**UNCLASSIFIED**

<table>
<thead>
<tr>
<th><strong>AD NUMBER</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>AD836153</td>
</tr>
</tbody>
</table>

**NEW LIMITATION CHANGE**

**TO**

Approved for public release, distribution unlimited

**FROM**

Distribution authorized to U.S. Gov’t. agencies and their contractors; Foreign Government Information; DEC 1963. Other requests shall be referred to Department of the Army Fort Detrick, Attn: Technical Release Branch [TID], Frederick, MD 21701.

**AUTHORITY**

SMUFD d/a ltr, 8 Feb 1972

**THIS PAGE IS UNCLASSIFIED**
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 hydraside ("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 peroxide 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 hydraside 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 2.10^{-5}\% hydrogen peroxide could barely be observed in
the darkroom. Each drop weighed approximately 0.06 gram.
We could, therefore, still detect 0.012\gamma 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 de-
tection 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 hydro-
chloride 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 re-
gion. Nevertheless, a distinct luminescence remained, fin-
ally, throughout the liquid when 3-aminoxindole (0.1 gram
chlorhydrate 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 aminoxin-
dole 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 peroxide. 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 \( \alpha \)-aminocarboxylic acids 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:

\[
\begin{align*}
\text{CO}_2\text{H} + \text{H}_2\text{N} \cdot \text{CH} \rightarrow \text{CO}_2\text{H} \\
\text{CO}_2\text{H} \\
\text{CO}_2\text{H} + \text{H}_2\text{O} \rightarrow \text{CO}_2\text{H}
\end{align*}
\]

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 autooxidation 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


4. See also N. Harvey, loc cit.

5. W. Langenbeck, Berichte der deutschen chemischen Gesell-

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

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


- END -