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This Week's Citation Classic

Klebanoff S J. Antimicrobial mechanisms in neutrophilic polymorphonuclear leukocytes. *Semin. Hematol.* 12:117-42, 1975.
[Dept. Medicine, Univ. Washington Sch. Med., Seattle, WA]

The antimicrobial systems of neutrophils are divided into those dependent on oxygen and those which are not. The former include the myeloperoxidase-H_2O_2-halide system and highly reactive oxygen radicals, and the latter include granule cationic proteins, lysozyme, lactoferrin, and possibly a fall in intraphagosomal pH. [The *SCI*® indicates that this paper has been cited in over 355 publications since 1975.]

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"From 1957 to 1962, I was at Rockefeller University, an endocrinologist by trade and a thyroidologist by research interest. I had two projects under way with a graduate student, Cecil Yip, both involving peroxidases. One dealt with the mechanism of action of the thyroid hormones; thyroxine by virtue of its phenolic hydroxyl group was found to greatly stimulate reactions catalyzed by peroxidase.[1] The second project dealt with the biosynthesis of thyroxine, a reaction which required a thyroid peroxidase to iodinate the tyrosine residues of thyroglobulin.[2] This interest in peroxidases and their role in the mechanism of thyroxine action prompted a search for biologically important peroxidases which could be stimulated by thyroxine. Granulocytes are rich in peroxidase. We purified this enzyme (myeloperoxidase) and found that it, like horseradish peroxidase, was stimulated by thyroxine and, like thyroid peroxidase, iodinated proteins. Another peroxidase, lactoperoxidase, present in milk and saliva, was purified and found to react similarly.

"At the same time that this work was going on, Zanvil Cohn and James Hirsch at Rockefeller University had characterized the cytoplasmic granules of rabbit granulocytes[3] and demonstrated the release of their contents into the phagosome as a prelude to the death of the ingested organisms. I therefore approached Hirsch with a tube of green myeloperoxidase and a proposal that we determine if this granule enzyme could kill bacteria. If so, this biological action of a peroxidase might be stimulated by thyroxine. We found that myeloperoxidase was ineffective alone or when combined with H_2O_2. It was, however, known from thyroxine synthesis that peroxidase and H_2O_2 oxidize iodide to iodine, a well-known germicidal agent. So we added iodide; the solution turned light yellow and the bacteria were killed, all according to expectation. However, the key experiment, the stimulation of this reaction by thyroxine, was negative. We lost interest.

"The next several years were spent at the University of Washington on other things until I was made aware by Ray Luebke, an endodontics trainee, of an incompletely understood antimicrobial system in saliva, which required a heat-stable dialyzable component (thiocyanate ions) and an unknown heat-labile nondialyzable component. We demonstrated that the latter was salivary peroxidase and that H_2O_2 was an additional requirement.[4] This rekindled an interest in the antimicrobial properties of myeloperoxidase, which was found to have potent antimicrobial activity when combined with H_2O_2 and a halide (iodide, bromide, chloride). Evidence was found implicating this as one of the antimicrobial systems of phagocytes. Unfortunately, we were unable to come full circle and demonstrated a stimulation of this peroxidase-dependent reaction by thyroxine. Over the years these studies have been punctuated by reviews of the antimicrobial systems of phagocytes. The paper indicated here is one of these, and has been highly cited as it appeared at a time of exploding interest in the role of oxygen metabolites in the cytocidal mechanisms of phagocytes (see reference five for a more recent review of this area)."

1. **Klebanoff S J.** An effect of thyroxine and related compounds on the oxidation of certain hydrogen donors by the peroxidase system. *J. Biol. Chem.* 234:2437-42, 1959.
2. **Klebanoff S J, Yip C & Kessler D.** The iodination of tyrosine by beef thyroid preparations. *Biochim. Biophys. Acta* 58:563-74, 1962.
3. **Cohn Z A & Hirsch J G.** The isolation and properties of the specific cytoplasmic granules of rabbit polymorphonuclear leucocytes. *J. Exp. Med.* 112:983-1004, 1960.
4. **Klebanoff S J & Luebke R G.** The antilactobacillus system of saliva. Role of salivary peroxidase. *Proc. Soc. Exp. Biol. Med.* 118:483-6, 1965.
5. **Klebanoff S J.** Oxygen-dependent cytotoxic mechanisms of phagocytes. (Gallin J I & Fauci A S, eds.) *Advances in host defense mechanisms.* New York: Plenum Press, 1982. Vol. 1. p. 111-62.