

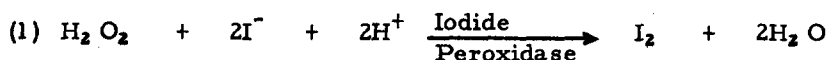
CATALASE INHIBITION OF THE PEROXIDATIC REACTION IN THYROID TISSUE*

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It has been concluded that a peroxidase in thyroid tissue oxidizes iodide ion as depicted in equation (1), because iodide incorporation into tyrosine and protein by thyroid preparations in vitro is stimulated by H_2O_2



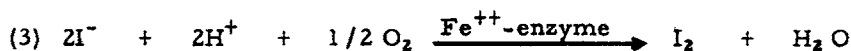
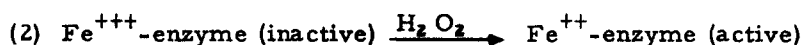
and inhibited by catalase, azide, cyanide, 3-amino-1,2,4-triazole, thiouracil, etc. (Alexander, 1959). Hydrogen peroxide can be added directly (Serif and Kirkwood, 1958), generated enzymatically (Alexander, 1959) or furnished by the autoxidation of substances such as riboflavin and flavin nucleotides (Tong et al, 1957; Alexander, 1960). In addition, manometric data (Alexander, 1959) are consistent with equation (1). Earlier experiments (Schachner et al, 1943) had indicated that carbon monoxide inhibited iodide utilization by thyroid slices but more recent work (Serif and Kirkwood, 1958) has refuted this result.

These findings are reminiscent of similar studies on the oxidation of L-tryptophan (Knox, 1954) which was also stimulated by H_2O_2 and inhibited by catalase, carbon monoxide, azide, and cyanide. A coupled peroxidase-oxidase system was suggested, but subsequently it was shown (Tanaka and Knox, 1958) that catalase added after hydrogen peroxide did not inhibit tryptophan oxidation. It was apparent that H_2O_2 converted inactive

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ferri-enzyme to active ferro-enzyme which then utilized oxygen to oxidize tryptophan to formylkynurenine. Catalase had no inhibitory effect after the ferro-enzyme was formed.

Iodide oxidation by thyroid tissue could possibly occur by a similar mechanism, whereby an inactive ferri-enzyme is reduced by H_2O_2 to an active ferro-enzyme that oxidizes iodide as presented in (2) and (3). If this



mechanism were operative, catalase added after H_2O_2 would have no effect on the utilization of iodide by thyroid homogenates. But if catalase inhibited peroxidase activity by competing for H_2O_2 substrate, inhibition of iodide utilization will occur the instant that catalase is added because the H_2O_2 will be decomposed to oxygen and water. In Table I it is seen that when catalase was added 5 minutes after the H_2O_2 generating system (incubation 3), human thyroid homogenate utilized iodide to a much smaller extent than the incubation (no. 2) in which no catalase was added. Progressively less inhibition was observed when catalase was added after 10 and 20 minutes. Oxidation of iodide had stopped when catalase was added since the utilization of iodide was essentially the same as in the corresponding incubations where the extract had been denatured with trichloroacetic acid (compare incubations 3 and 4, 5 and 6, 7 and 8). Similar results were obtained with another human thyroid homogenate (colloid goiter) and pig and rat tissues. These results are thus interpreted to mean that iodide is directly oxidized by H_2O_2 according to equation (1) and exclude the alternate mechanism outlined in (2) and (3).

It is possible that H_2O_2 is non-specifically oxidizing an unknown thyroid

TABLE I

INHIBITION OF IODIDE UTILIZATION BY HUMAN THYROID
HOMOGENATE BY ADDING CATALASE AFTER H_2O_2

Conditions: Each flask contained 0.1 μ mole NaI^{131} (0.1 μ curie), 0.1 ml. (2 mg. protein) of a 20% human thyroid homogenate from a hyperplastic goiter which had been dialyzed against 2 changes of 40 volumes of 0.11% KCl, 100 μ moles pH 7.4 sodium phosphate buffer, 2.5 μ moles L-tyrosine, and H_2O_2 generated by 2.5 μ moles D-glucose and 60 μ g. of glucose oxidase in a total volume of 1 ml. The reaction mixtures were incubated for 30 minutes at 37° with shaking and 0.5 mg. crystalline catalase or 0.2 ml. of 30% trichloroacetic acid (TCA) were added as indicated. Preparation of the homogenate and analysis for I^{131} in protein and moniodotyrosine have been described (Alexander 1959, 1961).

Incubation No.	Additions	% I^{131} Incorporated Into:	
		Protein	Moniodotyrosine
1*	None	0.1	0.1
2	None	4.2	65.4
3	Catalase after 5 min.	1.5	19.6
4	TCA after 5 min.	1.5	15.5
5	Catalase after 10 min.	3.0	39.7
6	TCA after 10 min.	2.8	37.8
7	Catalase after 20 min.	4.0	59.8
8	TCA after 20 min.	3.9	59.8

*Boiled thyroid homogenate was used.

electron carrier which is reduced by iodide, although this possibility is unlikely because H_2O_2 cannot be replaced by numerous other oxidants and known biological electron carriers (Alexander 1960, 1961). Some quinones (Tong and Chaikoff, 1960) have been reported to activate iodide utilization but this may be the result of H_2O_2 production through autoxidation of the quinones rather than a direct oxidation-reduction reaction between iodide and the quinone. Tong and Chaikoff (1960) also reported that cytochrome c stimulated the incorporation of iodide into protein by a mixture of microsomes, mitochondria and supernatant fractions. However, no activation by cytochrome c was observed by the authors (1960, 1961) with similar preparations from human, pig and rat tissue.

REFERENCES

- Alexander, N. M., J. Biol. Chem., 234, 1530 (1959).
- Alexander, N. M., Fed. Proc., 19, 173 (1960); Endocrinology, in press (1961).
- Knox, W. E., Biochim. et Biophys. Acta, 14, 117 (1954).
- Schachner, H., Franklin, A. L., and Chaikoff, I. L., J. Biol. Chem., 151, 191 (1943).
- Serif, G. and Kirkwood, S., J. Biol. Chem., 233, 109 (1958).
- Tanaka, T. and Knox, W. E., J. Biol. Chem., 234, 1162 (1958).
- Tong, W. and Chaikoff, I. L., Biochim. et Biophys. Acta, 37, 189 (1960).
- Tong, W., Taurog, A., and Chaikoff, I. L., J. Biol. Chem., 227, 773 (1957).