Decomposition of Hydrogen Peroxide by Catalase

WE have postulated previously that the catalytic decomposition by catalase of hydrogen peroxide to molecular oxygen and water is accompanied by changes in the valency of catalase iron[1]. This supposition is strongly supported by spectroscopic and manometric experiments carried out on azide-catalase. As additional evidence in support of this view, we brought forward the results of experiments carried out in Barcroft differential manometers, showing that under certain conditions even the activity of free catalase is inhibited when oxygen is completely removed from the surrounding medium and replaced by pure nitrogen. Our manometric experiments were repeated by Weiss and Weil-Malherbe[2] who, using Warburg manometers, failed to obtain this inhibition. Johnson and van Schouvenburg[3] also failed to confirm our results using luminescent bacteria as indicators of decomposition of hydrogen peroxide by catalase in complete absence of oxygen. However, we repeated our experiments from time to time, using different enzyme preparations, and invariably confirmed our previous results. The failure by other workers to obtain similar inhibition was explained by us[4,5] as being due probably either to some difficulty in complete elimination of oxygen from Warburg manometers or to some defects in their enzyme preparations.

However, two considerations induced us to reinvestigate the whole problem: (1) the ease with which our experiments were confirmed in our laboratory by Dr. H. Laser, who used for this purpose ordinary Warburg manometers; and (2) a private communication from Dr. Dickens informing us that he was completely satisfied with the experiments by Weiss and Weil-Malherbe carried out in his laboratory.

After a careful analysis of every step of our manipulation, special attention was paid to the nitrogen used in our experiments, with the view of detecting in it some impurities which could be responsible for the effects we invariably obtained.

The nitrogen in our experiments was purified by passage through a combustion tube filled with wire-form copper oxide reduced *in situ* with hydrogen and heated to redness in an electric furnace. The view that our purified nitrogen contained traces of impurities such as oxides of nitrogen was confirmed in various ways which can be summarized as follows:

(1) Incubation of a very dilute enzyme preparation with nitrogen purified as shown above, followed by evacuation and refilling the Barcroft manometers with air before mixing the enzyme with peroxide, already had a strong inhibitory effect upon the enzyme.

(2) The nitrogen from the furnace bubbled through sulphanilic acid – α-naphthylamine reagent at the rate of 100 ml. per minute for 20 minutes, together with a stream of oxygen, gave a positive colour reaction corresponding to 2 μgm. sodium nitrite.

(3) We have shown previously[6] that catalase combines with nitric oxide forming a well-defined compound.

(4) Decomposition of hydrogen peroxide in air by catalase is strongly inhibited by sodium nitrite in a concentration about ten times higher than those which could be detected in our experimental flasks, assuming that, in these flasks, 100 ml. of nitrogen comes into contact with our dilute enzyme preparations. On the other hand, the diazo reaction may be incomplete under these conditions.

(5) At the conclusion of all experiments in which inhibition was obtained, the liquid in the flask gave a positive brucine test for $-NO_3$.

(6) Passing the purified nitrogen to the manometers via a charcoal trap completely abolished the inhibitory effect of nitrogen.

(7) On lowering the temperature of the furnace so that within the combustion tube it reached only 400° C. and near the outlet somewhat lower, the inhibitory effect due to nitrogen together with the reaction for the oxides of nitrogen were completely abolished.

These experiments clearly demonstrate that the inhibition of catalase activity ascribed previously to the absence of oxygen was due to traces of some oxides of nitrogen formed during the purification of nitrogen owing to the high temperature of the furnace. It is interesting to note that out of many enzymes tested with this 'purified' nitrogen, so far only catalase, perhaps on account of high dilutions, seems to be affected by such minute traces of impurities. These results, as will be shown separately, do not invalidate our view that the catalytic decomposition of hydrogen peroxide by catalase is accompanied by reversible changes in the valency of its iron; a view shared by several other workers[7,8,9]. They only show that conditions have not yet been found for the detection of the ferrous state of free catalase during its activity owing to its much too rapid re-oxidation.

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Mechanism of the Butanol-Acetone Fermentation

THE mechanism of sugar breakdown in alcoholic fermentation by yeast, lactic acid fermentation by lactic acid bacteria and glycolysis by animal tissues has been elucidated, but the intermediate steps of the butanol-acetone fermentation are not yet known. The main problem, the transformation of C_6-compounds into C_4-compounds (like butyric acid or butanol), has not yet been solved. Two facts indicate that this fermentation follows a different scheme: (1) the normal C_3-compounds, which are doubtlessly intermediate products in the other fermentations, do *not* yield C_4-compounds; (2) *pentoses* cannot be fermented by yeast whereas they are fermented by action of butyric organisms to the *same* C_4 products as are obtained starting with C_6-compounds. As method of approach to this problem a systematic research on the *structural conditions of the substrate molecule* leading to C_4-compounds through the bacterial enzymes has been carried out in continuation of previous experiments[1].