MECHANISM OF INCREASE IN AMYLASE ACTIVITY DURING AUTOLYSIS OF BARLEY POWDER.

By R. H. Ramachandra Rau, M.Sc.,
Department of Biochemistry, Indian Institute of Science, Bangalore.

Received April 9, 1935.
(Communicated by Prof. V. Subrahmanyan, D.Sc., F.I.C.)

It has been suggested by a number of earlier workers that development of amyloclastic power in germinating barley takes place as a consequence of and with the co-operation of the proteoclastic system of enzymes of the seed (Baker and Hulton, 1922; Syniewski, 1925; 1925-26; 1927-28; Polak and Tychowski, 1927-28). Lüers (1920), on the other hand, maintained that the formation of the so-called β-diastase in malt is a process of oxidation of α-diastase of barley. More recent investigations of Waldschmidt-Leitz and Purr (1931) show that increase in amyloclastic power follows the activation of the seed proteinases. According to these authors, the essential difference between the barley amylose system and the malt system consists in the formation of amylokinase, an activator of organic nature which originates during the process of sprouting.

Baker and Hulton (loc. cit.) have shown that by auto-digestion of barley powder, the amyloclastic activity is increased. Similar increase is observed on treatment with papain. From a critical examination of the literature, it is not very clear whether the increased activity on autolysis is due to liberation of larger quantities of enzyme (or its active group) from adsorptive combination with proteins or whether any specific activator of the type of amylokinase arises and causes a change in activity of the enzyme. The observation that during germination activation of the seed proteinase precedes the formation of the activator would suggest that it is formed either as a product of decomposition of proteins or is liberated from them from an adsorbed state. Since it is known that proteoclastic enzymes play a prominent rôle in autolytic processes, it was thought possible that a similar type of activator would arise during autolysis and be responsible for the observed increase in activity. It was decided, therefore, to investigate whether the mechanism of autolysis in the physiological process of germination as it affects the amyloclastic system is similar to that occurring in remains of dead cells or their extracts. With a view to testing out this, the amyloclastic
activity of digests of powdered barley allowed to autolyse for different periods of time was determined.

Preparation of samples.—Malting barley (5 g.) was powdered to pass the 40-mesh sieve and mixed with 25 c.c. of distilled water and 5 c.c. of toluene in a centrifuge tube. The specimen to be examined on the first day was shaken for 2 hours in a shaker, centrifuged for 5 mins., and the almost clear supernatant filtered through dry filter paper. The extract was preserved with 1 c.c. of toluene. The other samples were allowed to autolyse for 48, 72, 96 hours and 8 days respectively. Each day the samples were shaken occasionally for a few minutes. At the end of the period of autolysis they were treated as before.

Determination of amylase activity.—To 50 c.c. lots of a solution of soluble starch (Zulkowski) containing 250 mg. were added 10 c.c. of Walpole’s acetate buffer (pH 5.1) and 0.5 c.c. of the enzyme extract obtained as described above and the sugar estimated at stated intervals (after stopping the enzyme action with 30 c.c. of N/10 caustic alkali) according to Willstätter and Schudel (1918). The results given in Table I show the percentage of maltose formed at different intervals of time (calculated on the basis of 650 mg. of maltose from 1 g. of starch).

The values for \( x/t \) in Table I go on decreasing in all cases except that of the extract obtained by shaking for two hours where there is a tendency for the ratio to be constant for a short period. The monomolecular constants also tend to decrease and as could be seen from Fig. 1, the enzyme from fresh barley follows reaction of the first order for a short period while with the other extracts the activity proceeds very much slower. These results would show that the enzymes obtained by autolytic processes are comparatively less stable.

The above results also show that during autolysis no activator of the type of amylokinase is formed. In this connection it is interesting to compare the behaviour of malt amylase as well as the system barley amylase plus amylokinase studied by Waldschmidt-Leitz and Purr (loc. cit.). For the saccharification of amylose by malt amylase those authors found a direct proportionality between time and action; the monomolecular constant showed a corresponding ascent. With fresh barley amylase, the hydrolysis was found to proceed much slower than a reaction of the first order. The course of saccharification with barley amylase activated by amylokinase, on the other hand, corresponded to that of the malt enzyme. These considerations when compared with the result obtained with the enzyme extracts from autolysed barley digests would preclude the possibility of the formation of amylokinase during autolysis.