·2 mg. present in the water and the salt-treated specimens respectively. This observation and the previous experiments suggest that the apparently higher diastatic power of the extract from electrically treated malt is due to a greater concentration of the enzyme therein.

Liquefaction and saccharification of starch.—During recent years, considerable evidence has shown that vegetable amylases contain at least two components, the liquefying and the saccharifying enzymes, which (*a*) can be at least partially separated from each other and (*b*) respond differently to a variety of physical and chemical treatments (Wijsman, *Rec. trav. chim.*, 1890, **9**, 1; Pottevin, *Ann. Inst. Pasteur*, 1899, **13**, 665; Frankel and Homburg, *Biochem. Z.*, 1906, **8**, 389; Fricke and Kaja, *loc. cit.*, Narayanamurti and Norris, *J. Indian Inst. Sci.*, 1928, **12A**, 134). To ascertain whether electrical treatment had favoured the increased development of either or both the liquefying and the saccharifying components, the following experiment was made :—To specimens (5 g.) of treated and untreated malt, 150 c.c. of water was added, and after being vigorously shaken, the mixtures were treated with toluene, allowed to stand for 12 hours, filtered and the filtrates centrifuged from suspended matter. Aliquot parts (10 c.c.) of the enzyme solutions were added to mixtures of 40 c.c. of potato starch (2 per cent.), 10 c.c. of Walpole's acetate buffer (P_H 4·6) and 20 c.c. of water. The reaction mixtures were then maintained at 34° and their changes in viscosity as well as sugar content determined in the manner described by Patwardhan and Norris (*J. Indian Inst. Sci.*, 1928, **11A**, 123).

The results (Fig. V) show that the amylase from treated barley effected liquefaction and saccharification of starch more rapidly than that from untreated grain. The effect of the electrical treatment was slightly more pronounced in the case of the liquefying than the saccharifying component, particularly in the later stages. The differences were not however so great as to suggest that the liquefying enzyme was either stimulated to a greater extent or present in larger proportion than the saccharifying component. In view of the previous observation relating to the concentration of the enzyme, the results of the present experiment suggest that the increased diastatic activity observed in the case of electrically treated malt was due to larger amounts of both components.

Effect of electrical treatment on nitrogen transformations during malting.—The observations of Bishop (Ph. D. thesis, Cambridge; *J. Inst. Brewing*, 1929, **35**, 323) have shown that during both the development of the barley grain on the plant and the subsequent process of malting, there is a definite relationship between the total nitrogen of the grain and its distribution into various forms. In view of the above and the earlier observations regarding the relationship of nitrogen to quality in malt, it was considered desirable to determine the effect of electrical treatment of barley on the nitrogen contents of malts prepared at different stages.

The germination of barley as well as the drying of the specimens were carried out in the manner described already. The nitrogen contents of the different preparations of malt were determined by the kjeldahl method (A.O.A.C., 1925). The results (Fig. VI) show that the electrical treatment had a marked effect on the nitrogen content of malt. Whereas the water and the salt-soaked specimens lost nitrogen to the extent of only 0·23 and 0·26 per cent., respectively, in the course of 96 hours, the treated malt lost 0·58 per cent. within the same period. Since none of the nitrogen could have passed outside the plant system and since the plumules and the radicles derived their nutrition exclusively from the seed, it may be inferred that the nitrogen lost by the malt was actually gained by the seedlings (Table I).

TABLE I.

Percentages of barley nitrogen passing into the seedlings at different stages during germination.

Time in hours	Percentage of total nitrogen		
	Treated	Untreated	
		Electrolyte	Water
20	11·0	6·1	3·9
48	14·3	6·1	5·5
72	...	6·1	...
96	32·0	17·0	12·8

The movement of nitrogen (Fig. VI) proceeded comparatively slowly during the first three days, the rate being almost insignificant between 24 and 48 hours. After that period, it became suddenly rapid and the quantity passing out into the plumule and the radicle within the next 24 hours was more than the aggregate passing out during the three previous days. Under the experimental conditions, the movement proceeded about twice as fast in case of the treated seed. In view of the fact that the uptake of the essential nutrients, particularly nitrogen, by the seedling determines the various physiological activities such as assimilation, growth and tillering, it may be inferred from the above that the electrical treatment should prove helpful at least in the early stages of plant life.

It is not yet possible to explain the mechanism of increased uptake of nitrogen by the seedling. It may be that (*a*) there was greater proteolytic activity in the treated barley than in the untreated, resulting in quicker breakdown of proteins and transport of the products into the seedling, or (*b*) the electric current imported a physical stimulus or introduced a physico-chemical condition resulting in the seedling germinating from treated barley exerting greater osmotic pressure. If the latter be the case, then the effect may be expected to persist longer and lead to the seedling raised from electrically treated barley responding better to manurial treatment on the seed-bed than the one from untreated barley. Further work is needed to settle this point.

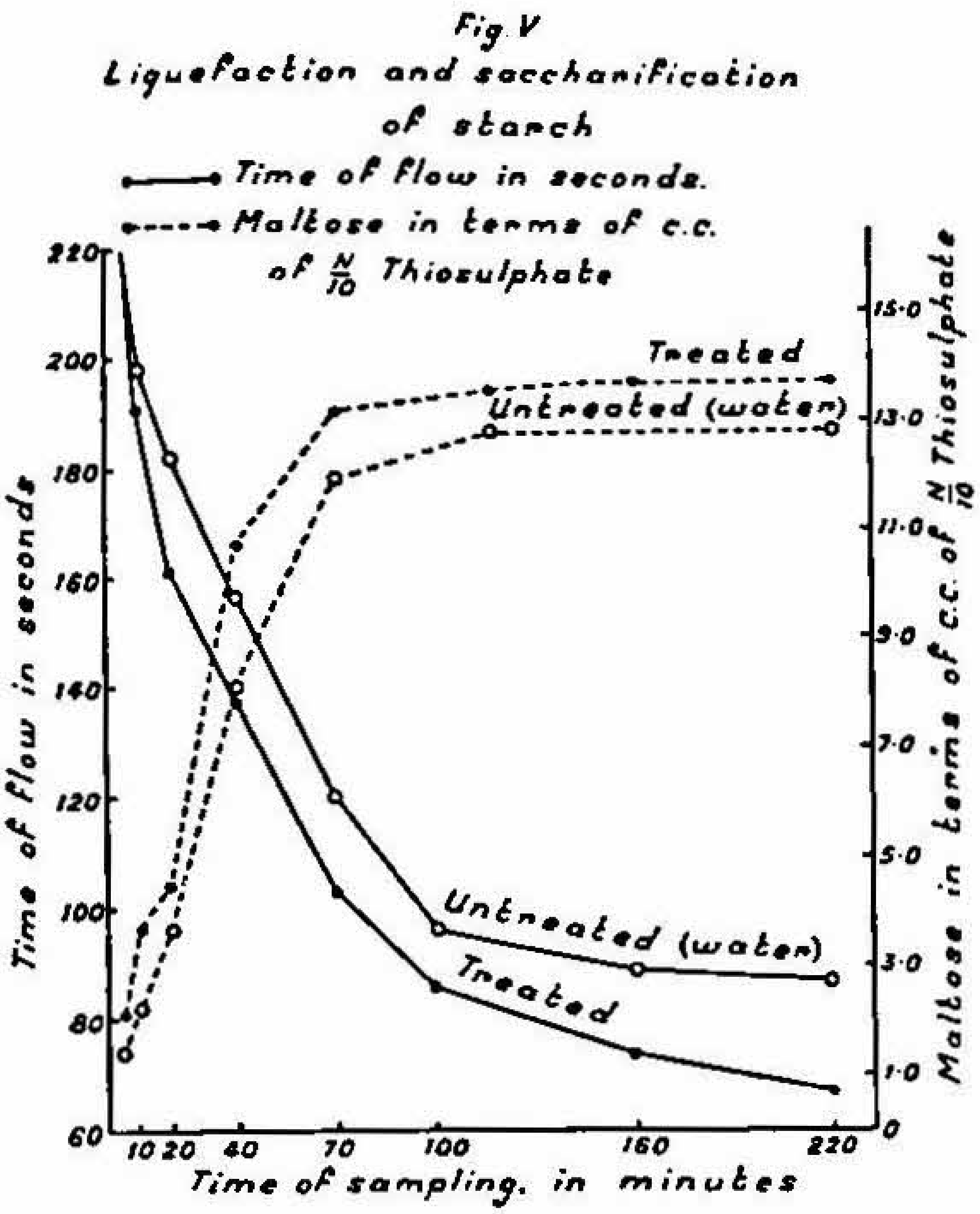
Fig. V
Liquefaction and saccharification of starch
Time of flow in seconds.
Maltose in terms of c.c. of N/10 Thiosulphate
Treated
Untreated (water)
Untreated (water)
Treated
Time of flow in seconds
Maltose in terms of c.c. of N/10 Thiosulphate
Time of sampling, in minutes
220
200
180
160
140
120
100
80
60
15·0
13·0
11·0
9·0
7·0
5·0
3·0
1·0
0
10 20 40 70 100 160 220

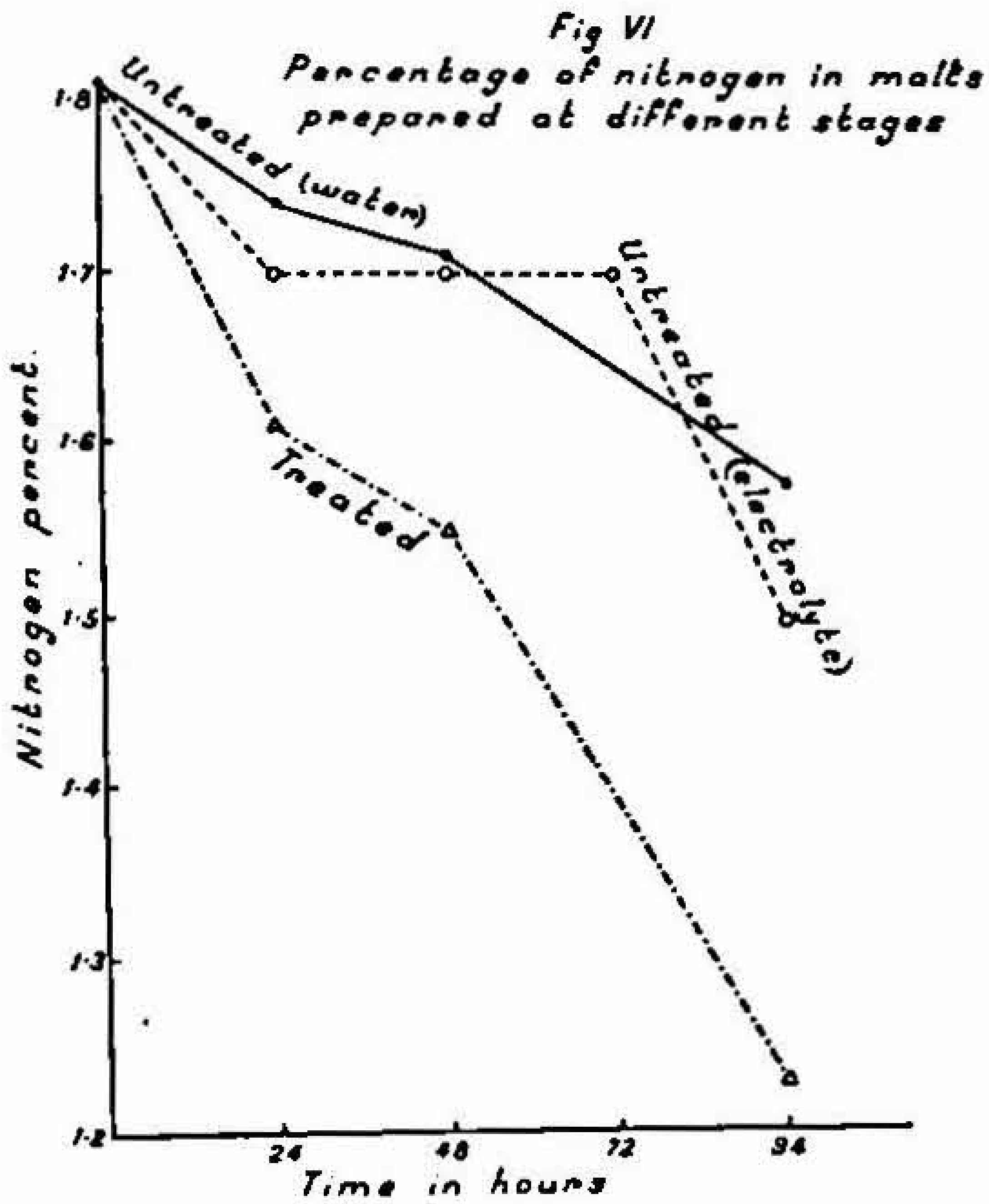
Fig VI
Percentage of nitrogen in malts prepared at different stages
Untreated (water)
Untreated (electrolyte)
Treated
Nitrogen percent.
Time in hours
1·8
1·7
1·6
1·5
1·4
1·3
1·2
24 48 72 94

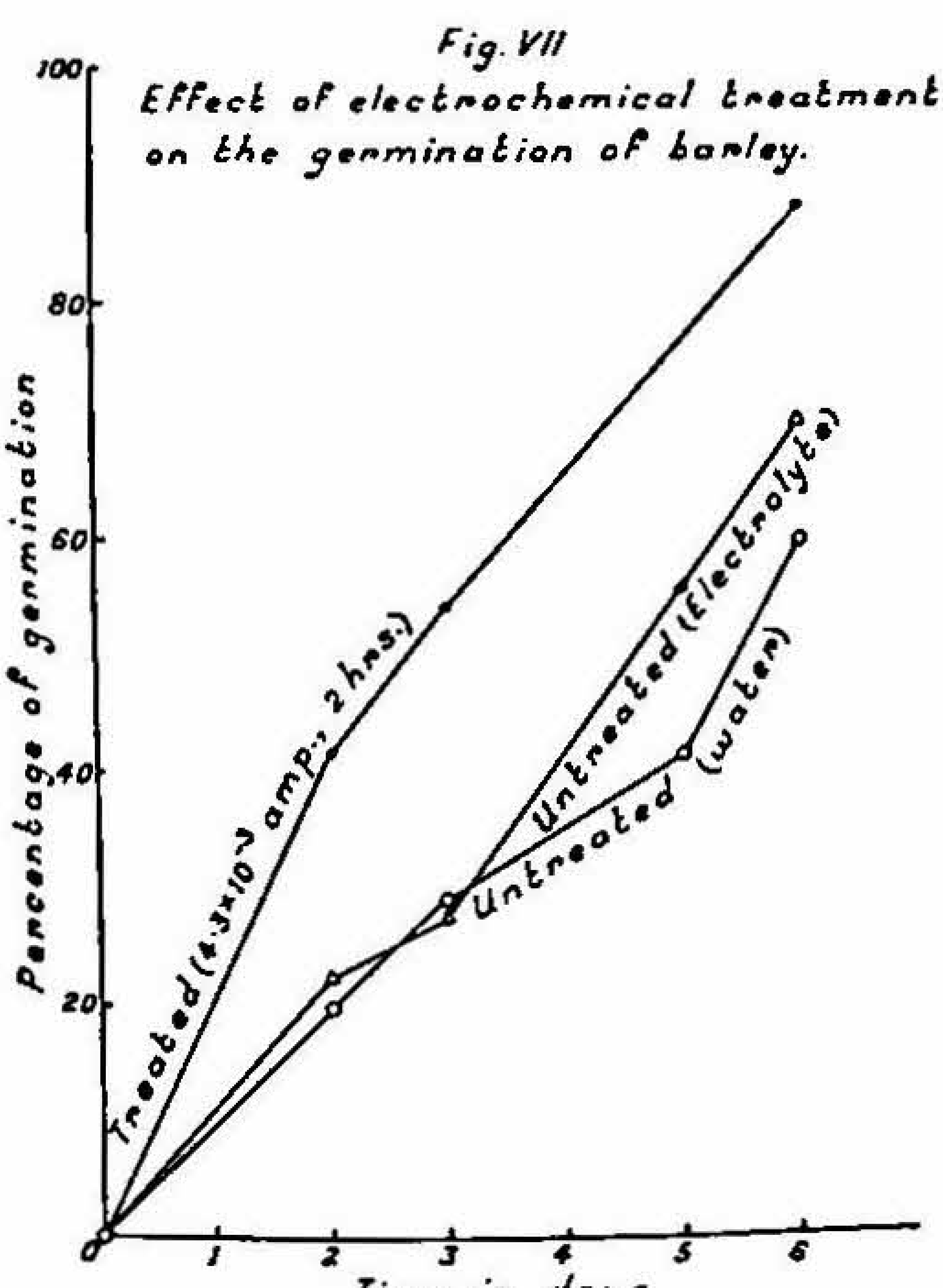
Fig. VII
Effect of electrochemical treatment on the germination of barley.
Treated (4·3×10⁻³ amp., 2 hrs.)
Untreated (Electrolyte)
Untreated (water)
Untreated
Percentage of germination
Time in days
100
80
60
40
20
0
1 2 3 4 5 6

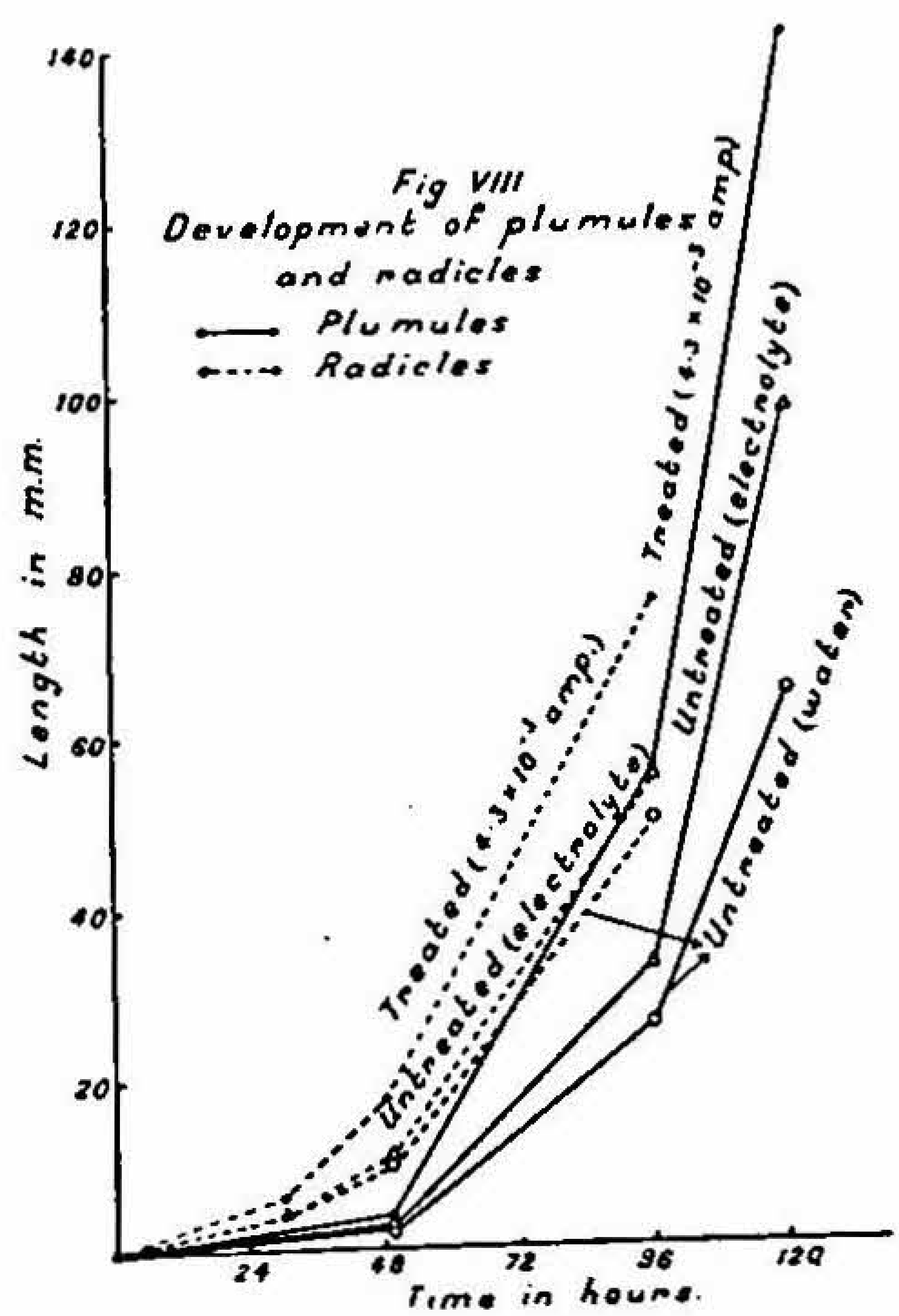
Fig VIII
Development of plumules and radicles
Plumules
Radicles
Treated (4·3×10⁻³ amp)
Untreated (electrolyte)
Untreated (water)
Treated (4·3×10⁻³ amp.)
Untreated (electrolyte)
Untreated
Length in m.m.
Time in hours.
140
120
100
80
60
40
20
24 48 72 96 120

The researches carried out under the auspices of the Institute of Brewing have already shown that when adopting the usual system of steeping and malting, the nitrogen content of malt is largely determined by the amount in the original barley. It has thus been found that the comparatively low percentages of nitrogen found in some superior malts are due to the fact that the latter are from barleys whose seeds were carefully selected, the plants being grown on sparsely manured soil to avoid excessive uptake of nitrogen. The results of the present experiment show that the quality attained after so much trouble and expense can be secured more easily and at much less cost by electrical treatment of the seeds previous to germination. The nitrogen content of the malt was reduced to such an extent as would have been possible only by starting with a barley of quality superior to that used in the experiment. Barley responds readily to nitrogenous manures; but since a grain of high nitrogen content is obtained together with the increased yield, the farmer is often obliged to sacrifice higher yield to attain better quality. The present observations show that by suitable manuring on the field followed by electrical treatment of seed, a high yield of grain as well as superior quality of malt can be secured.

Proteolytic enzymes of malt.—Recent investigations (Linderstrom-Lang and Sato, *Compt. rend. Trav. Lab. Carlsberg*, 1929, **17**, 17; Hopkins, *Biochem. J.*, 1929, **23**, 1022) have shown barley malt contains at least one protease and one peptidase. To determine whether the rapid movement of nitrogen observed in the case of electrically treated barley was due either to the activation, or increased production of the proteolytic enzymes, extracts from treated as well as untreated malts were prepared according to Mill and Linderstrom-Lang (*Compt. rend. Trav. Lab. Carlsberg*, 1928, **17**, 9) and their action on different substrates determined by the method of Linderstrom-Lang and Sato (*loc. cit.*). The results did not, however, bring out any difference between the activities of the enzymes from the two sources. The titration figures were almost identical at all stages, thereby suggesting that there was no appreciable increase in proteolytic activity as the result of electrical treatment.

Effect of electrical treatment on the germination of barley.—Some of the previous workers (*e.g.* Dunn, *loc. cit.*, Bennet, *loc. cit.*, Nehru, *Proc. Indian Sci. Congress*, 1930, 38) reporting on different methods of electrical treatment of seeds have stated that quick and efficient germination of seed together with rapid development of the seedling may be expected. To determine whether (*a*) similar advantages follow as the result of electrical treatment of barley under the optimum conditions, and (*b*) the increased enzyme activity observed during germination bears any relation to the development of the seedling, the following experiment was made:—The seeds were steeped successively in water and electrolyte in the manner described already and a current of $4 \cdot 3 \times 10^{-3}$ amp. passed through them for 2 hours. They were then washed carefully and germinated on moist sand at a uniform range of temperature (23—25°). The rate of germination was determined by counting the number of seeds whose plumules had just emerged at each stage of observation. The development of plumules and radicles was studied by measuring at stated intervals the respective length in a large number of representative specimens. The data thus obtained were compared with those for seeds merely soaked in water and electrolyte respectively.

A study of the percentages of germination at different stages (Fig. VII) shows that the electrically treated seeds sprouted very much more quickly than the untreated. Up to the end of the third day, the treated seeds germinated at nearly twice the rate of the untreated; after that period, the latter began sprouting at a relatively higher rate than the former and although the percentages of their germination were, at no time, so high as in the case of treated seeds, the observations suggest that the effect of electrical treatment was essentially one of hastening germination. It is possible that the treatment helped the germination of a small percentage of seeds which would not otherwise have emerged, but the results of the present experiment show that such an effect is very much less pronounced than that of quickened germination of seeds which would otherwise have sprouted more slowly.

Further examination of the results of germination at various intervals in the different cases shows that about 50 per cent. of the total number of seeds germinated between the first and the fifth days and about 30 per cent. between the fifth and the sixth days. The effect of the electrical treatment appears to have been chiefly one of hastening the germination of the seeds that sprouted during the first of the two periods mentioned.

It is not possible to state from the above observations whether the early germinating seeds favoured by the electrical treatment were in any way different from the others. Since it is known that even among mature grains collected from the same ear, some germinate less readily than others, it is hardly likely that there could be any measurable physical or physico-chemical difference between the seeds that respond readily to the treatment and those that do not.

The results of the germination studies explain in part the increased diastatic activity observed in the case of electrically treated malts. The investigations of Brown and Morris and other earlier workers have shown that, depending on the prevailing climatic and other conditions the diastatic power of germinating barley increases up to a certain period after which it decreases with considerable rapidity. There is also marked loss in the dry weight of malt owing to the major part of the solid matter having passed out into the seedlings. To secure the best results, the maltster is obliged to stop the germination of a batch of seeds on a particular day whether the germination of all individual grains is sufficiently advanced or not. The specimen of malt which is kilned at the desired point consists, therefore, of a certain percentage of seeds whose germination has proceeded up to the maximum diastatic power, and of seeds which have either over, or under germinated or not germinated at all. The diastatic power and the general quality of the malt will be determined more by the proportion of seeds which have germinated up to the desired point rather than by the others. The results of the foregoing trials lead to the inference that at the time of arresting germination on the fourth day, over 40 per cent. of the electrically treated seeds would have developed the maximum concentration of active enzyme, while in the case of the untreated the corresponding percentage should have been about 20. It thus follows that by effecting a quicker and more uniform germination, the electrical treatment helps to secure a malt with greater concentration of enzyme than would otherwise be possible. The increased diastatic power is also largely explained in this way.

The length measurements of plumules and radicles were carried out with seeds, whether treated or not, which germinated on the same day. The results may, therefore, be regarded as indicating the rates of development of the respective seedlings as distinct from the percentages of germination described in the previous experiment.

A study of the rates of development of the radicles (Fig. VIII) shows that those of the electrically treated barley grow about 50 per cent. more quickly than those of the untreated grain. In the case of the plumules, the difference is not appreciable up to the end of 48 hours, after which the treated grains develop much more quickly than the untreated, and maintain the difference up to the end of 120 hours. The correlation of development-rates of plumules and radicles between themselves, and with the simultaneous intake of nitrogen is positive, but not close. Thus whereas the intake of nitrogen within the first 20 hours is very rapid, there is no proportionate development of plumules and radicles. Between 48 and 72 hours the movement of nitrogen is very slow, but the development of the seedling proceeds rapidly during this period. A scrutiny of the results, suggests that there is a lag of about 24 hours between the intake of nitrogen and its visible effect on the development of the seedling. Such a period is presumably required for the movement of the nitrogenous compounds into the different parts of the plumules and radicles, and their re-synthesis into proteins. The effect of the nitrogen intake is first seen in the case of the radicles, which respond almost immediately, while in that of the plumules it follows only about 20 hours later. Allowing for the lag in each case, it may be noted that there is striking agreement between the intake of nitrogen and the subsequent development of the seedling.

The foregoing observations suggest that in addition to increasing the efficiency of germination, the electrical treatment brings about quicker movement of nitrogen into the seedling followed by more rapid development of the plumules and radicles than in the case of the untreated grain. Since the better formation of the seedling will favour the conditions for rapid photosynthesis as well as assimilation of mineral nutrients from the soil, it may be inferred that the electrical treatment of the seed will prove beneficial in the early stages of plant life.

There is striking agreement between the diastatic activity of malt at different stages and the corresponding development of seedlings. The correlation is closer in the case of the plumules than in that of the radicles. Thus, it may be noted that up to the end of 48 hours the diastatic power of treated malt is not significantly different from that of the untreated, and the same applies to development of the plumules. After that period, however, the diastatic power of treated malt increases more rapidly than that of the untreated, and similarly the plumules of treated barley develop faster than those of the untreated grain.

In the early stages of plant life, the seedling is dependent on the parent seed for its nutrition. The movement of the food materials into the seedling is rendered possible by the activity of the different digestive ferments, particularly that of amylase on starch. Since the electrical treatment leads to the development of a greater concentration of diastase in the germinating grain, it may be inferred that there is quicker and more efficient conversion of starch into soluble carbohydrates in the case of the treated grain than in that of the untreated. Since the rapid movement and consequent assimilation of the nutrients connotes more favourable development of the seedlings, it follows that the better growth of the electrically treated seedlings is due to the greater activity of the different enzymes, particularly that of amylase, in the germinating seed.

In the mature plant system, the nitrogenous as well as the mineral nutrients collected from the soil proceed from the roots to the different parts of the plant, while the carbohydrates and other products of photosynthesis move in the reverse direction. A similar order of movement appears to prevail in the seedling. The correlation studies suggest that the movement of nitrogen is first to the radicle and then to the plumule, while that of the carbohydrate is in the opposite direction.

Effect of electrical treatment of malting barley on the specific gravity and nitrogen content of wort.—Specimens of malts prepared from treated as well as untreated barley were ground to coarse powder, mixed with five times their weight of water and maintained at 55° for 6 hours. The mixtures were then boiled for 1 hour, allowed to cool, filtered, the solid washed and the filtrates made up to the same value in each case. The worts were compared for colour, aroma, nitrogen content and specific gravity. The experiments were repeated many times and the observations representing ranges of values have been presented in Table II.

TABLE II.

Source of extract : treatment of seed	Colour	Odour	Nitrogen per cent.	Specific gravity
Treated with $4 \cdot 3 \times 10^{-3}$ amp. for 2 hours ...	Orange ...	Highly aromatic ...	0·140–0·163	1·063–1·064
Untreated : Soaked in electrolyte ...	Light yellow.	Faintly aromatic ...	0·198–0·201	1·045–1·046
Untreated : Soaked in water	Pale yellow...	do.	0·207–0·233	1·042–1·045

It is rather difficult, at this stage, to explain the differences in colour and aroma though the starting materials and details of malting were the same in all the cases. The observations suggest that some caramelisation has occurred in the case of the treated specimen, but since the conditions of drying the malt and boiling the wort were identical, it is hardly probable that the differences observed were due to greater heating during those stages. Since the only difference between the various worts consisted in the original treatments of the barleys concerned, it may be inferred that the electrical treatment had produced an effect allied to kilning at a relatively high temperature. In this connection, it should be noted that strong heating as in the case of mild ale or porter malts tends to lower the diastatic power while the electrical treatment actually helps to increase it. The effects of the two treatments cannot therefore be regarded as identical.

The results of the replicate determinations of the nitrogen contents of the different preparations vary so considerably among themselves that it is not possible to draw any definite conclusion from them. It may, however, be said in a general way, that wort prepared from electrically treated barley contains less nitrogen than that from untreated grain.

The differences between the specific gravity of the different worts are more striking, that from treated barley corresponding to a malt extract of about 15·7 per cent. while those from untreated grain approximate to only 10·9 per cent. The maltose and dextrin contents vary in a similar manner and, reckoned according to the Brown and Morris formula, they would be 16·9 and 11·7 per cent. for the worts from treated and untreated barleys, respectively.

The foregoing observations raise various points of academic interest and practical importance. The deep colour and the sweet odour of the wort suggest that the electrical treatment had produced certain physico-chemical effects akin to that of strong heating without impairing the activity of the enzyme. The low nitrogen and the high sugar contents appear to be related and suggest that by the rapid removal of the nitrogenous compounds to the plumules and radicles, quick and efficient hydrolysis of starch and other polysaccharides by the different enzymes was rendered possible.

DISCUSSION.

The researches of Lintner, Osborne and other earlier workers have shown that vegetable amylases occur in association with proteins, chiefly albumins, from which it is not possible to separate them without impairing the enzymic activity. Sherman and Gettler observed that the distribution of nitrogen in a number of preparations of animal and vegetable amylases was the same as in proteins and concluded therefrom that the enzymes are proteins in nature (*J.A.C.S.*, 1913, **35**, 1790). Lüers (*Biochem. Z.*, 1920, **104**, 71) as well as Baker and Hulton (*J.C.S.*, 1922, **121**, 1929) obtained evidence to suggest that barley malt amylase is derived from the proteins of the grain. The observations of many of the later workers (e.g., Van Laer, *Z. Ges. Brauw.* 1914, **36**, 489; Willstätter, Waldschmidt-Leitz and Hesse, *Z. physiol. Chem.*, 1923, **126**, 143; Fricke and Kaja, *Ber.*, 1924, **57**, 765; Narayanamurti and Norris, *J. Indian Inst. Sci.*, 1928, **11A**, 134) did not support such views. No definite evidence has however been, so far, obtained either to establish the chemical nature of the enzyme or to exclude the need for its association with proteins.

It is generally known that malts derived from barleys rich in nitrogen possess high diastatic power (Rothamsted Conference on Malting barley, *loc. cit.*). The observations of Bishop (*loc. cit.*) have shown that the percentage distribution of nitrogen in barley is the same for all specimens belonging to a particular variety. It may be inferred from the above that the actual quantities of the different proteins present in the seed as well as in the derived malt would be proportional to the total nitrogen content of the former. Assuming that barley malt amylase is a protein, it would follow that its quantity as well as its activity would depend on the nitrogen content of the seed. The results of the present investigation do not, however, lend support to such a view: the malt prepared from electrically treated barley contains less nitrogen but more active enzyme than the one from untreated grain. Since the lower nitrogen content of the electrically treated malt is due to the rapid movement of the former into the seedling, it would, therefore, appear that the amylase protein (if such it is) differs from the ordinary seed protein in that it cannot be hydrolysed by the proteolytic enzymes present in the seed and thus be transported to the seedling.

It has been estimated that during steeping, the barley grain absorbs about 50 per cent. of its weight of water (Ross-Mackenzie, Brewing and Malting, 1926) which causes the proteins and the starch to swell up to soft masses holding water and salts in their interstices. In such a condition the seed offers a low resistance to the passage of electricity and it may therefore be assumed that during the electrical treatment, quite a considerable part of the energy passed through the seed.

The barley grain is rich in albumins which are more sensitive to physical and physico-chemical changes than the other proteins and in view of the observations of Hardy, (*J. Physiol.*, 1899, **24**, 288) it may be inferred that even a current of relatively low density would have denatured quite a large part of the albumins and the other associated proteins of the seed.

The observations of Mendel and Lewis (*J. Biol. Chem.*, 1913–14, **16**, 55), Bateman (*J. Biol. Chem.*, 1916, **26**, 263), Finks and Johns (*Am. J. Physiol.*, 1921, **57**, 61) and later workers have established that proteins are digested by proteolytic enzymes very much more readily when denatured than if raw. It would therefore follow that the proteins of the electrically treated barley would be hydrolysed more readily than those of the untreated grain during germination. This explanation accounts for the quick transport of nitrogen into the seedlings of electrically treated barley, but casts considerable doubt on the possibility of malt amylase being a protein allied to albumins. It may be expected that a treatment leading to the denaturation of proteins would have inactivated, at least partly, an enzyme belonging to the same class of compounds: but the increased activity observed in the case of malt amylase shows that although the enzyme may occur associated with proteins, and may even have the same distribution of nitrogen as the seed protein, its structure and properties are different from those of proteins.

It is well known that malt derived from barley rich in nitrogen has a high diastatic power, but yields a wort of low specific gravity, while the reverse is true of grain containing less of that element. Diastatic power being a measure of the initial rate of action of the enzyme on the substrate, it would appear from the above that the presence of nitrogenous compounds is favourable to the hydrolysis of starch by amylase in the early stages. The low gravity of the wort obtained after prolonged action of the enzyme suggests on the other hand that, in presence of excess of nitrogen, the conversion of starch into maltose and dextrins does not proceed to completion. The present investigation has already shown that (*a*) malt amylase acts independently of the nitrogenous compounds of the grain, and (*b*) rapid removal of nitrogen from the seed helps to increase the efficiency of the hydrolysis. The foregoing observations lead to the inference that although malt amylase occurs in association with the nitrogenous compounds of the grain, yet these, in some way hitherto obscure, hinder the prolonged action of the enzyme on starch.

In the initial stages of preparing a wort, much of the water-soluble as well as the salt-soluble proteins of the malt would pass into solution, the quantities thus peptised depending on (*a*) the quantities of inorganic salts present in the water, and (*b*) the total nitrogen of the grain. As the enzyme action progresses, the relatively high temperature, together with the increasingly acid reaction would bring about the denaturation and coagulation of the dissolved proteins, the quantities thus precipitated increasing at logarithmic rate as shown by Chick and Martin (*J. Physiol.*, 1910 **40**, 404; 1911, **43**, 1; 1912, **45**, 61, 261). The coagulated proteins would naturally settle on the particles of unattacked grist, the thickness of this protective layer depending on the quantity of protein originally in solution. At the same time, increasing quantities of hordein from incompletely malted grains would dissolve, the change in reaction facilitating their ready peptisation. Though hordein is not readily coagulated by heat, it may be expected that its occurrence around the unattacked particles of malt would add to the protective action exercised by the coagulated proteins.

Although the precise nature of the action of amylase on starch is still obscure, yet it is now definitely known that the hydrolysis is preceded by the adsorption of the enzyme on the surface of the starch gel. The occurrence of coagulated protein around the particles of unattacked grist would naturally interfere with the adsorption of the enzyme on the substrate, the extent of such hindrance depending on the thickness of the layer of protein. As already mentioned, the protein content of malt is proportional to its total nitrogen so that the extent of the hydrolysis of starch and, consequently, the specific gravity of the wort would be determined by the nitrogen content of the malt. It may, at the same time, be expected that some of the hordein and other resistant proteins which are not fully coagulated even on boiling the wort would find their way to the fermenting vessel, where, with the greatly increased acidity, saturation with carbon dioxide, lowered surface tension and viscosity and decreasing specific gravity, they would pass into the suspensoid condition and thereby (*a*) prevent mechanically the active functioning of the yeast and (*b*) introduce cloudiness in the fermented liquid. The extent to which such effects are produced will depend on the nitrogen content of the original barley for reasons stated already.

The above explanation would probably account for the various observations recorded by previous workers on the significance of the nitrogen content of malting barley to the brewing industry. In view of the technical importance of the subject, it is desirable that a further systematic investigation on the relation of different types and conditions of vegetable proteins to the action of amylase on starch be undertaken. Although the presence of suspended protein is detrimental to the success of the fermentation, yet the observations of Chapman have shown that the occurrence of useful amounts of dissolved nitrogen in the wort is necessary for the nutrition of yeast and the successful fermentation (Rothamsted Conference, *loc. cit.*). In view of the above and the fact that the malt protease is not generally sufficiently active to hydrolyse the superfluous amounts of proteins, it would appear that the addition of small quantities of highly active and thermo-resistant vegetable proteases like papain to either the mash prior to boiling, or the wort during cooling, may help to achieve the desired end.

The available data do not throw any light on the varied effects of different (*a*) concentrations of electrolyte, (*b*) densities of current and (*c*) periods of treatment. Further study of the energy distribution between seed and electrolyte, and the attendant physical and physico-chemical changes should be made before the significance of such observations can be understood.

The conditions relating to the steeping of seed prior to the electrical treatment also deserve further study. Thus, an investigation of the effects of varying (*a*) the periods of steeping in water and electrolyte respectively and (*b*) the nature, the concentration and the reaction of the electrolyte, may lead to useful results. Owing, probably, to the prolonged preliminary steeping in water, or the quick movement of the absorbed nitrate into the seedling, none of the electrolyte could be detected in the malts prepared by us. It is, however, possible that under other conditions, considerable amounts of the electrolyte may be present in the seed, and thereby influence (*a*) the germination of the seed and the development of the seedling and (*b*) the quantity of the derived malt.

The results of the present investigation also have a practical interest. The importance of the different observations to the brewing industry has already been outlined. Similar advantages may be expected to follow in the manufacture of malt syrup, malt vinegar and industrial alcohol from starchy materials. It may, perhaps, be argued that electrically treated malt will have a low nutritive value owing to its reduced nitrogen content, and therefore be unsuitable for the manufacture of malted foods. Since, however, most of such food materials contain large quantities of evaporated milk or such other products which are rich in proteins, it is hardly likely that their nutritive value would thereby be appreciably impaired : on the other hand, the high concentration of amylase and sweet flavour would commend the use of electrically treated malt in such industries.

The possibility of the application of electrical treatment to the manufacture of active preparations of various digestive ferments also deserves systematic investigation. Although our preliminary observations indicated that castor-seed lipase may not respond to the treatment, it is yet possible that the amylases, lipases, proteases and other characteristic enzymes present in a large number of other seeds, or in plant and animal tissues, would be readily activated under conditions similar to those described in the present study.

The results of this investigation indicate that the electrical treatment of seed may prove beneficial in the early stages of plant life. In view, however, of the reports of Brenchley, Goddard and others (*loc. cit.*) regarding the ineffectiveness of the seeds treated by the Wolfryn process, it is desirable that further systematic field trials should be conducted. It is possible that factors such as age and previous condition of seed, season and soil conditions, may influence the efficacy of the electrical treatment of seeds. Further laboratory work should also be pursued along lines already suggested for malting barley, so that the maximum benefit may be derived at minimum cost ; this has been begun in the biochemical laboratories of the Indian Institute of Science ; but in view of the variety in (*a*) soil and climatic conditions of different countries, and (*b*) technical details relating to malting and related industrial operations, it is desirable that similar investigations should be conducted concurrently in other parts of the world.

SUMMARY.

The effects of (*a*) concentration of electrolyte, (*b*) current density, and (*c*) period of treatment of barley on the diastatic power of malt prepared from it were studied. It was found that preliminary soaking in water for 24 hours followed by steeping in a 0·1 per cent. solution of sodium nitrate and passage of alternating current (25 cycles, 210 V.,

and density, $4{\cdot}3 \times 10^{-3}$ amp.) for 2 hours led to over 100 per cent. increase in the diastatic power of malt.

A study of the various factors leading to the activation showed that (*a*) the activity of the enzyme unit was the same as in malt prepared from untreated barley, (*b*) no evidence of any change in either the properties of barley starch or the nature of enzyme activators could be obtained, (*c*) under the experimental conditions, even traces of the electrolyte could not be detected in the treated malt and (*d*) evidence was obtained to show that the concentration of the enzyme as well as of its liquefying and saccharifying components was greater in the treated malt than in the untreated.

The movement of nitrogen into the seedling was more rapid in the case of electrically treated barley than in the untreated grain. There was, however, no evidence to show that the protease activity of the former was greater than that of the latter.

The treated grains germinated in greater proportion and with more uniformity than the untreated. The seedlings of the former also developed faster than those of the latter.

The wort from electrified malt had a deeper colour, better aroma, less nitrogen and about 50 per cent. more sugar than the preparation from untreated malt.

There is evidence to show that although the enzyme amylase occurs in association with the proteins of the grain, it is not itself a protein. The observations suggest that, when present in excess, the proteins of the malt form protective layers around the starch and thus hinder the action of amylase from proceeding to completion.

The academic as well as the practical significance of the foregoing and allied observations have been discussed and future lines of work indicated.

Our thanks are due to Mr. T. J. Mirchandani for valuable suggestions with regard to the technique of the treatment.

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[Accepted, 7–7–31.]

PRINTED AND PUBLISHED
AT THE DIOCESAN PRESS, POST BOX 455, VEPERY,
MADRAS—1931. C2529.