Fig. 1. Several fibroblasts have phagocytized carmine granules. Two cells are in anaphase. Aceto-carmine stain. ($\times 450$)

Regardless of the truth of this statement, our experiments show that a phagocytic cell may ingest large amounts of foreign material and afterwards undergo division. By analogy, it lends support to those who believe that the phagocytic reticulo-endothelial cell may ingest a foreign protein antigen and retain the ability to divide. If a reticulo-endothelial cell does differentiate into a plasma cell, these observations also support the unified concept of ingestion of antigen and production of antibody within a single cell.

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Inhibitory Action of Amino-Acid Esters on the Production of Extracellular Amylase by *Penicillium chrysogenum*

WASHED mycelium of *Penicillium chrysogenum* will not synthesize amylase except in the presence of starch. Production cannot be increased by adding amino-acids to the medium.

Amylase production by the mycelium of washed *Penicillium chrysogenum* strain Wis. 49-133 was studied. The mould was grown for 48 hr. in a medium containing soluble starch. The dry-matter content was 0.62 gm./100 ml. of incubated medium. The experimental conditions were as follows: *Penicillium chrysogenum* was grown at 24° C. in 200 ml. of medium in a 500-ml. Erlenmeyer flask on a rotary shaker (300 r.p.m., 2 cm. radius). The medium for the inoculum was composed of (per cent) glucose, 2; sodium nitrate, 3; potassium dihydrogen phosphate, 0.1; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05; potassium chloride, 0.05; it was adjusted to pH 6.5 before sterilization and 0.5 per cent calcium carbonate was added before inoculation. The flasks were inoculated with a spore suspension. Mycelium was

produced in the same medium. The energy source varied, but the concentration was always 2 per cent. Inoculations were carried out with 3-day-old inoculum. Mycelium production lasted for 48 or 72 hr., depending on the source of energy. Washing was done with 6 vol. of distilled water with filtering. The dry-matter content was determined by drying at 105° C. after filtering and washing with acetic acid. Enzyme production proceeded on a rotary shaker at 24° C. Mycelium suspended in 50 ml. of distilled water and 25 ml. of distilled water, or the indicated substance dissolved in distilled water, was added to 25 ml. of 0.4 per cent potassium dihydrogen phosphate and 0.4 per cent soluble starch (adjusted with potassium hydroxide to pH 6.5 before sterilization), in a 500-ml. Erlenmeyer flask. Amylase was determined in filtered samples of the incubated medium at 28° C. using the method of Smith and Roe¹. Every step of the experiments was carried out under aseptic conditions and was afterwards tested for sterility.

Table 1 illustrates enzyme production as a function of time. The results are typical of induced enzyme synthesis. Identical results were obtained with mycelium grown on a wide variety of energy sources (glucose, lactose, sorbitol and glycerol), and most strikingly, even when the mould had been grown in a medium containing starch. Amino-acid esters have been found to be capable of inhibiting this enzyme production. Table 1 shows experiments carried out with glycine ethyl ester. Results similar to these were obtained with D-phenylalanine methyl ester and L-isoleucine methyl ester. A mixture of glycine and ethanol corresponding to a 10^{-2} M concentration of glycine ethyl ester, 10^{-2} M glycine amide and 10^{-2} M glycylglycine ethyl ester, were found to have no effect on enzyme production. The washed mycelia of mould, grown on various energy sources, all displayed the same behaviour in our inhibition experiments.

Table 1

Time (hr.)	Amylase (U/100 ml.)				
	Glycine ethyl ester hydro- chloride (M)				Glycine and ethanol (see text)
	10^{-2}	5×10^{-3}	10^{-3}	5×10^{-4}	
0	3	1	1	1	0
2	0	1	2	0	0
4	1	2	20	27	28
6	4	13	92	139	148
8	9	22	127	155	207
10	9	24	114	166	198
12	8	19	105	164	198
14	10	20	108	176	206
16	7	19	98	163	206

The activated carboxyl groups of the amino-acids should take part in the synthesis of peptides. These activated carboxyl groups are capable of reacting with hydroxylamine giving rise to hydroxamic acid^{2,3}. Like hydroxylamine, the amino-group of the amino-acid esters possesses a marked reactive capacity.

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