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## EXPERIMENTS WITH LUMINOL

by

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1937, pp 367-369.

The particularly vigorous chemical luminescence occurring on oxidation of 3-aminophthalic acid hydrazide ("luminol") was discovered by W. Langenbeck and subsequently investigated in greater detail by several researchers<sup>1,2</sup>. The oxidizing agent most frequently used was a mixture of sodium hypochlorite and hydrogen peroxide. A considerable ~~step~~ was made by K. Gleu and K. Pfannstiel<sup>3</sup>. They showed that a ~~very~~ <sup>great</sup> chemical luminescence is obtained when luminol is oxidized with hydrogen peroxide alone in the presence of some hemin as catalyst. It is thus highly indicated that this reaction be utilized for the detection of hydrogen peroxide, and our experiments revealed that the "luminol test" in fact belongs to the most sensitive reactions for hydrogen peroxide.

### Sensitivity of the Luminol Test

The reagent used was a solution of 0.1 g pure 3-aminophthalic acid hydrazide<sup>1</sup> and 2 mg hemin (recrystallized according to the pyridine method) in 100 cm<sup>3</sup> 1% soda solution. By means of a pipette a few drops were placed near to each other on a white enameled porcelain plate and each treated with a drop of the progressively diluted hydrogen-peroxide solutions. On viewing in the dark chamber, the most dilute solution with which a marked chemical luminescence was still obtained contained  $2 \times 10^{-5}\%$  hydrogen peroxide. Each drop weighed about 0.06 g. Hence we were just able to detect 0.012% H<sub>2</sub>O<sub>2</sub>.

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1) Cf.: E.O. Lorentz, Zoschr. physik. Chem. (Journal of Physical Chemistry) 29, 302 (1926); N. Harvey, J. Phys. Chem. 33, 1455 (1929); E.H. Huntress, E.N. Stanley and A.S. Parker, J. Am. Chem. Soc. 56, 241 (1934); L. Harris and A.S. Parker, J. Am. Chem. Soc. 57, 1939 (1935).

2) Ann. prakt. Chem. (Journal of Practical Chemistry) [2] 146, 137 (1935)

3) K. Gleu and K. Pfannstiel, loc cit.

## Luminol Test for Other Peroxides

The luminol test is not quite specific for hydrogen peroxide<sup>4)</sup>. Luminescence is obtained also with peroxybenzoic acid and ammonium persulfate, though not at very high dilutions. The two peroxides give a luminescence with luminol-hemin that is just visible, only in solutions of about 0.01%. Thus the phenomenon cannot be based on a primary formation of hydrogen peroxide by hydrolysis, since on addition of completely dry sodium peroxybenzoate the luminescence ceases immediately.

### Detection of Hydrogen Peroxide in the Auto-Oxidation of Diacindole and 3-Amino-Oxindol

Even though the luminol test is not strictly specific, it still has an advantage compared with other reactions for hydrogen peroxide: it permits the detection of peroxides even in the presence of reducing agents. The question whether auto-oxidation of certain reduction products of isatin leads also to the formation of hydrogen peroxide has interested us for a long time. We did not succeed in carrying out the detection by means of the usual reagents. This failure, however, could have been caused also by the fact that the excess of reducing substances decomposed the hydrogen peroxide very rapidly. The luminol test showed that this, in fact, is the case. In this test the hydrogen peroxide may be detected at the moment of its formation by its luminescence.

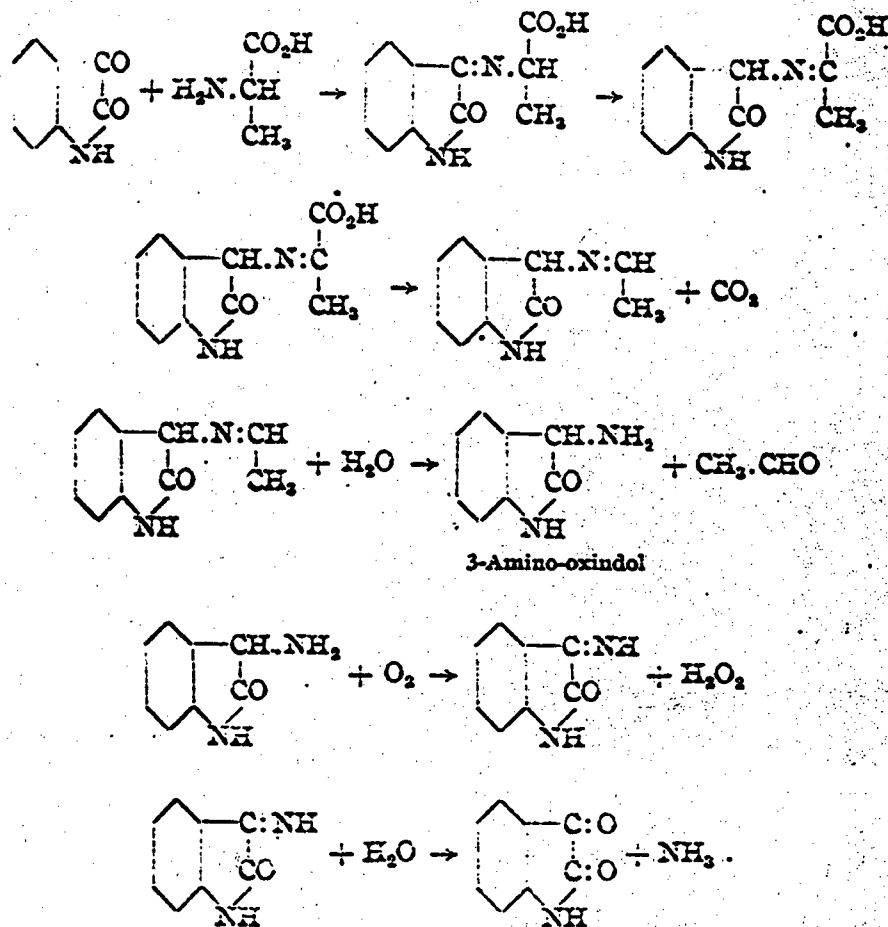
One gram of diacindol and 3-amino-oxindol hydrochloride were each dissolved in 100 cm<sup>3</sup> 1% soda. Both solutions gave in the spot test with luminol-hemin a clear luminescence even at a 1:1000 dilution. When working with larger amounts of solution it was distinctly noted that the luminescence was limited to the surface of the liquid. Only on shaking did the interior of the solution light up, only to become dark soon again. There the hydrogen peroxide was again rapidly reduced. At any rate a clear luminescence of the whole liquid was finally obtained after prolonged shaking of 3-amino-oxindol (0.1 g hydrochloride in 100 cc<sup>3</sup> 1% sodium bicarbonate solution, 5 minutes) with luminol-hemin in oxygen. Apparently in this case all the amino-oxindol was oxidized. This example demonstrated that the compound formed was indeed hydrogen peroxide and not another peroxide. The solutions were made slightly acid with sulfuric acid and distilled in vacuo. The distillate gave a marked luminescence with luminol-hemin. Hence it contained a volatile peroxide which could only have been hydrogen peroxide. The second possibility, ozone, could be ruled out, since a current of air passed over a solution of amino-oxindol gave no luminescence with luminol-hemin.

3-Amino-oxindol occurs as an intermediate substance when oxamine acids are hydrogenated with isatin as catalyst<sup>5)</sup>. The solution may again be hydrogenated to isatin directly with oxygen (isatin could be readily isolated from the auto-oxidized solution of 3-amino-oxindol after

<sup>4)</sup> Cf. also H. Harvey, loc. cit.

<sup>5)</sup> H. Langenbeck, B. 60, 930 (1927); B. 61, 942; W. Franke, *Monatsh. Chem.* (Berl.), 64, 295 (1933); W. Langenbeck, *Die organischen Katalysatoren* (Organic Catalysts), p 45, Berlin, 1935.

acidification, as the phenylhydrazone) or may be transformed with isatin and water to isatyd, e.g.:



As is well known the action of dehydrogenases leads to hydrogen peroxide as reaction product, as is anticipated by Wieland's dehydrogenation theory. The detection of hydrogen peroxide in the auto-oxidation of 3-amino-oxindol thus furnishes another parallel between the dehydrogenases and our dehydrogenase models<sup>6)</sup>. Apparently in the case of dehydrogenases the amounts of H<sub>2</sub>O<sub>2</sub> obtained are greater than in the case of our models since in the presence of natural enzyme the formation of the peroxide is very strongly activated compared with its reductive decomposition.

Parallelism is also found also with the enzyme luciferase<sup>7)</sup>; the latter, however, has a strictly reversible action, as opposed to luminol.

6) Cf. in this connection also: W. Langenbeck, Chem. Ber. (Chemical Journal) 60, 953 (1927).

7) E.H. Harvey, Ann. N.Y. Acad. Sci. (Enzyme Research) 4, 365 (1955).