SUMMARY

AND

CONCLUSIONS
1) Different fungal cultures available in the Departmental Collection were screened for glucoamylase activity.

2) The fungus *Aspergillus niger* NRRL 330 has been used for studies on glucoamylase formation under different nutritional and other growth conditions.

3) Maximum activity is produced 5 days after inoculation. Most of the enzyme formation occurs after growth phase ends.

4) Among the cereals tested, jowar and ragi provide the best medium. 14 per cent jowar (acid or amylase-treated for thinning) seems to be the best substrate for enzyme formation.

5) An initial pH of the medium of 3.5 to 5 and a growth temperature of 30 to 35° seems optimal for enzyme formation.

6) Growth in about 50 ml of medium in 250 ml Erlenmeyer flasks incubated on a rotary shaker gives good yields of enzyme.

7) Spores or 10 per cent of 24/48 hr vegetative inocula give good results in 120/96 hr.

8) Ammonium sulphate and urea were the best among inorganic sources of nitrogen. The latter were generally inferior to complex nitrogen sources such as peptone and yeast extract. But jowar was
better than even peptone-starch in promoting enzyme yields.

9) Among the sugars tested, only starch and maltose induced enzyme formation. Glucose was rather poor while galactose, lactose, etc. were ineffective.

10) B-complex vitamins and inorganic compounds such as phosphates and trace metals were not found to be essential. Perhaps they were present in sufficient quantities in the starch.

11) Based on the above findings, fermentations were carried out in 15 litre batches in stirred jar fermenters using 14 per cent acid-thinned jowar. Maximal yields of enzyme (18 units/ml) were obtained with aeration at 3 litres of air/litre media/min and agitation at 510 rpm, after 96 hr growth.

12) Hydrolysis of starch at different concentration by acids, by malt-enzyme or bacterial amylase, by malt followed by acid and by bacterial amylase followed by acid was studied. The results show that at 30 per cent starch concentration, bacterial amylase-acid combination hydrolysed starch to the extent of 91 per cent.

13) Digestion of 35 – 40 per cent starch with glucoamylase for 48 hrs at 60° and pH 4 subsequent to the action of bacterial amylase resulted in 96 per cent conversion to glucose.
14) The strain *A. niger* NBRiL 350 has been found to form three separable glucoamylase fractions on jowar media. The three fractions have been purified and found to be homogeneous by disc gel electrophoresis and only of them fraction B was tested ultracentrifugally and found homogeneous. Some of the properties of these enzymes have been studied.

15) The three fractions comprise 31 per cent, 33 per cent and 5.3 per cent of the total glucoamylase activity respectively.

16) The temperature and pH optima for activity seem similar to those of enzymes described in literature, viz. about 60° and 4.5 respectively.

17) In the absence of the substrate, the enzymes are stable at pH 2 - 2.5 at 25° for 2 - 3 days at 25° and pH 4 for a month and at 4° and pH 4 for about 12 months. They lose activity when heated to 50° or higher for more than 5 minutes.

18) The three fractions contain 8.2 per cent, 18 per cent and 6.2 per cent carbohydrates respectively; the nature of the carbohydrate present has not been investigated. Thus one of them resembles glucoamylase II of Pazur (1969) and Lineback (1972) since all have 18 per cent carbohydrate and the others seem different from the ones described in literature.
19) Mild periodate (0.02 M) treatment results in a partial loss of activity, whereas at 0.1 M, 96 per cent activity is lost in 10 min. It is not clear whether the loss in activity is due either to the lability/stability of the enzymes in the absence of the bound carbohydrate or whether a certain quantity of carbohydrate is essential for activity.

20) Treatment with urea (8 M) has no effect on the activity of the enzymes whereas guanidine hydrochloride (0.5 to 2 M) affects the activity. The results of SDS-polyacrylamide gel electrophoresis, and urea effects indicate that the enzyme molecules do not have subunits.

21) The molecular weights of the three fractions have been determined by gel filtration on Sephadex G-100 and by the SDS-polyacrylamide procedures. They are found to be 91,000 (81,300), 71,000 (69,200) and 72,000 (70,700) respectively; the figures in parenthesis were obtained by the SDS-polyacrylamide procedure.

22) Photooxidation of the enzyme in the presence of (0.01 per cent) methylene blue or Rose Bengal at pH 4 has hardly any effect on the activity of the enzymes. But at pH 8.9, there is 40 to 50 per cent loss in activity. The amino acid analysis has not been conducted after photooxidation and therefore the decrease in activity cannot as yet be specifically attributed to the destruction of any amino acids.
23) The chelating agent EDTA, the sulfhydryl-blocking reagents such as pCMB, iodoacetamide and NEM, the compounds which combine with tryptophane, and NBS had no effect on the activity of any of the fractions under the conditions of test. It is possible that divalent cations such as Ca++, serine, histidine, tryptophane and cysteine (SH groups) are not involved in the activity of the enzymes.

24) Trypsin digestion (4 per cent w/w) at 30° at pH 7.8 for as long as 3 hr does not still abolish the activity. Fraction C loses 75 per cent activity under these conditions whereas A and B show about 40 per cent the original activity. It would be instructive to determine the molecular weight of the digested active components since they may give an idea of the minimum molecular weight needed for activity.

25) The N-terminal amino acid of the three fractions seems to be common, namely, phenylalanine. This is another distinguishing feature since the glucoamylase I of fineback (1969) contains alanine as the N-terminal amino acid. The C-terminal amino acid also seems to be leucine/isoleucine in all three cases.