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Chemical Nature of Catalase.

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The chemical nature of catalase has been studied by a number of authors. The experiments of Zeile[1] and of Stern[2] indicate that the catalase molecule is probably a chromoprotein in which the hemin group is related to that of the natural blood pigments. Recently Agner[3] reported that if horse liver catalase is dialyzed against N/10 or N/100 HCl the enzyme splits into 2 inactive components, one of a low molecular weight which dialyses through the cellophane membrane and which is possibly hemin and another component which remains within the bag and which is a protein. If the 2 neutralized components are mixed, an active preparation is again obtained, according to Agner.

Having the intention of making certain studies on the basis of Agner's report, we first attempted to repeat his experiment. The technique is simple but we were not able to confirm his results. The only difference between his and our technique is in the species of animal from which the liver was derived. Horse liver, which Agner used, is practically unobtainable in our locality. We have, however, tried beef, rabbit, and rat liver, respectively. We followed the experiments of Agner in every detail, dialyzing the purified catalase against N/10 or N/100 HCl, for 10 to 48 hours. No splitting (inactivation) of the catalase could be obtained. With stronger HCl irreversible inactivation of the catalase took place, due to a marked decrease in pH inside the dialyzing bag.

Catalase comprises at least a hemin group and a protein group. Waenting[4] reported that catalase solutions could be digested by trypsin. This we can confirm and we have carried out the digestion in the following manner. To a beef liver catalase solution of pH 6.4 an equal volume of a 0.3% solution of trypsin (Fairchild Bros. and Foster) of the same pH is added. Within 3 hours at 35° the catalase is completely digested (inactivated). The catalase solution used was of such strength that it decomposed 50% of 10 cc. of a

1 Zeile, K., *Erg. Enzymforschung*, 1934, **3**, 265.
2 Stern, K. G., *Nature*, 1935, **136**, 302.
3 Agner, K., *Z. Physiol. Chem.*, 1935, **235**, II.
4 Waenting, P., *Fermentforschung*, 1916, **1**, 165.

0.02 N H_2O_2 solution per cc. at 0° in 10 minutes in the presence of 2 cc. 0.05 M phosphate-borate buffer of pH 6.4. Enzyme action was discontinued by the addition of 2 cc. of 20% H_2SO_4 and the undecomposed H_2O_2 titrated with N/10 $KMnO_4$.

To one volume of completely digested beef catalase, which had been boiled to destroy the protease, and containing the unchanged hemin group was added one volume of catalase which had been inactivated by bubbling H_2S through and the excess of H_2S removed with N_2. To another sample of digested catalase solution was added some catalase which had been treated with the minimum inhibitive amount of KCN solution. The mixed samples (containing the digested-boiled and the inactivated enzyme) were incubated for 2 hours. No reactivation of the catalase took place in either case.

We have now attempted to determine whether the protein shown to be present can be replaced by another. To some of the digested and boiled catalase containing the hemin component normal human plasma, egg albumin and milk respectively was added in order to replace the "carrier" of the enzyme. In no case could even a partial catalytic effect be observed.

It is interesting to note that about 30 years ago Battelli and Stern[5] found that catalase activity is influenced by a number of substances such as (a) *anticatalase* which is checked by (b) a thermolabile *philocatalase*. The philocatalase is activated by (c) a special thermostable *activator*. Philocatalase has the ability to activate inactivated catalase. This system of activators and inhibitors has been overlooked by practically all workers and it is quite possible that Agner's results may have been affected by these substances.

Our experiments do not exclude the possibility that the catalase molecule is a hemoglobin compound. It is undoubtedly of protein nature.

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[5] Reviewed by Stern, L., *Biochem. Z.*, 1927, **182**, 139.