hydrolyzed protein. The difference is shown in one typical experiment illustrated in Fig. 1. The presence of stainable cytoplasm in the liver of the animal receiving hydrolyzed protein is striking and is similar to previous studies in which this same change was observed following the ingestion of protein nourishment by mouth (*2*).

It would seem, from the observations reported here, that the intravenous injection of an amino acid mixture (hydrolyzed protein) leads to a deposition of tissue protein in the liver.

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Radical Reactions With Certain Nitrogen Compounds: The Conversion of Benzene (Toluene, etc.) in Other Compounds at Low Temperature

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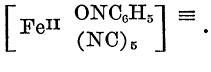
The conversion of benzene with nitrosyl, NOH, radical and air or oxygen (H_2O_2) into *o*-nitrosophenol has been reported upon by the writer in several papers (*2–6*). The very short-lived radical, NOH, is stabilized in the following paramagnetic copper complex $[\mathrm{Cu^I}\,(\overset{+}{\mathrm{NO}})]$ (*5*). The unpaired electron is on the nitrogen atom. Using the so-called L solution (Cu metal + $NH_2OH \cdot HCl$ + air), one can easily demonstrate that even frozen solid benzene is converted into *o*-nitrosophenol or into its red copper salt (B reaction). New reactions with iron nitric oxide complexes are reported here, and the results help in understanding the conversion of benzene into *o*-nitrosophenol at 0° C.

The best-known nitroso iron complexes are the nitroprussides. It has been known for a long time (*7*) that aqueous solutions of sodium nitroprusside become blue on standing in daylight, with the formation of Prussian blue compounds. The reaction is not very sensitive, and in weak daylight it may take hours before the solution becomes blue. Nitroprusside solutions were considered less light sensitive than ferrocyanide or pentacyano carbon monoxide solutions. The writer found, however, that nitroprusside solutions are just as light sensitive as ferrocyanide solutions. Even on winter days, nitric oxide, NO, is split off from the complex immediately (indicated by a positive Griess-reaction), but no color change takes place as in ferrocyanide solutions (pale yellow $\underset{\text{dark}}{\overset{\text{light}}{\leftrightarrows}}$ intensive yellow). The writer found new color reactions with which one can demonstrate that nitroprusside solutions are decomposed even in daylight and at 0° temperature.

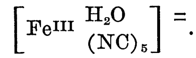
Dilute methyl alcoholic solution of nitroprusside, to which a very small amount of iron carbonyl, $Fe(CO)_5$, is added in the darkroom, gives a pale yellowish solution which, on standing, is unchanged in the dark but which changes color in a few seconds in daylight and becomes black in a few minutes—at the same time bubbles of CO are visible. The nitric oxide split off in daylight attaches itself immediately to the iron carbonyl complex, and black compounds are formed.

If a small amount of cupferron is dissolved in a dilute nitroprusside solution (darkroom), the pale yellowish-red solution remains entirely unchanged in the dark. If, however, it is exposed to daylight, a bluish tint appears in a few seconds, and usually in 1 min the solution is deep violet-blue. The violet-blue compound is



$$\left[\mathrm{Fe^{II}}\ \begin{matrix}\mathrm{ONC_6H_5}\\ \mathrm{(NC)_5}\end{matrix}\right]^{\equiv}.$$

A mixture of nitroprusside, H_2O_2, and sodium azide (NaN_3) made in the darkroom becomes pale blue in daylight in a few seconds and deep violet in a few minutes. The same mixture in the darkroom remains unchanged for hours. The violet compound is ferri-aquo salt



$$\left[\mathrm{Fe^{III}}\ \begin{matrix}\mathrm{H_2O}\\ \mathrm{(NC)_5}\end{matrix}\right]^{=}.$$

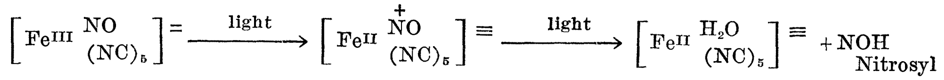
If thiourea is added to a nitroprusside solution in the darkroom, the solution retains its pale yellowish-red color for weeks and months. The mixture, however, becomes blue in the daylight in a few seconds, deep blue in a minute or so. If the nitroprusside solution without thiourea is exposed to light only for a few minutes, the illuminated solution which has not changed at all to the eye becomes immediately blue on adding thiourea. The light reaction with thiourea differs entirely in its mechanism from the other three reactions just described, in which NO is split off in the course of the reaction. In the thiourea reaction NO remains in the complex and the thiourea molecule attaches itself to the NO, thus forming a deep blue sulfur and nitrogen-containing complex. Since the color change does not take place in the dark at all, light must have changed NO in the original nitroprusside and made it reactive. This assumption is supported by an interesting light reaction, namely, the conversion of benzene into *o*-nitrosophenol in daylight at low temperature by short exposure of an H_2O_2-containing nitroprusside solution covered with benzene.

All solutions for this experiment were made in the darkroom. Five gm of sodium nitroprusside was dissolved in 100 cc of water and 2 cc of Perhydrol added. The ruby-red solution was put in an Erlenmeyer flask with ground-glass stopper and covered with 50 cc of benzene. The well-shaken mixture remains entirely unchanged in the dark for weeks or months. If, however, the Erlenmeyer flask is exposed to winter sunlight (outside air temperature was −15° C) for 5–10 min, the benzene becomes a beautiful green color. The aqueous part remains unchanged in color. The green benzene pure *o*-nitrosophenol. On shaking with dilute $CuSO_4$ solution, the aqueous layer becomes deep red, while the benzene becomes entirely colorless. From the red Cu salt of *o*-nitrosophenol all the other metal salts can be layer was separated, washed well with water, and dried with anhydrous Na_2SO_4. The green benzene contains easily prepared (*5*).

The photochemical synthesized *o*-nitrosophenol in benzene solution is unstable, and longer exposure discolors the green benzene to yellow or brown. Instead of using pure benzene, a mixture of ligroin and benzene can be used. The conversion of benzene into *o*-nitrosophenol in such heterogeneous systems as benzene (or benzene and ligroin) with aqueous phases at low temperature is unexpected.

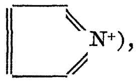
The reason that nitroprusside (I) is diamagnetic is explained by the Welo and Baudisch rule (*8*). If, however, nitroprusside is exposed to light, the central ferric iron atom is reduced to the divalent form, and a paramagnetic nitroprusside (II) with the Effective Atomic Number 37 is formed. The unpaired electron is attached to the nitrogen of the -NO grouping, which becomes extremely reactive. In this transitory condition it reacts with the sulfur atom in thiourea or with carbon in ethylene linkages.

The light reaction might be expressed schematically in the following manner:

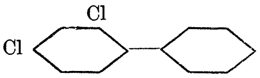


$$\left[\mathrm{Fe^{III}}\,\begin{matrix}\mathrm{NO}\\ \mathrm{(NC)_5}\end{matrix}\right]^{=} \xrightarrow{\text{light}} \left[\mathrm{Fe^{II}}\,\begin{matrix}\overset{+}{\mathrm{N}}\mathrm{O}\\ \mathrm{(NC)_5}\end{matrix}\right]^{\equiv} \xrightarrow{\text{light}} \left[\mathrm{Fe^{II}}\,\begin{matrix}\mathrm{H_2O}\\ \mathrm{(NC)_5}\end{matrix}\right]^{\equiv} + \underset{\text{Nitrosyl}}{\mathrm{NOH}}$$

I (diamagnetic) II (paramagnetic) III (paramagnetic)

In the paramagnetic nitroprusside (II) the NO is very loosely linked to divalent iron (*3*). The nitrosyl, NOH, radical formed from hydroxylamine by oxidation attaches itself easily to ferro-aquo salt III (amminsalt in acid solution), forming a transitory paramagnetic nitroprusside compound (II) which contains the unpaired electron on the nitrogen just as in the B reaction, where NO is attached to monovalent copper (Cu^{I}—NO). It is for the octahedral arrangement in the iron complexes that NO (or O_2) attaches itself easily to the metal central atom. The nature of the bond type is of extreme importance and determines the reactivity of the nitrogen atom. Since any nitrogen atom under circumstances can become tetravalent (for instance, in pyrrole ), the new reaction is of general interest. These complexes may be transitory, lasting just long enough to permit an interaction with the substrate.

In the reactions just described between copper and iron nitroso complex compounds and benzene, the paramagnetic nitrogen atom enters the benzene ring and a new N-containing compound is formed.

Other low-temperature reactions with benzene are known in which radicals are formed which unite while the nitrogen goes off as nitrogen gas (*1*). In these experiments 2,4,6-trichloraniline was used instead of aniline, treating pure trichloraniline diazonium nitrite with dilute NaOH at −25° C. The brilliant yellow diazo oxide or diazo anhydride formed is somewhat less explosive than the aniline compound. It decomposes very rapidly, however. After the violent reaction with benzene at −20° is finished from the reaction mixture  was isolated. One chlorine atom was split off during the reaction and could be detected as HCl. Both diazo oxides mentioned here were much too unstable and explosive to permit magnetic measurements. The writer found that diazotized *p*-anizidine gives a diazo oxide which is much less explosive and could be used for measurements.

Diazotized *p*-anizidine is dissolved in NaCl containing ice water and treated slowly with 20% ice-cold NaOH solution. A brilliant orange-colored crystalline precipitate is formed which can be filtered and washed with ice water. As long as the crystals are wet they are not explosive. If dried on an ice-cold porous plate, the remaining yellow powder must be handled with utmost care. If scratched with the platinum spatula, it explodes. With benzene a violent reaction takes place with the formation of nitrogen. The crystals are paramagnetic.

If we try to correlate these reactions with compounds extremely reactive to benzene, we find that all three contain paramagnetic nitrogen $[\mathrm{Cu}=\overset{+}{\mathrm{N}}=\mathrm{O}]$, $[\mathrm{Fe}=\overset{+}{\mathrm{N}}=\mathrm{O}]$, $[\text{-}\mathrm{N}=\overset{+}{\mathrm{N}}=\mathrm{O}]$. In view of these new observations, the formula of diazo oxides may be written schematically in the following way:

$$\mathrm{R}-\mathrm{N}=\overset{+}{\mathrm{N}}=\mathrm{O} \longleftrightarrow \mathrm{R}\,\overset{+}{\mathrm{N}}\equiv\mathrm{N}\Rightarrow\mathrm{O}.$$

The resemblance of the compounds to tetravalent nitrogen is obvious, but further experimental material is necessary to support the hypothesis.

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