

The uteri of rats in every group that received an injection of PMS, irrespective of DXM administration, increased in weight significantly ( $p < 0.05$ ) as compared to vehicle-injected control rats (table).

**Discussion.** Ovaries from PMS-treated rats which were given dexamethasone, had matured follicles or follicles approaching maturity, but ovulation had been almost entirely prevented since ova could be found in these follicles and corpora lutea were rarely present. Ovaries from PMS-treated control rats contained many well developed corpora lutea.

- 1 H. H. Feder, K. Brown-Grant and C. S. Corker, *J. Endocr.* 50, 29 (1971).
- 2 S. Y. Ying and R. K. Meyer, *Endocrinology* 84, 1466 (1969).
- 3 N. Hagino and J. W. Goldzieher, *Neuroendocrinology* 17, 27 (1975).
- 4 J. Vernikos-Danellis, P. Berger and J. S. Brachas, *Prog. Brain Res.* 39, 301 (1973).
- 5 M. L. Simon and R. George, *Neuroendocrinology* 17, 125 (1975).

The results indicate that luteinization should have occurred unless dexamethasone had inhibited either the hypophysis or centers in the central nervous system which influence the function of the hypophysis and thereby had blocked the release of endogenous LH. To further test these proposals, a group of rats was given PMS and DXM, in addition, HCG, which has LH-like activity, was administered. Dexamethasone did not suppress exogenous LH activity on the ovary in this group of rats.

DXM suppression of ovulation might be due to its central effect rather than its blocking effect on ACTH release, since ACTH administration did not overcome the inhibiting effect of DXM on ovulation. It is possible that DXM effect on ovulation was brought about by its direct effect on the brain and the hypothalamic biogenic amines. An inverse relationship has been indicated between circulating glucocorticoid levels and brain serotonin levels in the hypothalamus<sup>4</sup>, striatum, cortex and amygdala<sup>5</sup>.

## PRO EXPERIMENTIS

### A turbidimetric method for the screening of amylase-producing mutants of *Aspergillus niger*

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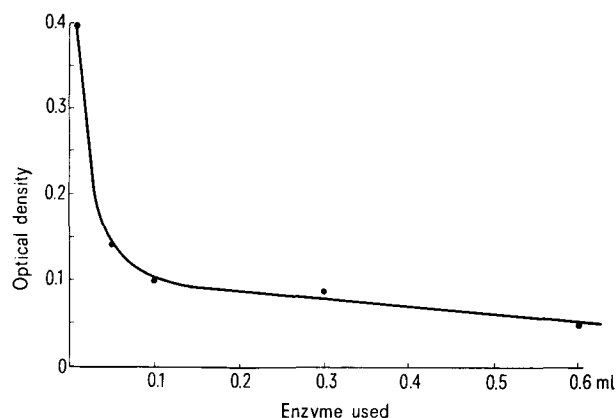
**Summary.** The method is based on the assumption that extracellular amylase, which is produced by strains of *Aspergillus niger* in liquid culture, hydrolyses the starch in the media and brings about a corresponding decrease in the turbidity of the media. Mutant strains which produced different quantities of amylase exhibited different degrees of decrease in turbidity of the media. The results showed that a greater degree of decrease in turbidity was observed for a higher quantity of amylase produced.

According to a recent FAO estimate<sup>1</sup>, about 95 million tons of starch from cassava were produced in 1970. It has been reported that the fungus, *Aspergillus niger*, produces amylases which hydrolyse starch<sup>2</sup>. It is the particular object of the present research to obtain mutant strains of *Aspergillus niger* with high amylolytic activity.

In this communication, we report a convenient turbidimetric method for the screening of amylase producing

mutants of *Aspergillus niger*. A turbidimetric method for the determination of  $\alpha$ -amylase has already been described by PERALTA and REINHOLD<sup>3</sup>. The present method is based on the assumption that extracellular amylase produced by the organism in culture in a liquid suspension containing starch hydrolyses the starch and this brings about a decrease in the turbidity of the culture media. The degree of turbidity of the suspension is related to the quantity of extracellular amylases produced by the particular strain in culture.

Tapioca flour liquid media used to cultivate *A. niger* contained 2 g NaNO<sub>3</sub>, 2 g tapioca flour and 100 ml water in 250 ml Erlenmeyer flasks. One loopful of conidiospores of each of 20 strains tested was inoculated into the media and incubated for 4 days at 34°C. The mycelial mat which formed on the surface of the media was removed and the resultant growth media were subjected to turbidity measurement at 550 nm. The blank in this case was water and the control was tapioca flour liquid medium prior to inoculation with fungal spores. 4 replicates were used in each case.



Measurement of turbidity of tapioca flour media containing specific quantities of  $\alpha$ -amylase. Optical density at 550 nm.

<sup>1</sup> Food and Agriculture Organisation, Rome. Production Yearbook (1971).

<sup>2</sup> G. T. BANKS, F. BINNS and R. L. CUTCLIFFE, *Progr. indust. Microbiol.* 6, 95 (1976).

<sup>3</sup> O. PERALTA and J. G. REINHOLD, *Clin. chim. Acta* 1, 157 (1955).

Relative quantities of  $\alpha$ -amylase (extracellular) produced by each strain of *A. niger* obtained by comparison with the standard curve

Fungal strain	Nutrient requirement	Volume of $\alpha$ -amylase enzyme corresponding to optical density from standard curve	Enzyme activity
551	none	0.625	16656.25
<i>An</i> (wild type)	none	0.07	1865.5
P4	none	0.41	10926.5
83	none	0.15	3997.5
32	none	0.07	1865.5
758	Arginine*	0.085	2265.25
111	Leucine*	0.19	5063.5
346	Methionine*	0.15	3997.5
862	Leucine*	0.11	2931.5
554	Methionine	0.06	1599.0
115	Methionine*	0.05	1332.5
16	Methionine*	0.05	1332.5
596	none	0.05	1332.5
E2	none	0.07	1865.5
E3	none	0.19	5063.5
E4	none	0.11	2931.5
P1	none	0.025	666.25
P3	none	0.015	399.75
56	none	0.03	799.5
57	none	0.01	266.5

\*Tapioca flour liquid media supplemented with respective amino acids at a final concentration of 1 g/l.

In another series of experiments, a similar procedure was repeated with the introduction into the tapioca liquid medium of known volumes of pure,  $\alpha$ -amylase in the place of the fungal culture. 4 replicates were used in each case. The Figure summarizes the results obtained in the form of a graph.

A comparison of the mean optical density readings for the strains for the first experiment and the values of the standard curve gives information on the respective relative quantities of  $\alpha$ -amylase produced by each strain of *A. niger* (Table).

Mutant strains of *Aspergillus niger* used in the analysis were obtained from the wild type strain *An*, by mutagenic treatment.  $\gamma$ -ray irradiation of conidiospores of the wild type strain using a  $\text{Co}^{60}$  source to give a dose of 50 Kr was administered. This gave a survival of about 2%. Mutant strains that were obtained differed from the wild type in specific nutrient requirements, morphology and ability to utilize starch. High producing strain, 551, is a prototroph which conidiates freely. Strain P4 is another prototroph which conidiates freely. 4 auxotrophic mutants have improved amylase activity when compared with the wild type strain and 3 have a slightly lower level of enzyme activity. From the results, some of the strains with very low activity seemed to be prototrophic.

This turbidimetric method provides a convenient means for determining the starch utilization capability of strains of the fungus.

## A simple system for inspection of microelectrophoretic patterns

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**Summary.** An inexpensive device for inspection of electrophoretic patterns on microgels is described. A common slide frame is modified in order to hold inside microgels immersed in liquid, so that the gels may then be projected on paper screens. The electrophoretic patterns may be drawn on the basis of the gel images. Staff-meeting presentation of microelectrophoretic patterns seems to be another application of the system.

Separation of macromolecules on acrylamide microgels with diameters down to 0.2 mm permits analysis on minute tissue samples down to single cells<sup>2</sup>. Such methods, like corresponding standard electrophoretic macromethods, also allow the determination of molecular parameters of the resolved bands by comparing their relative electrophoretic mobilities with those of suitable standards<sup>3</sup>. Thus, it is important to have an adequate means of determining the microelectrophoretic pattern.

In the sequence of analytical steps, the inspection and evaluation of the separated bands may cause trouble due to the small dimensions of and between the bands<sup>4</sup>. Densitometry may be performed directly on the gels, requiring in this particular case sophisticated instrumentation, or the gels may be photographed and subsequent inspection and analysis is made on the film. This introduces a time-consuming step and a loss of information. An alternative way involves the use of amicroscope, fitted with a drawing apparatus. We describe a simple and inexpensive method for visualization of electrophoretic patterns on microgels by projection of the stained gels on a screen.

A common slide frame is modified so that it can hold microgels completely immersed in liquid (figure 1). A rectangular rubber packing, thickness 1–2 mm, is positioned as in figure 1 and secured to the glass with glue. 2 rectangular pieces of glass, of a thickness slightly less than the rubber packing, are fastened to the glass of the frame, inside the rubber packing, so that a slit-shaped trough is produced, the width suitably being between 0.5 and 1 mm. The gel can be placed in the dry slit. If the gel tends to float when liquid is poured upon it, a cover-slip may be placed above the 'slit' with the gel, and by capillary force the trough will be filled with liquid, added to fill the space inside the rubber packing. The slide frame is then closed, resulting in a liquid-containing space inside the packing with the gel in the slit-trough. The frame is placed in any type of slide projector, and to allow only projection of the gel, the exterior of the frame is masked with razor blades to permit light to pass only through the slit-trough.

Projected on a suitable paper screen (figure 2), the electrophoretic pattern can be drawn on the basis of the gel image. It is then possible to determine the electrophoretic