Peroxidase and Catalase in Saliva.*† (23236)

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(Introduced by T. F. Paine, Jr.)
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Earlier work on peroxidase in saliva was qualitative with conflicting reports as to the source and the peroxidatic or oxidatic nature of the enzyme(1-5). Eggers-Lura(6) demonstrated presence of peroxidase in the parotid secretion and whole saliva. He noted the high peroxidase activity of the salivary sediment and suggested that this activity might be due to presence of leukocytes. Mosimann and Sumner(7) reported a strong peroxidase activity in whole saliva and salivary sediment. Catalase in saliva has received incidental mention by a number of investigators but only a few specific studies have been made. Deakins(8,9) investigated the effect of storage on catalase in saliva and the catalase activity of 14 samples of whole saliva. Eggers-Lura(6) claimed that catalase is present in the parotid gland secretion.

The present study extends these investigations and describes activities, properties, and sources of peroxidase and catalase in saliva.

Methods. For peroxidase determination the technic proposed by Sumner and Gjessing (10) was utilized unchanged except for a modification in final H_2O_2 concentration and for omission of the ether filtration. One ml of saliva was used in all determinations and the activity was calculated as milligrams of purpurogallin formed per ml of enzyme source. The color development was read in a Klett Summerson photoelectric colorimeter and compared with a standard curve. Pyrogallol was of reagent grade quality, and fresh solutions were prepared daily and maintained at 4°C. The cytochemical nickle-ammonium-sulfate benzidine stain for peroxidase was used essentially as it was described(11) except for the change in final hydrogen peroxide concentration from 0.015% to 0.0025%. This change shortened the staining time from 2 min to 30 sec. The counterstain employed was freshly filtered carbol-fuchsin. The guaiacol stain for peroxidase consisted of 99.5 ml of a 1.0% guaiacol solution and 0.5 ml of 0.5% H_2O_2. The staining time was 30 sec and the counterstain employed was carbol-fuchsin. Indicators used for determination of bacterial peroxidase were 2-7-diaminofluorene (Eastern Chemical Corp.) and benzidine. The reaction mixture consisted of 3.0 ml of a saturated solution of either indicator, 1.0 ml of saliva and 1.0 ml of H_2O_2. The final concentration of H_2O_2 ranged from 0.001% to 1.0%. After 4 hr at room temperature, tubes were read visually and recorded on a relative basis. Catalase activity was measured by the method of Feinstein(12). Activity was recorded as meq of perborate destroyed per ml of enzyme source. On the basis of Feinstein's data the relation of perborate units to the classical *Kat. f.* would be approximately 1:100. Duplicate samples and appropriate controls were used in all determinations. Whole saliva was collected by paraffin stimulation several hours after a meal. The parotid secretion was collected with a Curby cap(13) and the submaxillary secretion was collected with Schneyer's segregator(14). One percent acetic acid applied to the dorsum of the tongue was used for stimulation in both instances. Contamination of secretions by whole saliva was eliminated by thorough rinsing of the mouth and drying of the mucosa adjacent to the salivary ducts before placing the appliances. Whole saliva and glandular secretions were refrigerated at 4°C immediately after collection, and activity determinations were performed within 2 hrs.

Results. Peroxidase. Average peroxidase activity of whole saliva at 11:00 a.m. was 1.198 ± 0.3004 mg of purpurogallin formed

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TABLE I. Mean Effect of Inhibitors on Peroxidase Activity in 10 Samples of Whole Saliva and Glandular Secretions.

Compound	Molarity 1 ×	% Inhibition
Sodium fluoride	10^{-2}	42
"	10^{-1}	85
azide	10^{-3}	92
"	10^{-2}	100
Potassium cyanide	10^{-3}	85
" "	10^{-2}	100

per ml(15). When this activity was calculated on a dry weight basis, it was found that whole saliva had a P.Z.[‡] number ranging from 0.072 to 0.121. (For this calculation average dry weight of whole saliva was taken to be 12.4 mg/ml(16)). Peroxidase activity in whole saliva and glandular secretions was inhibited by several compounds (Table I). Temperature inhibition of peroxidase activity was determined by heating saliva for 10 min. When using the pyrogallol method the activity of both whole saliva and glandular secretions was rapidly lost above 80°C with total inhibition at 90°C.

Optimal substrate concentration for the peroxidases in saliva was determined by addition of graded concentrations of standardized H_2O_2 to the reaction mixture. Eleven samples of whole saliva and 2 samples of glandular secretion (1 parotid, 1 submaxillary) were used in the determination. The relationship of enzyme activity to optimum substrate concentration followed mass action principles in that samples with higher peroxidase activity had a higher optimum substrate concentration. Fig. 1 summarizes the data obtained with the 11 samples of whole saliva. Average maximum peroxidase activity for both whole saliva and glandular secretions was observed at a final concentration of 50 μg of H_2O_2 per ml. This concentration was used throughout the present study. It corresponds to the normal range of peroxidatic activity(15).

This is the optimum substrate concentration for salivary peroxidase in the presence of catalase. Although pyrogallol greatly inhibits its catalase, a residual activity remains(17). Apparently the residual activity did not alter the results since the glandular secretions which are catalase free had the same optimum substrate concentration as whole saliva.

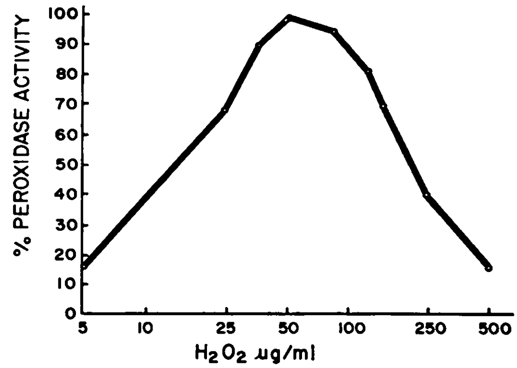

FIG. 1. Mean optimum substrate conc. for peroxidase in whole saliva.

Previous investigators(6,7) have reported that a portion of the peroxidase activity can be recovered in the sediment. When whole saliva from 25 subjects and 8 samples of glandular secretion (4 parotid, 4 submaxillary) were centrifuged at 1400 x Gravity for 20 min at a temperature of 8°C, the supernate of whole saliva was on an average 9.9 ± 6.7% less active than the uncentrifuged control. Glandular secretions showed no loss on centrifugation. The 2 most likely sources of activity in salivary sediment are leukocytic (myeloperoxidase(18)) and bacterial peroxidase.

Histochemical examination of the whole saliva sediment (Fig. 2) revealed a large num-

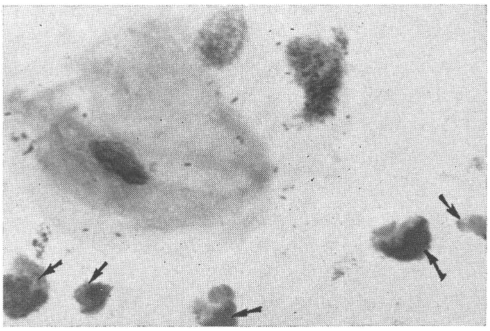

FIG. 2. Saliva sediment stained with NAS benzidine and carbol fuchsin. Epithelia and bacteria are stained red. Arrows point to blue peroxidase-positive granules in the cytoplasm of polymorphonuclear leukocytes. Original magnification 970 ×.

[‡] P.Z. or purpurogallin number is number of mg purpurogallin formed/mg of enzyme in 5 min at 20°C.

ber of peroxidase positive cells which on the basis of morphology and of intensity of the staining reaction were identified as polymorphonuclear leukocytes mainly of the neutrophilic series. They were seen infrequently in smears prepared from glandular secretions.

Bacterial peroxidase has been reported in a variety of organisms, particularly the streptococci. Bacterial peroxidase is said to be heat stable(19), while peroxidase in saliva was heat labile. Since pyrogallol is a poor indicator of bacterial peroxidase activity, 2-7-diaminofluorene and benzidine(20) were substituted in the search for a heat stable peroxidase. With these indicators a slight residual activity was detected after boiling saliva for 15 min. This activity was present not only in whole saliva but also in the glandular secretions. The secretions are relatively bacteria free and this fact suggested that not bacteria but traces of hemoglobin or its derivatives (pseudo-peroxidases) were the source of this weak heat stable peroxidase.

The activities of the 2 major glandular secretions were compared with the peroxidase activity of whole saliva by sampling whole saliva, parotid secretion, and submaxillary secretion as closely together in time as possible. The time required for collection was usually 20-30 min. The activity of the secretions was calculated in percentage of the activity of the corresponding whole saliva. Eighteen series were determined. The parotid secretion averaged 125.1 ± 19.6% and the submaxillary secretion averaged 86.8 ± 15.3%. The lower proportionate activity of the latter may be due to a greater content of inert matter, *e.g.* mucin.

Catalase. Average catalase activity of whole saliva at 11:00 a.m. was found to be 0.169 ± 0.0603 meq of perborate destroyed per ml(15). On a dry weight basis this was approximately 0.0137 meq of perborate destroyed per mg.

Relative catalase activity of centrifuged samples of whole saliva from 25 subjects was determined as for peroxidase. The average loss of catalase activity by whole saliva was 36.6 ± 16.0%. Catalase determinations in 40 samples of glandular secretion gave negative results with exception of one specimen contaminated by whole saliva.

Discussion. The source of *peroxidase* in saliva is predominantly glandular and a smaller amount of activity is contributed by the leukocytes. The percentage determined by centrifugation can only be taken as an approximation since it is possible that a large number of leukocytes have degenerated and released varying amounts of peroxidase into solution. This portion of leukocytic peroxidase would be recovered in the supernate. In addition, activity of the supernate would be increased relatively, on a unit volume basis, by sedimentation of inert material such as large masses of epithelia, bacteria and food residue which are devoid of peroxidase.

Preliminary biochemical and histochemical determinations of peroxidase in the human submaxillary gland indicate that the serous acinar cells of this gland are a rich source of tissue specific animal peroxidase and that they actively secrete it. This probably also holds true for the parotid gland. Plasma levels of peroxidase for all practical purposes can be considered to be non-existent.

Bacterial peroxidase is thought not to contribute to the peroxidase activity of saliva. The action of boiled saliva on benzidine and 2-7-diaminofluorene in the presence of H_2O_2 was slight and probably due to hemoglobin or its equally non-specific derivatives. Also, these indicators are highly sensitive and may give false positives(21,22) with various non-enzymatic materials. Hemoglobin has peroxidase-like action but is not specific for any one tissue of the body and is a constant source of contamination in studies of tissue specific peroxidases in animals. Hemoglobin was eliminated as a major source of peroxidase activity in saliva on the basis of the following evidence: The P.Z. of saliva on a dry weight basis has in some cases twice the peroxidase activity of crystalline oxyhemoglobin as determined by Bancroft and Elliott(17). The peroxidase activity of hemoglobin is relatively heat stable whereas the activity in saliva is heat labile. Microscopic examination of saliva utilizing histochemical stains for peroxidase failed to disclose any intact erythrocytes.

Absence of detectable hemoglobin and of

intact erythrocytes probably excludes the latter from consideration as a source of *catalase* in the healthy mouth. Since the glandular secretions are devoid of catalase it is presumed that the enzyme is primarily bacterial in origin. The fact that only 1/5 to 1/2 of the activity could be centrifuged out of solution may be explained by bacteria remaining in the supernate and by accumulated extracellular portion of the enzyme which would also remain in the supernate. Some authors (23,24) have reported that leukocytes contain catalase in the same quantity as erythrocytes. Agner(25) utilizing improved technics concluded that leukocytes are free of catalase.

Summary. 1. Peroxidase in normal saliva is predominantly of glandular origin with a smaller amount of activity contributed by the leukocytes. Hemoglobin may be present in trace amounts. Although saliva contains large numbers of streptococci said to contain bacterial peroxidase, the latter could not be demonstrated. 2. Catalase was absent from the glandular secretions in a large series of determinations and its origin in normal saliva is presumed to be bacterial. Leukocytes and erythrocytes probably contribute little if any catalase to normal saliva.

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