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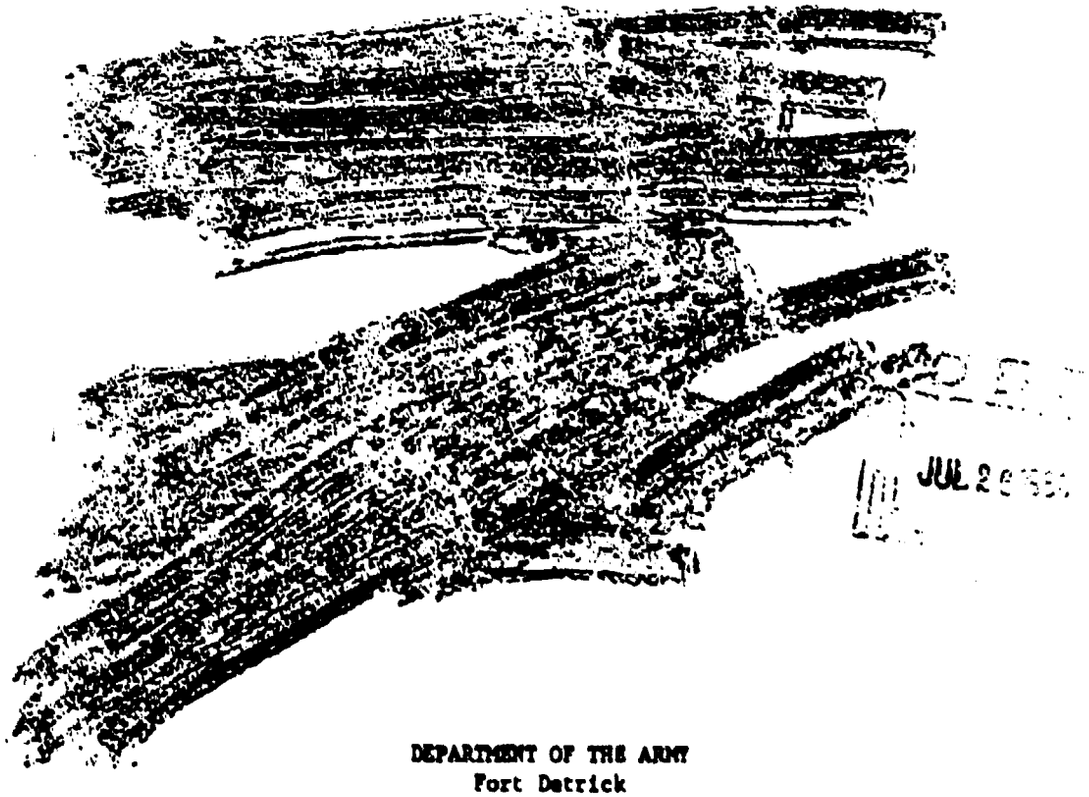


DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

SOME EXPERIMENTS WITH LUMINOL

(Received on 27 January 1937)

[Following is a translation of an article by W. Langenbeck and U. Ruge, Institute of Chemistry at the University of Greifswald, in the German-language periodical Berichte der deutschen chemischen Gesellschaft (Reports of the German Chemical Society), Vol 70, No 1, 1937, pages 367-369.]

As is known, W. Lommel discovered the particularly strong luminescence which occurs during oxidation of 3-aminophthalic acid hydrazide ("Luminol"). This phenomenon has been investigated in more detail by several researchers (1). In most cases a mixture of sodium hypochlorite and hydrogen peroxide was used as oxidizing agent. K. Gleu and K. Pfannstiel (2) made a substantial contribution. They showed that a very beautiful chemiluminescence is obtained when luminol is oxidized with hydrogen perioxide by itself in the presence of a little hemin as catalyst. This reaction appears to be useful for the detection of hydrogen peroxide and, indeed, in the course of our experiments we found that the "luminol test" belongs to the most sensitive tests for hydrogen peroxide.

Sensitivity of the Luminol Test

A solution of 0.1 gram pure 3-aminophthalic acid hydrazide hydrochloride (3) 2 milligram hemin (recrystallized according to the pyridine method) in 100 cubic centimeter 1% soda solution served as reagent. A few drops of the solution were dispensed through a pipette and placed next to each other on a white glazed porcelain plate. Each drop was treated with one of stepwise diluted hydrogen peroxide solutions. The chemiluminescence of the solution with a content

of $2 \cdot 10^{-5}$% hydrogen peroxide could barely be observed in the darkroom. Each drop weighed approximately 0.06 gram. We could, therefore, still detect 0.012γ H_2O_2.

Luminol Test for Other Peroxides

The luminol test is not quite specific for hydrogen peroxide (4). With perbenzoic acid and ammonium persulfate a luminescence is obtained, but not in highly diluted solutions. Both peroxides exhibited a distinct luminescence with luminol-hemin only in solutions of approximately 0.01%. This effect is probably not caused by the primary formation of hydrogen peroxide by hydrolysis, because the luminescence occurs immediately upon addition of completely dry sodium perbenzoate.

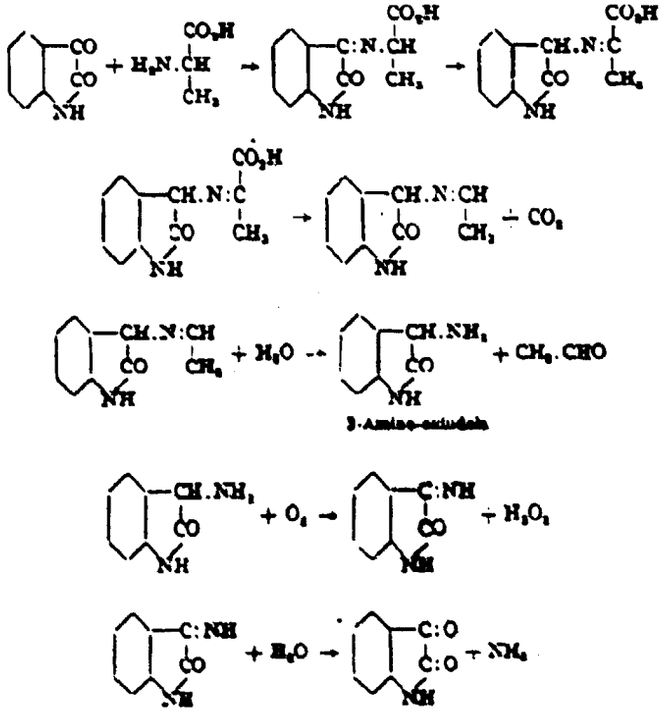
Detection of Hydrogen Peroxide by Autoxidation of Dioxindole and 3-Aminoxindole

Even if the luminol test is not strictly specific, it has an advantage compared with other hydrogen peroxide tests: it permits the detection of peroxides in the presence of reducing agents. For some time we have been interested in knowing whether hydrogen peroxide is formed during the autoxidation of certain reduction products of isatin. No detection was possible with conventional reagents. However, it was possible that this was due to the excess of reducing agents which might rapidly decompose any hydrogen peroxide formed. The luminol test proved that this is, in fact, the case. This test permits, by means of the luminescence, the recognition of any hydrogen peroxide as it is being formed.

One gram dioxindole and 1 gram 3-aminoxindole hydrochloride were each dissolved in 100 cubic centimeter 1% soda solution. Both solutions gave even on 1 : 1000 dilution a noticeable luminescence during the drop test with luminol-hemin. When larger volumes of liquids were taken, it could be distinctly observed that the luminescence was limited to the surface of the solution. Only upon shaking was there any luminescence in the interior, but it disappeared rapidly. The hydrogen peroxide had been reduced rapidly in this region. Nevertheless, a distinct luminescence remained, finally, throughout the liquid when 3-aminoxindole (0.1 gram chlorohydrate in 100 cubic centimeter 1% sodium bicarbonate solution, five minutes) was shaken for a while with luminol-hemin in oxygen. Apparently the entire amount of aminoxindole had been oxidized. With this example it could be shown that hydrogen peroxide itself had actually been formed and

not another peroxide. The solutions were slightly acidified with sulfuric acid and distilled in vacuo. The distillate exhibited distinct light emission with luminol-hemin. It contained therefore, a volatile peroxide which can only be hydrogen pericxide. The second possibility, the presence of ozone, could be excluded, because an air current flowing through an aminoxindole solution did not cause any luminescence when luminol-hemin was added.

3-Aminoxindole occurs as an intermediary when α-aminoacids are dehydrated (5) with isatin as catalyst. The compound can either be directly dehydrated to isatin (5a) by means of oxygen or it can react with isatin and water to form isatyde. For instance:



As is known, hydrogen peroxide is found as reaction product during reaction of dehydrases as can be predicted

from Wieland's dehydration theory. Therefore, a new parallel between dehydrases and our dehydrase models has been found (6) by the detection of hydrogen peroxide during autoxidation of 3-aminoxindole. Apparently more hydrogen peroxide is obtained with the dehydrases than with our models, because with the natural fermentation agent the formation of the peroxide is greatly activated compared to its reductive decomposition.

There are also similarities with the fermentation agent luciferase, however this agent acts strictly on a reversible basis in contrast to luminol.

Literature References and Footnotes

1. cf H. O. Albrecht, Zeitschrift fur physikalische Chemie (Journal for Physical Chemistry), Vol 136, 1928, page 321; N. Harvey, Journal of Physical Chemistry, Vol 33, 1929, page 1456; E. H. Huntiess, L. N. Stanley and A. S. Parker, Journal of the American Chemical Society, Vol 56, 1934, page 241; L. Harris and A. S. Parker, Journal of the American Chemical Society, Vol 57, 1935, page 1939.

2. Journal fur praktische Chemie (Journal for Practical Chemistry), series 2, Vol 146, 1936, page 137.

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

4. See also N. Harvey, loc cit.

5. W. Langenbeck, Berichte der deutschen chemischen Gesellschaft (Reports of the German Chemical Society), Vol 61, 1928, page 942, W. Franke, Biochemische Zeitschrift (Biochemical Journal), Vol 258, 1933, page 295; W. Langenbeck, Die organischen Katalysatoren (The Organic Catalysts), Berlin 1935, page 45.

5.a Isatin could easily be isolated from the autoxidized solution of 3-aminoxindole after acidification.

6. See also W. Langenbeck, Chemiker-Zeitung (Chemists' Journal), Vol 60, 1936; page 953.

7. E. N. Harvey, Ergebnisse der Enzymforschung (Results of Enzyme Research), Vol 4, 1935, page 365.

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