strength to slowly destroy the enzyme has been worked out and its conclusions are found to agree with the results of the experiments. In this way it has been possible to measure the activity of invertase in 50 and 70 per cent. alcohol, where the destruction plays an important role. Invertase can be precipitated by alcohol without much destruction, provided the strength of alcohol in the final solution is high, approximately 90 per cent. By this method of precipitation, working at room temperature, a solid preparation was obtained which had 78 per cent. of the activity of the original solution. If cane sugar is present, invertase can be precipitated with no important destruction by even 70 per cent. alcohol; this method of precipitation gave a recovery of 94 and 96 per cent. of the original activity.

[From the Storrs Agricultural Experiment Station.]

THE CATALASE OF MOLDS.

By Arthur W. Dox.

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An enzyme which accelerates the decomposition of hydrogen peroxide with the liberation of oxygen in a molecular form seems to be almost universally present in living tissues. Its presence in animal organs, in blood, and in milk has been widely investigated. In the vegetable kingdom its occurrence in green plants and in certain fungi is well known.

Many of the basidiomycetes, as well as the yeasts and bacteria, have been found by numerous investigators to contain catalase. The molds, however, have received but little attention with reference to their catalase content. Bach and Chodat[1] found catalase in *Sterigmatocystis nigra*, and Pringsheim[2] noted its presence in the press juice of fifteen out of the seventeen species of mold examined by him. No other investigators seem to have given any attention to the catalase of this important group of fungi.

Catalase is quite distinct from the oxidizing enzymes known as oxidase and peroxidase, though both of these are probably dependent for their oxidizing power upon the presence of some peroxide, either in the plant itself or added in the form of hydrogen peroxide. In the case of the oxidizing enzymes, the oxygen seems to be liberated in a nascent or active form, which readily acts upon oxidizable substances, such as leuco bases, guaiaconic acid, phenolphthalin, etc., and the reaction at once made apparent by the color of the product of oxidation.

Although the molds investigated by the writer showed only slight evidence of oxidase or peroxidase, catalase was generally present in greater concentration. Many of these fungi produce colored spores

[1] *Ber.*, **36**, 1756 (1893).

[2] *Z. physiol. Chem.*, **62**, 386 (1909).

or secrete a colored substance into the medium, making the color reactions for oxidizing enzymes rather difficult of demonstration. With guaiaconic acid little or no production of color was noticed, though hydroquinone seemed to be acted upon somewhat more readily. Catalase was first observed in the desiccated mycelium which furnished the basis of the studies of other enzymes by the writer.[1]

Experimental.

Most of these experiments were conducted with *Penicillium camemberti*, the well-known mold which ripens Camembert cheese. The organism was grown in pure culture upon a synthetic fluid containing *d*-glucose and inorganic salts. One hundred cc. of the medium were placed in each of a series of 300 cc. Erlenmeyer flasks. The flasks were closed with a cotton plug, then sterilized in an autoclave and inoculated with spores of *P. camemberti*. Determinations were made on successive days, a fresh culture being used each time. To distinguish between intra- and extracellular enzymes, both the mycelium and the filtered medium were tested. The mycelium was ground to a fine paste, suspended in 100 cc. water and 10 cc. taken for the determination. The same proportion of the medium (10 cc.) was taken for the extracellular determinations.

The form of apparatus used for measuring the gas liberated was an ordinary Schiff nitrometer. This was connected by means of a rubber tube with a small glass cylinder containing the enzyme solution and a small dish of hydrogen peroxide (5 cc.) floating on the solution. By shaking the cylinder the enzyme and peroxide are brought in contact, and the gas liberated displaces the water in the nitrometer. This method of measuring directly the volume of gas liberated in a given time was found more satisfactory in this case than Sentner's method of titrating the excess of peroxide with potassium permanganate. Owing to the presence of sugar and perhaps other reducing substances in the medium, the blank that had to be deducted when Sentner's method was used was so high as to introduce considerable error.

The peroxide used was the commercial preparation known as "Dioxygen." One cubic centimeter reduced 22.4 cc. of decinormal permanganate solution, showing that the strength was 3.81 per cent. by weight or 12.5 volume per cent.

In addition to the enzyme determinations, the nitrogen in an aliquot portion of the mycelium was determined, also the optical rotation of the medium, in order to compare the enzymatic activity with the stage of development of the fungus and the supply of nutriment remaining in the medium. The results for *P. camemberti* are tabulated below. The gas volumes are expressed at 0° and 760 mm. pressure.

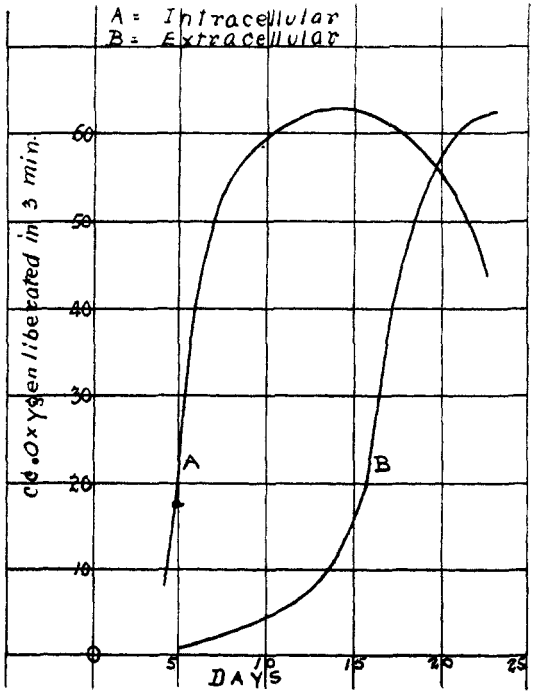
[1] *Bulletin No.* 120, *Bureau of Animal Industry*, Washington, 1910.

Age of culture. Days.	Catalase. Intracellular C_2 in 3 min. cc.	Catalase. Extracellular O_2 in 3 min. cc.	Nitrogen in mycelium. Gram.	Rotation. f medium, Ventzke.
0	0.0	0.0	0.000	9.5
4	8.1	0.0	0.009	8.8
5	17.1	0.5	0.012	8.2
6	30.7	1.2	0.018	7.4
7	52.0	1.9	0.025	5.5
8	54.0	2.9	0.030	4.3
9	58.0	3.7	0.030	3.1
10	60.1	4.1	0.031	2.7
11	61.5	5.1	0.031	2.2
12	62.0	6.5	0.031	1.8
13	63.3	7.5	0.031	1.5
14	63.1	12.7	0.031	0.07
15	61.7	13.1	0.030	0.7
16	61.4	21.5	0.030	0.3
17	60.7	37.4	0.029	0.1
18	60.7	47.8	0.028	0:0
19	59.4	50.5	0.027	...
20	56.1	57.2	0.027	...
21	52.8	60.9	0.027	...
22	50.8	61.1	0.026	...
23	43.0	60.7	0.026	...
24	42.5	61.1	0.025	...
25	41.2	60.9	0.023	...

As soon as the mold made its appearance on the surface of the culture medium, the mycelium was found to contain a very active catalase. The increase on succeeding days does not represent a percentage increase, but is due rather to the greater amount of mold as shown by the appearance of the culture as well as by the nitrogen determinations. After about fifteen days, when the culture is mature, the intracellular enzyme gradually decreases. This change is accompanied by a loss of nitrogen from the mycelium and a gradual decrease of turbidity of the fungus cells. In fact, after the culture is a month old the mycelium can be disintegrated by simply shaking the flask. The decrease in intracellular catalase is not to be regarded, therefore, as an actual loss in activity of the enzyme, but rather as the change from the intra- to the extracellular form.

The enzyme in the medium or extracellular enzyme increased steadily during the entire time that the cultures were under observation. This increase continued after the enzyme in the mycelium had begun to decrease and the carbohydrate was completely exhausted from the medium. The medium was always perfectly clear after filtering off the mold. Its activity was not appreciably impaired when it was freed from the mycelium and preserved two weeks with toluene. But on heating the liquid to boiling its activity was completely destroyed.

The relation between intra- and extracellular catalase can best be shown graphically. As the amount of oxygen approaches the theoretical yield, however, the differences in enzyme activity from day to day, as shown by the figures recorded, are much less striking than is actually



the case. Unless this is borne in mind the following curve, where the amount of oxygen liberated is plotted against the time during which the culture has grown, is apt to be somewhat misleading. The curve in the main shows the relation between age of culture and enzyme activity, except that the summit is unduly flattened.

Both the intra- and extracellular catalase are somewhat diffusible through parchment and collodion membranes. In performing the test with the endo enzyme, the cellular débris was not completely removed, and there is a possibility that during the course of the experiments, some of this enzyme may have changed to the extracellular form and passed through the membrane as such. Conclusive experiments as to the diffusibility of the intracellular enzyme can best be made with the so-called press-juice, the means of preparing which were not at the writer's command.

In order to determine how widely catalase is distributed in this group of fungi, cultures of a number of species were made, and the catalase

in the medium determined at the end of two months. The molds were grown in test tubes on 10 cc. of the fluid medium. The liquid was filtered, and 5 cc. diluted with 10 cc. water were tested with 2 cc. peroxide.

Species.	Cc. oxygen in 3 min.	Species.	Cc. oxygen in 3 min.
Penicillium duclauxi	25.3	*Aspergillus glaucus*	21.1
biforme	25.0	*fumigatus*	19.6
spinulosum	22.4	*clavatus*	13.2
decumbens	17.4	*nidulans*	10.1
camemberti	13.7	*varians*	7.4
italicum	11.1	*flavus*	6.5
chrysogenum	10.0	*ostianus*	4.5
stoloniferum	8.6	*ochraceus*	3.9
intricatum	5.2	*oryzae*	2.4
atramentosum	3.5	*candidus*	1.2
lilacinum	2.8	*wentii*	0.6
citrinum	2.6	*niger*	0.0
expansum	2.0		
divaricatum	1.2		
rugulosum	0.9		
roseum	0.8		
africanum	0.8		
claviforme	0.3		
pinophilum	0.2		
luteum	0.2		
roqueforti	0.2		
granulatum	0.0		

In this experiment the formation of extracellular catalase took place much less rapidly on account of the greater depth of the culture medium as compared with the mold-covered surface. In no case was the sugar completely used up, for the filtrate invariably gave a heavy precipitate of cuprous oxide on boiling with Fehling's solution. The above table shows, however, that nearly all of the species examined secrete a catalase into the medium. Nevertheless there are striking differences in the amount produced by the various species. These variations can hardly be attributed to differences in the vigor of the cultures, for all of the species grew well upon this medium and formed a dense mycelium with normal fruiting bodies.

From the experiments described in this paper it is evident that molds contain the enzyme catalase, first in the intracellular form, then gradually allow it to escape into the medium as an extracellular enzyme. Just how this change is effected is not definitely known as yet, but it seems probable that some of the fungus cells undergo disintegration, or at least a loss of vitality, by which an opportunity is afforded the enzymes for diffusion or mechanical release into the medium.