PHARMACOLOGICAL REVIEWS Vol. 18, No. 3
 Printed in U.S.A.

THE BIOCHEMICAL PRODUCTION OF FERRIHEMOGLOBIN-FORMING DERIVATIVES FROM AROMATIC AMINES, AND MECHANISMS OF FERRIHEMOGLOBIN FORMATION

MANFRED KIESE

Pharmakologisches Institut der Universität München[1]

TABLE OF CONTENTS

I. INTRODUCTION

The formation of ferrihemoglobin[2] (hemiglobin, methemoglobin) in human beings after the absorption of various substances was of major interest in a past

[1] Nussbaumstrasse 26, 8 München 15, Germany.

[2] Nomenclature. After it had been established by Conant and Scott (93) that a difference in the valence of the iron atom distinguishes the derivative named methemoglobin from hemoglobin, Pauling and Coryell (398) replaced the rather futile name "methemoglobin" by "ferri-hemoglobin." This term is very appropriate but a little unwieldy. Kiese (272) therefore proposed to indicate the valence state of iron in the two proteins by the names haem*o*globin and haem*i*globin; see also Lemberg and Legge (323). Recently, the Enzyme Commission of the International Union of Biochemistry (213) has

phase of our civilization, when aniline workers were known as "blue boys" on account of the cyanosis caused by ferrihemoglobin, and when children's health was endangered by the use of chlorate in the treatment of diphtheria. Ferrihemoglobin formation played a role in the beginning of one of the great achievements of modern medicine: antibacterial chemotherapy. The results of earlier research in this field have been described in reviews by Heubner (184), von Oettingen (382), and Bodansky (42). Literature concerning some of the problems discussed here has been compiled by Uehleke (479).

Although some future life-saving drug might cause formation of ferrihemoglobin, and this drug may have to be tolerated for some time, as was once the case with sulfanilamide, ferrihemoglobin formation has become a less frequent side effect of drugs. Thanks to changes in technical production methods, ferrihemoglobin formation has become a rare experience in industrial toxicology. However, not long ago new pathways by which nitrite induces ferrihemoglobinemias were discovered.

Apart from its limited practical importance, the formation of ferrihemoglobin is of current interest as a model in biochemical and molecular pharmacology. Aromatic amines are among the most frequent causes of ferrihemoglobin formation *in vivo*, but, except for diamines and aminophenols, they do not oxidize hemoglobin unless they undergo certain biochemical changes. The elucidation of these reactions led to the discovery that, among the biochemical derivatives of aromatic amines, the aminophenols are, with few exceptions (Section II F), of less importance in the formation of ferrihemoglobin *in vivo* than the N-hydroxyderivates (Section II H). This development was paralleled by investigations of the carcinogenic action of aromatic amines. It was recently discovered that N-hydroxy derivatives, rather than aminophenols, are, in many cases, proximate metabolites in carcinogenesis after the absorption of carcinogenic aromatic amines (Section II I). Still another type of derivatives of aromatic amines is capable of forming ferrihemoglobin, namely, N-oxides. These have also been found to be carcinogenic, but it is not known whether the N-oxide group is comparable with the hydroxylamino or nitroso group in carcinogenesis. Certainly, the production of tumors and of ferrihemoglobin are different actions, but the same "active structures" of the nitrogen seem to be involved in both cases.

This review deals primarily with the formation of ferrihemoglobin. The reactions of ferrihemoglobin-forming substances with hemoglobin will be discussed first. The properties of ferrihemoglobin will be described only as far as they relate to the problems dealt with in this review. The oxidation-reduction potentials of the system ferrihemoglobin-ferrohemoglobin has been investigated repeatedly (see 12).

In vivo the increase in ferrihemoglobin concentration by some substances can

---

recommended that the valence state of iron in hemoproteins should be indicated by the prefix ferro- or ferri-. We are following this recommendation. The name hemoglobin designates the protein without consideration of the state of iron.

The following abbreviations are used: Hb, hemoglobin; Hb^{II}, ferrohemoglobin; Hb^{III}, ferrihemoglobin; HbO_2, oxyhemoglobin; NAD, NADH, nicotinamide adenine dinucleotide and its reduced form; NADP, NADPH nicotinamide adenine dinucleotide phosphate and its reduced form.

be influenced by the enzymatic reduction of ferrihemoglobin to ferrohemoglobin in red cells, in particular if the rate of ferrihemoglobin formation is low and that of ferrihemoglobin reduction is high, as is the case in the red cells of rabbits and guinea pigs. The enzymatic reduction of ferrihemoglobin has been reviewed recently by Jaffé (224). Only the relationship of reducing enzymes to the enzymatic cycle of ferrihemoglobin formation will be investigated.

II. FERRIHEMOGLOBIN-FORMING REACTIONS

A. Autoxidation of hemoglobin

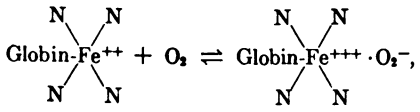
Neill and Hastings (377) observed that the autoxidation of ferrohemoglobin is influenced by the degree of oxygen saturation. Its rate of autoxidation is maximal at a fairly low oxygen pressure (71, 72). The pressure is close to that at which ferrohemoglobin is half saturated with oxygen. The reaction velocity is proportional to the concentration of deoxygenated ferrohemoglobin and to a function of the oxygen pressure $p/1 + bp$, where p is the oxygen pressure and b is a constant. Partial oxidation of hemoglobin slows down the oxidation of the remaining equivalents of ferrohemoglobin (293, 308).

After 3 hemes in the hemoglobin molecule have been oxidized, the reaction velocity is proportional to the concentration of ferrohemoglobin. The same kinetics has been observed with myoglobin, which contains only 1 heme per molecule (144). The oxidation of 3 hemes in the hemoglobin molecule shifts the maximum of autoxidation rate to lower oxygen pressures. This may be due to the increase in affinity for oxygen which is caused by the partial oxidation of hemoglobin (115, 273, 328). Myoglobin, which has a much higher affinity for oxygen than hemoglobin, shows the maximal rate of autoxidation at a much lower oxygen pressure (145). The observations that the maximal reaction rate occurs at half saturation of hemoglobin with oxygen led Legge (322) to suppose that the oxidation of the ferrohemes occurs within a molecule 2 hemes of which are occupied by oxygen. Brooks (73) has shown, however, that the change of autoxidation rate with the oxygen pressure is not parallel to the change of the concentration of ferrohemoglobin molecules carrying 2 molecules of oxygen. At oxygen pressures above 400 mm Hg the rate of autoxidation is many times faster than would be expected from Legge's hypothesis. Furthermore myoglobin, which has only 1 heme in its molecule, undergoes autoxidation, and so does hemoglobin with 3 ferriheme and only 1 ferroheme group. With both proteins the reaction velocity passes through a maximum with increasing oxygen pressure. There is no indication that in principle the reaction mechanism is not the same as with ferrohemoglobin. The high velocity of autoxidation under oxygen pressures which saturate only part of the ferrohemoglobin points to the deoxygenated ferroheme group as the state in which the iron is most readily oxidized.

No satisfactory explanation has yet been found for the effect of oxygen pressure on the velocity of the autoxidation. Under oxygen pressures above 700 mm Hg the velocity was still one fourth of the maximal with hemoglobin (72, 293) and half the maximal with myoglobin (145) or with hemoglobin which had previously been oxidized to 75% (293). Brooks (72) and George and Stratmann (145) designed functions to describe the effect. Because the concentration of deoxygen-

ated ferrohemoglobin under high oxygen pressure is too low to account for the reaction velocity, a formation of ferrihemoglobin in the oxygenated ferrohemes has to be assumed. A radical mechanism of the autoxidation of hemoglobin which involves the existence of an electron-accepting group in the hemoglobin molecule has been conjectured by George (143).

The understanding of the autoxidation mechanism can possibly be promoted by Weiss' conception of the reaction of molecular oxygen with ferrous ions in solution (501) and with ferrohemoglobin (502). The primary process in the autoxidation of ferrous ions is, according to Weiss (501), the reversible formation of a complex $Fe^{++} + O_2 \rightleftharpoons (Fe^{+++} \cdot O_2^-)$. In the presence of suitable ions, *e.g.*, F^-, the complex is stablilized and the reverse reaction to the initial state inhibited. The new complex may eventually decay into $(Fe^{+++} \cdot F^-) + O_2^-$. Weiss (502) presumed that oxyhemoglobin has a structure similar to the complex formed from ferrous ions and molecular oxygen



the iron being in the ferric state. The oxygen molecule has gone over into the O_2^-, which is then taken up in the coordination shell of ferric ion. This assumption would explain the magnetic and optical properties of oxyhemoglobin.

If oxyhemoglobin has the structure $(Hb^+ \cdot O_2^-)$ it should be able, under suitable conditions, to exert oxidizing effects due to the O_2^- or the HO_2 radical which would be formed with an H^+. This radical could yield hydrogen peroxide ($2\ HO_2 \rightarrow H_2O_2 + O_2$) or OH radicals. While these mechanisms may be useful in the elucidation of the ferrihemoglobin formation by "coupled oxidations" their role in the autoxidation of ferrohemoglobin cannot yet be assessed. In experiments by Kikuchi *et al.* (308), catalase inhibited a little the rate of autoxidation of hemoglobin and increased the amount of molecular oxygen liberated from oxyhemoglobin during the process, but Keilin (253) did not observe an effect of catalase on the autoxidation of hemoglobin. The application of various sensitive methods did not yield any indication as to the production of hydrogen peroxide by the autoxidation of hemoglobin.

During the autoxidation of hemoglobin and myoglobin, other oxidations occur besides the oxidation of the iron in the hemoproteins. More than 0.25 mole of oxygen is used up by either protein while 1 equivalent of iron is oxidized (144, 308). As much as 2.5 moles of oxygen are used for each mole of ferrimyoglobin formed.

In the blood of patients with idiopathic ferrihemoglobinemia and otherwise normal hemoglobin, the enzymatic reduction of herrihemoglobin is too slow to keep the ferrihemoglobin concentration low while autoxidation of ferrohemoglobin proceeds with normal velocity (147). In one of the rare cases of ferrihemoglobinemia where the abnormal hemoglobin M is present (205, 206), the rate of ferrihemoglobin reduction was normal. The cause of the ferrihemoglobinemia appears to be a higher rate of autoxidation of hemoglobin M (274).

B. Oxidation of hemoglobin by ferricyanide

Biologically the formation of ferrihemoglobin by ferricyanide is of no interest, because ferricyanide does not pass the intact red cell membrane. The oxidation of ferrohemoglobin by ferricyanide appears to be the simplest among the ferrihemoglobin-forming reactions so far investigated. In the case of deoxygenated ferrohemoglobin it is a reversible reaction that proceeds very rapidly (416). The kinetics of the reaction has been investigated only recently by Antonini *et al.* (11). If ferricyanide acts upon hemoglobin under carbon monoxide (457) or air (411, 413) the formation of ferrihemoglobin proceeds slowly, and ferricyanide also reacts with SH-groups of hemoglobin. The latter reaction is delayed if hemoglobin and ferricyanide react in acid solution (369). In the absence of oxygen, ferrihemoglobin is formed much more rapidly. No side reactions between ferrohemoglobin and ferricyanide were observed by Antonini *et al.* (11) in dilute solutions. The kinetics of the oxidation of ferrohemoglobin by ferricyanide does not correspond to a simple bimolecular reaction. At pH values below 8 the reaction velocity decreases as the fraction of hemoglobin oxidized increases. At pH 7.2 the velocity constant for the oxidation of the final ferrohemoglobin equivalents is only half of that for the initial reaction. On the other hand at pH 9.2 the final reaction velocity is about 60% higher than the initial one. Hydrogen ions increase the reaction rate. At pH 6 the initial reaction velocity is nearly 10 times higher than at pH 9.2. As is described above, in acid solution a decrease in the rate of ferrihemoglobin formation as the fraction of ferrihemoglobin increases is also observed with the autoxidation of ferrohemoglobin (293).

The formation of intermediates, *i.e.*, hemoglobin molecules with 3 Fe^{++} + Fe^{+++}, 2 Fe^{++} + 2 Fe^{+++}, *etc.*, in the oxidation of ferrohemoglobin is demonstrated by the change in affinity for oxygen caused by partial oxidation (115, 273). The existence of intermediates has been confirmed by electrophoretic separation from a mixture of carbon monoxy hemoglobin solutions in which the hemoglobin had been oxidized by ferricyanide to various degrees. The experiments of the authors also show that there is no quick exchange of electrons between hemoglobin molecules with unequal content of ferrihemes (211a, 221).

Betke *et al.* (37, 38) found the velocity of ferrihemoglobin formation by ferricyanide under air to vary widely with various species. These differences did not correspond to the differences in affinity for oxygen, *i.e.*, to the concentration of deoxygenated ferrohemoglobin (27, 455).

C. Oxidation of hemoglobin by hydrogen peroxide

Another compound capable of oxidizing ferrohemoglobin in a presumably simple reaction is hydrogen peroxide. It is produced during the formation of ferrihemoglobin by nitrite and by reducing agents in the presence of oxygen (Section II D). Its role as an essential intermediary product in these reactions is not yet clear. Hydrogen peroxide probably produces the ferrihemoglobin observed after X-ray irradiation of red cells or hemoglobin solutions. Laser (321) as well as Barron and Johnson (26) assumed that hydroxyl radicals produced by ionizing radiation oxidize ferrohemoglobin. In the absence of oxygen they observed the reduction of ferrihemoglobin by X-irradiation. Warburg *et al.* (487)

showed that the active agent produced by irradiation is more stable than hydroxyl radicals and that it is decomposed by catalase. If hemoglobin was added to an irradiated phosphate solution the same fraction of hemoglobin was oxidized as was oxidized during the irradiation of a solution containing hemoglobin, and the irradiated phosphate solution did not oxidize hemoglobin if catalase had been added before ferrohemoglobin. These observations do not disprove the formation of short-lived radicals. The hydrogen peroxide may have originated from them. Hydrogen peroxide as a mediator in the production of ferrihemoglobin by X-rays is also confirmed by the observation that the efficiency of X-ray irradiation in forming ferrihemoglobin is inversely proportional to the catalase content of red cells (3, 487).

Heininger and Aebi (178) irradiated suspensions of red cells and solutions of hemoglobin. They observed catalase to protect the hemoglobin in red cells against ferrihemoglobin formation by X-rays and to be of little effect in hemoglobin solutions. Therefore another mechanism, besides hydrogen peroxide formation, is also involved in the oxidation of ferrohemoglobin by X-rays. In red cells hemoglobin is protected against the action of hydrogen peroxide by two mechanisms: the reduction of the hydrogen peroxide by glutathione peroxidase (368) and its decomposition by catalase. Hydrogen peroxide added to red cells in higher concentrations is readily decomposed by catalase, but catalase does not protect hemoglobin against the continuous action of hydrogen peroxide in low concentration (2, 87, 88, 255). Such low concentrations of hydrogen peroxide as may develop metabolically are detoxified by glutathione peroxidase, provided reduced glutathione is regenerated in sufficient concentrations.

It may be doubted that catalase is quite ineffective against low concentrations of hydrogen peroxide. If hydrogen peroxide is generated continuously in suspensions of normal red cells and red cells lacking catalase, a 20 to 200 times higher rate of hydrogen peroxide formation is needed for ferrihemoglobin formation in normal red cells than in those lacking catalase (4). In red cells with a very low catalase content the formation of ferrihemoglobin by enzymatically produced hydrogen peroxide begins after a lag phase. Its length and the reaction velocity in the following phase of steady ferrihemoglobin formation depend upon the rate of hydrogen peroxide formation (2). The lag phase is ascribed to the oxidation of glutathione, which prevents hydrogen peroxide from reacting with the iron of ferrohemoglobin. The participation of glutathione perioxidase, catalase, and possibly SH-groups of hemoglobin render the analysis of the reaction between hydrogen peroxide and the hemoglobin iron rather difficult.

The mechanism of the oxidation of ferrohemoglobin by hydrogen peroxide has not been studied. It may be related to the reaction of hydrogen peroxide with ferrous ions, which has been discussed by Weiss (500).

D. Formation of ferrihemoglobin by nitrite

Cyanosis in infants due to the absorption of nitrite has become a matter of major interest. Comly (92) has shown that the ferrihemoglobinemia "of unknown origin" in infants observed by previous authors (458), may be due to nitrate in well water, which is reduced to nitrite in the intestine (34). The role of

nitrite as a cause of the ferrihemoglobinemia in infants was confirmed by a long series of publications (423, 447). Lethal intoxications have been observed even in these days (503). More recently nitrite was found to be the cause of ferrihemoglobinemia in infants fed with spinach (198, 435, 436). Whereas in fresh spinach nitrite is not found or found only in traces, on its being left to stand part of the high nitrate content, about 2 g nitrate per kg, may be reduced by bacteria (33, 433). Among various possibilities of intake of dangerous amounts of nitrite, the adulteration of fish with sodium nitrite may be mentioned (434). Meat-curing salt continues to be the source of only occasional nitrite poisoning (32, 77, 468).

Peculiarities occur in the kinetics of ferrihemoglobin formation by nitrite *in vitro* (16). With low concentrations of nitrite the reaction was very slow. After a certain amount of nitrite had been added to a given preparation of oxyhemoglobin without recognizable early effect, further adding of nitrite promptly produced ferrihemoglobin. Marshall and Marshall (345) showed that a quick formation of ferrihemoglobin by nitrite occurred after an "induction period" the length of which depended on the nitrite concentration. The course of the ferrihemoglobin formation is "autocatalytic" and strongly affected by the concentration of nitrite and hydrogen ions (238, 410). The mechanism of the reaction, however, could not be further elucidated. No evidence was found of ferrihemoglobin having a catalytic effect on the formation of ferrihemoglobin by nitrite (39, 238, 410). Betke *et al.* (39) confirmed the S-shaped curve which results if the increase in ferrihemoglobin concentration in a hemoglobin solution on the addition of nitrite is plotted against time. The initial retardation of the reaction is not due to a reduction of ferrihemoglobin by nitrite as in the case of phenylenediamines (282) (Section II G). Ascorbic acid delays the onset of rapid ferrihemoglobin formation (39, 342). It is uncertain whether it reduces the slowly formed ferrihemoglobin or whether it reacts with the nitrite (134). Kakizaki *et al.* (240) found that at pH 7 and constant oxyhemoglobin concentration the length of the induction period (lag phase) is inversely proportional to the square of nitrite concentration.

For an understanding of the reaction mechanism the findings of Cohen *et al.* (91) may prove useful. By applying the irreversible inhibition of catalase by 3-amino-1,2,4-triazol as an indicator for hydrogen peroxide they demonstrated the generation of hydrogen peroxide during the reaction of nitrite with oxyhemoglobin. By means of electron spin resonance measurements, Rein *et al.* (409) detected nitric oxide hemoglobin besides ferrihemoglobin as a product of the reaction between nitrite and red cells under air. The ratio between the proportion of hemoglobin oxidized to ferrihemoglobin and that combined with nitric oxide was found to vary widely with the species.

If hemoglobin is partially (36, 240) or fully (268) devoid of oxygen, the formation of ferrihemoglobin by nitrite proceeds more slowly than under air. Unlike the reaction of ferricyanide with oxyhemoglobin, the formation of ferrihemoglobin by nitrite is not accompanied by liberation of all the oxygen bound to hemoglobin into the gas phase (240, 345, 354).

A long disputed problem about the spectral changes accompanying the reaction of nitrite in high concentration with oxyhemoglobin was settled by Jung and

Remmer (238). They observed that ferrihemoglobin forms a compound with excess nitrite, its dissociation constant at pH 6.7 and 20°C being $10^{-2.6}$. Their results and conclusions were confirmed by van Assendelft and Zijlstra (15).

Differences occur in the reaction velocities of nitrite with the oxyhemoglobin of various species (39, 397, 442) and with the oxyhemoglobin of fetal or adult hemoglobin of the same species (318). These differences are related neither to the oxygen affinity of the hemoglobins, *i.e.*, to the concentration of deoxygenated ferrohemoglobin under the oxygen pressure of air (27, 455), nor to differences of species found with the formation of ferrihemoglobin by ferricyanide. Only slight differences in reaction rate were observed between the hemoglobin of young and adult rabbits (445). Martin and Huisman (346) isolated various types of human hemoglobin and found their rates of reaction with nitrite to differ substantially. The hemoglobins composed of α-chains and normal or abnormal δ-chains react more slowly with nitrite than Hb-A. Hb-Barts is oxidized at an extremely high rate.

The kinetics of ferrihemoglobin formation *in vivo* by nitrite was investigated in dogs and mice (298, 305). The reaction is slower in the mouse than in the dog. Under reduced pressure, corresponding to an altitude of about 8500 m, nitrite produces as much ferrihemoglobin in dogs as under normal barometric pressure (444). The action of nitrite in red cells appears to be limited to an increase in ferrihemoglobin concentration. No oxidation of glutathione was observed in the presence of an active pentose phosphate pathway if red cells were incubated for 1 hr with concentrations of nitrite many times higher than the hemoglobin concentration (165).

The occurrence of nitric oxide hemoglobin in the blood following fatal nitrite poisoning was confirmed in a boy, aged 2 years, who died after the intake of nearly 20 g sodium nitrite (28).

Under conditions which have not yet been demonstrated *in vivo*, nitrite may cause other chemical changes of the hemoglobin molecule than the oxidation of its iron or the formation of nitric oxide hemoglobin. Havemann (173, 174) observed the formation of a green hemoglobin derivative if hydrogen peroxide acted on a solution of hemoglobin containing an excess of nitrite or if nitrite was added to an acid solution of hemoglobin. The change in color caused by the action of nitrite and hydrogen peroxide was shown to be a structural change of the porphyrin (8, 258–260). The isolated porphyrin has a spectrum of the rhodo-type with bands displaced to the red. Because of spectral changes observed after treatment with hydroxylamine (oxime formation) and further change of the spectrum when the oxime was heated in acetic anhydride (nitrile formation), the formation of a formyl group in the porphyrin by the action of nitrite and hydrogen peroxide was assumed. Fox and Thomson (137) more recently studied the green pigment produced from hemoglobin and myoglobin by nitrite in acid solution, which had first been observed by Meier (354). The hemin split from the green proteins was found to be optically different from protohemin. Because of the higher nitrogen and lower iron content, the authors assumed the green hemin to be protohemin modified by the addition of a nitrogenous compound (nitroso derivative). Removal of the iron from the green hemin yielded protopophyrin IX, a result quite

different from what was observed with the green hemin produced by the action of nitrite and hydrogen peroxide on hemoglobin (8).

E. The reaction of redox dyes with hemoglobin

The appearance of hemolysis in infants treated with menadione has aroused interest in the oxidation of hemoglobin by redox dyes, in particular by menadione. There has been discussion whether the formation of ferrihemoglobin is an essential step in the changes caused by menadione in red cells (40, 65, 164, 420–422, 516).

Scudi and Buhs (426) found that menadione increases the ferrihemoglobin concentration of blood *in vitro*. After huge doses (about 250 mg/kg) had been given by stomach tube to rats, marked ferrihemoglobinemia was also observed *in vivo*. The reactions of some dyes in low concentrations with hemoglobin in solution and in red cells were studied by Kiese (257, 261). In the presence of oxygen and absence of reducing systems Blindschedlers green and menadione oxidized hemoglobin much more rapidly and to a higher extent than the thiazine and oxazine dyes tested. Each molecule of the dye produced several equivalents of ferrihemoglobin by catalytic transfer of electrons from the hemoglobin iron to molecular oxygen. The ferrihemoglobin concentration at which the velocity of ferrihemoglobin formation in the presence of the dye approaches the velocity of the uncatalyzed oxidation of hemoglobin by molecular oxygen seems to be determined by the ratio between the reaction velocity of the leuco dye or its semiquinone with oxygen and that with ferrihemoglobin.

Hoffmann-Ostenhof *et al.* (195, 196) studied the relationship of ferrihemoglobin formation by some quinones to their oxidation-reduction potential. It is not surprising that they missed a strong influence of the oxidation-reduction potential on the rate of ferrihemoglobin formation and the "equilibria" obtained, because the reactions which determine the velocity of ferrihemoglobin formation and the "equilibrium" concentration of ferrihemoglobin in the presence of oxygen are different from the electrode reactions in the absence of oxygen which determine the oxidation-reduction potential.

Jung and Witt (239) studied the action of some polyphenols *in vivo*. After the intraperitoneal injection of fairly large, in some cases lethal, doses, up to 40 and 50% of the hemoglobin was oxidized to ferrihemoglobin by resorcinol or pyrogallol and up to 10% by hydroquinone, catechol, or *p*-quinone. The activity of resorcinol appears the more remarkable, as it is said not to produce ferrihemoglobin *in vitro*. Apart from ferrihemoglobin, verdoglobin was found in the blood of the animals. Magos (341) observed the denaturation of hemoglobin by *p*-quinone.

In red cells containing glucose the reaction of the dyes is modified by their enzymatic reduction. Then other reactions may occur apart from the oxidation of hemoglobin by the oxidized dye. It is not known which part of the dye in red cells is maintained enzymatically in the reduced state. It certainly varies with the dye and the species. The reduction of the dyes is due mainly to the reaction with a diaphorase which accepts electrons from NADPH (ferrihemoglobin reductase$_{NADP}$), the NAD-system being of little effect. This conclusion, drawn from

experiments with various substrates and determinations of metabolic products (147, 168, 257, 262, 295, 484), was confirmed by the observation of decreased methylene blue activity in human red cells with low content of glucose-6-phosphate dehydrogenase and, therefore, diminished NADPH production (116, 165, 168, 334, 414, 485). Taking the increase in pyruvic acid as a measure of NADH oxidized, Hoffmann *et al.* (197) calculated the fraction of the methylene-blue-stimulated oxygen uptake in rabbit's red cells which is used to oxidize NADH as amounting to about 25%. Since the rapid reduction of ferrihemoglobin in rabbit's red cells is not allowed for in this calculation, the true share of NADH in the methylene blue reduction is probably even smaller.

The enzymatically produced leuco dye may react either with oxygen or with ferrihemoglobin, which has been produced by the oxidized form of the dye or by some other way. This reaction is the basis of the catalytic effect of dyes on the reduction of ferrihemoglobin in red cells. A dye can have a strong catalytic effect on the reduction of ferrihemoglobin in red cells in the presence of oxygen if its reduced form does not react faster with molecular oxygen than with ferrihemoglobin, as was observed with toluidine blue by Kiese and Waller (304). Several thiazine and oxazine dyes show this catalytic effect and keep the ferrihemoglobin concentration in red cells at a rather low level. The steady state concentration of ferrihemoglobin does, however, increase with the concentration of the dye in the red cells (304). Bock (41) has shown that methylene blue in high concentration causes a substantial increase of ferrihemoglobin concentration in red cells. The system that reduces dyes like toluidine blue is of maximum efficiency at rather low concentrations of the dye, 5×10^{-4} M (304), higher concentrations being inhibitory. The higher the dye concentration, then, the more it acts as if there were no enzymatic reduction of the dye.

Although menadione accelerates the reduction of ferrihemoglobin in red cells in the absence of oxygen more than methylene blue or toluidine blue does (257), its effect is negligible in the presence of oxygen (165, 226, 261), the cause being the rapid reaction of the reduced menadione with oxygen. In stimulating the oxygen uptake of red cells, menadione is as effective as methylene blue (81, 261). Broberger *et al.* (67), as well as Harley and Mauer (163), confirmed the fairly rapid oxidation of hemoglobin by menadione under air with human hemoglobin and studied the kinetics in more detail. Using the irreversible inhibition of catalase by 3-amino-1,2,4-triazole as an indicator for hydrogen peroxide, Cohen and Hochstein (89, 90) have shown that hydrogen peroxide is produced by the reaction of menadione in red cells *in vitro* and *in vivo*. In the formation and reduction of ferrihemoglobin in human red cells, 1,2-naphthoquinone is much like menadione (167, 168, 257).

Chemical changes in red cells which cause enzyme inhibition and eventually hemolysis have also been observed with dyes like toluidine blue or methylene blue in high concentration (304, 453). Denaturation of hemoglobin by menadione as the result of a more drastic reaction with the hemoglobin has also been observed (163), 164). The same action of methylene blue in high concentrations had been demonstrated by Kikuth and Schilling (309), who observed the forma-

tion of Heinz bodies in mice after injecting large doses of methylene blue. Bock (41) produced Heinz bodies with large doses of methylene blue in mice, rats, guinea pigs, rabbits, cats, and dogs.

Under certain conditions, endogeneously produced benzoquinones may cause ferrihemiglobinemia in men. In scorbutic patients with ferrihemiglobinemia, Fishberg (130, 131) found an increased excretion of benzoquinone acetic acid, which *in vitro* forms ferrihemoglobin. After the administration of ascorbic acid, the excretion of the benzoquinone derivative and the ferrihemoglobin concentration decreased.

F. Formation of ferrihemoglobin by aminophenols in the presence of oxygen

In vitro. In contrast to ferricyanide, hydrogen peroxide, and the oxidized forms of reversible dyes, aminophenols and the substances dealt with in the following sections cannot oxidize hemoglobin in the absence of oxygen. They can all reduce ferrihemoglobin. With some substances, *e.g.*, phenylenediamines, the rate of this reaction is so high that it delays the increase in ferrihemoglobin concentration in the presence of oxygen. With other substances like aminophenols and arylhydroxylamines the reduction of ferrihemoglobin affects its increase only at higher concentrations. The mechanism of ferrihemoglobin formation by reducing substances will be discussed in Section II H 1 after the facts have been presented.

p-Aminophenol, the first metabolic product of aniline discovered (456), was observed a long time ago to produce ferrihemoglobin *in vivo* (193). *o*- and *m*-Aminophenol, which were later found also to be metabolic products of aniline (225, 395, 440) or of other substances (160, 474), are also capable of forming ferrihemoglobin.

The studies of Heubner (183, 189) more than 70 years ago unveiled some fundamental facts concerning the formation of ferrihemoglobin by aminophenols. No ferrihemoglobin is formed by the aminophenols in the absence of oxygen. *o*-Aminophenol was found to react more rapidly than *p*-aminophenol. *m*-Aminophenol is very much less active. *o*- and *p*-Aminophenol produce several equivalents of ferrihemoglobin per mole of aminophenol added to blood or hemoglobin solution. From these results it was concluded that aminophenol is oxidized to quinonimine and that this is the compound which oxidizes hemoglobin.

Determinations of the decrease in *p*-aminophenol concentrations in suspensions of red cells, hemoglobin solutions, and phosphate solutions of the same pH showed that *p*-aminophenol, as determined by the indophenol reaction, disappears the more rapidly the faster hemoglobin is oxidized (276, 279). *p*-Quinonimine oxidized hemoglobin very quickly (229). In suspensions of red cells the rate of decrease in *p*-aminophenol concentration and increase in ferrihemoglobin concentration were slower under oxygen than under air (248). These results point to hemoglobin enhancing the formation of quinonimine or other active products. Quinonimine then oxidizes ferrohemoglobin and thereby returns to aminophenol for another catalytic cycle (276, 375). *m*-Aminophenol, which does not form a quinonimine, is inactive.

Free or oxygenated iron in hemoglobin is essential for the rapid disappearance

of aminophenol in a solution of hemoglobin and aminophenol. The blockade of the iron in hemoglobin by carbon monoxide inhibits the effect of hemoglobin on the oxidation of *p*-aminophenol by oxygen (248). Blood serum proteins do not affect this reaction (276). The more detailed study by Kampffmeyer and Kiese (248) has shown the reaction rate of hemoglobin with *p*-aminophenol and oxygen to be proportional to the concentration of hemoglobin and *p*-aminophenol at constant oxygen pressure. *p*-Aminophenol reduces ferrihemoglobin to hemoglobin, the velocity of the reaction being proportional to the concentration of *p*-aminophenol and to the square of the ferrihemoglobin concentration. Therefore, on adding *p*-aminophenol to red cells *in vitro*, an equilibrium concentration of ferrihemoglobin develops, no matter whether at the outset the hemoglobin was in the ferro-form or almost completely oxidized to ferrihemoglobin (279).

On reduction of the oxygen pressure, the rate of ferrihemoglobin formation by *p*-aminophenol increases only a little. In suspensions of red cells taken from cattle blood and suspended in Krebs-Ringer phosphate solution of pH 7.4 at 23°C maximum reaction velocity occurs at 50 mm Hg oxygen (248). About 80% of the hemoglobin is saturated with oxygen at this oxygen pressure, temperature, and pH. The inhibition of the reaction by high oxygen pressures reflects the role of deoxygenated ferrohemoglobin in the reaction. Contrary to the "coupled oxidation" of hemoglobin and phenylhydroxylamine (see below), the concentration of oxygen seems to affect the reaction rate more strongly than the concentration of deoxygenated ferrohemoglobin does.

Large species differences have been observed in the reaction velocity. The hemoglobins of cats and dogs react with *p*-aminophenol and oxygen 15 times more rapidly than cattle hemoglobin (276).

In a study of several aminophenols, Kiese and Rachor (279) confirmed the higher reactivity of *o*-aminophenol as compared with that of *p*-aminophenol and found N-methyl- and N-butylaminophenol to be more reactive too. *p*-Dimethylaminophenol exceeds *p*-methylaminophenol in the rate of ferrihemoglobin formation (283). The substitution of an acetyl residue on the ring (2-acetyl-4-aminophenol) substantially decreases the reactivity of *p*-aminophenol (279). *o*-Aminophenol is virtually inactivated by the substitution of a sulfonamide group in para position to the amino group (471, 472).

The oxidation products of aminophenols react not only with the iron of hemoglobin but also with proteins, but compounds with nucleic acids have not been detected (310).

In vivo. The functioning of aminophenols as metabolites active in the formation of ferrihemoglobin after the absorption of certain aromatic amines is strongly hinted at in cases where the concentration and activity of N-hydroxy derivative or N-oxide in the blood is too low to account for the observed rate of ferrihemoglobin formation and where aminophenols of high activity may be formed. This holds for the ferrihemoglobin formation caused by N-alkylanilines with longer alkyl residues like N-butylaniline, by N,N-dialkylanilines (200, 283) or by phenetidine (20). Quantitative data concerning the formation of ferrihemoglobin by aminophenols in various species are available: *o*-Aminophenol: cat (279, 400),

dog (159, 305), mouse (305), rabbit (6); *p*-aminophenol: cat (175, 220, 279, 459), dog (20, 35, 103, 444, 481), rat (330, 450); *m*-aminophenol: dog (279); 2-acetyl-4-aminophenol: dog (267); *p*-methylaminophenol: cat, dog (279); *p*-butylaminophenol: cat (279); *p*-dimethylaminophenol: dog (283); 1-hydroxy-2-aminonaphthalene: cat (180); 6-hydroxy-2-aminonaphthalene: cat (180). A comparison of the effects of various agents on ferrihemoglobin formation after the intravenous injection into cats shows *o*-aminophenol, *p*-methylaminophenol, and, particularly, *p*-butylaminophenol to be more active than *p*-aminophenol. In a study of the dose-effect relationship with these 4 aminophenols injected intravenously into cats, the maximal ferrihemoglobin concentration attained was proportional to the logarithm of the dose (279). The same is the case with *p*-dimethylaminophenol intravenously injected into dogs (283). In the dog this compound is several times more active than *p*-methylaminophenol. Although *p*-butylaminophenol has not been tried in the dog, from the ratio between *p*-methyl- and *p*-butylaminophenol activity observed in the cat, *p*-butylaminophenol can also be expected to be less active than *p*-dimethylaminophenol.

After intraperitoneal injection of *p*-aminophenol into rats, Lester, Greenberg, and Shukowski (330) found the ferrihemoglobin concentration not to increase beyond 35% of the total hemoglobin.

The effect of *p*-aminophenol has been studied under special conditions. Spicer and Neal (444) found it to produce only half as much ferrihemoglobin in dogs under reduced atmospheric pressure simulating an altitude of about 8500 m as under normal pressure. In partially hepatectomized rats, Scheff (450) found that intraperitoneal injection of *p*-aminophenol produced more ferrihemoglobin than in normal rats. After the application of 250 mg of *o*-aminophenol per kg Akahori (6) could not detect ferrihemoglobin in rabbits. Even one fourth of the dose, however, produced large amounts of ferrihemoglobin if the portal vein had been ligated previously.

G. Formation of ferrihemoglobin by phenylenediamines in the presence of oxygen

Although the reaction of phenylenediamines with hemoproteins has been known for a long time from their use for reducing cytochrome c in experiments with cytochrome oxidase ("*p*-phenylenediamine oxidase"), the reaction with hemoglobin has been investigated by few authors. Afanassiew (5) as well as Stadelmann (460) noticed the dark brown discoloration of the blood of dogs dosed with *m*-tolylenediamine. Since they did not mention ferrihemoglobin, they probably did not realize that ferrihemoglobin was the cause of the discoloration. Matsumoto (351) observed the formation of ferrihemoglobin by *o*-, *m*-, and *p*-phenylenediamine *in vitro*, but he did not find ferrihemoglobin in the blood of cats and dogs on injection of *p*-phenylenediamine. Erdmann and Vahlen (125) in experiments with *p*-phenylenediamine on dogs and Meissner (355) with *o*-, *m*-, and *p*-phenylenediamine on cats were looking for ferrihemoglobin, but did not find any. Meissner noticed that triaminobenzene and triaminotoluene produce large amounts of ferrihemoglobin in cats.

Kiese and Seipelt (296) observed fatal doses of *p*-phenylenediamine to produce

little ferrihemoglobin in dogs and *m*-tolylenediamine (2,4-diaminotoluene) to be much more active. The authors also noticed the formation of a verdoglobin by *m*-tolylenediamine in dogs.

Jung (237), using cats, confirmed that *m*-phenylenediamine produces substantial amounts of ferrihemoglobin. "Tolylenediamine," which according to Stahl's thesis (461) was 1-methyl-2,4-diaminobenzene, was found by Stahl and Jung (462) to be about as active as *m*-phenylenediamine. In a more thorough investigation into the mechanism of ferrihemoglobin formation by phenylenediamines, Kiese and Rauscher (282) observed peculiar kinetics. With all 3 isomers and some ring-substituted derivatives, the rate of ferrihemoglobin formation increased with time and later slowed. For a detailed study of the kinetics, the authors used durenediamine. Duroquinonediimine and, to a less extent, duroquinone produced by hydrolysis of the diimine were found to be proximate ferrihemoglobin-forming agents. It is still unknown whether the formation of duroquinonediimine is furthered by hemoglobin or whether it is produced only by "autoxidation." The delay in the increase in ferrihemoglobin concentration at the outset of the reaction of durenediamine with oxygen and ferrihemoglobin is due to two reactions. Duroquinonediimine, as soon as it is formed, is rapidly reduced to the diamine by SH-groups. The previous treatment of red cells or hemolysates with *p*-chloromercuribenzoate, N-ethylmaleimide, or iodoacetamide increases the rate of ferrihemoglobin formation by the diimine, as well as by durenediamine, and diminishes the initial delay in the increase in ferrihemoglobin concentration observed after adding durenediamine to the solution of oxyhemoglobin. The other reaction which delays the increase in ferrihemoglobin concentration is the reduction of ferrihemoglobin by the diamine. This reaction is much more rapid than the formation of ferrihemoglobin by durenediamine. These results do not explain the initial delay in the increase in ferrihemoglobin concentration with all phenylenediamines. *m*-Phenylenediamine probably produces ferrihemoglobin by different mechanisms. The velocity of ferrihemoglobin formation by *m*-phenylenediamine in hemoglobin solutions is increased by adding liver microsomes, NADP, and an NADP-reducing system. The ferrihemoglobin formation by *o*-phenylenediamine and *p*-phenylenediamine is not enhanced by the presence of microsomes and NADPH (282).

H. Formation of ferrihemoglobin by arylhydroxylamines in the presence of oxygen

1. *Reaction with hemoglobin in solution.* Experiments by Heubner *et al.* (190) had already shown that phenylhydroxylamine, hemoglobin, and oxygen react in a "coupled oxidation." Nitrosobenzene is the reaction product of phenylhydroxylamine (113). Whereas hemoglobin and phenylhydroxylamine alone in aqueous solution are slowly oxidized by molecular oxygen, both react rapidly with oxygen if present in the same solution. Kiese and von Ruckteschell (291) confirmed this observation and studied the catalytic effect of hemoglobin on the oxidation of phenylhydroxylamine as measured by the oxygen uptake. In high concentrations of phenylhydroxylamine, the rate of oxygen uptake is proportional to the hemoglobin concentration. With a low constant hemoglobin con-

centration, the rate of oxygen uptake is proportional to the logarithm of the phenylhydroxylamine concentration.

The kinetics of the ferrihemoglobin formation by phenylhydroxylamine and oxygen in hemoglobin solutions was studied by Kiese and Reinwein (284). This reaction does not seem to be affected by the reversible system phenylhydroxylamine-nitrosobenzene (340, 437) or by the reduction of ferrihemoglobin by phenylhydroxylamine (see below). At constant oxygen pressure the initial reaction velocity is proportional to the concentrations of hemoglobin and phenylhydroxylamine. The yield of ferrihemoglobin varies with the ratio of hemoglobin concentration to phenylhydroxylamine concentration. Under air and a 10-fold excess of hemoglobin, the yield is one equivalent of ferrihemoglobin per mole of phenylhydroxylamine. It may be even higher under other conditions. Within the red cell the yield of ferrihemoglobin does not seem to be quite so high. Of the oxygen which is used up during the "coupled oxidation" of hemoglobin and phenylhydroxylamine only about one fourth is used for oxidizing the iron of hemoglobin (303). Like the oxidation of hemoglobin by molecular oxygen (71, 72, 293, 377), with increasing oxygen pressure the formation of ferrihemoglobin by phenylhydroxylamine and oxygen passes through a maximum at rather low oxygen pressures. In solutions of low concentrations of hemoglobin and phenylhydroxylamine at pH 6.8 and 24°C a maximum of the velocity of ferrihemoglobin formation was observed by Brinkmann and Kiese (66) at an oxygen pressure of 40 mm Hg. However, at this oxygen pressure the reaction rate is only 50% higher than under 700 mm Hg oxygen. The importance of deoxygenated ferrohemoglobin in the reaction is demonstrated also by experiments with muscle hemoglobin. This protein, which has a higher affinity for oxygen than hemoglobin, is oxidized at pH 6.8 and 24°C by phenylhydroxylamine and oxygen most rapidly at an oxygen pressure of 9 mm Hg or less.

The mechanism of the reaction between hemoglobin, phenylhydroxylamine, and oxygen has been speculated on before. Heubner (186) presumed that phenylhydroxylamine is oxidized to nitrosobenzene with a radical as an intermediate and that this radical takes an electron from ferrohemoglobin, thereby returning to phenylhydroxylamine. From measurements of paramagnetic resonance absorption, Wahler *et al.* (483) concluded that radicals appear in solutions of hemoglobin, arylhydroxylamines, and oxygen. Free radicals derived from nitrosobenzene and phenylhydroxylamine have been obtained by one-electron reduction or oxidation in aqueous solution (155) and were observed in condensation reactions (418). The autoxidation of phenylhydroxylamine is assumed to proceed through a one-electron transfer to molecular oxygen (383). Thermodynamic data are not in favor of a hypothesis which assumes that a radical produced by transfer of an electron from phenylhydroxylamine to oxygen is the active intermediate which takes the electron from the hemoglobin iron. The oxidation-reduction potential of ferri-ferro-hemoglobin at pH 7 is about +150 mV (12) and that of nitrosobenzene-phenylhydroxylamine at pH 7 is about −100 mV (437).

A catalytic mechanism of ferrihemoglobin formation by a one-electron transfer has also been presumed by Lenk and Wahler (326) for the oxidation of hemo-

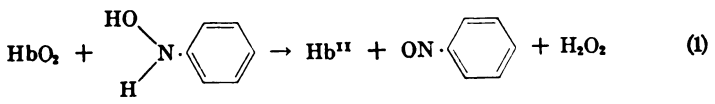
globin by diphenylnitrogen oxide. They observed each mole of this substance to oxidize several equivalents of hemoglobin in the presence of oxygen. Diphenylnitrogen oxide must be capable of transferring the electron accepted from hemoglobin to oxygen.

Heubner *et al.* (191) found that each mole of N-acetylphenylhydroxylamine produces several equivalents of ferrihemoglobin in red cells. Since the authors believed that N-acetylphenylhydroxylamine could not be oxidized to the nitroso level they took their observation as a proof of a radical of N-acetylphenylhydroxylamine (and phenylhydroxylamine) being the derivative which accepts the electron from ferrohemoglobin and thereby returns to the hydroxylamine. This mechanism, of course, should also work outside the red cell with hemoglobin. Ziegler's (512) data show that in a solution of crystallized hemoglobin N-acetylphenylhydroxylamine does not react repeatedly. She expressly stated that enzymes in the red cell are necessary for a repeated reaction of N-acetylphenylhydroxylamine. The experiments with N-acetyl- and N-benzoylphenylhydroxylamine on cats are of no consequence for the problem. The N-acylphenylhydroxylamines are rapidly split *in vivo*. Therefore the administration of an N-acylphenylhydroxylamine produces a slightly delayed phenylhydroxylamine effect (212, 278). Benzoylphenylhydroxylamine was found by Kiese and Plattig (278) to react with horse hemoglobin in solution only once and very slowly. Hustedt and Kiese (212) observed acetylphenylhydroxylamine to react with oxyhemoglobin much less rapidly than phenylhydroxylamine. This reaction probably produces nitrosobenzene from the N-acylphenylhydroxylamines as was observed in the reaction of N-acylphenylhydroxylamines with lead tetraacetate (30). A re-investigation by Baerwolff *et al.* (21) of the oxidation of hemoglobin by prophyrexide (185) did not produce any evidence of catalytic ferrihemoglobin formation by this radical.

An attempt at describing the reaction mechanisms has to consider the following results. The velocity of ferrihemoglobin formation has a maximum at low oxygen pressures; the velocity is proportional to the phenylhydroxylamine concentration and, under constant oxygen pressure, to the hemoglobin concentration; phenylhydroxylamine is rapidly oxidized to nitrosobenzene; in the absence of hemoglobin the autoxidation of phenylhydroxylamine is slow.

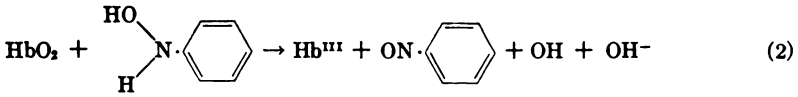
Since neither phenylhydroxylamine nor nitrosobenzene oxidizes ferrohemoglobin, a reactive intermediate, or several of them, must be formed. The intermediate which is produced in a bimolecular reaction of phenylhydroxylamine with oxyhemoglobin oxidizes deoxygenated ferrohemoglobin.

The active intermediate is probably a peroxide or a radical which originates from the oxygen of oxyhemoglobin. Possibly the peroxide produced in the reaction of phenylhydroxylamine with oxyhemoglobin



(1)

reacts at the site of its origin. Then ferrihemoglobin and an OH radical would be formed.



(2)

The same reaction products would be observed if the oxygen in oxyhemoglobin were ionized according to Weiss (502), and the O_2^- would react with phenylhydroxylamine, leaving the iron behind in the ferric state. The relatively high rate of ferrihemoglobin formation under high oxygen pressures, when the concentration of deoxygenated ferrohemoglobin is very low, gives rise to a suspicion that oxygenated hemes are transformed into ferrihemes.

The involvement of radicals or hydrogen peroxide in the formation of ferrihemoglobin by reducing substances in the presence of oxygen has been considered since Hoppe-Seylers' (203) early observation of ferrihemoglobin formation by palladium-hydrogen and oxygen. Using the luminol test Rostorfer and Cormier (415) observed hydrogen peroxide, or radicals, appearing in the formation of ferrihemoglobin by phenylhydroxylamine.

Strömme (466) used the oxidation of sulfite to sulfate for detecting free radicals (138) during the formation of ferrihemoglobin by reducing substances and oxygen. The formation of ferrihemoglobin by diethyldithiocarbamate was found to initiate the sulfite oxidation. On the other hand the radicals generated by the sulfite oxidation greatly increase the rate of ferrihemoglobin formation by diethyldithiocarbamate. This compound autoxidizes very slowly. Strömme (466) assumed a mechanism of its oxidation by oxyhemoglobin analogous to equation (2) with the disulfide as oxidation product. The appearance of OH radicals, or hydrogen peroxide formed from them, is also shown in experiments with human red cells lacking glucose-6-phosphate dehydrogenase. In these cells glucose does not affect the increase in ferrihemoglobin concentration caused by diethyldithiocarbamate, whereas in normal cells glucose slows the increase in ferrihemoglobin concentration. The generation of active intermediates like OH or O_2H_2 in the reaction of phenylhydroxylamine with oxyhemoglobin could easily explain the excess of oxygen taken up beyond that needed for the formation of ferrihemoglobin and nitrosobenzene; some of the intermediates react with glutathione, SH-groups, or unknown partners. Perhaps one of the side reactions, *i.e.* the decomposition of hydrogen peroxide by catalase, is decreased in the reactions with phenylhydroxylamine or *p*-aminophenol, because these substances in very low concentrations inhibit catalase (429).

There is thus circumstantial evidence for a mechanism delineated above in which OH radicals or hydrogen peroxide, or both, are active intermediates. The kinetics of the formation of ferrihemoglobin by aminophenols is in principle the same as that of its formation by arylhydroxylamines. The reaction has an opti-

mal oxygen pressure, and this means that deoxygenated ferrohemoglobin takes part in the reaction. The aminophenol is oxidized by oxyhemoglobin. The oxidation product differs from that of phenylhydroxylamine in that it may be reduced by ferrohemoglobin or other substances to its original state. It does not seem to be of great importance whether the oxidation product is the semiquinone or the quinoneimine.

The radical or hydrogen peroxide hypothesis in its simple form, however, cannot explain why hemoglobin is attacked differently by various "coupled oxidations." The reaction of hemoglobin and ascorbic acid with oxygen also produces ferrihemoglobin, but in addition a substantial proportion of the hemoglobin is transformed into a verdoglobin. Lemberg *et al.* (324, 325) have shown that, like the formation of ferrihemoglobin, the formation of verdoglobin by ascorbic acid has an optimal oxygen pressure. The coupled oxidation of phenylhydroxylamine or *p*-aminophenol does not produce noticeable amounts of verdoglobin.

Phenylhydroxylamine also reacts with ferrihemoglobin to form ferrohemoglobin and nitrosobenzene (254). The reaction was studied by Kiese and Reinwein (285). One mole of phenylhydroxylamine reduced 2 equivalents of ferrihemoglobin. The velocity of the rather slow reaction was proportional to the phenylhydroxylamine concentration and to nearly the square of the ferrihemoglobin concentration. Therefore this reaction has little inhibiting effect on the rate of increase in ferrihemoglobin concentration in red cells as long as the ferrihemoglobin concentration is low.

The nitrosobenzene produced in the coupled oxidation of phenylhydroxylamine and hemoglobin, or added to hemoglobin, combines with the iron of hemoglobin like carbon monoxide or nitric oxide (235, 254). The affinity of hemoglobin for nitrosobenzene was found by Murayama (373) to be higher and by Scheler (452) to be lower than that for oxygen. Substituents affect the binding of nitrosobenzene to hemoglobin (148, 373, 451, 452). Unless reduced to phenylhydroxylamine by an additional reaction, nitrosobenzene does not form ferrihemoglobin, or at least only very slowly.

2. Reaction in red cells in vitro. a. The enzymatic cycle of ferrihemoglobin formation. Whatever the role of a radical might be in the intimate mechanism of ferrihemoglobin formation during the coupled oxidation of hemoglobin and phenylhydroxylamine by molecular oxygen, it does not act as a catalyst which without additional effects (186) would produce the many equivalents of ferrihemoglobin per mole of phenylhydroxylamine observed *in vivo*. From his experiments on cats Issekutz (220) calculated that 1 mole of phenylhydroxylamine produces up to 50 equivalents of ferrihemoglobin.

The mechanism by which phenylhydroxylamine produces several equivalents of ferrihemoglobin has been elucidated by Kiese *et al.* (286). In red blood cells suspended in isotonic salt solution without substrate 10^{-4} M phenylhydroxylamine produced only a trace of ferrihemoglobin. After adding glucose to the red cells, there was a continuous production of ferrihemoglobin (fig. 1). In the course of 2 hr, nearly half the hemoglobin is oxidized by a cycle which obviously needs hydrogen-transferring enzymes reduced by metabolites of glucose in red cells.

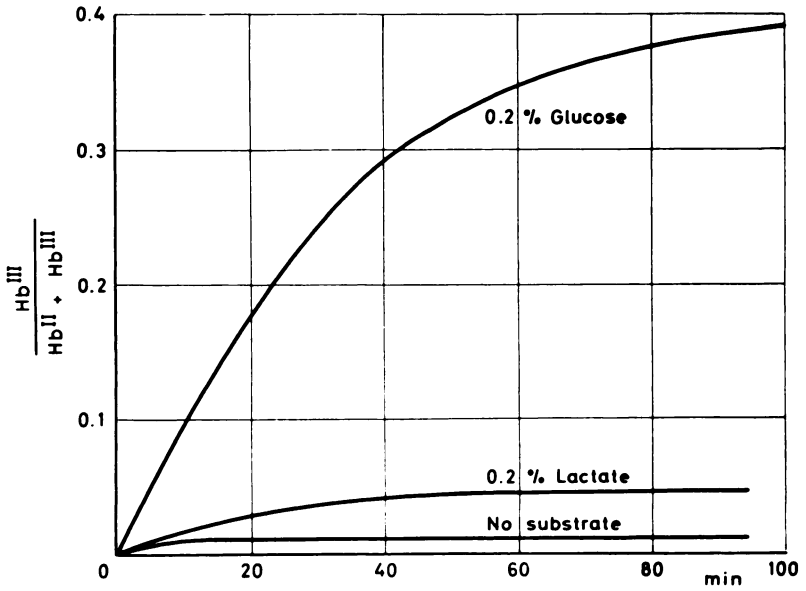


FIG. 1. Formation of ferrihemoglobin in washed red cells taken from dog blood. The cells were suspended in an equal volume of Ringer carbonate solution and kept at 37°C under a mixture of oxygen and 5% carbon dioxide. Phenylhydroxylamine was added to a concentration of 10^{-4} M. The substrates added are mentioned beside the curves. Kiese *et al.* (286).

Nitrosobenzene produced as much ferrihemoglobin as phenylhydroxylamine. The dependence of the rate of ferrihemoglobin formation and of the maximal ferrihemoglobin concentration on the concentration of phenylhydroxylamine or nitrosobenzene added has been investigated. Lactate and malate are much less active than glucose in supporting the enzymatic cycle.

In red cells which produced ferrihemoglobin after phenylhydroxylamine had been added Dannenberg and Kiese (113) found nitrosobenzene. The same authors (114) demonstrated that nitrosobenzene is enzymically reduced to phenylhydroxylamine in red cells.

Studies of the reduction of ferrihemoglobin in red cells (147, 257) have shown that there are mainly two electron-transporting systems which can donate electrons to ferrihemoglobin: 1) by the dehydrogenation of triosephosphate or lactate, NAD is reduced. A diaphorase, ferrihemoglobin reductase NAD, which Scott and McGraw (425) have purified and studied, transfers electrons from NADH to ferrihemoglobin. The metabolic product is pyruvate. 2) NADPH generated by the pentose phosphate pathway can reduce another diaphorase, ferrihemoglobin reductase NADP, which has been studied and purified by Kiese (257), Huennekens *et al.* (210, 211), and Kiese *et al.* (294). Carbon dioxide is produced by this system. Although the properties of purified ferrihemoglobin reductase NADP preparation from human red cells (210, 211, 294, 431) differ in some respects, all preparations were similar in accepting electrons from NADPH and

transferring them quickly to methylene blue; they did not react directly with ferrihemoglobin, or at least very slowly. Recently Scott *et al.* (424) have shown that there are at least two enzymes in red cells which oxidize NADPH and also two enzymes which oxidize NADH.

From experiments with various substrates (257) and determinations of metabolites (262, 295) the conclusion had to be drawn that within the red cell ferrihemoglobin reductase$_{NADP}$ is the enzyme transferring electrons from NADPH to methylene blue and similar dyes. The same enzyme was also found to reduce nitrosobenzene to phenylhydroxylamine. In solutions of purified enzyme together with catalytic concentrations of NADP, an NADP-reducing system, hemoglobin, and nitrosobenzene or phenylhydroxylamine, the enzymatic cycle of ferrihemoglobin formation in the red cell could be reproduced *in vivo* (290).

Again by determining metabolites Kiese and Waller (303) as well as Dannenberg and Kiese (114) have shown that in the red cell too the enzymatic cycle of ferrihemoglobin formation is driven by electrons supplied by the pentose phosphate pathway and transferred *via* NADP and ferrihemoglobin reductase$_{NADP}$ to nitrosobenzene. The production of carbon dioxide was found to increase so much during the cycle in dog red cells as to account for 90 % of the oxygen uptake due to the cycle, except the oxygen used for the oxidation of hemoglobin. The formation of pyruvate had only increased a little.

The lead of the pentose phosphate pathway and NADPH in the enzymatic cycle of ferrihemoglobin formation was confirmed by experiments conducted by Waller *et al.* (485) and Löhr and Waller (334) with human red cells lacking in glucose-6-phosphate dehydrogenase, which were discovered by Carson *et al.* (80). These cells, suspended in phosphate Ringer solution containing glucose, did not produce ferrihemoglobin when nitrosobenzene was added. Under the same conditions normal red cells were found to oxidize half their hemoglobin in 20 min. The enzymatic cycle of ferrihemoglobin formation may be briefly represented by the scheme of figure 2. Kiese and Schwarzkopff (295) as well as Spicer *et al.* (443) have observed that malate and fumarate can be used as substrates in ferrihemoglobin reduction in red cells. The first-mentioned authors did not observe a catalytic activity of methylene blue in this reaction, and Nossal (381) observed only a little increase in oxygen uptake by methylene blue in red cells to which fumarate or malate was offered as a substrate. In red cells taken from dog's blood, malate is much less active than glucose in supporting the enzymatic cycle of ferrihemoglobin formation by phenylhydroxylamine (303).

The reduction of nitrosobenzene is not confined to the red cell. If reducing enzymes, capable of reacting with nitrosobenzene, are available outside the red cells, they can increase the rate of ferrihemoglobin formation within the red cells (302). The role of the reduction of nitrosobenzene outside the red cell during ferrihemoglobin formation *in vivo* has to be further investigated.

At this point the reactions of nitrosobenzene (and phenylhydroxylamine) and redox dyes like methylene blue in red cells may be briefly compared. As stated above, both are reduced by ferrihemoglobin reductase$_{NADP}$. Both increase the oxygen uptake of the red cells; in red cells taken from dog's blood, 2.4×10^{-4} M

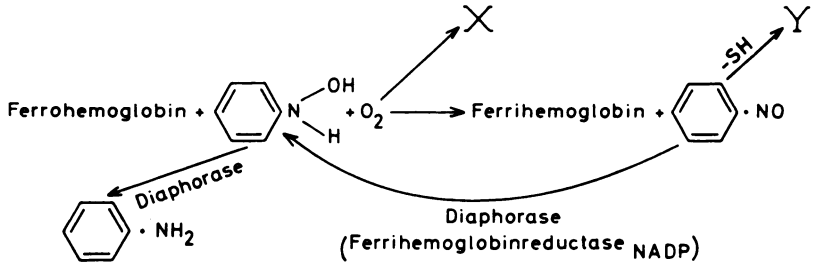


FIG. 2. Schematic representation of the enzymic cycle of ferrihemoglobin formation in red cells by phenylhydroxylamine and oxygen.

phenylhydroxylamine was found to be twice as active as toluidine blue (303, 304). The oxidation of reduced methylene blue by molecular oxygen does not simultaneously cause the oxidation of ferrohemoglobin. Reduced toluidine blue in red cells of dogs in concentrations below 10^{-6} M was found to react with ferrihemoglobin as quickly as with molecular oxygen (304). This relatively rapid reaction of the reduced dye with ferrihemoglobin is the basis of its catalytic effect on the ferrihemoglobin reduction. This effect is also observed in the presence of oxygen, but it is then smaller because oxygen and ferrihemoglobin compete for the reduced dye.

The reduction of nitrosobenzene by ferrihemoglobin reductase$_{NADP}$, on the other hand, produces phenylhydroxylamine. This can also reduce ferrihemoglobin as well as oxygen. As explained above, the reaction of phenylhydroxylamine with oxygen in the presence of hemoglobin quickly produces ferrihemoglobin.

Thus the same enzyme in red cells, ferrihemoglobin reductase$_{NADP}$, can support either a catalytic reduction of ferrihemoglobin or a catalytic formation of ferrihemoglobin depending on the type of catalyst.

The oxygen uptake by red cells caused by methylene blue proceeds evenly for quite a long time (169, 303). The rate of oxygen uptake caused by phenylhydroxylamine or nitrosobenzene decreases steadily, and so does the rate of ferrihemoglobin formation (303). This decrease in reaction velocity is not so much due to the decrease in ferrohemoglobin as to the disappearance of phenylhydroxylamine and nitrosobenzene. In experiments *in vitro* with red cells from oxen, Haan *et al.* (157) observed 4×10^{-4} M phenylhydroxylamine and nitrosobenzene to disappear in 3 to 4 hr. About a quarter of it was reduced to aniline. Under nitrogen the same concentration of nitrosobenzene disappears in about 1 hr and is recovered largely as aniline. *p*-Nitrosophenetol disappears in red cells from oxen under air more rapidly than nitrosobenzene, and a larger fraction is recovered as the amine (20).

The fate of the major part of phenylhydroxylamine in red cells is still unknown. Part of it probably forms irreversible compounds with hemoglobin. In experiments with radioactive *p*-iodophenylhydroxylamine on rats, Crick and Jackson (112) observed undiminished radioactivity of the blood after the ferrihemoglobin formation had ceased and the ferrihemoglobin concentration returned to normal

values. Red cells treated with radioactive *p*-iodophenylhydroxylamine and injected into rats retain radioactivity for many days (222). After the intravenous injection of N-hydroxy-2-acetylaminofluorene in rats a substantial amount is lightly bound to red cell proteins in a few minutes (491a).

Another reaction of phenylhydroxylamine or nitrosobenzene in the red cells may be with thiols (60). After adding phenylhydroxylamine to red cells, Wagner *et al.* (482) observed a rapid decrease in reduced glutathione. Lotlikar *et al.* (339) found that N-hydroxy-2-aminofluorene is not reduced by glutathione under nitrogen at pH 7.8. Under the same conditions 2-nitrosofluorene reacts quickly with glutathione; besides 2-aminofluorene a compound is formed which is not extracted into ether from neutral solution. This compound is decomposed in 0.1 N NaOH or HCl. For metabolic changes of arylhydroxylamines and nitroso compounds in other tissues see Section IV A.

While N-acylphenylhydroxylamines turned out to be unsuitable for a further study of the reaction between arylhydroxylamines and hemoglobin or the enzymatic cycle of ferrihemoglobin formation, Kiese and Plattig (277) found another N-substituted phenylhydroxylamine, benzylphenylhydroxylamine and its oxidation product, benzalphenylnitrone, to reproduce the effects of phenylhydroxylamine and nitrosobenzene almost quantitatively. Low concentrations, 2×10^{-4} M, of benzylphenylhydroxylamine in dog red cells produce little ferrihemoglobin in the absence of glucose. After the addition of glucose the enzymatic cycle of ferrihemoglobin formation begins and each mole of benzylphenylhydroxylamine produces about 40 equivalents of ferrihemoglobin. The oxygen uptake of the red cells increased to a rate corresponding to 3 to 4 equivalents of oxygen per equivalent of ferrihemoglobin formed. Benzalphenylnitrone was as active as benzylphenylhydroxylamine in these effects.

b. The activity of various arylhydroxylamines; species differences. In comparing the activity of phenylhydroxylamine derivatives in red cells *in vitro*, species differences have to be taken into account. From the data compiled in table 1, it may be seen that species differences are not the same if tested with various hydroxylamines. With phenylhydroxylamine, rabbit cells are most active, followed by cells from dogs and oxen. Whereas *p*-chlorophenylhydroxylamine was observed to be as active as phenylhydroxylamine in the cells taken from rabbits and oxen, it had only about one tenth the activity of phenylhydroxylamine in dog cells.

Results of experiments concerning the effect of substituents on the activity of phenylhydroxylamine, as determined by the initial velocity of ferrihemoglobin formation, are valid only for the species they are obtained with. The available data (table 1) do not offer much information. According to experiments with red cells taken from dog's blood *p*-substitution with chlorine inhibits the activity. The sulfonamide group in the *para*position does not affect the activity very much. This is the more remarkable as the sulfonamide group almost completely abolishes the quinonime formation of *o*-aminophenol (Section II F). The propionyl residue was found to increase the activity substantially if placed in the *para*position and to decrease the activity in the *meta*position. According to experiments with ox red cells an alkoxy residue in the *para*position slightly decreases the activity of phenylhydroxylamine.

TABLE 1

Formation of ferrihemoglobin in red cells by various arylhydroxylamines or the nitroso analogues

Species	Hydroxylamine or Nitroso Analogue	Concentration (M)	Initial Velocity of Ferrihemoglobin Formation Equivalent Hb^{III} $\times l^{-1} \times sec^{-1}$	Maximal $\frac{Hb^{III}}{Hb^{III} + Hb^{II}}$	Reference
Dog	Phenylhydroxylamine	10^{-5}	6×10^{-7}	0.2	(286)
		10^{-4}	2×10^{-6}	0.6	
		10^{-3}	10^{-5}	0.9	
Dog	Phenylhydroxylamine	3×10^{-5}	1.2×10^{-6}	0.4	(152)
Dog	Nitrosobenzene	3.7×10^{-4}	7×10^{-6}		(269)
Ox	Nitrosobenzene	3.7×10^{-4}	3×10^{-6}	0.8	(157)
Rabbit	Phenylhydroxylamine or nitrosobenzene	10^{-4}	4×10^{-6}	0.22	(31)
		2×10^{-4}		0.27	
Ox	2-Naphthylhydroxylamine	3.7×10^{-4}	3×10^{-6}	0.55	(180)
Ox	*p*-Nitrosophenetol	10^{-3}	3×10^{-6}	0.3	(20)
Ox	*p*-Chloronitrosobenzene	10^{-5}	8×10^{-7}	0.31	(267)
		10^{-4}	3×10^{-6}	0.71	
Dog	*p*-Chloronitrosobenzene	3.7×10^{-4}	8×10^{-7}		(269)
Rabbit	*p*-Chlorophenylhydroxylamine	10^{-4}	4×10^{-6}	0.28	(31)
Rat	*p*-Iodophenylhydroxylamine	1.6×10^{-4}		0.4	(112)
		8×10^{-4}		0.5	
		8×10^{-3}		0.6	
Dog	*p*-Hydroxylaminopropiophenone	3×10^{-5}	5×10^{-6}	0.75	(152)
Dog	*m*-Hydroxylaminopropiophenone	10^{-4}	2×10^{-7}	0.3	(281)
Dog	*p*-Hydroxylaminobenzenesulfonamide	10^{-4}	1.5×10^{-6}	0.35	(201)
Ox	*p*-Hydroxylaminobenzenesulfonamide	10^{-4}	2.6×10^{-6}	0.45	(201)
Dog	4-(2-Methoxy-ethoxy)-3-acetylphenylhydroxylamine	10^{-4}	10^{-6}	0.05	(281)
		10^{-3}	4×10^{-6}	0.2	
Man	N-Acetylphenylhydroxylamine	10^{-4}	10^{-7}		(191)
		10^{-3}	10^{-6}		
Ox	N-Acetylphenylhydroxylamine	2×10^{-4}	10^{-7}		(212)
		2×10^{-3}	1.4×10^{-6}		
Ox	Benzylphenylhydroxylamine or benzalphenylnitrone	2×10^{-4}	7.5×10^{-7}	0.72	(277)
		2×10^{-3}		0.90	

The red cells were suspended in isotonic saline solution containing glucose and incubated at 37°C.

This interpretation of activity does not consider the side reactions of the hydroxylamines which do not parallel the ferrihemoglobin-forming activity. They can affect the life of the hydroxylamine in the red cell differently, as is reflected by the maximal ferrihemoglobin concentration reached (table 1). The relationship between age and activity of the enzymic cycle of ferrihemoglobin formation

TABLE 2

Formation of ferrihemoglobin by arylhydroxylamines in vivo

Species	Hydroxylamine or Nitroso Analogue	Dose (mg/kg)	Maximal $\frac{Hb^{III}}{Hb^{III} + Hb^{II}}$	Reference
Cat	Phenylhydroxylamine	0.5 s.c.[a]	0.23	(220)
		1.0 s.c.	0.41	
		1.5 s.c.	0.47	
		2.0 s.c.	0.55	
Cat	Phenylhydroxylamine	2.5 i.p.[b]	0.78	(234)
	Nitrosobenzene	2.5 i.p.[b]	0.59	
Cat	Nitrosobenzene	5 s.c.[c]	0.5	(400)
Cat	Phenylhydroxylamine	0.5 s.c.	0.49	(454)
		1.0 s.c.	0.60	
		1.5 s.c.	0.68	
Cat	Phenylhydroxylamine	2.06 i.v.	0.58	(180)
Cat	Phenylhydroxylamine	2 i.v.	0.68	(212)
Dog	Phenylhydroxylamine or nitrosobenzene	0.1 i.v.	0.05	(297, 299)
		0.5 i.v.	0.15	
		1.5 i.v.	0.4	
Rabbit	Phenylhydroxylamine or nitrosobenzene	1.3 i.v.	0.12	(31)
		3 i.v.	0.33	
		5 i.v.	0.38	
		6 i.v.	0.47	
Rat	Phenylhydroxylamine	5 i.p.[b]	0.69	(234)
	Nitrosobenzene	5 i.p.[b]	0.46	
Rat	Phenylhydroxylamine	1 i.p.	0.1	(330)
		2 i.p.	0.3	
		5 i.p.	0.65	
		15 i.p.	0.65	
		35 i.p.	0.65	
Rat	Phenylhydroxylamine	5 i.p.	0.54	(112)
Cat	N-Acetylphenylhydroxylamine	10 i.v.	0.72	(212)
Dog	N-Acetylphenylhydroxylamine	40 i.v.	0.67	(212)
Dog	N-Benzoylphenylhydroxylamine[d]	2.5 i.v.	0.4	(278)
Dog	N-Benzylphenylhydroxylamine[e]	5.7 i.v.	0.39	(277)
Cat	*o*-Tolylhydroxylamine	1.13 s.c.	0.36	(454)
	m-Tolylhydroxylamine	1.13 s.c.	0.33	
	p-Tolylhydroxylamine	1.13 s.c.	0.39	
Dog	*p*-Chloronitrosobenzene	1.32 i.v.	0.15	(267)
Rabbit	*p*-Chlorophenylhydroxylamine	5 i.v.	0.44	(31)
Rat	*p*-Iodophenylhydroxylamine	10 i.p.	0.64	(112)

[a] The abbreviations used are: s.c. = subcutaneously, i.m. = intramuscularly, i.p. = intraperitoneally, i.v. = intravenously, p.o. = orally.

[b] Dissolved in oil.

[c] Dissolved in ether.

[d] Doses from 5 to 100 mg/kg have been studied.

[e] Doses from 1.9 to 10.45 mg/kg have been studied.

TABLE 2—*Continued*

Species	Hydroxylamine or Nitroso Analogue	Dose (mg/kg)	Maximal $\frac{Hb^{III}}{Hb^{III}+Hb^{II}}$	Reference
Dog	*p*-Hydroxylaminopropiophenone	0.5 i.v.	0.32	(152)
		1.0 i.v.	0.45	
Dog	*m*-Hydroxylaminopropiophenone	5 i.v.	0.43	(281)
Dog	*p*-Nitrosophenetol	5.65 i.v.	0.3	(20)
Dog	*p*-Hydroxylaminobenzenesulfonamide	10 i.v.	0.2	(201)
Cat	*m*-Nitrophenylhydroxylamine	1.41 s.c.	0.42	(181)
Cat	3-Nitro-4-methylphenylhydroxylamine	1.6 s.c.	0.37	(181)
Cat	3,5-Dinitro-4-methylphenylhydroxylamine	10 s.c.	0.33	(158)
		15 s.c.	0.5	
Dog	4-(2-Methoxy-ethoxy)-3-acetylphenylhydroxylamine	10 i.v.	0.06	(281)
Cat	2-Naphthylhydroxylamine	3 i.v.	0.21	(180)
Dog	2-Naphthylhydroxylamine	12 i.v.	0.5	(180)
Rat	N-hydroxy-4-acetyaminobiphenyl	61 i.p.	?	(367)

is illustrated by the higher rate of ferrihemoglobin formation in red cells taken from human umbilical cord blood than that in red cells from the blood of adults, when 10^{-5} M *p*-hydroxylaminobenzenesulfonamide is added to the cells (465).

3. Formation of ferrihemoglobin in vivo. Phenylhydroxylamine has been shown by Kiese (264) to be the metabolite which produces most of the ferrihemoglobin observed in the dog after the injection of aniline. If the increase in the concentration of phenylhydroxylamine and nitrosobenzene as observed after the injection of aniline is produced by slow intravenous infusion of phenylhydroxylamine, then the ferrihemoglobin concentration increases in the same manner as after the aniline injection. Therefore, other metabolites contribute little ferrihemoglobin. From the concentrations of N-hydroxy derivative determined in the blood and its ferrihemoglobin forming activity found *in vitro* and *in vivo* it may be concluded that the ferrihemoglobin formation caused by several aromatic amines is mainly due to the N-hydroxy derivative. As is explained above (Section II F) this, however, does not hold for any aromatic amine.

Table 2 contains data on the formation of ferrihemoglobin by various arylhydroxylamines in several laboratory animals. For a comparision of the activities, the maximal ferrihemoglobin concentration has been chosen. These data are strongly affected by the rate of hydroxylamine elimination from the blood. In some cases the ratios of activities as found in red cells *in vitro* (table 1) are substantially changed by these factors, *e.g.*, with hydroxylaminobenzenesulfona-

mide. Initial velocities of ferrihemoglobin formation may be calculated from data reported in the literature (20, 31, 153, 180, 201, 212, 277, 278, 281, 297).

In the cat, phenylhydroxylamine raises the ferrihemoglobin concentration equally high if injected subcutaneously or intravenously. Results of experiments with hydroxylamines injected into the blood and into tissues should nevertheless be compared with caution. Probably because of the intraperitoneal injection (234), the equal activity of phenylhydroxylamine and nitrosobenzene *in vivo* was not noticed. By intravenous injection, the high activity of nitrosobenzene, equalling that of phenylhydroxylamine, was demonstrated (297, 299). With these reservations in mind, one may conclude that the tolylhydroxylamines are about half as active as phenylhydroxylamine. Although a nitro group in the *meta*-position does not affect the activity of phenylhydroxylamine, an acyl residue in the same position diminishes it. The high activity of *p*-hydroxylaminopropiophenone is also observed *in vivo*.

I. Carcinogenic action of arylhydroxylamines

Carcinogenic aromatic amines, such as 4-aminobiphenyl (312), benzidine (1), 2-naphthylamine (123), and 2-aminofluorene (379) cause formation of ferrihemoglobin *in vivo*. The study of the mechanisms of ferrihemoglobin formation and carcinogenic action of aromatic amines has shown that in either case the same derivatives of the amines are the active agents. Firstly aminophenols were assumed to be the active derivatives in ferrihemoglobin formation, and the ortho-hydroxylation hypothesis of carcinogenic action (84–86) was a useful guide in the elucidation of carcinogenic action. Whereas the high ferrihemoglobin forming activity of arylhydroxylamines has been known for some time, it was only recently discovered that the N-hydroxy derivatives of carcinogenic aromatic amines are proximate carcinogens. Although carcinogenic action does not come within the scope of this review, table 3 lists the N-hydroxy derivatives of aromatic amines which have been found to be of higher carcinogenic activity than the parent amine, to differ from them by the capacity to produce tumors at the site of application, or both. N-Hydroxy-2-naphthylamine is carcinogenic on implantation as a pellet into mouse bladders or on intraperitioneal injection in rats, but it does not produce tumors on intraperitoneal injection in guinea pigs (54). However N-hydroxy-2-acetylaminofluorene causes local sarcomas on intraperitoneal injection in guinea pigs.

The N-oxides tested so far proved to be of low ferrihemoglobin-forming activity, but it may be mentioned that carcinogenic action has also been observed with 4-nitroquinoline-N-oxide (374, 428). Endo *et al.* (122) presumed that 4-nitroquinoline-N-oxide acts after the nitro group is reduced to the hydroxylamine. But the question of the essential structures is not yet settled. 4-Hydroxylaminonitroquinoline-N-oxide differs from the 4-nitro analogue in carcinogenic activity. It does not produce tumors in rats as a result of skin-painting and does not produce lung tumors, whereas 4-nitroquinoline-N-oxide produces lung tumors in rats after a single subcutaneous injection (430).

The relationship between ferrihemoglobin-forming and carcinogenic activity may point to some common feature in the primary process which starts the two

TABLE 3

N-Hydroxy derivatives of carcinogenic amines which are more active than the parent amine, produce tumors at the site of the application, or both

Species	Mode of Administration, Site of Tumors	Reference
	N-Hydroxy-2-acetylaminofluorene	
Rat	Forestomach, if fed; peritoneum, if injected	(357, 359, 364)
Mice Hamster Guinea pig	Local tumors in forestomach and intestine, if fed; and at the site of injection	(363)
Rabbit	Sarcomas after intraperitoneal injection	(510)
Rat	Fed with diet; liver carcinomas	(492)
Mouse	Bladder tumors following the implantation of cholesterol pellets containing N-hydroxy-2-acetylaminofluorene	(76)
	N-Hydroxy-2-benzoylaminofluorene	
Rat	45 mg/kg intraperitoneally 3 times weekly for 4 weeks or twice weekly for 6 weeks. All animals intraperitoneal sarcomas	(156)
	N-Hydroxy-2-aminofluorene	
Rat	Subcutaneous injection; a little lower incidence of mammary tumors than with the acetylated compound but more sarcomas at the site of the injection. The same was the case with 2-nitrosofluorene, N-methoxy- and N-acetoxy-2-acetylaminofluorene.	(357)
Mouse	Bladder cholesterol pellet; bladder carcinoma	(51)
Guinea pig	Intraperitoneal injection; sarcomas of peritoneum	(363)
	Metal chelates of N-hydroxy-2-acetylaminofluorene	
Rat	Cupric chelate — Sarcoma at the site of a single injection	(359, 362)
Rat	Cobaltous, Cupric, Ferric, Nickelous, Manganous, Zinc chelate — Sarcomas at the site of injection more frequently than after injecting N-hydroxyacetylaminofluorene	(406)
	N-Hydroxy-4-acetylaminobiphenyl	
Rat	Local tumors (forestomach), more ear duct and mammary tumors	(367)

TABLE 3—*Continued*

Species	Mode of Administration, Site of Tumors	Reference
	N-Hydroxy-2-naphthylamine	
Rat	Abdominal tumors after intraperitoneal injection	(52, 53)
Mouse	Bladder pellet; bladder carcinoma	(43)
Mouse	Bladder stearic acid pellet; bladder carcinoma	(51)
Mouse	Bladder cholesterol pellet; bladder carcinoma	(76)
	N-Hydroxy-2-acetylnaphthylamine	
Mouse	Bladder cholesterol pellet; bladder carcinoma	(76)
	N-Hydroxy-4-aminostilbene	
Rat	Sarcomas after intraperitoneal injection, more mammary tumors in female rats; carcinomas of ear duct, small intestine, liver, and papillomas of forestomach in male rats	(10)
	N-Hydroxy-4-acetylaminostilbene	
Rat	Fed with diet; ear duct carcinomas as frequent as with the parent amine; in addition tumors of the intestine	(25, 459a)
Rat	Local tumors forestomach, intestine or injection site; more ear duct and mammary tumors	(10, 10a)

actions. This could be true despite the fact that there is no close relationship between carcinogenic and ferrihemoglobin-forming activity of aromatic amines, as Neish (378, 379) has pointed out.

J. Formation of ferrihemoglobin by N-oxides

As a result of 3 studies, N-oxides of aromatic amines seemed possible proximate ferrihemoglobin-forming derivatives. Holzer and Kiese (200) observed that the formation of ferrihemoglobin in cats on the injection of N, N-dimethylaniline was caused not so much by phenylhydroxylamine as by another derivative. More recently observations made by Terayama (469, 470) suggested that the N-oxide of 4-dimethylaminoazobenzene can transform ferrohemoglobin to ferrihemoglobin *in vitro*. After Ziegler and Pettit (513) had demonstrated the biochemical formation of N,N-dimethylaniline-N-oxide from N,N-dimethylaniline, the role of N,N-dimethylaniline-N-oxide in the formation of ferrihemoglobin on the injection of N,N-dimethylaniline has been studied by Kiese *et al.* (283). They found N-oxide to be of little, if any, importance for the formation of ferrihemoglobin after the

injection of N,N-dimethylaniline. Although substantial amounts of the N-oxide appeared in the blood they did not produce ferrihemoglobin because of the low activity.

The reaction of hemoglobin and ferrihemoglobin with N,N-dimethylaniline-N-oxide *in vitro*, however, proved to be of interest with respect to the kinetics of ferrihemoglobin formation. The course of the ferrihemoglobin formation in red cells as well as in hemolysates is characterized by a lag phase and a rather sudden increase in reaction velocity. If the SH-groups of the hemoglobin are blocked with *p*-chloromercuribenzoate, N-ethylmaleimide, or iodoacetamide, the rate of decrease in N-oxide concentration as well as the rate of ferrihemoglobin formation is increased. The acceleration of ferrihemoglobin formation with time does not disappear. A reaction of the N-oxide with SH-groups does not seem to be an important factor in the kinetics of the reaction. Kiese (271) found that the ferrihemoglobin formation by N,N-dimethylaniline-N-oxide is an autocatalytic reaction. Partial oxidation of the hemoglobin before the N-oxide is added accelerates the formation of ferrihemoglobin and the addition of cyanide delays it. N,N-dimethylaniline-N-oxide does not oxidize ferrohemoglobin in the absence of oxygen; it reduces ferrihemoglobin in the absence of oxygen. No decrease in ferrihemoglobin concentration is observed if the N-oxide is added to ferrihemoglobin in the presence of oxygen. But the N-oxide disappears more rapidly than in the absence of oxygen. The formation of ferrihemoglobin by N,N-dimethylaniline-N-oxide is a "coupled oxidation" similar to the formation of ferrihemoglobin by phenylhydroxylamine. Reaction products of N,N-dimethylaniline-N-oxide are N-methylaniline, formaldehyde, and N,N-dimethylaniline (292).

K. Appendix

Kallner (241, 242) and Svartz and Kallner (448) assumed that aromatic amines, particularly sulfanilamides, can cause cyanosis by the formation of dark colored compounds between hemoglobin and the amines. The hypothesis was not confirmed by Gaede and Kiese (139) and Heubner and Kiese (187). In cases of sulfanilamide cyanosis, the authors observed ferrihemoglobin or verdoglobin in such concentrations in the blood of patients as fully explain the cyanosis.

The therapeutic use of ferrihemoglobin formation in cyanide poisoning has recently been discussed by Kiese and Weger (305).

III. THE BIOCHEMICAL PRODUCTION OF FERRIHEMOGLOBIN-FORMING DERIVATIVES FROM AROMATIC AMINES

Among the biochemical reactions that may be involved in the production of ferrihemoglobin-forming substances, only the proximate reactions are to be discussed here. More remote reactions like the hydrolysis of N-acylderivatives of aromatic amines or the splitting of azobenzenes will be dealt with only in some special cases.

A. Aminophenols

In vivo. The hydroxylation of aniline to aminophenols *in vivo* has been little investigated. By studying the metabolites of aniline found in the urine, it was

TABLE 4

Metabolic o- and p-hydroxylation of aniline by various animal species

Species	Sex	Oral Dose (mg/kg)	Ratio[a] *para/ortho*
Gerbil	F	250	15 (12–17)[3]
Guinea pig	F	250	11 (9–12)[3]
Golden hamster	F	250	10 (9–11)[3]
Rabbit[b]	F	160–500	6 (4–9)[10]
Rat	M	250	6 (5–6)[3]
	F	250	2.5 (2–3)[3]
Chicken	F	50	4 (4)[3]
Mouse	M	250	3 (2–4)[3]
Ferret	F	250	1 (1)[3]
Dog	F	200	0.5
Cat	F	200	0.4

Data reported in Parke (395).

[a] Figures are mean values; range is given in parenthesis and the number of experiments as indices.

[b] Piotrowski (404) using much smaller doses of aniline, 0.1 mg/kg intravenously, found the ratio of *p*- to *o*-aminophenol in rabbit's urine to be 12.

discovered that aniline is hydroxylated in the *ortho*, *meta*, and *para* positions by the rabbit and the dog (395, 440). The amounts of *m*-aminophenol found in the urine were always small, and the ratios of *o*- to *p*-aminophenol in the urine were observed to vary widely with various species (table 4). *p*-Aminophenol may also originate from acetanilide (68, 154, 438), *p*-phenetidine (441), or phenacetin (439).

A hint at the urinary excretion of *p*-dimethylaminophenol by rats after intraperitoneal injection of N,N-dimethylaniline was found by Elson *et al.* (121). The isolation of *p*-diethylaminophenol from the urine of dogs and rabbits after injection of N,N-diethylaniline has been described by Horn (209). Klutch *et al.* (314) found 2-hydroxy-4-ethoxyacetanilide in the urine of dog, cat and man after administration of acetophenetidine. They isolated the compound from dog's urine that had been incubated with β-glucuronidase. 2-Hydroxy-4-ethoxyaniline as the O-sulfate was found by Büch *et al.* (78) in the urine of people and rats dosed with acetophenetidine. The metabolite had previously been observed in the urine of rabbits fed with phenetidine (441).

Virtually nothing is known of the concentrations of free aminophenols in the blood of animals into which aniline or its derivatives had been injected. Lester and Greenberg (329) did not find free *p*-aminophenol in the blood of two men who had taken 1 g of acetanilid and in whom the ferrihemoglobin concentration had increased. The concentration must have been below 0.1 μg/ml, which is much less than what is needed for the formation of ferrihemoglobin (154). The minor part of *p*-aminophenol in the formation of ferrihemoglobin after absorption of aniline was also shown by Lester *et al.* (330). In their experiments with rats, even lethal doses of *p*-aminophenol did not increase the ferrihemoglobin concentration as high as was observed with aniline. Williams (505) observed a slow formation

TABLE 5

Comparison of the relative amounts of 1-, 3-, 5-, 7-, and 8-hydroxylated metabolites of 2-acetylaminofluorene, excreted as glucuronides by rats and guinea pigs

Metabolite	Rat	Guinea Pig
1-OH	5.9	0.09
3-OH	4.5	0.18
5-OH	42	2.1
7-OH	32	95
8-OH	3.1	1.9

The figures indicate per cent of the ether-extractable metabolites after incubation of the urine with β-glucuronidase.

Data reported in Weisburger *et al.* (498).

of ferrihemoglobin by *o*- and *p*-aminophenol glucosiduronic acids. There are no kinetic data which would make it possible to assess the role of this reaction *in vivo*.

Sulfanilamide and its derivatives are also hydroxylated to phenols. Williams (506) isolated 3-hydroxysulfanilamide (1-amino-2-hydroxybenzene-4-sulfonamide) from the urine of rabbits, where it appeared as the ethereal sulfate. Its presence in the urine of people treated with sulfanilamide was detected by means of a color reaction (507). Although the compound is an *o*-aminophenol, it produces ferrihemoglobin slowly *in vitro* only in concentrations of no interest to biology (472, 507). 2-Hydroxysulfanilamide forms no ferrihemoglobin even in high concentration (472).

Several hydroxy derivatives from other aromatic amines, in particular carcinogenic ones, are excreted in the urine. Most of them have not been tested for their ferrihemoglobin-forming activity. Heringlake *et al.* (180) found both 1-hydroxy- and 6-hydroxy-2-aminonaphthalene to have little activity in cats, the 1-hydroxy compound reacting more rapidly than the isomer. These aminonaphthols have been isolated from the urine of dogs, rats, rabbits, and monkeys fed with 2-naphthylamine (118, 343, 504). The excretion of 1-hydroxy-2-aminonaphthalene differs in various species (44). The manifold hydroxylation of 2-acetylaminofluorene, investigated by Weisburger *et al.* (493, 495–498), may be mentioned as an illustration of the species differences (table 5). Weisburger and Weisburger (494) studied factors affecting the metabolism of 2-acetylaminofluorene in rats. In the rabbit, Irving (214) found the excretion of the 1-hydroxy derivative to be even less than 0.1 % of the dose.

Brodie and Udenfriend (70) found a ferrihemoglobin-forming metabolite of pamaquine in the urine of man and dog and presumed it to be 5-hydroxypamaquine. 5-Hydroxylation of pamaquine was observed by Josephson *et al.* (232, 233). They isolated the 5,6-quinone of pamaquine from the droppings of chicken treated with this drug and found it to produce ferrihemoglobin.

In vitro. The enzymatic hydroxylation of aromatic amines *in vitro* was first observed by Booth *et al.* (48). They found rat liver slices to oxidize 2-naphthylamine to its 1-hydroxy and 6-hydroxy derivatives. Then Brodie *et al.* (69) dis-

TABLE 6

Ring-hydroxylation of aromatic amines observed in experiments with microsomes prepared from the livers of various species

Species	Positions at Which Hydroxylation Was Observed	Reference
	Aniline	
Rabbit	only 4	(370)
Rat	2 and 4	(45)
Cat	2 and 4 equal	(408)
Dog	2 and 4 equal	(408)
	Acetanilide	
Rabbit	2, 3, and 4	(370)
Rat	only 4	(45)
Cat	2 and 4 equal	(408)
Dog	2 and 4 equal	(408)
	2-Naphthylamine	
Rat	1 and 6	(45)
	2-Acetylaminonaphthalene	
Rat	only 6	(45)
	2-Aminofluorene	
Rat	7	(45)
	2-Acetylaminofluorene	
Rat	7	(45)
	1, 3, 5, 7	(104)
	7	(427)
Hamster	1, 3, 5, 7	(427)

covered that various metabolic changes of drugs known from studies *in vivo* are performed by isolated microsomes in the presence of oxygen and NADPH. Rabbit liver microsomes hydroxylate aniline and acetanilide in the *para*position; only small amounts of the *o*- and *m*-isomers were observed with acetanilide as substrate and none with aniline (370). With rat liver microsomes *o*-aminophenol and *p*-aminophenol appear when aniline is substrate, but only the *p*-hydroxy derivative with acetanilide; only the 6-hydroxy derivative is produced from 2-acetylaminonaphthalene, whereas 2-naphthylamine is hydroxylated in the 1-position too (45). In line with Parke's (395) results on urinary excretion of hydroxylation products Posner (408) found aniline to be evenly hydroxylated in 2- and 4-position by microsomes prepared from the livers of cats or dogs. Species differences in the microsomal hydroxylation of aromatic amines may be seen in table 6. The manifold hydroxylation of 2-acetylaminofluorene is of particular interest. Whereas Peters and Gutmann (399) observed only the hydroxylation in 7-position in fortified rat liver homogenates, Cramer *et al.* (104, 105) demonstrated the hydroxylation in 1, 3, 5 and 9 positions by rat liver microsomes, *i.e.*, in all positions observed by Weisburger *et al.* (496) *in vivo* except the 8-position. Only the 7-hydroxy derivative was detected in the chromatograms of the derivatives produced by rat liver microsomes from 2-acetylaminofluorene (45, 427). The appearance of unidentified aminofluorenols after the incubation of 7-fluoro-2-acetyl-

aminofluorene points to a hydroxylation at other positions than 7. No fluorenols were found if 7-fluoro-2-acetylaminofluorene was incubated with guinea pig liver microsomes.

The hydroxylation of aromatic amines in various positions is probably not due to the action of a single enzyme. Bauer and Kiese (29) observed differences between the *o*- and *p*-hydroxylation activity of rabbit liver microsomes in stability and susceptibility to inhibitors. The existence of different enzymes for *o*- and *p*-hydroxylation has also been shown (107, 109); in induction experiments with rats, the injection of 3,4-benzopyrene stimulated only the microsomal 2-hydroxylation of biphenyl, not its 4-hydroxylation, whereas the injection of phenobarbital stimulated both hydroxylations.

Little is known of factors which affect the ring-hydroxylation of aniline derivatives *in vivo*. In isolated microsomes prepared from the livers of guinea pigs deficient in ascorbic acid there was only one tenth of the activity observed in microsomes from normal animals with acetanilide as a substrate (117).

B. Arylhydroxylamines

In vivo. The biological N-hydroxylation of aromatic amines was suspected for several reasons. Except for the diamines and aminophenols, aromatic amines and nitro compounds do not form ferrihemoglobin *in vitro* but do so *in vivo*. A common derivative was assumed to be produced *in vivo* and to cause the ferrihemoglobin formation (333). The reduction of nitrobenzenes to the hydroxylamines by tissues *in vitro* was first observed by Lipschitz (332). 2,6-Dinitro-4-hydroxylaminotoluene occurs in the urine of rats given trinitrotoluene (82), and *m*-nitrophenylhydroxylamine in the urine of rabbits after the oral administration of C^{14}-labelled *m*-dinitrobenzene (396). Phenylhydroxylamine in small doses produces large amounts of ferrihemoglobin *in vivo* (Section II H 3).

Several attempts at demonstrating the N-hydroxylation of aromatic amines *in vivo* were unsuccessful. Whereas all other metabolites of aromatic amines were discovered in the urine, the products of N-hydroxylation were first observed in the blood (263, 265). Because of the quick reaction of phenylhydroxylamine with hemoglobin and oxygen (Section II H 1), a large part of the hydroxylation product is present in the blood as nitrosobenzene. In the procedure which led to the discovery of the N-hydroxylation, any hydroxylation product still present as hydroxylamine was also oxidized to nitrosobenzene (263, 265). This was separated from the amines by its low solubility in acidified water and identified by its characteristic ultraviolet spectrum in organic solvents. On diazotizing it and coupling the product with α-naphthylethylenediamine, it was determined even in low concentration in the blood (182). N-Alkylanilines were found to yield higher concentrations of phenylhydroxylamine and nitrosobenzene in the blood of dogs and cats than aniline (200, 265). The nitroso analogues of the aromatic amines listed in table 7 have been found in the blood of animals after the injection of the amine. Only such nitroso compounds are mentioned in the table as have been identified by their ultraviolet spectrum in organic solvents. After *p*-chloroaniline was in-

TABLE 7

N-Hydroxylation products of aromatic amines observed in the blood. They were identified and determined as the nitroso analogues

Aromatic Amine	Dose (mg/kg)	Species	Nitroso Analogue in the Blood (μg/ml)	Reference
Aniline	288	Dog	2.3	(263)
N-Methylaniline	11	Dog	2.2	(265)
N-Methylaniline	22	Cat	1.4	
N-Ethylaniline	25	Cat	2.2	(200)
N-Butylaniline	31	Cat	0.9	
2-Naphthylamine	500	Dog	0.5	(180)
	500	Cat	0.4	
p-Phenetidine	250	Dog	0.8	(20)
m-Toluidine	83	Dog	3.5	
p-Toluidine	83	Dog	1.3	
m-Chloroaniline	25	Dog	2.5	
p-Chloroaniline	25	Dog	4.5	(267)
	100	Dog	17	
N-Methyl-*p*-chloroaniline	28	Dog	3.2	
N-(β-Hydroxyethyl)-aniline	29	Cat	0.4	
p-Aminopropiophenone	12	Dog	0.4	(152)
	40	Dog	3.3	
	30	Rabbit	6.3	(228)
p-Chloroaniline	78	Rabbit	1.9	
p-Ethylaniline	50	Rabbit	0.3	
	50	Dog	6.3	
	75	Dog	10.5	(228)
2-Aminofluorene	100	Rabbit	1.7	
	100	Dog	1.1	
4-Aminobiphenyl	80	Dog	5.1	
	100	Rabbit	4.5	

Except for 2-naphthylamine, which was injected intraperitoneally, the amines were injected intravenously. The doses refer to the free bases.

jected, *p*-chlorophenylhydroxylamine and *p*-chloronitrosobenzene accumulated to high concentrations. They were isolated as *p*-chloronitrosobenzene to prove unequivocally the presence of N-hydroxylation products in the blood (287).

The biological N-hydroxylation of aromatic amines has also been proved by the demonstration of N-hydroxy derivatives of the amines in the urine of animals which had received the amine. Cramer *et al.* (106) isolated N-hydroxy-2-acetylaminofluorene from the urine of rats fed on a diet containing 2-acetylaminofluorene. The N-hydroxy derivative was excreted in conjugated form; before its isolation, the urine had been treated with β-glucuronidase and Taka-diastase. As a consequence of the daily administration of 2-acetylaminofluorene the amount of the N-hydroxy derivative in time exceeded 10 % of the daily ingested dose of the amine. Irving (214) found the rabbit to excrete as the N-hydroxy derivative as much as 30 % of the 2-acetylaminofluorene administered by mouth.

The observations of urinary excretion of N-hydroxylation products of aromatic amines are summarized in table 8.

The excretion of large amounts of N-hydroxylation products of some aromatic amines is the more remarkable as no N-hydroxylation product was observed in the urine of dogs after the injection of aniline (229). In studies of the metabolism of aniline in the rabbit, no evidence was found to support the view that aniline is converted to phenylhydroxylamine *in vivo* (395, 440).[3] Poirier *et al.* (405), using chromatographic analysis, did not detect N-hydroxylation products in the urine of dogs fed acetanilide (500 mg), *p*-vinylacetanilide (225 mg), *p*-fluoroacetanilide (150 mg), *p*-ethoxyacetanilide (500 mg), trans 4-acetylaminostilbene (100 mg), 2-propionylaminofluorene (300 mg), or 2-n-butyrylaminofluorene (200 mg). Von Jagow *et al.* (228) did not find phenylhydroxylamine in the urine and bile of rabbits given aniline by injection; but about 30 % of *p*-aminopropiophenone was excreted by rabbits as the N-hydroxylation product, partly conjugated, in the urine. The bile also contained conjugated hydroxylaminopropiophenone. *Para*substitution of the aniline seems to favor the excretion of the N-hydroxylation product in the urine, as may be illustrated by the data presented in table 8.

After the administration of 2-acetylaminofluorene, its N-hydroxy derivative appears in the urine not as such but as a compound which is split by β-glucuronidase. Irving (218) has shown that the conjugate excreted by the rabbit is the O-glucosiduronic acid of N-hydroxy-2-acetylaminofluorene. The N-hydroxy derivatives appearing in the urine of rabbits and guinea pigs after the injection of aromatic amines like *p*-aminopropiophenone or 2-aminofluorene were also excreted as conjugates. Since these conjugates are split on incubating the urine adjusted to a pH between 4 and 5 for 2 hr without adding an enzyme (228), they cannot be O-glucuronides but are probably N-glucuronides, which are known to decay in slightly acid solution.

Whereas the N-hydroxy derivative of 2-acetylaminofluorene is excreted in the urine of several species (table 8), it was not detected in the urine of guinea pigs fed on a diet containing 2-acetylaminofluorene or given the amine by injection (358, 363). However, the urine of such animals was found to contain N-hydroxy-2-aminofluorene (307). The amount was much smaller than that found after the injection of 2-aminofluorene. It cannot be determined whether the N-hydroxylation had occurred before or after deacetylation. 2-Acetylaminofluorene as well as its N-hydroxylation product is hydrolyzed by liver enzymes (219, 288). More N-hydroxylation product is found in the urine of guinea pigs if 2-aminofluorene is given in place of its N-acetyl derivative. The guinea pig, which is refractory to the carcinogenic action of 2-aminofluorene and 2-acetylaminofluorene (14, 64, 363, 480, 493), is thus capable of N-hydroxylating 2-aminofluorene. N-Hydroxy-2-acetylaminofluorene is excreted by rats (358) and also by rabbits (228) after the administration of 2-aminofluorene. The dog, however, excretes N-hydroxy-2-acetylaminofluorene only if 2-acetylaminofluorene has been given (405).

Using 2-(acetyl-1′-C^{14})aminofluorene, Miller *et al.* (358) in experiments with

[3] The urine of rabbits given nitrobenzene orally had previously been tested for phenylhydroxylamine by Parke (394), but he did not find any.

TABLE 8

N-Hydroxylation products found in the urine after the administration of various aromatic amines

Species	Dose and Application	Method of Identification and Determination	Fraction of Dose Excreted as N-Hydroxy Derivative	Reference
		2-Acetylaminofluorene		
Rat	0.03% in diet	Column partition chromatog.	14% after 6 weeks feeding	(106)
Rat	0.03% in diet	Paper chromatography	Traces in the first days; 15% after 18 weeks feeding	(358)
Rat	10 mg/kg intraperitoneally	Paper chromatog. spectrum	0.5%	(344)
Rat	5 to 22 mg	Paper chromatography	5–17%	(120)
Mouse	0.03% in diet	Paper chromatography; spectrophotometry	2.3% after 3 weeks	(358)
Mouse	0.05% in a grain diet	Column partition chromatog.	1%	(363)
Hamster	10–30 mg/kg intraperitoneally	Paper chromatography	5–15%	(363)
Hamster	Intraperitoneal injection of radioactive substance	Paper chromatography of the radioactive material	15–20%	(490)
Guinea pig	200 mg/kg intraperitoneally	Free N - hydroxy - 2 - aminofluorene. Oxidation to nitroso analogue, spectrum	0.03%	(307)
Rabbit	70 mg/day given orally	Cupric chelate	Increasing from 13% on the first to 30% on the third day	(214, 216)
Cat	Intraperitoneal injection of radioactive substance	Paper chromatography of the radioactive material	1.5%	(489)
Dog	300 mg given orally with horse meat	Cupric chelate; paper chromatography	5%	(405)
Dog	Fed 10–25 mg/kg/day		6%	(365)
Dog	55 mg/kg by stomach tube	Paper chromatography		(120)
Steppe lemming	?		Trace	(488)
Rhesus monkey	30 mg/kg/day fed with diet	Precipitation as cupric chelate	1.8%	(124)
Rhesus monkey	6–60 mg	Paper chromatography	"Moderate amounts"	(119)
Rhesus monkey	Tracer dose of carbon-14-labelled substance orally or intraperitoneally	Paper chromatography, autoradiography	up to 6%	(119a)
Rhesus monkey	Fed		2%	(365)
Man	Tracer dose of carbon-14-labelled substance by mouth	Chromatography isotope dilution	4–14%	(488)

2-Aminofluorene				
Rat	0.03% in diet	Paper chromatography	N - Hydroxy - 2 - acetyla - minofluorene. Similar to experiments with 2-acetyl-aminofluorene	(358)
Guinea pig	100 mg/kg intraperitoneally	Oxidation to nitroso analogue; spectrum; thin-layer chromatography	0.5%	(289)
Rabbit	100 mg/kg intraperitoneally	Oxidation to nitroso analogue; spectrum; thin-layer chromatography	2%	(228)
Dog	Same	Same	0.2%	
2-Naphthylamine				
Dog		Extraction as the ferrous complex of the N-nitroso derivative		(478)
Dog		Paper and cellulose column chromatography		(57, 59)
Rhesus monkey	30 mg/kg/day in banana or apple	Paper chromatography	Trace of N-hydroxy-2-acetylaminonaphthalene?	(124)
Man		Extraction as the ferrous complex of the N-nitroso derivative		(478)
Man	600 mg	Cupric chelate	N - hydroxy - 2 - acetyl - aminonaphthalene	(477)
2-Acetylaminonaphthalene				
Dog	500 mg	Paper chromatography	0.1%	(405)
Rhesus monkey	30 mg/kg/day in banana or apple	Paper chromatography	Trace?	(124)

TABLE 8—Continued

Species	Dose and Application	Method of Identification and Determination	Fraction of Dose Excreted as N-Hydroxy Derivative	Reference
		4-Aminobiphenyl		
Rhesus monkey	30 mg/kg/day fed with diet	Paper chromatography	About 0.6% as N-acetyl derivative	(124)
Guinea pig	100 mg/kg intraperitoneally	Oxidation to nitroso analogue, spectrum	4.7%	(228)
Rabbit	100 mg/kg intraperitoneally	Oxidation to nitroso analogue; spectrum	19%	(228)
Dog	80 mg/kg intravenously	Same	1%	
		4-Acetylaminobiphenyl		
Rat	0.04–0.08% in diet	Column partition chromatography; paper chromatography	3.9% in 2 weeks	(367)
	Single intraperitoneal injection of 40 mg/kg		0.2–0.5%	
Dog	20 mg/kg/day orally	Paper chromatography	7–20%	(367)
		Benzidine		
Man	200 mg	Cupric chelate		(477)
		4-Aminostilbene		
Rat	5 mg/rat/day intraperitoneally	Isolation as cupric chelate of N-hydroxy-4-acetylaminostilbene	0.5%	(10a)
		4-Acetylaminostilbene		
Rat		?		(25)
Rat	40 mg per kg of diet	Chromatography, color reactions	2%	(24a, 459a)
Rat	5 mg/rat/day intraperitoneally	Paper chromatography, spectrophotometry	0.4–0.7%	(10a)

		p-Aminopropiophenone		
Rabbit	1–40 mg/kg intravenously	Oxidation to nitroso analogue; spectrum	31%	(228)
Guinea pig	1–100 mg/kg intraperitoneally	Same	15%	
		m-Aminopropiophenone		
Rabbit	6 mg/kg intravenously	Oxidation to nitroso analogue, spectrum	4%	(228)
Guinea pig	25 mg/kg intraperitoneally	Same	3.2%	(228)
		p-Chloroaniline		
Rabbit	78 mg/kg intravenously	Oxidation to nitroso analogue; spectrum	0.4%	(228)
Dog	39 mg/kg intravenously	Same	0.04%	
		p-Ethylaniline		
Rabbit	30 mg/kg intravenously	Oxidation to nitroso analogue; spectrum	0.7%	(228)
Dog	50 mg/kg intravenously	Same	0.03%	
		4-Aminoazobenzene		
Rat	?	N-hydroxy-N-acetylaminoazobenzene	0.05–0.3%	(366)

rats and Poirier *et al.* (405) in experiments with dogs have demonstrated that 2-acetylaminofluorene retains its acetyl group during enzymic hydroxylation.

Some factors which affect the production and excretion of N-hydroxy derivatives of aromatic amines have been investigated. Cramer *et al.* (106, 358) observed an increase in the urinary excretion of N-hydroxy-2-aminofluorene by rats during the daily feeding of a diet containing 2-acetylaminofluorene. On addition of 3-methylcholanthrene to the diet in low concentration, the increase in the excretion of the N-hydroxy derivative was largely prevented. The fraction of a single test dose of 2-acetylaminofluorene which is excreted by rats as N-hydroxy derivative is increased not only by previous treatment with 2-acetylaminofluorene but also by feeding a diet containing 3-methyl-4-dimethylaminoazobenzene or ethionine (344). The same authors also observed a 7-fold increase in the excretion of N-hydroxy-2-acetylaminofluorene 1 and 2 days after partial hepatectomy of rats. Adrenalectomy of weanling male rats decreases the urinary excretion of N-hydroxy-2-acetylaminofluorene after the injection of 2-acetylaminofluorene by about 50 %. Injection of cortisone or deoxycorticosterone acetate restores the excretion of the N-hydroxy derivative to normal levels. When adrenals and pituitaries are removed, the excretion of N-hydroxy-2-acetylaminofluorene drops to about 30 % of the control level. Administration of cortisone restores the urinary excretion of the N-hydroxy derivative to 75 % of the control level. Growth hormone does not affect the excretion of N-hydroxy-2-acetylaminofluorene by adrenalectomized-hypophysectomized rats (337).

Unlike the case with 2-acetylaminofluorene, continuous feeding of 4-acetylaminobiphenyl to rats leads to a gradual decrease in urinary excretion of the N-hydroxy derivative (367). The fraction of *p*-aminopropiophenone excreted as N-hydroxy derivative by guinea pigs was the same over a wide range of doses (1 to 100 mg/kg) (228). N-Hydroxy-2-naphthylamine occurs in the urine of dogs but not of other animals dosed with 2-naphthylamine (55). It is excreted as a labile derivative, possibly as N-glucosiduronic acid. Besides the N-hydroxy derivative, 2-nitrosonaphthaline was detected in the urine of dogs. 1-Naphthylamine is also excreted by the dog partly as the N-hydroxy derivative.

In vitro. The elucidation of the biochemical N-hydroxylation of aniline was complicated by the failure to detect the N-hydroxy derivative or the nitroso analogue in tissue slices or homogenates incubated with aniline, although a sensitive method for determining the oxidation products was used (182). The rapid disappearance of added phenylhydroxylamine from liver homogenates, was probably the cause of this failure (199). Nor was N-hydroxy-2-acetylaminofluorene, which is copiously excreted by rats fed a diet containing 2-acetylaminofluorene, found in rat liver homogenate incubated with the amine (105, 358). On addition of red blood cells to slices or homogenates of rat liver incubated with N-methylaniline, the N-hydroxylation product was trapped and its content increased to measurable proportions (300).

The site of the N-hydroxylation of aromatic amines in liver cells was discovered by Kiese and Uehleke (300, 301) to be in the microsomes. For good yields of the hydroxylation product the microsomes had to be thoroughly washed in

order to remove traces of the soluble fraction of liver cells. The soluble fraction contains enzymes which in the presence of NADPH readily reduce nitrosobenzene and phenylhydroxylamine (302). Oxygen and reduced NADP are necessary for the microsomal N-hydroxylation. NADH was much less active than NADPH (300). The N-hydroxylation product was first identified, after being oxidized by means of ferricyanide, as nitrosobenzene by its ultraviolet absorption, distribution between aqueous acid and carbon tetrachloride, and formation of diazobenzene with nitrous aicd Later phenylhydroxylamine produced by rabbit liver microsomes from N-ethylaniline was isolated as the iron complex of its N-nitroso derivative (280).

Microsomes prepared from rabbit's lungs and kidneys also N-hydroxylate aniline and N-ethylaniline (244). In addition to the livers of rats (300), rabbits, and dogs (244) those of hamsters, cats, chickens (217), and guinea pigs (13) N-hydroxylate aromatic amines. The microsomes prepared from the livers of rabbits and guinea pigs hydroxylate aniline and some of its derivatives more rapidly than the microsomes from the livers of rats, cats, and dogs. A finer grading is not possible because the ratios of the activities vary with the substrate and the particular hydroxylation studied. The hydroxylating enzymes of various species also vary in such properties as sensitivity to inhibitors and affinity for oxygen and carbon monoxide (13). In addition to aniline, several of its derivatives are N-hydroxylated by liver microsomes. The compounds and the methods of detecting their N-hydroxylation product are listed in table 9. Many substitutions of the aniline increase the rate of its N-hydroxylation, *e.g.*, N-alkylation (300, 301), or *p*-substitution with chlorine, ethoxy, propionyl or phenyl residues (228, 245, 250, 289, 300). 7-Fluoro-2-acetylaminofluorene is N-hydroxylated more rapidly than 2-acetylaminofluorene (217). The enhancing effect of some substituents does not seem to be limited to the *para* position. *m*-Aminopropiophenone is N-hydroxylated more rapidly than aniline (281). However, *p*-aminopropiophenone reacts even faster (245).

C. N-Oxides

In vivo. Although the N-oxides so far studied do not play an important role as ferrihemoglobin-forming metabolites of the parent N,N-dialkylanilines, the biochemical production of N-oxides will be discussed briefly. Weak though the action of N,N-dimethylaniline-N-oxide may be, it is capable of producing ferrihemoglobin. There may be other N-oxides which play a more important role as proximate metabolites in ferrihemoglobin formation.

The biochemical production of N-oxides from tertiary amines was discovered by Lintzel (331) in experiments with trimethylamine in man. It was found to occur also in dogs, rabbits, guinea pigs, and rats (202, 372, 380). Chaykin and Bloch (83) discovered the formation of another N-oxide, nicotinamide-N-oxide, in rats. N-oxide formation *in vivo* was later shown with other tertiary amines, of which only chlorpromazine-N-oxide (133) and imipramine-N-oxide (132) need to be mentioned. The production of aniline-N-oxides was observed by Ziegler and Pettit (513) with liver microsomes *in vitro*. Kiese *et al.* (283) found that the N-

TABLE 9

Aniline derivatives which have been observed to be N-hydroxylated by NADPH-dependent enzymes in liver microsomes

Compound	Species	Method of Identifying the N-Hydroxylation Product	Reference
N-Methylaniline	Rat	Oxidation to nitroso analogue, spectrum	(301)
p-Chloroaniline	Rat	Oxidation to nitroso analogue, spectrum	(300)
p-Phenetidine	Rat	Oxidation to nitroso analogue, spectrum	(300)
2-Acetylaminofluorene	Rabbit	Paper chromatography spectrum	(215)
N-Ethylaniline	Rabbit	Isolation of phenylhydroxylamine as iron complex of the N-nitroso derivative	(280)
4-Aminobiphenyl	Rabbit	Thin-lay erchromatography, various color reagents	(47)
4-Acetylaminobiphenyl	Rabbit	Thin-layer chromatography, various color reagents	(47)
4-Acetylamino-4′-aminobiphenyl	Rabbit	Thin-layer chromatography, various color reagents	(47)
N-acetylbenzidine	Rabbit	Thin-layer chromatography, various color reagents	(47)
p-Aminopropiophenone	Rabbit	Oxidation to nitroso analogue, spectrum	(245)
7-Fluoro-2-acetylaminofluorene	Rabbit	Thin-layer chromatography	(217)
2-Acetylaminofluorene	Hamster Dog Cat Chicken	Thin-layer chromatography Thin-layer chromatography Thin-layer chromatography Thin-layer chromatography	(217)
m-Aminopropiophenone	Dog	Oxidation to the nitroso analogue, spectrum	(281)
4-(2-Methoxy-ethoxy)-3-acetylaniline	Dog Rabbit	Oxidation to the nitroso analogue, spectrum	(281)
2-Aminofluorene	Guinea pig	Oxidation to the nitroso analogue, spectrum	(289)
4-Acetylaminostilbene	Rat	Paper chromatography, electrophoresis	(24a)
m-Aminopropiophenone	Rabbit	Oxidation to nitroso analogue, spectrum	(228)
Sulfanilamide	Rat	Thin-layer chromatography	(471)

oxides of N,N-dimethylaniline and N,N-diethylaniline in the blood of dogs given the N,N-dialkylanilines by injection play no role in the ferrihemoglobin formation after the injection of N,N-dialkylanilines.

In vitro. The biochemical formation of N-oxides was found by Szara and Axelrod (449) to be located in the microsomes. They chromatographically separated and identified N,N-dimethyltryptamine-N-oxide in suspensions of rabbit liver microsomes incubated with the amine. Fish *et al.* (128) had previously observed

the same reaction to occur in mouse liver homogenates from which the nuclei and mitochondira had been separated. The biosynthesis of nicotinamide-N-oxide in slices of mouse liver is also located in the microsomes (311).

Baker and Chaykin (22, 23) studied the N-oxide formation from trimethylamine by hog liver microsomes in the presence of oxygen and NADPH. Using O_2^{18} and O^{18}-labelled water they found that the oxygen in the N-oxide stems from molecular oxygen. The microsomal synthesis of trimethylamine-N-oxide has a pH-optimum between pH 7.5 and 8.0; it is little inhibited by chloromercuribenzoate 5×10^{-5} M. Baker *et al.* (24) observed the formation of trimethylamine-N-oxide with the homogenates prepared from the livers of a wide variety of species.

Microsomal N-oxide formation from N,N-dimethylaniline has been described (513). A flavoprotein appears to be involved in the reaction. The activity of hog liver microsomes decreases if they are first suspended in a solution which effects dissociation of flavoproteins and then passed through a Sephadex column to remove flavins (401). The activity is restored by adding flavin adenine dinucleotide. A method for concentrating the enzyme from hog liver has been described (515).

The relationship between N-oxide formation and other microsomal hydroxylations is still unknown. Experiments with various inhibitors showed an inhibition pattern different from that determined for the N-hydroxylation or the N-dealkylation of N-alkylanilines (194).

IV. DISPOSAL OF FERRIHEMOGLOBIN-FORMING DERIVATIVES OF AROMATIC AMINES

A. Arylhydroxylamines

In vivo the intensity and the full extent of action of a ferrihemoglobin-forming agent is determined not only by the rate of its enzymic production from the parent aromatic amine and its intrinsic activity but also by its metabolic change to inactive or less active derivatives. The metabolism of aminophenols has been reviewed by R. T. Williams (508). More recent studies in this field are concerned mainly with aminophenols which have not been tested for ferrihemoglobin-forming activity. To our present knowledge, ring hydroxylation of aromatic amines cannot be reversed *in vivo* as is the case with N-hydroxylation. Very often the ring hydroxylation prevents the formation of a more active compound, *i.e.*, the N-hydroxy derivative. Together with a rapid conjugation it favors the elimination of the amines. The metabolism of the ferrihemoglobin-forming derivatives of aromatic diamines, the quinone diimines, has not been studied. Therefore only the metabolism of arylhydroxylamines and aromatic N-oxides are to be dealt with here.

In the blood a large part of the N-hydroxylation product is carried as the nitroso analogue. It originates from the reaction of the hydroxylamine with oxyhemoglobin and is partly bound to hemoglobin (Section II H 1).

The reduction of arylhydroxylamines to amines *in vivo* was discovered by Meyer (356). He found *p*-aminophenol in the urine of rabbits fed phenylhydroxylamine. Sieburg (432) observed the same biochemical change in the dog. Williams' (506) isolation of acetylsulfanilamide from the urine of rabbits given *p*-

hydroxylaminobenzenesulfonamide subcutaneously showed that substituted phenylhydroxylamines are also converted into the amines. The reduction of arylhydroxylamines to the amine *in vivo* was also observed by Kiese (20, 264). During the intravenous infusion of phenylhydroxylamine or *p*-nitrosophenetol into dogs the amines appear in the blood. Their concentration increases steadily as long as the infusion goes on. The net rate of elimination of arylhydroxylamines and their nitroso analogues has been measured by means of continuous intravenous infusion. It was proportional to the concentration of the N-hydroxylation products in the blood. The elimination of phenylhydroxylamine is more rapid in the rabbit than in the dog (31, 264). *p*-Hydroxylaminopropiophenone disappears more slowly from the blood of rabbits than phenylhydroxylamine. In view of the rapid microsomal N-hydroxylation of *p*-aminopropiophenone (Section III B), the slow decrease of the hydroxylamine concentration in the blood indicates a rapid cycle of N-hydroxylation and reduction (228). A rapid interconversion of the amine and its N-hydroxy derivative was also observed with 2-acetylaminofluorene in the rat liver (357). In the urine of rats given phenylhydroxylamine, Boyland *et al.* (61) found *p*-aminophenylmercapturic acid.

Among the arylhydroxylamines containing only one ring, N-hydroxy-*p*-aminopropiophenone was excreted to a large part in the urine (230). A substantial fraction of the N-hydroxy-*p*-aminopropiophenone excreted by rabbits and guinea pigs is in a conjugated form, from which it is liberated by incubation at pH 4.5 and 37°C for 2 hr. Incubation with β-glucuronidase does not increase the amount of N-hydroxy derivative determined. N-Glucosiduronic acid derivatives have been found to be metabolites of aromatic amines; the amines are liberated in slightly acid solution (19, 56, 62, 79). The labile conjugate of N-hydroxy-*p*-aminopropiophenone, therefore, may be its N-glucosiduronic acid.

Rabbits excrete about 30% of the compound administered as N-hydroxy-*p*-aminopropiophenone if *p*-aminopropiophenone is injected or its N-hydroxy derivative is infused intravenously (228). If either 4-acetylaminostilbene or its N-hydroxy derivative is orally administered with the diet, the same fraction (2%) is found in the urine of rats as N-hydroxy derivative. (24a).

Boyland *et al.* (59) found 2-amino-6-naphthol in the urine of rats given 2-naphthylhydroxylamine. Since 2-naphthylhydroxylamine does not yield 2-amino-6-naphthol *in vitro*, the metabolite must have arisen from the naphthylhydroxylamine after being reduced to 2-naphthylamine. The O-sulfate of 2-naphthylhydroxylamine occurs in the urine of dogs after oral or subcutaneous administration of N-hydroxy-2-naphthylamine (57). After intraperitoneal injection of N-hydroxy-2-naphthylamine into rats, the urine contains most of the metabolites found after the injection of 2-naphthylamine. Aminonaphthyl and aminophenyl mercapturic acids appear in the urine of some species after the administration of 2-naphthylamine or aniline. Boyland *et al.* (61) assumed that these mercapturic acids originate from the N-hydroxy derivatives of the amines, because they had found that phenylhydroxylamine and 2-naphthylhydroxylamine readily form *o*-aminophenylmercapturic acid and (2-amino-1-naphthyl) mercapturic acid, respectively, by reacting with thiols (60). In line with the hypothesis

of Miller and Miller (361) concerning the mechanism of *o*-hydroxylation, Boyland *et al.* (61) discussed the formation of 2-amino-1-naphthylhydrogen sulfate and (2-amino-1-naphthyl) mercapturic acid from a common precursor.

The metabolism of N-hydroxy-2-aminofluorene and its N-acetyl derivatives has been studied more often than that of any other arylhydroxylamine. N-Hydroxy-2-acetylaminofluorene injected intraperitoneally or mixed with the diet is excreted as N-hydroxy compound in the urine to an extent of up to 15% of the dose by the rat (358), up to 10% by the guinea pig and mouse, up to 20% by the hamster (363) and up to 24% by the rhesus monkey (124). Most of the compound is present in a conjugated form. In the urine of rats (358), rhesus monkeys (124), dogs (405), or hamsters (363) the same metabolites appear when 2-aminofluorene or N-hydroxy-2-aminofluorene is administered. In all cases a higher percentage of the dose is found as N-hydroxy derivative in the urine, when N-hydroxy-2-acetylaminofluorene has been administered for 8 weeks. Mice excrete 10 times more N-hydroxy-2-acetylaminofluorene after being fed this compound than after an equivalent dose of 2-acetylaminofluorene (363). Rats which had been kept for 8 weeks on a diet containing 2-acetylaminofluorene excreted 10 times more N-hydroxy-2-acetylaminofluorene than untreated animals after an intraperitoneal injection of a test dose of this substance. Hamster and guinea pig excreted the larger part of the N-hydroxy-2-acetylaminofluorene as 7-hydroxy-2-acetylaminofluorene. A corresponding amout of the N-hydroxy-2-acetylaminofluorene is hydroxylated in 7-position after (or before?) being reduced to the amine. Miller and Miller (361) found more 1-hydroxy-2-acetylaminofluorene in the urine of rats when N-hydroxy-2-acetylaminofluorene had been fed than after feeding 2-acetylaminofluorene. They assumed that the N-hydroxy derivative of 2-acetylaminofluorene is rearranged *in vivo* partly to the 1-hydroxy derivative, possibly in a manner analogous to the rearrangement of arylhydroxylamines in acid solution. This hypothesis was supported by Booth and Boyland (47), who found that the soluble fraction of rat or rabbit liver transforms the N-hydroxy derivatives of acetanilide, 2-acetylaminonaphthalene, 4-acetylaminobiphenyl, and 2-acetylaminofluorene into the *o*-hydroxy derivatives, NADH or NADPH being needed for the reaction. Heating to 100°C destroys the activity of the soluble fraction.

Several observations indicate that N-hydroxylation products of 2-acetylaminofluorene are excreted in the urine mainly as conjugates. The ratio of the various conjugates of N-hydroxy derivatives in the urine is known in only few cases. After injection of radioactive 2-acetylaminofluorene, the urine of male and female hamsters contains 25 and 31%, respectively, of the N-hydroxy-2-acetylaminofluorene as glucuronic acid conjugate (489). The rat excretes N-hydroxy-2-acetylaminofluorene only as glucuronic acid conjugate (358). Rhesus monkeys and rabbits also excrete N-hydroxy-2-acetylaminofluorene only as the glucuronic acid conjugate (119, 214). The structure of the glucuronide has been investigated by Irving (218).

The excretion of N-hydroxy derivatives of aromatic amines in the urine is affected by various physiological factors. Weisburger *et al.* (491), using the 9-

carbon-14-labelled N-hydroxy-2-acetylaminofluorene, discovered differences in its urinary excretion by male and female rats. The glucuronide fraction of the female urine contains twice as much N-hydroxy-2-acetylaminofluorene as the same fraction of the male urine. Rats fed on a diet containing 2-acetylaminofluorene before the experiments excrete more 1-hydroxy-2-acetylaminofluorene in the urine after the intraperitoneal injection of N-hydroxy-2-acetylaminofluorene than after the injection of 2-acetylaminofluorene (358).

Several of the factors that affect the urinary excretion of N-hydroxylation products after the administration of an aromatic amine influence the production of the N-hydroxylation products more than their excretion. Therefore they have been mentioned above (Section III B). Adrenalectomy of weanling male rats, for example, decreases the urinary excretion of N-hydroxy-2-acetylaminofluorene from 4.4 to 1.8% of the 2-acetylaminofluorene injected, but the excretion of injected N-hydroxy-2-acetylaminofluorene is the same with normal and adrenalectomized rats (337).

Although they do not strictly concern metabolism, the results of Bryan *et al.* (75) on the elution of some arylhydroxylamines and other compounds from pellets implanted into mouse bladder are mentioned here as an example of elimination from an artificial depot.

N-Acetylphenylhydroxylamine and N-benzoylphenylhydroxylamine are readily hydrolyzed *in vivo* (212, 278). The enzymatic hydrolysis has also been studied *in vitro*. A comparison of experiments by Irving (219) and by Kiese and Renner (288) shows that guinea pig liver microsomes hydrolyze N-hydroxy-2-acetylaminofluorene much more rapidly than 2-acetylaminofluorene. Deacetylation of N-hydroxy-2-acetylaminofluorene occurs also in rat liver and brain homogenates as well as its reduction to 2-acetylaminofluorene (153).

It is likely that the N-acetylation of arylhydroxylamines occurs *in vivo*. Von Jagow *et al.* (228, 230) found N-hydroxy-2-acetylaminofluorene in the urine of rabbits given 2-aminofluorene by injection. The possibility cannot be excluded, however, that the acetylation occurred prior to N-hydroxylation. The formation of conjugates with glucuronic and sulfuric acid is demonstrated by the appearance of these metabolites in the urine. Apart from the enzymatic reduction of arylhydroxylamines in red cells, a reduction occurs in other tissues. The presence in the soluble liver cell fraction of one or more enzymes which can reduce phenylhydroxylamine to aniline in the presence of NADPH has been demonstrated by Kiese *et al.* (302).

The reduction of an arylhydroxylamine to the amine is probably not the only pathway of its inactivation, but it is of particular interest because this metabolite can again be transformed into the hydroxylamine. In suspensions of liver microsomes which N-hydroxylate aromatic amines part of the hydroxylamine is removed soon after it is produced. This fraction is small with phenylhydroxylamine. In suspensions of rabbit liver microsomes containing 5 μg phenylhydroxylamine per ml, less than 10% is reduced to aniline in 40 min (320). But with a substituted phenylhydroxylamine, 4-(2-methoxy-ethoxy)-3-acetylphenylhydroxylamine, during 40 min of incubation of the amine rabbit liver microsomes produce 2 to 3

times more hydroxylamine than is found at the end of the incubation (281). The isolation from pork liver of a nitrosoreductase reducing *p*-nitrosophenol to *p*-aminophenol has been described (393).

Booth and Boyland (47) observed the reduction of N-hydroxy-4-aminobiphenyl and N-hydroxy-4-acetylaminobiphenyl to the amines in suspensions of rabbit liver microsomes. During 30 min of incubation of N-hydroxy-2-acetylaminofluorene with rabbit liver microsomes under hydroxylating conditions 40% of the compound disappears (217). Part of the compound was bound to protein. Most of the metabolized material was found in a nonacidic, nonpolar fraction in which 2-acetylaminofluorene and 2-nitrosofluorene were identified. The 10,000 × *g* supernatant disposes of the N-hydroxy-2-acetylaminofluorene much more quickly. In 30 min more than 90% is converted to protein-bound derivatives, 2-acetylaminofluorene, 2-aminofluorene, the O-glucuronide of N-hydroxy-2-acetylaminofluorene, and some unidentified compounds. Similar results occur with rabbit liver homogenates (153). The soluble liver fraction reduces N-hydroxy-2-acetylaminofluorene slightly less rapidly than the homogenate. Brain homogenate was less active than liver homogenate.

Lotlikar *et al.* (339) investigated the reduction of N-hydroxy-2-acetylaminofluorene in more detail. Cofactor requirements, activities of subcellular fractions, and the possible role of flavins were studied in addition to the activity of various tissues of rats and of the livers of various species. Homogenates prepared from liver reduced N-hydroxy-2-acetylaminofluorene more quickly than other homogenized tissues. A comparison of the livers of weanling males showed the highest reduction rate with hamster liver. Guinea pig liver was about half as active but more so than the livers of rabbits, rats, and mice. Most of the substrate reduced by guinea pig liver appeared as the deacetylated amine. The rapid reduction of the N-hydroxy compound by the guinea pig also appears of interest with regard to the failure of 2-aminofluorene to produce tumors in guinea pigs (14, 64), although it is readily N-hydroxylated by guinea pig liver microsomes (289). The reduction of N-hydroxy-2-acetylaminofluorene by homogenates prepared from livers of weanling rats is not affected by previous adrenalectomy of the animals. On the reduction of arylhydroxylamines in red cells see Section II H 2 a.

One metabolic reaction of arylhydroxylamines observed *in vitro* has been mentioned above in this section: N-Hydroxy acetanilide, N-hydroxy-2-acetylaminobiphenyl, and N-hydroxy-2-acetylaminofluorene are isomerized to the *o*-hydroxy amines by the soluble fraction of rabbit liver (47).

In addition to the reaction with glutathione (339), aromatic nitroso compounds react with (SH-groups of) proteins. Miller *et al.* (360) found that 2-nitrosofluorene, nitrosobenzene, or *o*-nitrosotoluene 10^{-4} M inhibits the amino acid incorporation in rat liver microsomes to 25 to 40% of the control value. The same concentration of phenylhydroxylamine or nitrosobenzene inhibits the oxygen uptake of diaphragm, kidney slices, and liver homogenate from rats (9).

B. N-Oxides

The N-oxide of trimethylamine is found in large amounts in human urine after

injection of trimethylamine or its N-oxide. N,N-Dimethylaniline-N-oxide has not been detected in the urine of dogs or rabbits after injection of N,N-dimethylaniline (207, 208). After the subcutaneous injection of very large doses of N,N-dimethylaniline-N-oxide into dogs or N,N-diethylaniline-N-oxide into rabbits the N-oxides were found in the urine (207, 209). This confirms Hildebrandt's supposition that N,N-dimethylaniline-N-oxide is metabolized in warm-blooded animals.

In addition to the demethylation and reduction in red cells (Section II J), N,N-dimethylaniline-N-oxide is demethylated in liver microsomes in a reaction which proceeds in the absence of oxygen and NADPH (402, 514). The N-oxide is likely to be an intermediate in biological demethylation of N,N-dimethylaniline (514). It has also been discussed as an intermediate in trialkylamine dealkylation (128, 129). However, rat liver microsomes demethylate propoxyphene much more rapidly than its N-oxide (353). Therefore, N-oxide formation does not seem to be always a prerequisite to demethylation.

Quinoline-N-oxide is not excreted unchanged by the rabbit. After the intramuscular injection of several grams of the compound, mainly hydroxyquinolines appear in the urine, besides derivatives which still contain the N-oxide group (161). The reduction of nicotinamide-N-oxide by hog liver homogenates has been observed (83).

V. THE ENZYMES THAT CATALYZE THE OXIDATION OF AROMATIC AMINES TO FERRIHEMOGLOBIN-FORMING DERIVATIVES

A. Components and mechanisms

All three types of ferrihemoglobin-forming compounds derived from aromatic amines, *i.e.*, the aminophenols, the hydroxylamines, and the N-oxides, are produced by enzymes located in the endoplasmic reticulum of the cell. After homogenation and centrifugal fractionation they are found in the microsomal fraction. The enzymes are bound to particulate structures. Attempts at separating and solubilizing these enzymes have not been successful.

This type of enzyme was discovered by Axelrod (17, 18). He found that rabbit liver microsomes oxidatively deaminate ephedrine if NADPH is present. Then Brodie *et al.* (69) observed that microsomes oxidatively transform a variety of substances in different ways if oxygen and NADPH are available. Among other reactions the dealkylation of alkylamines (319) and the *p*-hydroxylation of aniline and acetanilide were demonstrated (370). Baker and Chaykin (22, 23) found that NADPH-dependent microsomal enzymes oxidize trimethylamine to the N-oxide, and Ziegler and Pettit (513) showed that arylalkylamines like N,N-dimethylaniline are also transformed into the N-oxide. Kiese and Uehleke (300, 301) discovered that NADPH-dependent microsomal enzymes N-hydroxylate aromatic amines.

The tissue distribution of the hydroxylating enzymes considered here has attracted scant attention. Seal and Gutmann (427) did not detect any hydroxylation of 2-acetylaminofluorene by microsomes prepared from rat testis, kidney, or bladder. Mitoma *et al.* (370) found no hydroxylation of acetanilide by microsomes

prepared from rabbit brain, kidney, lung, or muscle. On the other hand Kampffmeyer and Kiese (244) showed that microsomes prepared from rabbits lungs or kidneys N- and *p*-hydroxylate aniline and N-ethylaniline and dealkylate the latter compound. Microsomal electron transport enzymes in several tissues have been studied by Garfinkel (141). The 7-hydroxylation of coumarin has been observed only with the microsomes prepared from rabbit's liver. Rat liver microsomes do not catalyze the reaction (108, 110, 315).

The hydroxylating microsomal enzymes,[4] which also catalyze a number of other reactions not discussed here, have other features in common apart from the need of NADPH. The oxygen found in the hydroxyl or N-oxide group stems from atmospheric oxygen (23, 177, 407); microsomal hydroxylations may be inhibited by carbon monoxide (13, 126, 249, 390, 419). These observations called attention to a CO-binding protein previously described by Klingenberg (313) and Garfinkel (140), whose CO-compound shows an extinction maximum at 450 mμ. Omura and Sato (384–386) showed this protein to be a cytochrome, provisionally named it P-450, and studied its properties. Solubilization by the action of desoxycholate or phospholipase A modifies cytochrome P-450. The absorption maximum of the carbon monoxide compound shifts to 420 mμ, and the modified protein is accordingly called P-420.

By means of electron-spin resonance spectroscopy Hashimoto *et al.* (172) noticed another hemoprotein, which they named "microsomal Fe_x." Its reduced form readily autoxidizes. On further investigation of the protein, which can be separated from rabbit liver microsomes by tryptic digestion, some relationships between cytochromes P-450, P-420, and microsomal Fe_x were disclosed. Fe_x may be an intermediate in the degradation of cytochrome P-450 to P-420. Like P-450, microsomal Fe_x is transformed to P-420 by *p*-chloromercuribenzoate. Mason *et al.* (348) suggested that microsomal Fe_x is the sulfide of P-420 and P-450 is the phospholipid complex of microsomal Fe_x.

The likelihood of the participation of cytochrome P-450 in the microsomal hydroxylation reactions has increased considerably as a result of studies by Estabrook *et al.* (126) and Cooper *et al.* (101). Investigating the light reversal of the carbon monoxide inhibition of steroid C_{21}-hydroxylation by bovine adrenal microsomes and of acetanilide *p*-hydroxlyation or methlyaminoantipyrine demethylation by rat liver microsomes, they obtained a photochemical action spectrum of the carbon monoxide compound of the inhibited enzyme which is identical with the spectrum of the carbon monoxide compound of P-450 or very similar to it.

The mechanism of NADPH-dependent hydroxylation by enzymes in liver microsomes can only be speculated on, in relation to experiences with other enzymes.

[4] These enzymes belong to the group of oxygenases (176), *i.e.*, oxygen activating enzymes. They differ from "true" oxygenases (176) [or oxygen transferases (347)], which catalyze the incorporation of two atoms of atmospheric oxygen into one molecule of substrate, in that they catalyze the incorporation of only one atom of atmospheric oxygen into a substrate and the reduction of the other one by hydrogen. Mason (347) therefore named them mixed-function oxidases. The term hydroxylases (349) does not comprise all the enzymes of this group. NADPH-dependent microsomal enzymes also catalyze the formation of N-oxides (22, 23), sulfoxides (151), and epoxides (376, 511).

Liver microsomes so far have resisted any attempt at solubilizing and separating active proteins with which a hydroxylating system could have been reconstructed.

The participation of a flavoprotein in microsomal oxidations is indicated by the observation (401) that flavin nucleotides can be dissociated from pork liver microsomes by potassium chloride at pH 5.8 and can be separated by passing the microsome suspension through a G-25 Sephadex column. The capacity of the microsomes to produce N,N-dimethylaniline-N-oxide from the amine is completely lost after this procedure and can be partially restored by adding flavin adenine dinucleotide. In view of these observations and results reported in the next paragraph, it is very likely that microsomal NADPH-cytochrome c reductase is part of the electron transport system involved in the microsomal hydroxylation reactions. This flavoprotein has been separated from liver microsomes and purified (403, 509). Several data suggest that it is identical with Horecker's (204) NADPH-cytochrome c reductase isolated from liver. The reduction of the enzyme by NADPH and its reactions with various electron acceptors have been studied (243, 350). Coenzyme Q has been isolated from rat liver microsomes (327), but it is not known whether it is an essential component in the electron transport from NADPH to the terminal oxygenase.

NADPH-cytochrome c reductase is probably part of the microsomal NADPH oxidizing system, which Gillette *et al.* (150) discovered. The oxidase of this system must be different from the microsomal mixed function oxidases. The reaction is not inhibited by carbon monoxide (388). Phenobarbital treatment of rats was found to increase 4- to 5-fold the amidopyrine-demethylating and NADPH-cytochrome c reductase activities as well as the concentration of cytochrome P-450, but not to change the rate of NADPH oxidation in liver microsomes (391).

Important results have been reported by Omura *et al.* (387) concerning the analysis of the electron transport in a hydroxylating enzyme system in which cytochrome P-450 also functions as the "oxygen activating enzyme." From beef adrenal cortex mitochondria, which contain cytochrome P-450 (162) and hydroxylate deoxycorticosterone in 11 β-position, they obtained a particulate fraction containing cytochrome P-450 devoid of other hemoproteins, and furthermore a purified flavoprotein and a nonheme iron protein, which has many properties remarkably similar to spinach ferredoxin. The flavoprotein does not posses NADPH-cytochrome reductase activity unless the highly purified nonheme iron protein is added. By mixing the 3 isolated components, *i.e.*, the flavoprotein, the nonheme iron protein, and the particulate fraction containing cytochrome P-450, a reconstruction of the steroid C_{11} hydroxylation of deoxycorticosterone is obtained. On the basis of these results Omura *et al.* (387) drew a scheme of the electron transport for cytochrome P-450 reduction, which is reproduced in figure 3. One may presume that the same or a very similar scheme will be found some time to depict the process in liver microsomes.

Whereas some hypothesis may be developed soon concerning the microsomal electron transport supplying 1 oxygen atom with 2 electrons, the mechanism by which the other oxygen atom is incorporated into the substrate is still obscure. Another problem is whether there exists only one uniform oxygenase or several

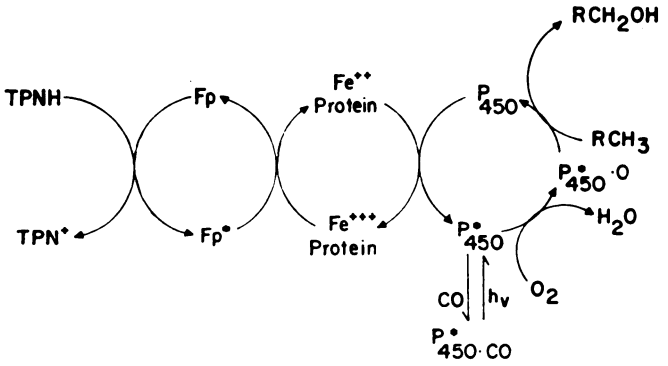


FIG. 3. Schematic representation of the pathway of electron transport for cytochrome P-450 reduction and the activation of oxygen for hydroxylation reactions. Omura *et al.* (387).

with different specificities. In the first case, only the reactivity of a certain structure of an aromatic amine and the chance that it hits the fully unspecific oxygenase would determine whether the oxygen is incorporated at the nitrogen or any of the carbon atoms. Species differences in the preference of the hydroxylation of one atom to another could be explained by species differences in the protein structure of the oxygenase.

In several properties studied, the enzymatic hydroxylations of various aromatic amines in various positions are similar. There is circumstantial evidence, however, of the existence of several oxygen-incorporating enzymes (or directing systems) with different specificities. Experiences with other microsomal hydroxylations support this view.

B. Factors affecting the incorporation of oxygen into various positions of the same substrate

In some respects the C-hydroxylation, N-hydroxylation, N-oxide formation, and N-dealkylation of aromatic amines display a fairly similar behavior or show only few clearly defined differences.

The pH-optima of the C-hydroxylation of aromatic compounds (45, 46, 105, 108, 110, 111, 245, 256, 370, 392, 427), N-hydroxylation (217, 245), N-dealkylation (245), N-oxide formation (23), NADPH oxidation (463), and cytochrome c reduction (403) are in the pH range from 7 to 8. Only the 4-hydroxylation of biphenyl is optimal in more alkaline solution, and the N-hydroxylation of N-ethylaniline by rabbit liver microsomes increases with the hydrogen ion concentration below pH 6.5 (245).

Investigations into other properties of the microsomal reactions have resulted in a colorful picture. The affinities of the substrates for the catalysts as expressed by the Michaelis constants vary widely. Kampffmeyer and Kiese (246) observed differences in the oxygen affinity of the rabbit liver microsomal systems hydroxylating aniline in various positions. The Michaelis constants, K_M, for oxygen were

found to be 0.7×10^{-7} with the *p*-hydroxylation and dealkylation of N-ethylaniline, 7×10^{-6} with the N-hydroxylation of aniline, and 28×10^{-6} with the N-hydroxylation of N-ethylaniline. With the 7-hydroxylation of coumarin the same affinity for oxygen was determined as with the *p*-hydroxylation of aniline, $K_M = 10^{-6}$ (315). Differences in the oxygen affinity between the enzyme systems N-hydroxylating N-ethylaniline have been demonstrated by Appel *et al.* (13). Michaelis constants with aromatic amines have been determined only for the *p*-hydroxylation of acetanilide, $K_M = 6 \times 10^{-4}$ (316) and the dealkylation of N-ethylaniline, $K_M = 9 \times 10^{-4}$ (245). No Michaelis constants could be calculated for some reactions of major interest like the *p*-hydroxylation and N-hydroxylation of aniline and N-ethylaniline by rabbit liver microsomes, because higher concentrations of the substrates are inhibiting (245). The same holds for the ring hydroxylation of 2-acetylaminonaphthalene by rat liver microsomes (45). The affinity of NADPH for the enzyme which accepts its electrons was found to vary with the substrate which is hydroxylated, between $K_M = 10^{-7}$ for the 7-hydroxylation of coumarin (315) and $K_M = 5 \times 10^{-4}$ for the N-hydroxylation of 2-acetylaminofluorene (217). Michaelis constants for other substrates vary between 6×10^{-6} for coumarin (7-hydroxylation) (315) and 10^{-3} for N-methylephedrine (demethylation) (352).

The microsomal ring hydroxylation of aniline and other aromatic compounds is inhibited by compounds like α,α'-bipyridyl (108, 110, 111, 256, 408, 427), 8-hydroxyquinoline (245), *p*-chloromercuribenzoate, and N-ethylmaleimide (13, 105, 245, 256). The enzymes from various species and the reactions with various substrates differ in their sensitivity to the same inhibitor. Differences in the sensitivity of the *o*- and *p*-hydroxylation of aniline occur with either semicarbazide or copper chloride as inhibitor (29). In contrast to their inhibiting effect on hydroxylations, α,α'-bipyridyl, *o*-phenanthroline, and 8-hydroxyquinoline strongly enhance the microsomal oxidation of NADPH (464).

The site of action of heavy-metal-complexing agents and SH-reagents cannot yet be assessed, but carbon monoxide probably competes with oxygen for the terminal oxygenase. The data listed in table 10 show not only species differences in the ratio between the affinities for oxygen and carbon monoxide but also differences in this ratio between the hydroxylations of the same molecule at various positions.

An investigation of the effect of several substances on two or three different hydroxylations of the same substrate by rabbit liver microsomes was carried out by Kampffmeyer and Kiese (244, 245, 247). The reactions studied were the N- and *p*-hydroxylation of aniline and N-alkylanilines and the dealkylation of N-alkylanilines. The results reproduced in table 11 show that *p*-hydroxylation and N-dealkylation are affected in a rather similar way by a variety of substances. A difference between the two reactions was observed in the sensitivity to magnesium ions. In line with observations on other N-dealkylations (74, 245, 319, 371, 446) and some other hydroxylations (475, 476), the dealkylation of N-alkylanilines is accelerated by magnesium ions whereas the *p*-hydroxylation of aniline is not affected by these ions.

TABLE 10

The effect of carbon monoxide on some NADPH-dependent hydroxylations and oxidations of liver microsomes

Species	Reaction	CO (%)	O_2 (%)	% Inhibition	Reference
Rat	NADPH-oxidation	40	4	0	(388)
Rat or rabbit	NADPH-oxidation by oxidase I	10	5	38	(464)
Rat	*p*-Hydroxylation of acetanilide	10	10	60	(101)
Rat	N-Demethylation of amidopyrine	40	4	85	(388)
Rat	N-Demethylation of amidopyrine	40	4	>90	(390)
Rabbit	N-Hydroxylation of aniline and N-ethyl-aniline	99	1	0	(249)
Rabbit	*p*-Hydroxylation of aniline and N-ethyl-aniline, dealkylation of N-ethylaniline	90	10	50	(249)
Guinea pig	N-Hydroxylation	98.5	1.5	20	(13)
	p-Hydroxylation of N-ethylaniline	98.5	1.5	90	
Dog	N-Hydroxylation	98.5	1.5	30	(13)
	p-Hydroxylation of N-ethylaniline	98.5	1.5	75	
Rat	N-Hydroxylation	98.5	1.5	63	(13)
	p-Hydroxylation of N-ethylaniline	98.5	1.5	67	
Rabbit	7-Hydroxylation of coumarin	10.9	4.5	90	(315)
		42	21	88	

The N-hydroxylation of aniline is less sensitive than the *p*-hydroxylation to the inhibiting effect of some substances. The N-hydroxylation of N-alkylanilines behaves quite differently. It is activated, in some cases to a large extent, by substances which react with SH-groups and inhibit other hydroxylations. An activation by *p*-chloromercuribenzoate has also been observed with microsomal NADPH-cytochrome c reductase (350, 403). Possibly this effect is involved in the increase caused by SH-reagents in the N-hydroxylation of N-alkylanilines. Moreover, it shows that the inhibitory effect of SH-reagents on other hydroxylations is located more closely to the oxygen incorporating reaction. Results by Appel *et al.* (13) show that some "inhibitors" affect enzymes in microsomes from various species quite differently.

The diversity of effects on various hydroxylations observed with the same "inhibitor" suggests that it either acts on an enzyme which reacts directly with the substrate to be hydroxylated, or affects a system which directs the substrates to the unspecific hydroxylase. An action on a more remote catalyst in the system,

TABLE 11

The effect of several substances upon the N- and C-hydroxylation of aniline and N-alkylanilines and on the dealkylation of N-alkylanilines by rabbit liver microsomes

Substance Added		Aniline		N-Ethylaniline		N-Methylaniline			N-Butylaniline		
		N-Hydroxylation	*p*-Hydroxylation	N-Hydroxylation	*p*-Hydroxylation	N-Hydroxylation	*p*-Hydroxylation	Dealkylation	N-Hydroxylation	*p*-Hydroxylation	Dealkylation
2,4-Dichlorophenol	$2 \cdot 10^{-3}$ M	0	−69	+89	−62	+63	−75	−83	+139	−65	−36
p-Chloromercuribenzoate	10^{-3} M	−12	−55	+237	−38	+40	−83	−89	+284	−69	−29
N-Ethylmaleimide	$5 \cdot 10^{-3}$ M	−27	−66	+120	−50	+26	−80	−81	+111	−71	−43
Semicarbazide	10^{-2} M	+18	−3	−61	+9	−60	−13	−16	−51	−4	+5
Iproniazid	10^{-3} M	−71	−36	−3	−8						
8-Hydroxyquinoline	10^{-3} M	−67	−88	+10	−57						
D-Penicillamine	$5 \cdot 10^{-3}$ M	−3	+24	−20	+54						
o-Phenanthroline	10^{-3} M	−27	−78	+27	−47						
Potassium cyanide	10^{-3} M	−14	+12	−8	−7						

With regard to the rather wide deviation in the activity of the microsomes taken from various animals, the inhibiting or accelerating effect was calculated in per cent of the unaffected activity. The figures indicate the means of 4 or more experiments. Data from Kampffmeyer and Kiese (244, 245, 247).

for example in the electron transport chain, should cause a more uniform effect on various hydroxylations.

Although the conclusiveness of some inhibition experiments is diminished by contradictory results obtained with the same substrate, the same inhibitor, and liver microsomes of the same species, there is a body of results which hints at the existence of several hydroxylating enzymes. The circumstantial evidence it offers is increased by observations on species differences. For example coumarin is 7-hydroxylated by rabbit liver microsomes but not by rat microsomes (108, 110, 315). Liver microsomes of many species hydroxylate biphenyl in 4-position. Whereas the livers of mice, hamsters, and cats also hydroxylate biphenyl in 2-position, the ability to form 2-hydroxybiphenyl is almost absent from the livers of rabbits, rats, and guinea pigs (108, 111). A sex difference in microsomal hydroxylations has been observed with rat liver microsomes. Kato and Gillette (251, 252) found that this difference varies with the substrate used. The demethylation of amidopyrine and the hydroxylation of hexobarbital are 3 times more rapid with male microsomes than with female ones. But no sex difference exists in the *p*-hydroxylation of aniline. Several factors, like starvation, hypoxia, castration, ACTH, and thyroxine, influence the enzyme activities in male and female microsomes differently. However, no inhibition experiment and no species difference can definitively prove the existence of two enzymes with different specificities as catalysts of the hydroxylation of a substrate in different positions. Proteins with similar functions are known to be of different structure in different species. The reaction of an enzyme of low specificity with an "inhibitor" may modify the enzyme in such a way as allows an unchanged or even increased catalytic effect on the hydroxylation of one substrate, or one position, while the effect on another hydroxylation is largely decreased. Similarly the differences in oxygen and carbon monoxide affinity (see above and table 10) observed with the hydroxylation of the same substrate in different positions could be explained by an unequal oxygen and carbon monoxide affinity of the different enzyme-substrate complexes.

None of these objections, however, is valid in regard to the results of induction experiments. Several investigations have demonstrated that a large number of substances can induce the formation of microsomal hydroxylating enzymes. But the reaction to the inducing agents is not uniform. Conney *et al.* (98) tested various actions, hydroxylations, demethylations, *etc.*, of rat liver microsomes. Pretreatment of the rats with 3,4-benzopyrene stimulated the reactions studied to an unequal extent. Later Conney *et al.* (96) noticed that the treatment of rats with 3,4-benzopyrene differs from that with phenobarbital (412) in the type of microsomal reactions it stimulates. Pretreatment with thyroxin inhibits the microsomal metabolism of hexobarbital but not that of zoxazolamine (97). The differences in the induction capacity of various substances have been discussed by Conney and Burns (94, 95) and Gillette (149). S-, N-, and O-Demethylations by rat liver microsomes are stimulated unequally by pretreatment with phenobarbital or 3,4-benzopyrene (179). Treatment of rats with chlordane increases the side-chain oxidation of hexobarbital and the demethylation of amidopyrine

by liver microsomes much more than the sulfoxidation of chlorpromazine (135). 4,4′-Dichlorodiphenyl-trichloroethane (DDT) stimulates the side-chain oxidation of hexobarbital by rat liver microsomes less than the demethylation of amidopyrine (170). The α-, β-, and γ-isomer of hexachlorocyclohexane induce the enzymes metabolizing various substrates in rat liver microsomes to an unequal extent (146). Experiments by Fouts and Rogers (136) illustrate the different sensitivities to the inducing effect of hydrocarbons like 3,4-benzopyrene and 3-methylcholanthrene on the one hand and other substances like phenobarbital or chlordane on the other hand (170, 171). Microsomal steroid hydroxylation is stimulated by the treatment with phenobarbital or phenylbutazone but not by treatment with 3-methylcholanthrene or 3,4-benzopyrene (99, 100). Phenobarbital pretreatment does not affect the rate of *l*-ephedrine demethylation by rat liver microsomes but increases the demethylation of N-methylephedrine about 4-fold (352). Creaven *et al.* (107, 109) showed that *o*- and *p*-hydroxylation of aromatic compounds by liver microsomes from rats and mice can be stimulated differently. Pretreatment with phenobarbital, nikethamide, or meprobamate increased the 4-hydroxylation of biphenyl and had little effect on the 2-hydroxylation. In contrast, 3,4-benzopyrene, 20-methylcholanthrene, and 1,2,5,6-dibenzanthracene chiefly stimulated the 2-hydroxylation of biphenyl. Differences in the stimulation of microsomal N- and C-hydroxylation of aromatic amines were discovered by Lotlikar *et al.* (338) and by Lange (320). The former observed a 5-fold increase in the N-hydroxylation of 2-acetylaminofluorene by hamster liver microsomes after treatment with 3-methylcholanthrene, the ring-hydroxylation remained unchanged. Lange found that pretreatment of young rabbits with DDT increased the microsomal *p*-hydroxylation of N-ethylaniline by 300%, the N-dealkylation by only 50%, and the N-hydroxylation not at all (−7%). In the same experiments the *p*-hydroxylation of aniline was not stimulated (+14%) by DDT-pretreatment, but the N-hydroxylation increased by nearly 200%. The treatment of rabbits with phenobarbital, in principle, causes similar changes in hydroxylating activities. The stimulation of the *p*-hydroxylation of N-ethylaniline and the N-hydroxylation of aniline, however, was much higher than after DDT treatment. In rat liver microsomes pretreatment with DDT likewise does not stimulate the N-hydroxylation of N-ethylaniline; the dealkylation is increased more than the *p*-hydroxylation. Orrenius (389) attaches great importance to results which show that NADPH-cytochrome reductase, cytochrome P-450, and amidopyrine demethylase increase to the same extent in rat liver microsomes after treatment of rats with amidopyrine, 3-methylcholanthrene, or nikethamide. But this is no serious argument against the experiments which prove an unequal stimulation of various hydroxylations.

The results of the induction experiments strongly suggest that the N-hydroxylation of aniline and N-alkylanilines, their *o*- and *p*-hydroxylation, and the N-dealkylation are catalyzed by different microsomal factors.

REFERENCES

1. Adler, O.: Die Wirkung und das Schicksal des Benzidins im Tierkörper. Arch. exp. Path. Pharmak. **58**: 167, 1908.
2. Aebi, H., Heiniger, J. P. und Lauber, E.: Methämoglobinbildung in Erythrocyten durch Peroxideinwirkung.

Versuche zur Beurteilung der Schutzfunktion von Katalase und Glutathionperoxidase. Helv. chim. Acta **47**: 1428, 1964.

3. AEBI, H., HEINIGER, J. P. UND SUTER, H.: Methämoglobinbildung durch Röntgenstrahlen in normalen und katalasefreien Erythrocyten des Menschen. Experientia **18**: 129, 1962.
4. AEBI, H. UND SUTER, H.: Wirkung peroxydbildender Cytostatika auf Methämoglobin- und Glutathiongehalt normaler und akatalatischer Erythrocyten. Helv. physiol. Acta **23**: 9, 1965.
5. AFANASSIEW, M.: Über Icterus und Hämoglobinurie, hervorgerufen durch Toluylendiamin und andere Blutkörperchen zerstörende Agentien. Z. klin. Med. **6**: 281, 1883.
6. AKAHORI, W.: Detoxication of *o*-aminophenol in the liver with special reference to methemoglobin formation. Folia pharmacol. jap. **50**: 112, 1954; Chem. Abstr. **49**: 10517, 1955.
7. ALBAUM, H. G., TEPPERMAN, J. AND BODANSKY, O.: The *in vivo* inactivation by cyanide of brain cytochrome oxidase and its effect on glycolysis and on the high energy phosphorus compounds in brain. J. biol. Chem. **164**: 45, 1946.
8. ALSLEV, J. UND KIESE, M.: Darstellung und Eigenschaften von Verdoglobinen. IV. Kristalliner Verd-NO_2-porphyrindimethylester. Arch. exp. Path. Pharmak. **207**: 525, 1949.
9. ALSLEV, J. UND KIESE, M.: Die Wirkung von Phenylhydroxylamin und Nitrosobenzol auf den Sauerstoffverbrauch von Gewebsschnitten und Gewebsbrei. Arch. exp. Path. Pharmak. **213**: 249, 1951.
10. ANDERSON, R. A., ENOMOTO, M., MILLER, J. A. AND MILLER, E. C.: Carcinogenesis and tumor inhibition by trans N-hydroxy-4-acetylaminostilbene. Proc. Amer. Ass. Cancer Res. **4**: 2, 1963.

10a. ANDERSEN, R. A., ENOMOTO, M., MILLER, E. C. AND MILLER, J. A.: Carcinogenesis and inhibition of the Walker 256 tumor in the rat by trans-4-acetylaminostilbene, its N-hydroxy metabolite, and related compounds. Cancer Res. **24**: 128, 1964.

11. ANTONINI, E., BRUNORI, M. AND WYMAN, J.: Studies on the oxidation-reduction potentials of heme proteins. IV. The kinetics of oxidation of hemoglobin and myoglobin by ferricyanide. Biochemistry **4**: 545, 1965.
12. ANTONINI, E., WYMAN, J., BRUNORI, M., TAYLOR, J. F., ROSSI-FANELLI, A. AND CAPUTO, A.: Studies on the oxidation-reduction potentials of heme proteins. I. Human hemoglobin. J. biol. Chem. **239**:907, 1964.
13. APPEL, W., GRAFFE, W., KAMPFFMEYER, H. AND KIESE, M.: Speciegdifferences in the hydroxylation of aniline and N-ethylaniline by liver microsomes. Arch. exp. Path. Pharmak. **251**: 88, 1965.
14. ARGUS, M. F. AND RAY, F. E.: Effect of 2-aminofluorene and related compounds on rats, mice, and guinea pigs. Proc. Amer. Ass. Cancer Res. **2**: 92, 1956.
15. ASSENDELFT, O. W. VAN AND ZIJLSTRA, W. G.: The formation of hemiglobin using nitrites. Clin. chim. Acta. **11**: 571, 1965.
16. AUSTIN, J. H. AND DRABKIN, D. L.: Spectrophotometric studies. III. Methemoglobin. J. biol. Chem. **112**: 67, 1935.
17. AXELROD, J.: An enzyme for the deamination of sympathomimetic amines: properties and distribution. J. Pharmacol. **110**: 2, 1954.
18. AXELROD, J.: The enzymatic deamination of amphetamine. J. biol. Chem. **214**: 735, 1955.
19. AXELROD, J., INSCOE, J. K. AND TOMKINS, G. M.: Enzymatic synthesis of N-glucuronic acid conjugates, Nature **179**: 538, 1957.
20. BAADER, H., GIRGIS, S., KIESE, M., MENZEL, H. UND SKROBOT, L.: Der Einfluß des Lebensalters auf Umsetzungen von Phenacetin, *p*-Phenetidin, N-Acetyl-*p*-aminophenol, *p*-Aminophenol und Anilin im Hunde. Arch. exp. Path. Pharmak. **241**: 317, 1961.
21. BAERWOLFF, E., HEUBNER, W. UND KORANSKY, W.: Über die Reaktion von Blutfarbstoff mit Porphyrexid. Arch. exp. Path. Pharmak. **225**: 479, 1955.
22. BAKER, J. AND CHAYKIN, S.: The biosynthesis of trimethylamine-N-oxide. Biochim. biophys. Acta **41**: 548, 1960.
23. BAKER, J. R. AND CHAYKIN, S.: The biosynthesis of trimethylamine-N-oxide. J. biol. Chem. **237**: 1309, 1962.
24. BAKER, J. R., STRUEMPLER, A. AND CHAYKIN, S.: A comparative study of trimethylamine-N-oxide biosynthesis. Biochim. biophys. Acta **71**: 58, 1963.

24a. BALDWIN, R. W. AND SMITH, W. R. D.: N-Hydroxylation in aminostilbene carcinogenesis. Brit. J. Cancer **19**: 433, 1965.

25. BALDWIN, R. W., SMITH, W. R. D. AND SURTEES, S. J.: Carcinogenic action of N-hydroxy-4-acetylaminostilbene. Nature **199**: 613, 1963.
26. BARRON, E. S. G. AND JOHNSON, P.: X-Irradiation of oxyhemoglobin and related compounds. Radiat. Res. **5**: 290, 1956.
27. BARTELS, H. UND HARMS, H.: Sauerstoffdissoziationskurven des Blutes von Säugetieren. (Mensch, Kaninchen, Meerschweinchen, Hund, Katze, Schwein, Rind und Schaf). Pflüg. Arch. ges. Physiol. **268**: 334, 1959.
28. BARTON, G. M. G.: A fatal case of sodium nitrite poisoning. Lancet **1**: 190, 1954.
29. BAUER, S. AND KIESE, M.: Heterogeneousness of the microsomal enzymes effecting the *o*- and *p*-hydroxylation of aniline. Arch. exp. Path. Pharmak. **247**: 144, 1964.
30. BAUMGARTEN, H. E., STAKLIS, A. AND MILLER, E. M.: Reactions of amines. XIII. The oxidation of N-acyl-N-arylhydroxylamines with lead tetraacetate. J. org. Chem. **30**: 1203, 1965.
31. BAYERL, P. AND KIESE, M.: The low activity of aniline in producing hemiglobin in rabbits. Arch. exp. Path. Pharmak. **251**: 212, 1965.
32. BECKER, E.: Nitrit-Vergiftung. Dtsch. med. Rundschau **3**: 900, 1949.
33. BECKER, K. F.: Nitrat- und Nitritgehalt im Spinat. Bundesgesundheitsblatt **8**: 246, 1965.
34. BERGH, A. A. H. VAN DEN: Enterogene Cyanose. Dtsch. Arch. klin. Med. **83**: 86, 1905.
35. BERNHEIM, F., BERNHEIM, M. L. C. AND MICHEL, H. O.: The action of *p*-aminophenol on certain tissue oxidations. J. Pharmacol. **61**: 311, 1937.

38. BETKE, K.: Der menschliche rote Blutfarbstoff bei Fetus und reifem Organismus. Springer-Verlag, Berlin, 1954.
37. BETKE, K.: Über experimentelle Oxydation menschlicher und tierischer Hämoglobine. Folia haemat. Lpz. **75:** 242, 1957.
38. BETKE, K., GREINACHER, I. UND HECKER, F.: Oxydation menschlicher und tierischer Oxyhämoglobine durch Kaliumferricyanid. Arch. exp. Path. Pharmak. **229:** 207, 1956.
39. BETKE, K., GREINACHER, I. UND TIETZE, O.: Oxydation menschlicher und tierischer Oxyhämoglobine durch Natriumnitrit. Arch. exp. Path. Pharmak. **229:** 220, 1956.
40. BEUTLER, E.: Drug-induced hemolytic anemias and the mechanism and significance of Heinz body formation in red blood cells. Nature **196:** 1095, 1962.
41. BOCK, M.: Heinz-Körper- und Methämoglobinbildung durch Methylenblau. Arch. exp. Path. Pharmak. **204:** 595, 1947.
42. BODANSKY, O.: Methemoglobinemia and methemoglobin-producing compounds. Pharmacol. Rev. **3:** 144, 1951.
43. BONSER, G. M., BOYLAND, E., BUSBY, E. R., CLAYSON, D. B., GROVER, P. L. AND JULL, J. W.: A further study of bladder implantation in the mouse as a means of detecting carcinogenic activity: use of crushed paraffin wax or stearic acid as the vehicle. Brit. J. Cancer **17:** 127, 1963.
44. BONSER, G. M., CLAYSON, D. B. AND JULL, J. W.: An experimental inquiry into the cause of industrial bladder cancer. Lancet **II:** 286, 1951.
45. BOOTH, J. AND BOYLAND, E.: The biochemistry of aromatic amines. 3. Enzymic hydroxylation by rat-liver microsomes. Biochem. J. **66:** 73, 1957.
46. BOOTH, J. AND BOYLAND, E.: Metabolism of polycyclic compounds. XIII. Enzymic hydroxylation of naphthalene by rat liver microsomes. Biochem. J. **70:** 681, 1958.
47. BOOTH, J. AND BOYLAND, E.: The biochemistry of aromatic amines. 10. Enzymic N-hydroxylation of arylamines and conversion of arylhydroxylamines into *o*-aminophenols. Biochem. J. **91:** 362, 1964.
48. BOOTH, J., BOYLAND, E. AND MANSON, D.: Metabolism of polycyclic compounds. 9. Metabolism of 2-naphthylamine in rat tissue slices. Biochem. J. **60:** 62, 1955.
49. BÖTTCHER, G. UND KIESE, M.: Oxydation von Anilin zu Nitrosobenzol durch Peroxydasen. Naturwissenschaften **47:** 157, 1960.
50. BOYLAND, E.: The mechanism of tumor induction by aromatic amines and other carcinogens. Z. Krebsforsch. **65:** 378, 1963.
51. BOYLAND, E., BUSBY, E. R., DUKES, C. E., GROVER, P. L. AND MANSON, D.: Further experiments on implantation of materials into the urinary bladder of mice. Brit. J. Cancer **18:** 575, 1964.
52. BOYLAND, E., DUKES, C. E. AND GROVER, P. L.: Carcinogenicity of 2-naphthylamine and 2-naphthylhydroxylamine. A. R. Brit. Emp. Cancer Campgn. **39:** 81, 1961.
53. BOYLAND, E., DUKES, C. E. AND GROVER, P. L.: Carcinogenicity of 2-naphthylhydroxylamine and 2-naphthylamine. Brit. J. Cancer **17:** 79, 1963.
54. BOYLAND, E., DUKES, C. E. AND GROVER, P. L.: Failure to induce tumors with 2-naphthylamine and 2-naphthylhydroxylamine in guinea pigs. A. R. Brit. Emp. Cancer Campgn. **42:** 29, 1964.
55. BOYLAND, E., GORROD, J. W. AND MANSON, D.: Metabolism of aromatic amines. A. R. Brit. Emp. Cancer Campgn. **42:** 27, 1964.
56. BOYLAND, E. AND MANSON, D.: The metabolites of 2-naphthylamine produced by rats and rabbits. Biochem. J. **60:**11, 1955.
57. BOYLAND, E. AND MANSON, D.: Metabolism of 2-naphthylamine and 2-naphthylhydroxylamine. A. R. Brit. Emp. Cancer Campgn. **39:** 81, 1961.
58. BOYLAND, E. AND MANSON, D.: Metabolism of 2-naphthylamine and its derivatives. A. R. Brit. Emp. Cancer Campgn. **41:** 39, 1963.
59. BOYLAND, E., MANSON, D. AND NERY, R.: Metabolism of 2-naphthylamine and aniline. A. R. Brit. Emp. Cancer Campgn. **38:** 52, 1960.
60. BOYLAND, E., MANSON, D. AND NERY, R.: The reaction of phenylhydroxylamine and 2-naphthylhydroxylamine with thiols. J. chem. Soc. p. 606, 1962.
61. BOYLAND, E., MANSON, D. AND NERY, R.: Biochemistry of aromatic amines. Mercapturic acids as metabolites of aniline and 2-naphthylamine. Biochem. J. **86:** 263, 1963.
62. BOYLAND, E., MANSON, D. AND ORR, S. F. D.: The conversion of arylamines into arylsulfamic acids and arylamine-N-glucosiduronic acids. Biochem. J. **65:** 417, 1957.
63. BOYLAND, E. AND NERY, R.: Arylhydroxylamines. IV. Their colorimetric determination. Analyst **89:** 95, 1964.
64. BREIDENBACH, A. W. AND ARGUS, M. F.: Attempted tumor induction in guinea pigs. Quart. J. Fla. Acad. Sci. **19:** 68, 1956.
65. BREWER, G. J., TARLOV, A. R., KELLERMEYER, R. W. AND ALVING, A. S.: The hemolytic effect of primaquine. XV. Role of methemoglobin. J. Lab. clin. Med. **59:** 905, 1962.
66. BRINKMANN, F. UND KIESE, M.: Oxydation von Hämoglobin und Muskelhämoglobin durch Phenylhydroxylamin und Sauerstoff; Abhängigkeit vom Sauerstoffdruck. Biochem. Z. **326:** 218, 1955.
67. BROBERGER, O., ERNSTER, L. AND ZETTERSTRÖM, R.: Oxidation of human haemoglobin by vitamin K_3. Nature **188:** 316, 1960.
68. BRODIE, B. B. AND AXELROD, J.: The fate of acetanilide in man. J. Pharmacol. **94:** 29, 1948.
69. BRODIE, B. B., AXELROD, J., COOPER, J. R., GAUDETTE, L., LA DU, B. N., MITOMA, C. AND UDENFRIEND, S.: Detoxication of drugs and other foreign compounds by liver microsomes. Science **121:** 603, 1955.
70. BRODIE, B. B. AND UNDENFRIEND, S.: Metabolites of pamaquine in urine. Proc. Soc. exp. Biol., N. Y. **74:** 845, 1950.

71. Brooks, J.: The oxidation of hemoglobin to methemoglobin by oxygen. Proc. Roy. Soc. B **109:** 35, 1932.
72. Brooks, J.: The oxidation of hemoglobin to methemoglobin by oxygen. II. The relation between the rate of oxidation and the partial pressure of oxygen. Proc. Roy. Soc. B **118:** 560, 1935.
73. Brooks, J.: The oxidation of haemoglobin to methaemoglobin by oxygen. J. Physiol. **107:** 332, 1948.
74. Brown, R. R., Miller, J. A. and Miller, E. C.: The metabolism of methylated aminoazo dyes. IV. Dietary factors enhancing demethylation *in vitro*. J. biol. Chem. **209:** 211, 1954.
75. Bryan, G. T., Brown, R. R., Morris, C. R. and Price, J. M.: *In vivo* elution of tryptophan metabolites and other aromatic nitrogen compounds from cholesterol pellets implanted into mouse bladders. Cancer Res. **24:** 586, 1964.
76. Bryan, G. T., Brown, R. R. and Price, J. M.: Mouse bladder carcinogenicity of certain tryptophan metabolites and other aromatic nitrogen compounds suspended in cholesterol. Cancer Res. **24:** 596, 1964.
77. Büch, O.: Massenvergiftung durch Natriumnitrit. Sammlung v. Vergiftungsfällen **14:** 53, 1952.
78. Büch, H., Häuser, H., Pfleger, K. und Rüdiger, W.: Über die Ausscheidung eines noch nicht beschriebenen Phenacetinmetaboliten beim Menschen und bei der Ratte. Arch. exp. Path. Pharmak. **253:** 25, 1966.
79. Bushby, S. R. M. and Woiwod, A. J.: The identification of the major diazotizable metabolite of 4:4′-diaminodiphenylsulfone in rabbit urine. Biochem. J. **63:** 406, 1956.
80. Carson, P. E., Flanagan, C. L., Ickes, C. E. and Alving, A. S.: Enzymatic deficiency in primaquine-sensitive erythrocytes. Science **124:** 484, 1956.
81. Carver, M. J. and Ryan, W. L.: Stimulation of erythrocyte metabolism by menadione. Proc. Soc. exp. Biol. **104:** 473, 1960.
82. Channon, H. J., Mills, G. T. and Williams, R. T.: The metabolism of 2,4,6-trinitrotoluene (α-T.N.T.) Biochem. J. **38:** 70, 1944.
83. Chaykin, S. and Bloch, K.: The metabolism of nicotinamide-N-oxide. Biochim. biophys. Acta **31:** 213, 1959.
84. Clayson, D. B.: A working hypothesis for the mode of carcinogenesis of aromatic amines. Brit. J. Cancer **7:** 460, 1953.
85. Clayson, D. B.: Chemical carcinogenesis. J. and A. Churchill, London, 1962.
86. Clayson, D. B. and Ashton, M. J.: The metabolism of 1-naphthylamine and its bearing on the mode of carcinogenesis of the aromatic amines. Acta Un. int. Cancr. **19:** 539, 1963.
87. Cohen, G. and Hochstein, P.: Glucose-6-phosphate dehydrogenase and detoxification of hydrogen peroxide in human erythrocytes. Science **134:** 1756, 1961.
88. Cohen, G. and Hochstein, P.: Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. Biochemistry **2:** 1420, 1963.
89. Cohen, G. and Hochstein, P.: Generation of hydrogen peroxide by hemolytic agents. Biochemistry **3:** 895, 1964.
90. Cohen, G. and Hochstein, P.: *In vivo* generation of H_2O_2 in mouse erythrocytes by hemolytic agents. J. Pharmacol. **147:** 139, 1965.
91. Cohen, G., Martinez, M. and Hochstein, P.: Generation of hydrogen peroxide during the reaction of nitrite with oxyhemoglobin. Biochemistry **3:** 901, 1964.
92. Comly, H. H.: Cyanosis in infants caused by nitrates in well water. J. Amer. med. Ass. **129:** 112, 1945.
93. Conant, J. B. and Scott, N. D.: A spectrophotometric study of certain equilibria involving the oxidation of hemoglobin to methemoglobin. J. biol. Chem. **76:** 207, 1928.
94. Conney, A. H. and Burns, J. J.: Factors influencing drug metabolism. Adv. in Pharmacol. **1:** 31, 1962.
95. Conney, A. H. and Burns, J. J.: Induced synthesis of oxidative enzymes in liver microsomes by polycyclic hydrocarbons and drugs. Adv. Enzyme Regulation **1:** 189, 1963.
96. Conney, A. H., Davison, C., Gastel, R. and Burns, J. J.: Adaptive increases in drug metabolizing enzymes induced by phenobarbital and other drugs. J. Pharmacol. **130:** 1, 1960.
97. Conney, A. H. and Garren, L.: Contrasting effects of thyroxin on zoxazolamine and hexobarbital metabolism. Biochem. Pharmacol. **6:** 257, 1961.
98. Conney, A. H., Gillette, J. R., Inscoe, J. K., Trams, E. R. and Posner, H. S.: Induced synthesis of liver microsomal enzymes which metabolize foreign compounds. Science **130:** 1478, 1959.
99. Conney, A. H. and Klutch, A.: Increased activity of androgen hydroxylases in liver microsomes of rats pretreated with phenobarbital and other drugs. J. biol. Chem. **238:** 1611, 1963.
100. Conney, A. H. and Schneidman, K.: Enhanced androgen hydroxylase activity in liver microsomes of rats and dogs treated with phenyl butazone. J. Pharmacol. **146:** 225, 1964.
101. Cooper, D. Y., Levin, S., Narasimhulu, S., Rosenthal, O. and Estabrook, R. W.: Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems. Science **147:** 400, 1965.
102. Cowan, W. K. and Price, D. A.: Primaquine and methemoglobin. Clin. Pharmacol. Ther. **5:** 307, 1964.
103. Cox, W. W. and Wendel, W. B.: The normal rate of reduction of methemoglobin in dogs. J. biol. Chem. **143:** 331, 1942.
104. Cramer, J. W., Miller, J. A. and Miller, E. C.: The stimulatory effect of 3-methylcholanthrene on the hydroxylation of 2-acetylaminofluorene by rat liver. Proc. Amer. Ass. Cancer Res. **2:** 288, 1958.
105. Cramer, J. W., Miller, J. A. and Miller, E. C.: The hydroxylation of the carcinogen 2-acetylaminofluorene by rat liver: Stimulation by pretreatment *in vivo* with 3-methylcholanthrene. J. biol. Chem. **235:** 250, 1960.
106. Cramer, J. W., Miller, J. A. and Miller, E. C. N-Hydroxylation: A new metabolic reaction observed in the rat with the carcinogen 2-acetylaminofluorene. J. biol. Chem. **235:** 885, 1960.
107. Creaven, P. J. and Parke, D. V.: The stimulation of hydroxylation by carcinogenic and non-carcinogenic compounds. Biochem. Pharmacol. **15:** 7, 1966.
108. Creaven, P. J., Parke, D. V. and Williams, R. T.: Aromatic hydroxylation by liver microsomes. Biochem. J. **85:** 5P, 1962.

109. Creaven, P. J., Parke, D. V. and Williams, R. T.: Differential stimulation of the *o*- and *p*-hydroxylation of biphenyl by liver microsomes. Biochem. J. **91**: 12P, 1964.
110. Creaven, P. J., Parke, D. V. and Williams, R. T.: A spectrofluorimetric study of the 7-hydroxylation of coumarin by liver microsomes. Biochem. J. **96**: 390, 1965.
111. Creaven, P. J., Parke, D. V. and Williams, R. T.: A fluorimetric study of the hydroxylation of biphenyl *in vitro* by liver preparations of various species. Biochem. J. **96**: 879, 1965.
112. Crick, J. and Jackson, H.: The selective localization of *p*-radioiodophenylhydroxylamine in red cells; its relation to methemoglobinemia. Brit. J. Pharmacol. **8**: 87, 1953.
113. Dannenberg, H. und Kiese, M.: Nachweis von Nitrosobenzol in roten Zellen bei der Hämiglobinbildung durch Phenylhydroxylamin. Arch. exp. Path. Pharmak. **211**: 102, 1950.
114. Dannenberg, H. und Kiese, M.: Reduktion von Nitrosobenzol in roten Zellen. Arch. exp. Path. Pharmak. **211**: 410, 1950.
115. Darling, R. C. and Roughton, F. J. W.: The effect of methemoglobin on the equilibrium between oxygen and hemoglobin. Amer. J. Physiol. **137**: 56, 1942.
116. Dawson, J., Thayer, W. and Desforges, J.: Acute hemolytic anemia in the newborn infant due to naphthalene poisoning: report of two cases, with investigations into the mechanism of the disease. Blood **13**: 1113, 1958.
117. Degkwitz, E. und Staudinger, H.: Untersuchungen zur Hydroxylierung von Acetanilid mit Lebermikrosomen normaler und skorbutischer Meerschweinchen. Hoppe-Seyl. Z. **342**: 63, 1965.
118. Dobriner, K., Hofmann, K. and Rhoads, C. P.: The metabolism of β-naphthylamine by rats, rabbits and monkeys. Science **93**: 600, 1941.
119. Dyer, H. M. and Kelley, M. G.: The metabolism of N-2-fluorenylacetamide in the rhesus monkey. Acta Un. int. Cancr. **19**: 502, 1963.
119a. Dyer, H. M., Kelley, M. G. and O'Gara, R. W.: Lack of carcinogenic activity and metabolic fate of fluorenylacetamides in monkeys. J. nat. Cancer Inst. **36**: 305, 1966.
120. Dyer, H. M., Sherwin, B. E. and Morris, H. P.: Observations on the metabolism of N-2-fluorenylacetamide in dogs and rats. J. nat. Cancer Inst. **34**: 363, 1965.
121. Elson, L. A., Goulden, F. and Warren, F. L.: The metabolism of aromatic amines. Biochem. J. **40**: XXIX, 1946.
122. Endo, H., Ishizawa, M. and Kamiya, T.: Induction of bacteriophage formation in lysogenic bacteria by a potent carcinogen, 4-nitroquinoline-1-oxide, and its derivatives. Nature **198**: 195, 1963.
123. Engel, H.: Über die Ausscheidung von subkutan zugeführtem Alpha- und Betanaphthylamin beim Hunde. Zbl. Gew. Hyg. **1924**: 35 and 68.
124. Enomoto, M., Lotlikar, P., Miller, J. A. and Miller, E. C.: Urinary metabolites of 2-acetylaminofluorene and related compounds in the rhesus monkey. Cancer Res. **22**: 1336, 1962.
125. Erdmann, E. und Vahlen, E.: Über die Wirkungen des *p*-Phenylendiamins und Chinondiimins. Arch. exp. Path. Pharmak. **53**: 401, 1905.
126. Estabrook, R. W., Cooper, D. Y. and Rosenthal, O.: The light reversible carbon monoxide inhibition of the steroid C_{21}-hydroxylase system of the adrenal cortex. Biochem. Z. **338**: 741, 1963.
127. Fischbach, E.: Die Bildung von Hämiglobin (Methämoglobin) durch Plasmochin beim Menschen. Arch. exp. Path. Pharmak. **212**: 284, 1951.
128. Fish, M. S., Johnson, N. M., Lawrence, E. P. and Horning, E. C.: Oxidative N-dealkylation. Biochim. biophys. Acta **18**: 564, 1955.
129. Fish, M. S., Sweeley, C. C., Johnson, N. M., Lawrence, E. P. and Horning, E. C.: Chemical and enzymic rearrangements of N,N-dimethylamino acid oxides. Biochim. biophys. Acta **21**: 196, 1956.
130. Fishberg, E. H.: Excretion of a methemoglobin-forming substance in urine. Proc. Soc. exp. Biol., N. Y. **56**: 24, 1944.
131. Fishberg, E. H.: Excretion of benzoquinoneacetic acid in hypovitaminosis C. J. biol. Chem. **172**: 155, 1948.
132. Fishman, V. and Goldenberg, H.: Identification of a new metabolite of imipramine. Proc. Soc. exp. Biol., N. Y. **110**: 187, 1962.
133. Fishman, V., Heaton, A. and Goldenberg, H.: Metabolism of chlorpromazine. III. Isolation and identification of chlorpromazine-N-oxide. Proc. Soc. exp. Biol., N. Y. **109**: 548, 1962.
134. Floren, W. und Heite, H. J.: Über die Wirkung methämoglobinbildender Gifte auf einige körpereigene reduzierende Stoffe. Arch. exp. Path. Pharmak. **197**: 338, 1941.
135. Fouts, J. R.: Factors influencing the metabolism of drugs in liver microsomes. Ann. N. Y. Acad. Sci. **104**: 875, 1963.
136. Fouts, J. R. and Rogers, L. A.: Morphological changes in the liver accompanying stimulation of microsomal drug metabolizing enzyme activity by phenobarbital, chlordane, benzpyrene or methyl cholanthrene in rats. J. Pharmacol. **147**: 112, 1965.
137. Fox, J. B. and Thomson, J. S.: The formation of green heme pigments from metmyoglobin and methemoglobin by the action of nitrite. Biochemistry **3**: 1323, 1964.
138. Fridovich I,. and Handler, P.: Detection of free radicals generated during enzymic oxidations by the initiation of sulfite oxidation. J. biol. Chem. **236**: 1836, 1961.
139. Gaede, D. und Kiese, M.: Die Cyanose bei der Behandlung mit Sulfonamiden. Klin. Wschr. **23**: 92, 1944.
140. Garfinkel, D.: Studies on pig liver microsomes. I. Enzymic and pigment composition of diffeent microsomal fractions. Arch. Biochem. **77**: 493, 1958.
141. Garfinkel, D.: A comparative study of electron transport in microsomes. Comp. Biochem. Physiol. **8**: 367, 1963.
142. Gaudette, L. E. and Brodie, B. B.: Relation between lipide solubility of drugs and their oxidation by liver microsomes. Biochemc. Pharmacol. **2**: 89, 1959.

143. George, P.: The specific reactions of iron in some hemoproteins. Advanc. Catalys. 4: 367, 1952.
144. George, P. and Stratmann, C. J.: The oxidation of myoglobin to metmyoglobin. Biochem. J. 51: 103, 1952.
145. George, P. and Stratmann, C. J.: The oxidation of myoglobin to metmyoglobin by oxygen. 2.) The relation between the first order rate constant and the partial pressure of oxygen. Biochem. J. 51: 418, 1952.
146. Ghazal, A., Koransky, W., Portig, J., Vohland, H. W. und Klempau, I.: Beschleunigung von Entgiftungsreaktionen durch verschiedene Insecticide. Arch. exp. Path. Pharmak. 249: 1, 1964.
147. Gibson, Q. H.: The reduction of methemoglobin in red blood cells and studies on the cause of idiopathic methemoglobinemia. Biochem. J. 42: 13, 1948.
148. Gibson, Q. H.: The reactions of some aromatic C-nitroso compounds with haemoglobin. Biochem. J. 77: 519, 1960.
149. Gillette, J. R.: Factors that affect the stimulation of the microsomal drug enzymes induced by foreign compounds. Advanc. Enzyme Regulation 1: 215, 1963.
150. Gillette, J. R., Brodie, B. B. and La Du, B. N.: Oxidation of drugs by liver microsomes: role of reduced triphosphopyridine nucleotide and oxygen. J. Pharmacol. 119: 532, 1957.
151. Gillette, J. R. and Kamm, J. J.: The enzymatic formation of sulfoxides: the oxidation of chlorpromazine and 4,4'-diaminodiphenylsulfide by guinea pig liver microsomes. J. Pharmacol. 130: 262, 1960.
152. Graffe, W., Kiese, M. and Rauscher, E.: The formation *in vivo* of *p*-hydroxylaminopropiophenone from *p*-aminopropiophenone and its action *in vivo* and *in vitro*. Arch. exp. Path. Pharmak. 249: 168, 1964.
153. Grantham, P. H., Weisburger, E. K. and Weisburger, J. H.: Dehydroxylation and deacetylation of N-hydroxy-N-2-fluorenylacetamide by rat liver and brain homogenates. Biochim. biophys. Acta 107: 414, 1965.
154. Greenberg, L. A. and Lester, D.: The metabolic fate of acetanilid and other aniline derivatives. II. The role of *p*-aminophenol in the production of methemoglobinemia after acetanilid. J. Pharmacol. 90: 150, 1947.
155. Gutch, C. J. W. and Waters, W. A.: Free radicals derived from nitrosobenzene and phenylhydroxylamine. Proc. chem. Soc. p. 230, 1964.
156. Gutmann, H. R., Galitski, S. B. and Foley, W. A.: N-Hydroxy-2-fluorenylbenzamide, an arylhydroxamic acid with high carcinogenic activity. Nature 209: 202, 1966.
157. Haan, J., Kiese, M. und Werner, A.: Reduktion von Nitrosobenzol zu Anilin in roten Blutzellen. Arch. exp. Path. Pharmak. 235: 365, 1959.
158. Haas, E.: Studien über Methämoglobinbildung. 28. Mitteilung. Dinitrotolylyhydroxylamin. Arch. exp. Path. Pharmak. 204: 130, 1946.
159. Halpern, B. N. et Dubost, P.: Rélation entre l'équilibre acidobasique du sang et la formation de la méthémoglobine. La Médecine 19: 729, 1938.
160. Hankes, L. V., Schmaeler, M. and Rai, K.: *o*-Aminophenol: A urinary product of tryptophan metabolism in the human. Proc. Soc. exp. Biol., N. Y. 110: 420, 1962.
161. Hara, M.: Biochemical studies on quinoline derivatives. V. Metabolic products of quinoline N-oxide. Nihon Univ. J. Med. 2: 295, 1960.
162. Harding, B. W., Wong, S. H. and Nelson, D. H.: Carbon monoxide-combining substances in rat adrenal. Biochim. biophys. Acta 92: 415, 1964.
163. Harley, J. D. and Mauer, A. M.: Studies on the formation of Heinz bodies. I. Methemoglobin production and oxyhemoglobin destruction. Blood 16: 1722, 1960.
164. Harley, J. D. and Robin, H.: Hemolytic activity of vitamin K_3: evidence for a direct effect on cellular enzymes. Nature 193: 478, 1962.
165. Harley, J. D. and Robin, H.: The effect of menadione on the reduction of methemoglobin. Aust. J. exp. Biol. med. Sci. 40: 473, 1962.
166. Harley, J. D. and Robin, H.: The effect of the nitrite ion on intact human erythrocytes. Blood 20: 710, 1962.
167. Harley, J. D. and Robin, H.: Adaptive mechanisms in erythrocytes exposed to naphthoquinones. Aust. J. exp. Biol. med. Sci. 41: 281, 1963.
168. Harley, J. D. and Robin, H.: Role of the pentose phosphate pathway in the regulation of methemoglobinemia. Nature 198: 397, 1963.
169. Harrop, G. A. and Barron E. S. G.: The effect of methylene blue and other dyes upon the oxygen consumption of mammalian and avian erythrocytes. J. exp. Med. 48: 207, 1928.
170. Hart, L. G. and Fouts, J. R.: Further studies on the stimulation of hepatic microsomal drug metabolizing enzymes by DDT and its analogs. Arch. exp. Path. Pharmak. 249: 486, 1956.
171. Hart, L. G. and Fouts, J. R.: The possible mechanism by which chlordane stimulates hepatic microsomal drug metabolism in the rat. Biochem. Pharmacol. 14: 263, 1965.
172. Hashimoto, V., Yamano, T. and Mason, H. S.: An electron spin resonance study of microsomal electron transport. J. biol. Chem. 237: 3843, 1962.
173. Havemann, R.: Untersuchungen über Verdohämochromogene. I. Biochem. Z. 308: 1, 1941.
174. Havemann, R.: Verdoglobinstudien. Klin. Wschr. 23: 179, 1944.
175. Hauschild, F.: Die Wirkung des Katalysins bei der Methämoglobinvergiftung. Arch. exp. Path. Pharmak. 184: 458, 1937.
176. Hayaishi, O.: Oxygenases. Academic Press, Inc., New York, 1962.
177. Hayaishi, O., Rothberg, S. and Mehler, A. H.: Studies on enzymatic oxygenation. 130th Am. chem. Soc. Meeting 1956: 53.
178. Heiniger, J. P. und Aebi, H.: Methämoglobinbildung durch Röntgen-Bestrahlung in Hämolysat und intakten Erythrocyten von verschiedenem Katalasegehalt. Helv. chim. Acta 46: 255, 1963.
179. Henderson, J. F. and Mazel, P.: Studies of the induction of microsomal S-, N- and O-demethylases. Biochem. Pharmacol. 13: 1471, 1964.

180. HERINGLAKE, R., KIESE, M., RENNER, G. UND WENZ, W.: N-Oxydation von 2-Naphthylamin *in vivo* und Wirkungen von Oxydationsprodukten des 2-Naphthylamins. Arch. exp. Path. Pharmak. **239:** 370, 1960.
181. HERKEN, H.: Studien über Methämoglobinbildung, 27. Mitteilung: Nitrierte aromatische Hydroxylamine und 3,3'-dinitroazoxybenzol. Arch. exp. Path. Pharmak. **202:** 70, 1943.
182. HERR, F. UND KIESE, M.: Bestimmung von Nitrosobenzol im Blute. Arch. exp. Path. Pharmak. **235:** 351, 1959.
183. HEUBNER, W.: Studien über Methämoglobinbildung. Arch. exp. Path. Pharmak. **72:** 241, 1913.
184. HEUBNER, W.: Methämoglobinbildende Gifte. Ergebn. Physiol. **43:** 9, 1940.
185. HEUBNER, W.: Reaktionsformen einiger Blutgifte. Klin. Wschr. **21:** 961, 1942
186. HEUBNER, W.: Giftung aromatischer Nitroverbindungen. Arch. exp. Path. Pharmak. **205:** 310, 1948.
187. HEUBNER, W. UND KIESE, M.: Die Cyanose bei der Behandlung mit Sulfonamiden. Bemerkung zu der Veröffentlichung von S. Kallner, Schweiz. med. Wschr. **1947:** 292; Schweiz. med. Wschr **77:** 1337, 1947,
188. HEUBNER, W., KIESE, M., STUHLMANN, M. UND SCHWARZKOPFF-JUNG, W.: Der Hämiglobingehalt normalen Blutes. Arch. exp. Path, Pharmak. **204:** 313, 1947.
189. HEUBNER, W. UND MEIER, R.: Studien über Methämoglobinbildung. IV. Arch. exp. Path. Pharmak. **100:** 137, 1923.
190. HEUBNER, W., MEIER, R. UND RHODE, H.: Studien über Methämoglobinbildung. V. Phenylhydroxylamine. Arch. exp. Path. Pharmak. **100:** 149, 1923.
191. HEUBNER, W., WAHLER, B. UND ZIEGLER, C.: Über die Bildung von Hämiglobin durch acylierte Phenylhydroxylamine. Hoppe-Seylers Z. **295:** 397, 1953.
192. HILDEBRANDT, H.: Über das biologische Verhalten von Phenylalkylaminen und Phenylalkylammoniumbasen. Beitr. chem. Physiol. Path. **9:** 470, 1907.
193. HINSBERG, O. UND TREUPEL, G.: Über die physiologische Wirkung des *p*-Aminophenols und einiger Derivate desselben. Arch. exp. Path. Pharmak. **33:** 216, 1894.
194. HLAVICA, P. AND KIESE, M.: Unpublished experiments, 1965.
195. HOFFMANN-OSTENHOF, O., WEIS, W. UND KRAUPP, O.: *In-vitro*-Versuche über die Methämoglobinbildung durch Chinonderivate. Experientia **3:** 414, 1947.
196. HOFFMANN-OSTENHOF, O., WEIS, W. UND KRAUPP, O.: Untersuchungen über bakteriostatische Chinone und andere Antibiotika. III. Versuche über die durch verschiedene Benzochinon- und Naphthochinonderivate verursachte Methämoglobinbildung *in vitro*. Monatshefte f. Chem. **77:** 86, 1947.
197. HOFMANN, E. C. G., v.KREPL, H. UND PESCHKE, R.: Der Stoffwechsel von Monosacchariden in roten Blutkörperchen. Fol. Haematol. **78:** 481, 1962.
198. HÖLSCHER, P. M. UND NATZSCHKA, J.: Methämoglobinämie bei jungen Säuglingen durch nitrithaltigen Spinat. Dtsch. med. Wschr. **89:** 1751, 1964.
199. HOLZER, N. AND KIESE, M.: Unpublished experiments. Tübingen, 1958.
200. HOLZER, N. UND KIESE, M.: Bildung von Nitrosobenzol, Anilin und Hämiglobin in Katzen und Hunden nach intravenöser Injektion von N-Alkylanilinen. Arch. exp. Path. Pharmak. **238:** 546, 1960.
201. HOLZER, N. AND KIESE, M.: The formation of hemiglobin by *p*-hydroxylaminobenzenesulfonamide *in vivo* and *in vitro*. Arch. exp. Path. Pharmak. **251:** 222, 1965.
202. HOPPE-SEYLER, A. F.: Untersuchungen über Alkylamine. Ber. ges. Physiol. **81:** 392, 1934.
203. HOPPE-SEYLER, F.: Vorläufige Mitteilungen. Hoppe-Seyl. Z. **1:** 396, 1878.
204. HORECKER, B. L.: Triphosphopyridine nucleotide-cytochrome *c* reductase in liver. J. biol. Chem. **183:** 593, 1950.
205. HÖRLEIN, H. UND WEBER, G.: Über chronische familiäre Methämoglobinämie und eine neue Modifikation des Methämoglobins. Dtsch. med. Wschr. **73:** 476, 1948.
206. HÖRLEIN, H. UND WEBER, G.: Chronische familiäre Methämoglobinämie. Z. ges. inn. Med. **6:** 197, 1951.
207. HORN, F.: Über den Abbau des Dimethylanilins und des Dimethylanilinoxyds im Tierkörper. Hoppe-Seyl. Z. **238:** 84, 1936.
208. HORN, F.: Über den Abbau des Dimethylanilins bei Pflanzenfressern. Hoppe-Seyl. Z. **242:** 23, 1936.
209. HORN, F.: Über den Abbau des Diäthylanilins und Diäthylanilinoxyds im Tierkörper. Hoppe-Seyl. Z. **249:** 82, 1937.
210. HUENNEKENS, F. M., CAFFREY, R. W., BASFORD, R. E. AND GABRIO, B. W.: Isolation and properties of methemoglobin reductase. J. biol. Chem. **227:** 261, 1957.
211. HUENNEKENS, F. M., LIU, L., MYERS, H. A. P. AND GABRIO, B. W.: Erythrocyte metabolism. III. Oxidation of glucose. J. biol. Chem. **227:** 253, 1957.
211a. HUISMAN, T. H. J.: Studies on the heterogeneity of hemoglobin. XI. Chromatographic studies of intermediate forms of oxy- and ferrihemoglobin. Arch. Biochem. **113:** 427, 1966.
212. HUSTEDT, G. UND KIESE, M.: Umsetzungen von Acetanilid und Acetylphenylhydroxylamin im Organismus. Arch. exp. Path. Pharmak. **236:** 435, 1959.
213. International Union on Biochemistry: Enzyme nomenclature. Amsterdam, 1965.
214. IRVING, C. C.: N-Hydroxylation of 2-acetylaminofluorene in the rabbit. Cancer Res. **22:** 867, 1962.
215. IRVING, C. C.: N-Hydroxylation of the carcinogen 2-acetylaminofluorene by rabbit liver microsomes. Biochim. biophys. Acta **65:** 564, 1962.
216. IRVING, C. C.: Carcinogenicity and metabolism of 2-acetylaminofluorene in the rabbit. Acta Un. int. Cancr. **19:** 507, 1963.
217. IRVING, C. C.: Enzymatic N-hydroxylation of the carcinogen 2-acetylaminofluorene and the metabolism of N-hydroxy-2-acetylaminofluorene-9-^{14}C *in vitro*. J. biol. Chem. **239:** 1589, 1964.
218. IRVING, C. C.: On the structure of the glucuronide of N-hydroxy-2-acetylaminofluorene. J. biol. Chem. **240:** 1011, 1965.

219. Irving, C. C.: Deacetylation of N-hydroxy-2-acetylaminofluorene by guinea pig liver microsomes. Fed. Proc. **24**: 152, 1965.
220. Issekutz, B. von: Studien über Methämoglobinbildung. 15. Mitt. Arch. exp. Path. Pharmak. **193**: 551, 1939.
221. Itano, H. H. and Robinson, E.: Electrophoretic separation of intermediate compounds in two reactions of ferrihemoglobin. Biochim. biophys. Acta **29**: 545, 1958.
222. Jackson, H.: Studies with erythrocytes labelled with radioactive *p*-iodophenylhydroxylamine. Nature **172**: 80, 1953.
223. Jackson, H. and Thompson, R.: The reaction of hemoglobin and some of its derivatives with *p*-iodophenylhydroxylamine and *p*-iodonitrosobenzene. Biochem. J. **57**: 619, 1954.
224. Jaffé, E. R.: Metabolic processes involved in the formation and reduction of methemoglobin in human erythrocytes. In: The Red Blood Cell, ed. by C. Bishop and D. M. Surgenor, Academic Press, Inc., New York, 1964.
225. Jaffé, M. und Hilbert, P.: Über Acetanilid und Acettoluid und ihr Verhalten im thierischen Stoffwechsel. Hoppe-Seyl. Z. **12**: 295, 1888.
226. Jaffé, E. R. and Neumann, G.: A comparison of the effect of menadione, methylene blue and ascorbic acid on the reduction of methemoglobin *in vivo*. Nature **202**: 607, 1964.
227. Jagow, R. von, Kampffmeyer, H. and Kiese, M.: The preparation of microsomes. Arch. exp. Path. Pharmak. **251**: 73, 1965.
228. Jagow, R. von, Kiese, M. and Renner, G.: Urinary excretion of N-hydroxylation products of some aromatic amines by dogs, rabbits, and guinea pigs. In preparation, 1966.
229. Jagow, R. von, Kiese, M., and Rauscher, E.: Unpublished experiments, 1964.
230. Jagow, R. von, Kiese, M., Renner, G. und Wiedemann, I.: Ausscheidung von N-Hydroxylierungsprodukten aromatischer Amine in Harn und Galle. Arch. exp. Path. Pharmak. **253**: 47, 1966.
231. Jandorf, B. J. and Bodansky, O.: Therapeutic and prophylactic effect of methemoglobinemia in inhalation poisoning by hydrogen cyanide and cyanogen chloride. J. Ind. Hyg. Toxicol. **28**: 125, 1946.
232. Josephson, E. S., Greenberg, J., Taylor, D. J. and Bami, H. C.: A metabolite of pamaquine from chickens. J. Pharmacol. **103**: 7, 1951.
233. Josephson, E. S., Taylor, D. J., Greenberg, J. and Ray, A. P.: A metabolic intermediate of pamaquine from chicken. Proc. Soc. exp. Biol., N. Y. **76**: 700, 1951.
234. Jung, F.: Studien über Methämoglobinbildung. 18. Der Kreisprozess Phenylhydroxylamin-Nitrosobenzol. Arch. exp. Path. Pharmak. **195**: 208, 1940.
235. Jung, F.: Hämoglobin und Oxydationsprodukte des Anilins (Nitrosobenzolhämoglobin). Biochem. Z. **305**: 248, 1940.
236. Jung, F.: Nitrosobenzolhämoglobin. Naturwissenschaften **28**: 264, 1940.
237. Jung, F.: Studien über Methämoglobinbildung. XXIX. Chlor- und Amino-Nitrobenzole. Arch. exp. Path. Pharmak. **204**: 133, 1947.
238. Jung, F. und Remmer, H.: Über die Umsetzung zwischen Nitrit und Hämoglobin. Arch. exp. Path. Pharmak. **206**: 459, 1949.
238a. Jung, F. und Stoytchev, Tz.: Oxydation des Hämoglobins durch Pentacyanoaquoferrat(III) Acta biol. med. germ. **14**: 482, 1965.
239. Jung, F. und Witt, P.: Studien über Methämoglobinbildung. XXX. Versuche mit Polyphenolen. Arch. exp. Path. Pharmak. **204**: 426, 1947.
240. Kakizaki, T., Sato, M. and Hasegawa, H.: Oxidation of oxyhemoglobin by sodium nitrite. Ind. Health (Japan) **2**: 124, 1964; Chem. Abstr. **62**: 12068, 1965.
241. Kallner, S.: The cyanosis developing during treatment with sulfanilamide preparations. Acta med. scand. suppl. **130**, 1942.
242. Kallner, S.: Die Cyanose bei der Behandlung mit Sulfonamiden. Schweiz. med. Wschr. **77**: 292, 1947.
243. Kamin, H., Masters, B. S. S., Gibson, Q. H. and Williams, C. H.: Microsomal TPNH-cytochrome-c-reductase. Fed. Proc. **24**: 1164, 1965.
244. Kampffmeyer, H. und Kiese, M.: Einige Eigenschaften der Anilin-hydroxylierenden Enzyme in Mikrosomen. Arch. exp. Path. Pharmak. **244**: 375, 1963.
245. Kampffmeyer, H. and Kiese, M.: Further factors affecting the hydroxylation of aniline and some of its derivatives. Arch. exp. Path. Pharmak. **246**: 397, 1964.
246. Kampffmeyer, H. and Kiese, M.: The hydroxylation of aniline and N-ethylaniline by microsomal enzymes at low oxygen pressures. Biochem. Z. **339**: 454, 1964.
247. Kampffmeyer, H. and Kiese, M.: Unpublished experiments, 1964.
248. Kampffmeyer, H. and Kiese, M.: Kinetics of the reaction of *p*-aminophenol with hemoglobin and oxygen. In preparation, 1965.
249. Kampffmeyer, H. and Kiese, M.: The effect of carbon monoxide on the hydroxylation of aniline and N-ethylaniline by microsomal enzymes. Arch. exp. Path. Pharmak. **250**: 1, 1965.
250. Kampffmeyer, H. und Kiese, M.: Unterschiede in der Hydroxylierung durch Lebermikrosomen verschiedener Tierarten. Arch. exp. Path. Pharmak. **251**: 112, 1965.
251. Kato, R. and Gillette, J. R.: Effect of starvation on NADPH-dependent enzymes in liver microsomes of male and female rats. J. Pharmacol. **150**: 279, 1965.
252. Kato, R. and Gillette, J. R.: Sex differences in the effects of abnormal physiological states on the metabolism of drugs by rat liver microsomes. J. Pharmacol. **150**: 285, 1965.
253. Keilin, D.: Reactions of haemoproteins with hydrogen peroxide and the supposed formation of hydrogen peroxide during the autoxidation of haemoglobin. Nature **191**: 769, 1961.

254. KEILIN, D. AND HARTREE, E. F.: Reactions of haemoglobin and its derivatives with phenylhydroxylamine and nitrosobenzene. Nature 151: 390, 1943.
255. KEILIN, D. AND HARTREE, E. F.: Properties of catalase. Catalysis of coupled oxidation by alcohols. Biochem. J. 39: 293, 1945.
256. KERÉKJÁRTÓ, B. V., KRATZ, F. UND STAUDINGER, H.: Hydroxylierung von Cumarin mit Lebermikrosomen von Kaninchen. 1) Direkte spektralfluorometrische Messung der enzymatischen Aktivität. Biochem. Z. 339: 460, 1964.
257. KIESE, M.: Die Reduktion des Hämiglobins. Biochem. Z. 316: 264, 1944.
258. KIESE, M.: Spirographishäm, die prosthetische Gruppe des Verdoglobin NO_2. Naturwissenschaften 33: 123, 1946.
259. KIESE, M.: Darstellung und Eigenschaften von Verdoglobinen. Arch. exp. Path. Pharmak. 204: 385, 1947.
260. KIESE, M.: Darstellung und Eigenschaften von Verdoglobinen. II. Zur Konstitution des Verdoglobin NO_2. Arch. exp. Path. Pharmak. 204: 439, 1947.
261. KIESE, M.: Die katalytische Wirkung einiger Farbstoffe auf die Reduktion des Hämiglobins in roten Zellen. Arch. exp. Path. Pharmak. 204: 288, 1947.
262. KIESE, M.: Die Reduktion des Hämiglobins. VI. Die von reversibel reduzierbaren Farbstoffen katalysierte Reduktion. Arch. exp. Path. Pharmak. 207: 99, 1949.
263. KIESE, M.: Oxydation von Anilin zu Nitrosobenzol im Hunde. Arch. exp. Path. Pharmak. 235: 354, 1959.
264. KIESE, M.: Die Bedeutung der Oxydation von Anilin zu Nitrosobenzol für die Hämiglobinbildung nach Aufnahme von Anilin in den Organismus. Arch. exp. Path. Pharmak. 235: 360, 1959.
265. KIESE, M.: Oxydative Entmethylierung von N-Methylanilin *in vivo*. Naturwissenschaften 46: 384, 1959.
266. KIESE, M.: TPNH-abhängige Fermente in Mikrosomen, die Gifte oxydieren. Tagung der Deutschen Gesellsch. f. physiol. Chem und der Österreichischen Biochem. Ges. in Wien 1962. Symposium Redoxfunktionen cytoplasmatischer Strukturen, S.1.
267. KIESE, M. The effect of certain substituents upon the N-oxidation of aniline *in vivo*. Arch. exp. Path. Pharmak. 244: 387, 1963.
268. KIESE, M.. Unpublished experiments, 1963.
269. KIESE, M.: The reduction of *p*-chloronitrosobenzene in the red cells of dogs. Arch. exp. Path. Pharmak. 245: 484, 1963.
270. KIESE, M : Relationship of drug metabolism to methemoglobin formation. Ann. N. Y. Acad. Sci. 123: 141, 1965.
271. KIESE, M.: Autocatalytic formation of ferrihemoglobin by N,N-dimethylaniline-N-oxide. In preparation, 1966.
272. KIESE, M. UND KAESKE, H.: Verbindungen des Muskelhämoglobins. Biochem. Z. 312: 121, 1942.
273. KIESE, M. UND KLINGMÜLLER, G.: Die Sauerstoffbindung partiell oxydierten Blutfarbstoffs. Arch. exp. Path. Pharmak. 207: 655, 1949.
274. KIESE, M., KURZ, H. UND SCHNEIDER, C.: Chronische Hämiglobinämie durch pathologischen Blutfarbstoff. Klin. Wschr. 35: 957, 1956.
275. KIESE, M. UND MENZEL, H.: Hämiglobinbildung im Blute des Menschen nach Einnahme von Phenacetin und N-Acetyl-*p*-aminophenol. Arch. exp. Path. Pharmak. 242: 511, 1962.
276. KIESE, M. UND PEKIS, M.: The reaction of *p*-aminophenol with hemoglobin and oxygen *in vivo* and *in vitro*. Arch. exp. Path. Pharmak. 246: 413, 1964.
277. KIESE, M. UND PLATTIG, K. H.: Kreisprozess der Hämiglobinbildung durch Benzylphenylhydroxylamin und Benzalphenylnitron. Arch. exp. Path. Pharmak. 233: 484, 1958.
278. KIESE, M. UND PLATTIG, K. H.: Hämiglobinbildung durch Benzoylphenylhydroxylamin. Arch. exp. Path. Pharmak. 235: 373, 1959.
279. KIESE, M. AND RACHOR, M.: The reaction of several aminophenols with hemoglobin and oxygen *in vitro* and *in vivo*. Arch. exp. Path. Pharmak. 249: 225, 1964.
280. KIESE, M. AND RAUSCHER, E.: Isolation of phenylhydroxylamine produced from N-ethylaniline by microsomal enzymes. Biochem. Z. 338: 1, 1963.
281. KIESE, M. AND RAUSCHER, E.: The failure of some aromatic amines to produce hemiglobin *in vivo* in spite of microsomal N-hydroxylation. Arch. exp. Path. Pharmak. 251: 201, 1965.
282. KIESE, M. AND RAUSCHER, E.: The mechanism of ferrihemoglobin formation by phenylenediamines. In preparation, 1965.
283. KIESE, M , RAUSCHER, E. AND WEGER, N.: The role of N,N-dimethylaniline-N-oxide in the formation of hemiglobin following the absorption of N,N-dimethylaniline. Arch. exp. Path. Pharmak. 254: 253, 1966.
284. KIESE, M. UND REINWEIN, D.: Kinetik der Hämiglobinbildung. VIII. Die Oxydation von Hämoglobin durch Phenylhydroxylamin und Sauerstoff. Arch. exp. Path. Pharmak. 211: 392, 1950.
85. KIESE, M. UND REINWEIN, I.: Die Reduktion von Hämiglobin durch Phenylhydroxylamin. Arch. exp. Path. Pharmak. 211: 402, 1950.
286. KIESE, M., REINWEIN, D. UND WALLER, H. D.: Die Hämiglobinbildung durch Phenylhydroxylamin und Nitrosobenzol in roten Zellen *in vitro*. Arch. exp. Path. Pharmak. 210: 393, 1950.
287. KIESE, M. AND RENNER, G.: The isolation of *p*-chloronitrosobenzene from the blood of dogs injected with *p*-chloroaniline. Arch. exp. Path. Pharmak. 246: 163, 1963.
288. KIESE, M. AND RENNER, G.: The hydrolysis of acetanilide and some of its derivatives by enzymes in the microsomal and soluble fraction prepared from livers of various species. Arch. exp. Path. Pharmak. 252: 480, 1966.
289. KIESE, M., RENNER, G. AND WIEDEMANN, I.: N-Hydroxylation of 2-aminofluorene in the guinea pig and by guinea pig liver microsomes *in vitro*. Arch. exp. Path. Pharmak. 252: 418, 1966.
290, KIESE, M., RESAG, K. UND SCHNEIDER, C.: Reduktion von Nitrosobenzol durch Hämiglobinreduktase und durch altes gelbes Ferment. Arch. exp. Path. Pharmak. 231: 170, 1957.

291. KIESE, M. UND RUCKTESCHELL, A. VON: Die katalytische Wirkung des Hämoglobins auf die Oxydation von Phenylhydroxylamin durch Sauerstoff. Arch. exp. Path. Pharmak. **213**: 128, 1951.
292. KIESE, M. AND SCHLAEGER, R.: The reaction of N,N-dimethylaniline-N-oxide with hemoglobin. In preparation.
293. KIESE, M. UND SCHNEIDER, C.: Oxydation von Hämoglobin durch Sauerstoff. Biochem. Z. **326**: 209, 1955.
294. KIESE, M., SCHNEIDER, C. UND WALLER, H. D.: Hämiglobinreduktase. Arch. exp. Path. Pharmak. **231**: 158, 1957.
295. KIESE, M. UND SCHWARTZKOPFF, W.: Die Reduktion des Hämiglobins. III. Reduktion des Hämiglobins und Stoffwechsel in roten Zellen. Arch. exp. Path. Pharmak. **204**: 267, 1947.
296. KIESE, M. UND SEIPELT, L.: Bildung und Elimination von Verdoglobinen. Arch. exp. Path. Pharmak. **200**: 648, 1943.
297. KIESE, M. UND SOETBEER, M.: Hämiglobinbildung durch Phenylhydroxylamin *in vivo*. Arch. exp. Path. Pharmak. **207**: 426, 1949.
298. KIESE, M. UND SOETBEER, M.: Hämiglobinbildung durch Nitrit *in vivo*. Arch. exp. Path. Pharmak. **207**: 437, 1949.
299. KIESE, M. UND SOETBEER, M.: Hämiglobinbildung durch Nitrosobenzol *in vivo*. Arch. exp. Path. Pharmak. **210**: 305, 1950.
300. KIESE, M. UND UEHLEKE, H.: Der Ort der N-Oxydation des Anilins in höheren Tier. Arch. exp. Path. Pharmak. **242**: 117, 1961.
301. KIESE, M. UND UEHLEKE, H.: Zwei Wege der Entmethylierung von N-Methylanilin durch Mikrosomen aus Rattenlebern. Naturwissenschaften **48**: 379, 1961.
302. KIESE, M., UEHLEKE, H. UND WEGER, N.: Extraerythrocytäre Einflüsse auf die Hämiglobinbildung durch Phenylhydroxylamin und Nitrosobenzol in roten Zellen. Arch. exp. Path. Pharmak. **242**: 130, 1961.
303. KIESE, M. UND WALLER, H. D.: Die Stoffwechselvorgänge in roten Zellen bei der Hämiglobinbildung durch den Kreisprozess Phenylhydroxylamin-Nitrosobenzol. Arch. exp. Path. Pharmak. **211**: 345, 1950.
304. KIESE, M. UND WALLER, H. D.: Die Reduktion von Hämiglobin und Sauerstoff durch reversibel reduzierbare Farbstoffe in roten Zellen. Arch. exp. Path. Pharmak. **213**: 44, 1951.
305. KIESE, M. AND WEGER, N.: The treatment of experimental cyanide poisoning by hemiglobin formation. Arch. Toxikol. **21**: 89, 1965.
306. KIESE, M. UND WEIS, B.: Die Reduktion des Hämiglobins in den Erythrocyten verschiedener Tiere. Arch. exp. Path. Pharmak. **202**: 493, 1943.
307. KIESE, M. AND WIEDEMANN, I.: Excretion of N-hydroxy-2-aminofluorene in the urine of guinea pigs injected with 2-acetylaminofluorene. In preparation, 1966.
308 KIKUCHI, G., SHUKUYA, R., SUZUKI, M. AND NAKAMURA, CH.: On the mechanism of the activation of molecular oxygen by hemoglobin. J. Biochem., Tokyo **42**: 267, 1955.
309. KIKUTH, W. UND SCHILLING, J.: Chemotherapeutische Versuche beim Fleckfieber (R. mooseri) mit Methylenblau. Zbl. Bakt. Abt. 1 Orig. **151**: 293, 1944.
310. KING, C. M. AND KRIEG, E.: The differential reactivity of the oxidation products of *o*-aminophenols towards protein and nucleic acid. Biochim. biophys. Acta **111**: 147, 1965.
311. KIRCHNER, J. AND CHAYKIN, S.: Biosynthesis of nicotinamide-N-oxide. Fed. Proc. **22**: 296, 1963.
312. KLINGENBERG, K.: Studien über Oxydation aromatischer Substanzen in thierischen Organismus. Diss. Rostock, 1891.
313. KLINGENBERG, M.: Pigments of rat liver microsomes. Arch. Biochem. **75**: 376, 1958.
314. KLUTCH, A., HARFENIST, M. AND CONNEY, A. H.: 2-Hydroxyacetophenetidine, a new metabolite of acetophenetidine. J. med. Chem. **9**: 63, 1966.
315. KRATZ, F. UND STAUDINGER, H.: Kinetische Untersuchungen zur Hydroxylierung von Cumarin mit Lebermikrosomen von Kaninchen. Hoppe-Seyl. Z. **343**: 27, 1965.
316. KRISCH, K. UND STAUDINGER, H.: Untersuchungen zur enzymatischen Hydroxylierung. Hydroxylierung von Acetanilid und deren Beziehung zur mikrosomalen Pyridinnucleotidoxydation. Biochem. Z. **334**: 312, 1961.
317. KUNTZMAN, R., LAWRENCE, D. AND CONNEY, A. H.: Michaelis constants for the hydroxylation of steroid hormones and drugs by rat liver microsomes. Mol. Pharmacol. **1**: 163, 1965.
318. KÜNZER, W., AMBS, E. UND SCHNEIDER, D.: Zur Wirkung von Natriumnitrit auf Nabelschnurerythrocyten. Klin. Wschr. **31**: 617, 1953.
319. LA DU, B. N., GAUDETTE, L., TROUSOF, N. AND BRODIE, B. B.: Enzymatic dealkylation of aminopyrine (pyramidon) and other alkylamines. J. biol. Chem. **214**: 741, 1955.
320. LANGE, G.: Unpublished experiments. München, 1965.
321. LASER, H.: Effect of ionizing radiation on haemoglobin and cytochrome c. Nature **176**: 360, 1955.
322. LEGGE, J. W.: Methemoglobin formation. J. and Proc. Roy. Soc. New South Wales **76**: 47, 1942.
323. LEMBERG, R. AND LEGGE, J. W.: Hematin compounds and bile pigments. New York and London, 1949.
324. LEMBERG, R., LEGGE, J. W. AND LOCKWOOD, W. H.: Coupled oxidation of ascorbic acid and haemoglobin. I. Biochem. J. **33**: 754, 1939.
325. LEMBERG, R., LEGGE, J. W. AND LOCKWOOD, W. H.: Coupled oxidation of ascorbic acid and haemoglobin. 3. Quantitative studies on choleglobin formation. Estimation of haemoglobin and ascorbic acid oxidations. Biochem. J. **35**: 339, 1941.
326. LENK, H. P. UND WAHLER, B. E.: Untersuchungen zum Mechanismus der Hämoglobinoxydation. Reaktionen N-substituierter Phenylhydroxylaminderivate. Arch. exp. Path. Pharmak. **238**: 50, 1960.
327. LEONHÄUSER, S., LEYBOLD, K., KRISCH, K. STAUDINGER, H., GALE, P. H., PAGE, A. C. AND FOLKERS, K.: On the presence and significance of coenzyme Q in microsomes. Arch. Biochem. **96**: 580, 1962.
328. LESTER, D. AND GREENBERG, L. A.: The comparative anoxemic effects from carbon monoxide hemoglobin and methemoglobin. J. Pharmacol. **81**: 182, 1944.

329. LESTER, D. AND GREENBERG, L. A.: The metabolic fate of acetanilide and other aniline derivatives. II. Major metabolites of acetanilide appearing in the blood. J. Pharmacol. **90:** 68, 1947.
330. LESTER, D., GREENBERG, L. A. AND SHUKOVSKY, E.: Limited importance of methemoglobinemia in the toxicity of certain aniline derivatives. J. Pharmacol. **80:** 78, 1944.
331. LINTZEL, W.: Untersuchungen über Trimethylammoniumbasen. III. Trimethylammoniumbasen im menschlichen Harn. Biochem. Z. **273:** 243, 1934.
332. LIPSCHITZ, W.: Mechanismus der Giftwirkung aromatischer Nitroverbindungen, zugleich ein Beitrag zum Atmungsproblem tierischer und pflanzlicher Zellen. Hoppe-Seyl. Z. **109:** 189, 1920.
333. LIPSCHITZ, W.: Giftung und Entgiftung aromatischer Nitroverbindungen. Arch. exp. Path. Pharmak. **205:** 305, 1948.
334. LÖHR, G. W. UND WALLER, H. D.: Biochemie und Pathogenese der enzymopenischen hämolytischen Anämien. Dtsch. med. Wschr. **86:** 27, 87, 1961.
335. LOISELEUR, J., CATINOT, L. ET PETIT, M.: Présence dans la rate d'un enzyme transformant l'hémoglobine en methémoglobine. C. R. Soc. Biol. (Paris) **155:** 253, 1961.
336. LOISELEUR, L. ET PETIT, M.: Charactéristiques le l'enzyme transformant l'hémoglobine en methémoglobine. C R. Soc. Biol. (Paris) **156:** 838, 1962.
337. LOTLIKAR, P. D., ENOMOTO, M., MILLER, E. C. AND MILLER, J.A.: The effects of adrenalectomy, hypophysectomy, and castration on the urinary metabolites of 2-acetylaminofluorene in the rat. Cancer Res. **24:** 1835, 1964
338. LOTLIKAR, P. D., ENOMOTO, M., MILLER, E. C., MILLER, J. A. AND NEWTON, H. P.: Increased N-hydroxylation of 2-acetylaminofluorene on pretreatment of rats or hamsters with 3-methylcholanthrene. Proc. Am. Ass. Cancer Res. **6:** 41, 1965.
339. LOTLIKAR, P. D., MILLER, E. C. MILLER, J. A. and MARGRETH, A.: The enzymatic reduction of the N-hydroxy derivatives of 2-acetylaminofluorene and related carcinogens by tissue preparations. Cancer Res. **25:** 1743, 1965.
340. LUTZ, R. E. AND LYTTON, M. R.: Oxidation-reduction potentials of a series of nitrosobenzene-phenylhydroxylamine systems. J. org. Chem. **2:** 68, 1937.
341. MAGOS, L.: Globin-hemochromogen formation caused by quinones. Biochim. biophys. Acta **90:** 55, 1964.
342. MAGOS, L., SZIZA, M. UND SZIRTES, M.: Die Wirkung von Ascorbinsäure auf durch Nitrit verursachte Methämoglobinämie. Naturwissenschaften **45:** 385, 1958.
343. MANSON, L. A. AND YOUNG, L.: Biochemical studies of toxic agents. 2. The metabolism of 2-naphthylamine and 2-acetamidonapthalene. Biochem. J. **47:** 170, 1950.
344. MARGRETH, A., LOTLIKAR, P. D., MILLER, E. C. AND MILLER, J. A.: The effects of hepatotoxic agents and of liver growth on the urinary excretion of the N-hydroxy metabolite of 2-acetyl-aminofluorene by rats. Cancer Res. **24:** 920, 1964.
345. MARSHALL, W. AND MARSHALL, C. R.: The action of nitrites on blood. J. biol. Chem. **158:** 187, 1945.
346. MARTIN, H. AND HUISMAN, T. H. J.: Formation of ferrihemoglobin of isolated human hemoglobin types by sodium nitrite. Nature **200:** 898, 1963.
347. MASON, H. S.: Mechanisms of oxygen metabolism. Science **125:** 1185, 1957.
348. MASON, H. S., NORTH, J. C. AND VANNESTE, M.: Microsomal mixed-function oxidations: the metabolism of xenobiotics. Fed. Proc. **24:** 1172, 1965.
349. MASSART, L. AND VERCAUTEREN, R.: Oxygenases and hydroxylases. Ann. Rev. Biochem. **28:** 527, 1959.
350. MASTERS, B. S. S., KAMIN, H., GIBSON, Q. H. AND WILLIAMS, C. H.: Studies on the mechanism of microsomal triphosphopyridine nucleotide-cytochrome *c* reductase. J. biol. Chem. **240:** 921, 1965.
351. MATSUMOTO, H.: Über die Giftwirkung des Paraphenylendiamins. Diss. Med. Würzburg, 1901.
352. MCMAHON, R. E.: Some observations on the *in vitro* demethylation of secondary N-methylamines by liver microsomes. Life Sci. **3:** 235, 1964.
353. MCMAHON, R. E. AND SULLIVAN, H. R.: Oxidative demethylation of 1-propoxyphene and 1-propoxyphrene N-oxide by rat liver microsomes. Life sci. **3:** 1167, 1964.
354. MEIER, R.: Studien über Methämoglobinbildung. VII. Nitrit. Arch. exp. Path. Pharmak. **110:** 241, 1925.
355. MEISSNER, R.: Physiologische Versuche mit aromatischen Diaminen. Biochem. Z. **93:** 149, 1919.
356. MEYER, E.: Über das Verhalten des Nitrobenzols und einiger anderer aromatischer Nitrokörper im Organismus. Hoppe-Seyl. Z. **46:** 497, 1905.
357. MILLER, E. C., COOKE, C. W., LOTLIKAR, P. D. AND MILLER, J. A.: The metabolism and carcinogenicity of N-hydroxy-2-acetylaminofluorene (N-HO-AAF) and some of its possible metabolites. Proc. Am. Ass. Cancer Res. **5:** 45, 1964.
358. MILLER, J. A., CRAMER, J. W. AND MILLER, E. C.: The N- and Ring-hydroxylation of 2-acetylaminofluorene during carcinogenesis in the rat. Cancer Res. **20:** 950, 1960.
359. MILLER, J. A., ENOMOTO, M. AND MILLER, E. C.: The carcinogenicity of small amounts of N-hydroxy-2-acetylaminofluorene and its cupric chelate in the rat. Cancer Res. **22:** 1381, 1962.
360. MILLER, E. C., MCKECHNIE, D. POIRIER, M. M. AND MILLER, J. A.: Inhibition of aminoacid incorporation *in vitro* by metabolites of 2-acetylaminofluorene and by certain nitroso compounds. Proc. Soc. exp. Biol., N.Y. **120:** 538, 1965.
361. MILLER, E. C. AND MILLER, J. A.: A mechanism of *ortho*-hydroxylation of aromatic amines *in vivo*. Biochim. biophys. Acta **40:** 379, 1960.
362. MILLER, E. C. AND MILLER, J. A.: The induction of tumors in rats with small amounts of N-hydroxy-2-acetylaminofluorene or its cupric chelate. Proc. Amer. Ass. Cancer Res. **3:** 344, 1962.
363. MILLER, E. C., MILLER, J. A. AND ENOMOTO, M.: The comparative carcinogenicities of 2-acetylaminofluorene and its N-hydroxy metabolite in mice, hamsters and guinea pigs. Cancer Res. **24:** 2018, 1964.

364. MILLER, E. C., MILLER, J. A. AND HARTMANN, H. A.: N-Hydroxy-2-acetylaminofluorene: A metabolite of 2-acetylaminofluorene with increased carcinogenic activity in the rat. Cancer Res. **21**: 815, 1961.
365. MILLER, J. A., POIRIER, L. A., ENOMOTO, M. AND LOTLIKAR, P.: The N-hydroxylation of 2-acetylaminofluorene in the dog and monkey. Proc. Amer. Ass. Cancer Res. **3**: 344, 1962.
366. MILLER, J. A., SATO, K., POIRIER, L. A. AND MILLER, E. C.: Synthesis and formation *in vivo* of N-hydroxy-N-acetyl-*p*-aminoazo dyes. Proc. Amer. Ass. Cancer Res. **5**: 45, 1964.
367. MILLER, J. A., WYATT, C. S., MILLER, E. C. AND HARTMANN, H. A.: The N-hydroxylation of 4-acetylaminobiphenyl by the rat and dog and the strong carcinogenicity of N-hydroxy-4-acetylaminobiphenyl in the rat. Cancer Res. **21**: 1465, 1961.
368. MILLS, G. C.: Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J. biol. Chem. **229**: 189, 1957.
369. MIRSKY, A. E. AND ANSON, M. L.: Sulfhydryl groups of native proteins—hemoglobin and the proteins of the crystalline lens. J. gen. Physiol. **19**: 439, 1936.
370. MITOMA, CH., POSNER, H. S., REITZ, H. C. AND UDENFRIEND, S.: Enzymatic hydroxylation of aromatic compounds. Arch. Biochem. **61**: 431, 1956.
371. MUELLER, G. C. AND MILLER, J. A.: The metabolism of 4-dimethylaminoazobenzene by rat liver homogenates. J. biol. Chem. **176**: 535, 1948.
372. MÜLLER, M. UND IMMENDORFER, I.: Ein Beitrag zum Verhalten des Trimethylamins und des Trimethylaminoxyds im Stoffwechsel. Hoppe-Seyl. Z. **275**: 267, 1942.
373. MURAYAMA, M.: The combining power of normal human hemoglobin for nitrosobenzene. J. biol. Chem. **235**: 1024, 1960.
374. NAKAHARA, W., FUKUOKA, F. AND SUGIMURA, T.: Carcinogenic action of 4-nitroquinoline-N-oxide. Gann **48**: 129, 1957.
375. NAKAJIMA, T. AND KUSUMOTO, S.: Methemoglobin formation by aromatic nitro and amino compounds; methemoglobin formation by aminophenol and diphenol *in vitro*. Ind. Health **1**: 12, 1963; Chem. Abstr. **61**: 4848, 1964.
376. NAKATSUGAWA, T., ISHIDA, M. AND DAHM, P. A.: Microsomal epoxidation of cyclodiene insecticides. Biochem. Pharmacol. **14**: 1853, 1965.
377. NEILL, J. M. AND HASTINGS, A. B.: The influence of the tension of molecular oxygen upon certain oxidations of hemoglobin. J. biol. Chem. **63**: 479, 1925.
378. NEISH, W. J. P.: Effect of size and age of female rats on their response to the methemoglobinemic action of 3'-methyl-4-dimethylaminoazobenzene. Nature **178**: 1350, 1956.
379. NEISH, W. J. P.: Lack of correlation between the methemoglobinemic activity of azo dyes and their carcinogenicity. Naturwissenschaften **46**: 535, 1959.
380. NORRIS, E. R. AND BENOIT, G. J., JR.: Studies on trimethylamine oxide. III. Trimethylamine oxide excretion by the rat. J. biol. Chem. **158**: 443, 1945.
381. NOSSAL, P. M.: The metabolism of erythrocytes. I. Respiration in the absence and presence of methylene blue. Aust. J. exp. Biol. med. Sci. **26**: 123, 1948.
382. OETTINGEN, W. F. VON: The aromatic amino and nitro compounds, their toxicity and potential dangers. Publ. Hlth, Bull. No. 271, Washington 1941.
383. OGATA, Y., SAWAKI, Y., MIBAE, J. AND MORIMOTO, T.: Kinetics of the autoxidation of phenylhydroxylamines to azoxybenzenes in methanol. J. Amer. chem. Soc. **86**: 3854, 1964.
384. OMURA, T. AND SATO, R.: A new cytochrome in liver microsomes. J. biol. Chem. **237**: PC1375, 1962.
385. OMURA, T. AND SATO, R.: The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. J. biol. Chem. **239**: 2370, 1964.
386. OMURA, T. AND SATO, R.: The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. J. biol. Chem. **239**: 2379, 1964.
387. OMURA, T., SATO, R., COOPER, D. V., ROSENTHAL, O. AND ESTABROOK, R. W.: Function of cytochrome P-450 of microsomes. Fed. Proc. **24**: 1181, 1965.
388. ORRENIUS, S.: On the mechanism of drug hydroxylation in rat liver microsomes. J. Cell Biol. **26**: 713, 1965.
389. ORRENIUS, S.: Induction of the drug hydroxylating enzyme system of liver microsomes. J. Cell Biol. **26**: 725, 1965.
390. ORRENIUS, S., DALLNER, G. AND ERNSTER, L.: Inhibition of the TPNH-linked lipid peroxidation of liver microsomes by drugs undergoing oxydative demethylation. Biochem. biophys. Res. Commun. **14**: 329, 1964.
391. ORRENIUS, S., ERICSON, J. C. E. AND ERNSTER, L.: Phenobarbital induced synthesis of the microsomal drug metabolizing enzyme system and its relationship to the proliferation of endoplasmic membranes. A morphological and biochemical study. J. Cell Biol. **25**: 627, 1965.
392. OTANI, T., AKAGI, K. AND SAKAMOTO, Y.: Indole hydroxylase and aniline hydroxylase. J. Biochem. (Tokyo) **52**: 428, 1962.
393. OTSUKA, S.: Studies on nitro-reducing enzymes of swine liver. Properties and cofactor requirements of nitro- and nitrosoreductases. J. Biochem. (Tokyo) **50**: 87, 1961.
394. PARKE, D. V.: The metabolism of [^{14}C] nitrobenzene in the rabbit and guinea pig. Biochem. J. **62**: 339, 1956.
395. PARKE, D. V.: The metabolism of [^{14}C] aniline in the rabbit and other animals. Biochem. J. **77**: 493, 1960.
396. PARKE, D. V.: The metabolism of *m*-dinitro [^{14}C] benzene in the rabbit. Biochem. J. **78**: 262, 1961.
397. PAULET, G. ET MENORET, P.: Etude comparée de la sensibilité du sang de l'homme (frais ou conservé) et du sang de chien á divers methémoglobinisants. C. R. Soc. Biol. (Paris) **148**: 1014, 1954.
398. PAULING, L. AND CORYELL, C. D.: The magnetic properties and structure of hemoglobin, oxyhemoglobin, and carbon monoxyhemoglobin. Proc. nat. Acad. Sci., Wash. **22**: 210, 1936.

399. PETERS, J. H. AND GUTMANN, H. R.: Stimulation of hydroxylation and protein binding of the carcinogen 2-acetylaminofluorene in rat liver homogenates. Arch. Biochem. 62: 234, 1956.
400. PETERSEN, CH.: Studien über Methämoglobinbildung. XX. Ortho-Aminophenol und Nitrosobenzol. Arch. exp. Path. Pharmak. 198: 675, 1941.
401. PETTIT, F. H., ORME-JOHNSON, W. AND ZIEGLER, D. M.: The requirement for flavin adenine dinucleotide by a liver microsomal oxygenase catalyzing the oxidation of alkylarylamines. Biochem. biophys. Res. Commun. 16: 444, 1964.
402. PETTIT, F. H. AND ZIEGLER, D. M.: The catalytic demethylation of N,N-dimethylaniline-N-oxide by liver microsomes. Biochem. biophys. Res. Commun. 13: 190, 1963.
403. PHILLIPS, A. H. AND LANGDON, R. G.: Hepatic triphosphopyridine nucleotide-cytochrome c reductase· Isolation, characterization, and kinetic studies. J. biol. Chem. 237: 2652, 1962.
404. PIOTROWSKI, J.: The metabolism of aniline (low dosage) in rabbits. Med. Pracy 12: 309, 1961; Chem. Abstr. 57: 2732, 1962.
405. POIRIER, L. A., MILLER, J. A. AND MILLER, E. C.: The N- and ring-hydroxylation of 2-acetylaminofluorene and the failure to detect N-acetylation of 2-aminofluorene in the dog. Cancer Res. 23: 790, 1963.
406. POIRIER, M. M., MILLER, J. A. AND MILLER, E. C.: Carcinogenic activity of N-hydroxy-2-acetylaminofluorene and its metal chelates as a function of retention at the injection site. Cancer Res. 25: 527, 1965.
407. POSNER, H. S., MITOMA, CH., ROTHBERG, S. AND UDENFRIEND, S.: Enzymic hydroxylation of aromatic compounds. III. Studies on the mechanism of microsomal hydroxylation. Arch. Biochem. 94: 280, 1961.
408. POSNER, H. S., MITOMA, C. AND UDENFRIEND, S.: Enzymic hydroxylation of aromatic compounds. II. Further studies of the properties of the microsomal hydroxylating system. Arch. Biochem. 94: 269, 1961.
409. REIN, H., RISTAU, O. UND JUNG, F.: Über den Nachweis von Nitrosohämoglobin mit der Elektronenspinresonanz bei der intrazellulären Oxydation des Hämoglobins mit Natriumnitrit. Folia haemat., Lpz. 82: 191, 1964.
410. REMMER, H.: Über die Reaktion des Nitritis mit Blutfarbstoff. Diss. Berlin, 1944.
411. REMMER, H.: Die Reaktion zwischen Globin und Ferricyanid. Biochem. Z. 330: 232, 1958.
412. REMMER, H.: Der beschleunigte Abbau von Pharmaka in den Lebermikrosomen unter dem Einfluss von Luminal. Arch. exp. Path. Pharmak. 235: 279, 1959.
413. REMMER, H. UND MEYER-WILMES, R. A.: Die Oxydation von Hämoglobin durch Ferricyanid. Biochem. Z. 330: 218, 1958.
414. ROSS, J. AND DESFORGES, J.: Erythrocyte glucose-6-phosphate dehydrogenase activity and methemoglobin reduction. J. Lab. clin. Med. 54: 450, 1959.
415. ROSTORFER, H. H. AND CORMIER, M. J.: Formation of "hydrogen peroxide" in the reaction of oxyhemoglobin with methemoglobin forming agents. Arch. Biochem. 71: 235, 1957.
416. ROUGHTON, F. J. W.: The kinetics of hemoglobin. VI. The competition of carbon monoxide and oxygen for hemoglobin. Proc. roy. Soc. B. 115: 473, 1934.
417. RUBIN, A., TEPHLY, T. R. AND MANNERING, G.: Kinetics of drug metabolism by hepatic microsomes. Biochem. Pharmacol. 13: 1007, 1964.
418. RUSSEL, G. A. AND GEEDS, E. J.: Paramagnetic intermediates in the condensation of nitrosobenzene and phenylhydroxylamine. J. Amer. chem. Soc. 87: 122, 1965.
419. RYAN, K. J. AND ENGEL, L. L.: Hydroxylation of steroids at carbon 21. J. biol. Chem. 225: 103, 1957.
420. SASS-KORTSAK, A., THALME, B. AND ERNSTER, L.: Mechanism of the vitamin K_3 induced glutathione instability in human red blood cells. Possible intermediary role of methemoglobin. Acta chem. scand. 14: 1847, 1960.
421. SASS-KORTSAK, A., THALME, B. AND ERNSTER, L.: Hemolytic activity of vitamin K_3: evidence for a direct effect on cellular enzymes. Nature 193: 480, 1962.
422. SASS-KORTSAK, A., THALME, B. AND ERNSTER, L.: Drug-induced hemolytic anemias and the mechanism and significance of Heinz body formation in red blood cells. Nature 196: 1096, 1962.
423. SATTELMACHER, P. G.: Methämoglobinämie durch Nitrate im Trinkwasser. Schriftenreihe d. Vereins f. Wasser-, Boden- und Lufthygiene Nr. 20 Fischer, Stuttgart, 1962.
424. SCOTT, E. M., DUNCAN, I. W. AND EKSTRAND, V.: The reduced pyridine nucleotide dehydrogenases of human erythrocytes. J. biol. Chem. 240: 481, 1965.
425. SCOTT, E. M. AND MCGRAW, J. C.: Purification and properties of diphosphopyridine nucleotide diaphorase of human erythrocytes. J. biol. Chem. 237: 249, 1962.
426. SCUDI, J. V. AND BUHS, R. P.: Reactions of 2-methyl-1,4-naphthoquinone with whole blood and plasma studied by means of a rapid colorimetric method. J. biol. Chem. 144: 599, 1942.
427. SEAL, U. S. AND GUTMANN, H. R.: The metabolism of the carcinogen N-(2-fluorenyl)acetamide by liver cell fractions. J. biol. Chem. 234: 648, 1959.
428. SEARLE, C. E. AND WOODHOUSE, D. L.: The carcinogenicity and reactivity to tissue constituents of 4-nitroquinoline-N-oxide. Acta Un. int. Cancr. 19: 519, 1963.
429. SEIDE, G.: Über die Wirkung von Blutgiften auf die Katalase. Biochem. Z. 308: 175, 1941.
430. SHIRASU, Y. AND OHTA, A.: A preliminary note on the carcinogenicity of 4-hydroxyaminoquinoline-1-oxide. Gann 54: 221, 1963.
431. SHRAGO, E. AND FALCONE, A. B.: Human-erythrocyte reduced triphosphopyridine nucleotide oxidase. Biochim. biophys. Acta 67: 147, 1963.
432. SIEBURG, E.: Über das Verhalten von Phenylhydroxylamin und dessen Nitrosoderivat im Organismus. Hoppe-Seyl. Z. 92: 331, 1914.
433. SIMON, C., MANZKE, H., KAY, H. UND MROWETZ, G.: Über Vorkommen, Pathogenese und Möglichkeiten zur Prophylaxe der durch Nitrit verursachten Methämoglobinämie. Z. Kinderheilk. 91: 124, 1964.

434. SINGLEY, T. L.: Secondary methemoglobinemia due to the adulteration of fish with sodium nitrite. Ann. intern. Med. 57: 800, 1962.
435. SINIOS, A.: Methämoglobinämie durch nitrithaltigen Spinat. Münch. med. Wschr. 106: 1180, 1964.
436. SINIOS, A. UND WODSAK, W.: Die Spinatvergiftung des Säuglings. Dtsch. med. Wschr. 90: 1856, 1965.
437. SMITH, J. W. AND WALLER, J. G.: Polarographic behavior of aromatic nitro compounds. II. Nitrosobenzene and N-phenylhydroxylamine. Trans. Faraday Soc. 46: 290, 1950.
438. SMITH, J. N. AND WILLIAMS, R. T.: Studies on detoxication. 16. The metabolism of acetanilide in the rabbit. Biochem. J. 42: 538, 1948.
439. SMITH, J. N. AND WILLIAMS, R. T.: The metabolism of phenacetin (*p*-ethoxyacetanilide) in the rabbit and a further observation on acetanilide metabolism. Biochem. J. 44: 239, 1949.
440. SMITH, J. N. AND WILLIAMS, R. T.: The fate of aniline in the rabbit. Biochem. J. 44: 242, 1949.
441. SMITH, J. N. AND WILLIAMS, R. T.: The metabolism of *p*-phenetidine (*p*-ethoxyaniline) with some observations on the anisidines (methoxyanilines). Biochem. J. 44: 250, 1949.
442. SPICER, S. S.: Species differences in susceptibility to methemoglobin formation. J. Pharmacol. 99: 185, 1950.
443. SPICER, S. S., HANNA, C. H. AND CLARK, A. M.: Studies *in vitro* on methemoglobin reduction in dog erythrocytes. J. biol. Chem. 177: 217, 1949.
444. SPICER, S. S. AND NEAL, P. A.: The effect of hypoxia on the *in vivo* formation of methemoglobin by aniline and nitrite. J. Pharmacol. 95: 438, 1949.
445. SPICER, S. S. AND REYNOLDS, H.: Individual and age variation in methemoglobin formation and reduction in rabbit erythrocytes. Amer. J. Physiol. 159: 47, 1949.
446. SPIRTES, M. A.: Further effects of Mg^{++} on drug and NADPH oxidation by rabbit liver microsomes. Fed. Proc. 23: 538, 1964.
447. SUBBOTIN, F. N.: Water and alimentary methemoglobinemia in children. Materialy Resp. Itog. Nauchn. Konf. po Gigiene, Leningrad, Sb. 1963: 138; Chem. Abstr. 62: 12359, 1965.
448. SVARTZ, N. ET KALLNER, S.: La cyanose dans le traitement par la sulfonamide. Acta med. scand. Suppl. 123: 44, 1941.
449. SZARA, S. AND AXELROD, J.: Hydroxylation and N-demethylation of N,N-dimethyltryptamine. Experientia 15: 216, 1959.
450. SCHEFF, G. J.: The influence of partial and complete hepatectomy on methemoglobin formation by aniline and *p*-aminophenol. J. Pharmacol. 70: 334, 1940.
451. SCHELER, W.: Die Bindung von Nitrosobenzolderivaten an menschliches Hämoglobin. Naturwissenschaften 47: 399, 1960.
452. SCHELER, W.: Die Bindung von Nitrosobenzol und Nitrosobenzolderivaten an Hämoglobin und Hämoglobin-Verbindungen. Acta biol. med. germ. 5: 382, 1960.
453. SCHEUCH, D., JACOBASCH, K. H., HÄNEL, A. UND WAGENKNECHT, L.: Der Einfluss von Methylenblau auf Glutathion und die Aktivität einiger SH-Enzyme. Acta biol. med. germ. 11: 616, 1963.
454. SCHLIMME, H.: Studien über Methämoglobinbildung. XXVI. Phenyl- und Tolylhydroxylamin. Arch. exp. Path. Pharmak. 202: 60, 1943.
455. SCHMIDT-NIELSEN, K. AND LARIMER, J. L.: Oxygen dissociation curves of mammalian blood in relation to body size. Amer. J. Physiol. 195: 424, 1958.
456. SCHMIEDEBERG, O.: Über das Verhältnis des Ammoniaks und der primären Monoaminbasen zur Harnstoffbildung im Thierkörper. Arch. exp. Path. Pharmak. 8: 1, 1877.
457. SCHÜLER, H.: Über die Oxydation des Hämoglobineisens durch Ferricyankalium und das Gleichgewicht der Reaktion. Biochem. Z. 255: 474, 1932.
458. SCHWARTZ, A. S. AND RECTOR, E. J.: Methemoglobinemia of unknown origin in a two week old infant. Amer. J. Dis. Child. 60: 652, 1940.
459. SCHWEDTKE, G.: Einfluss des Alkohols auf die Methämoglobinbildung durch Anilin. Arch. exp. Path. Pharmak. 188: 130, 1937.
459a. SMITH, W. R. D. AND BALDWIN, R. W.: Carcinogenicity of aminostilbenes: N-hydroxylation of aminostilbenes. Carcinogenicity of N-hydroxy-4-acetylaminostilbene. A. R. Brit. Empire Cancer Campgn. 40: 431, 1962.
460. STADELMANN, E.: Die chronische Vergiftung mit Toluylendiamin. Weitere Beiträge zur Lehre vom Ikterus. Arch. exp. Path. Pharmak. 23: 427, 1887.
461. STAHL, E. K.: Toxische Wirkungen von 2,4 *m*-Toluylendiamin insbesondere auf das Blut. Diss. Würzburg, 1950.
462. STAHL, K. E. UND JUNG, F.: Über Blutgiftwirkungen des Phenylendiamins und des Toluylendiamins. Arch. exp. Path. Pharmak. 220: 503, 1953.
463. STAUDINGER, H. UND ZUBRZYCKI, Z.: Zur Kinetik der mikrosomalen NADPH-Oxydation. Hoppe-Seyl. Z. 332: 109, 1963.
464. STAUDINGER, H. UND ZUBRZYCKI, Z.: Zur Kinetik der mikrosomalen NADPH-Oxydation bei verschiedenen Sauerstoffdrucken. Hoppe-Seyl. Z. 340: 191, 1965.
465. STÖFFLER, G., THAUER, R. K. UND UEHLEKE, H.: Methämoglobinbildung durch *p*-Hydroxylamino- und *p*-Nitrosobenzolsulfonamid in Nabelschnur- und Erwachsenen-Erythrocyten. Arch. exp. Path. Pharmak. 252: 359, 1966.
466. STRÖMME, J. H.: Methemoglobin formation induced by thiols. Biochem. Pharmacol. 12: 937, 1963.
467. TAKEMORI, A. E. AND MANNERING, G. J.: Metabolic N- and O-demethylation of morphine- and morphinan-type analgesics. J. Pharmacol. 123: 171, 1958.
468. TEPPERMAN, J., MARQUARDT, R., REIFENSTEIN, G. H., AND LOZNER, E. L.: Methemoglobinemic cyanosis. J. Amer. med. Ass. 146: 923, 1951.
469. TERAYAMA, H.: Aminoazo dye amino-oxide as a possible intermediate metabolite preceeding to N-demethylation and ortho-hydroxylation as well as azodye-protein binding. Acta Un. int. Cancr. 19: 534, 1963.

470. TERAYAMA, H.: Amino azo dye amine-N-oxide as a possible intermediate metabolite preceeding N-demethylation and ortho-hydroxylation, as well as azo dye-protein binding. Gann **54**: 195, 1963.
471. THAUER, R. K., STÖFFLER, G. UND UEHLEKE, H.: N-Hydroxylierung von Sulfanilamid zu p-Hydroxylaminobenzolsulfonamid durch Lebermikrosomen. Arch. exp. Path. Pharmak. **252**: 32, 1965.
472. THORPE, W. V. AND WILLIAMS, R. T.: The synthesis of some possible biological oxidation products of sulfanilamide. Biochem. J. **35**: 61, 1941.
473. TODD, J. R. AND THOMPSON, R. H.: Methemoglobin in chronic copper poisoning of sheep. Nature **191**: 89, 1961.
474. TOMPSETT, S. L.: The determination in urine of some metabolites of tryptophan-kynurenine, anthranilic acid, and 3-hydroxyanthranilic acid- and reference to the presence of *o*-aminophenol in urine. Clin. Chim. Acta **4**: 411, 1959.
475. TRIVUS, R. H.: Mg^{++}, a possible cofactor for the microsomal drug oxidation. Fed. Proc. **23**: 538, 1964.
476. TRIVUS, R. H. AND SPIRTES, M. A.: Mg^{++}, a requirement for some microsomal drug oxidation. Biochem. Pharmacol. **13**: 1679, 1964.
477. TROLL, W., BELMAN, S. AND RINDE, E.: N-Hydroxy acetylamino compounds, urinary metabolites of aromatic amines in man. Proc. Amer. Ass. Cancer Res. **4**: 68, 1963.
478. TROLL, W. AND NELSON, N.: N-Hydroxy-2-naphthylamine, a urinary metabolite of 2-naphthylamine in man and dog. Fed. Proc. **20**: 41, 1961.
479. UEHLEKE, H.: Biologische Oxydation und Reduktion am Stickstoff aromatischer Amino- und Nitroderivate und ihre Folgen für den Organismus. Fortschr. Arzneimittelforsch. **8**: 195, 1965.
480. URQUHART, M. E.: The metabolism of 2-acetamidofluorene in the guinea pig. Brit. J. Cancer **9**: 611, 1955.
481. VANDENBELT, J. M., PFEIFFER, C., KAISER, M. AND SIBERT, M.: Methemoglobinemia after administration of *p*-aminoacetophenone and *p*-aminopropiophenone. J. Pharmacol. **80**: 31, 1944.
482. WAGNER, J., HYKEŠ, P., JANATA, V. UND KÁCL, K.: Die Wirkung von Hydroxylamin und Phenylhydroxylamin auf die GSH-Konzentration und die Bedeutung für den Stoffwechsel der Erythrocyten. Acta biol. med. germ. Suppl. **3**: 312, 1964.
483. WAHLER, B. E., SCHOFFA, G. UND THOM, H. G.: Nachweis von Radikal-Zwischenstufen bei der Hämoglobinoxydation nach Einwirkung aromatischer Hydroxylamine. Arch. exp. Path. Pharmak. **236**: 20, 1959.
484. WAISMAN, H. A., BAIN, J. A., RICHMOND, J. B. AND MUNSEY, F. A.: Laboratory and clinical studies in congenital methemoglobinemia. Pediatrics **10**: 293, 1952.
485. WALLER, H. D., LÖHR, G. W. UND TABATABAI, M.: Hämolyse und Fehlen von Glucose-6-Phosphatdehydrogenase in roten Blutzellen. Klin. Wschr. **35**: 1022, 1957.
486. WARBURG, O., KUBOWITZ, F. UND CHRISTIAN, W.: Über die Wirkung von Phenylhydrazin und Phenylhydroxylamin auf den Stoffwechsel der roten Blutzellen. Biochem. Z. **242**: 170, 1931.
487. WARBURG, O., SCHRÖDER, W. UND GATTUNG, H. W.: Über die Wirkung von Röntgenstrahlen auf Hämoglobin. Z. Naturf. **15b**: 163, 1960.
488. WEISBURGER, J. H., GRANTHAM, P. H., VANHORN, E., STEIGBIGEL, N. H., RALL, D. P. AND WEISBURGER, E. K.: Activation and detoxification of N-2-fluorenylacetamide in man. Cancer Res. **24**: 475, 1964.
489. WEISBURGER, J. H., GRANTHAM, P. H. AND WEISBURGER, E. K.: The metabolism of N-2-fluorenylacetamide in the cat: evidence for glucuronic acid conjugates. Biochem. Pharmacol. **13**: 469, 1964.
490. WEISBURGER, J. H., GRANTHAM, P. H. AND WEISBURGER, E. K.: Metabolism of N-2-fluorenylacetamide in the hamster. Toxicol. appl. Pharmacol. **6**: 427, 1964.
491. WEISBURGER, E. K., GRANTHAM, P. H. AND WEISBURGER, J. H.: Differences in the metabolism of N-hydroxy-N-2-fluorenylacetamide in male and female rats. Biochemistry **3**: 808, 1964.
491a. WEISBURGER, J. H., GRANTHAM, P. A. AND WEISBURGER, E. K.: Transport of carcinogens. Rat blood plasma and red cell binding of isotope after N-hydroxy-N-2-fluorenyl acetamide. Life Sci. **5**: 41, 1966.
492. WEISBURGER, J. H., PAI, S. R. AND YAMAMOTO, R. S.: Pituitary hormones and liver carcinogenesis with N-hydroxy-N-2-fluorenylacetamide. J. nat. Cancer Inst. **32**: 881, 1964.
493. WEISBURGER, E. K. AND WEISBURGER, J. H.: Chemistry, carcinogenicity, and metabolism of 2-fluorenamine and related compounds. Advanc. Cancer Res. **5**: 331, 1958.
494. WEISBURGER, J. H. AND WEISBURGER, E. K.: Endogenous and exogenous factors in chemical carcinogenesis by N-2-fluorenylacetamide. Acta Un. int. Cancr. **19**: 513, 1963.
495. WEISBURGER, J. H., WEISBURGER, E. K., GRANTHAM, P. H. AND MORRIS, H. P.: N-(6-Hydroxy-2-fluorenyl)-acetamide, a urinary metabolite after intraperitoneal injection of N-2-fluorenylacetamide into rats. J. biol. Chem. **234**: 2138, 1959.
496. WEISBURGER, J. H., WEISBURGER, E. K. AND MORRIS, H. P.: Urinary metabolites of the carcinogen N-2-fluorenylacetamide. J. nat. Cancer. Inst. **17**: 345, 1956.
497. WEISBURGER, J. H., WEISBURGER, E. K. AND MORRIS, H. P.: Orientation of biochemical hydroxylation in aromatic compounds. Science **125**: 503, 1957.
498. WEISBURGER, J. H., WEISBURGER, E. K. AND MORRIS, H.: Differences in the metabolism of N-2-fluorenylacetamide in the guinea pig and rat. Cancer Res. **18**: 1039, 1958.
499. WEISBURGER, J. H., WEISBURGER, E. K., MORRIS, H. P. AND SOBER, H. A. Chromatographic separation of some metabolites of the carcinogen N-2-fluorenylacetamide. J. nat. Cancer Inst. **17**: 363, 1956.
500. WEISS, J.: The free radical mechanism in the reactions of hydrogen peroxide. Advanc. Catalys. **4**: 343, 1952.
501. WEISS, J.: The autoxidation of ferrous ions in aqueous solution. Experientia **9**: 61, 1953.
502. WEISS, J. J.: Nature of the iron bond in oxyhaemoglobin. Nature **202**: 82, 1964.
503. WERNER, U., THAL, W. UND W. WUTTKE, W.-D.: Schwerste und lethal verlaufene Methämoglobinämien durch nitrathaltiges Brunnenwasser bei jungen Säuglingen. Dtsch. med. Wschr. **90**: 124, 1965.

504. WILEY, F. H.: The metabolism of β-naphthylamine. J. biol. Chem. **124**: 627, 1938.
505. WILLIAMS, R. T.: The biosynthesis of aminophenyl- and sulfonamidoaminophenylglucuronides in the rabbit and their action on hemoglobin *in vitro*. Biochem. J. **37**: 329, 1943.
506. WILLIAMS, R. T.: The isolation and identification of 3-hydroxysulfanilamide as the oxidation product of sulfanilamide in the rabbit. Biochem. J. **40**: 219, 1946.
507. WILLIAMS, R. T.: The detection of 3-hydroxysulfanilamide in the urine of hospital patients treated with sulfanilamide. Biochem. J. **41**: 1, 1947.
508. WILLIAMS, R. T.: Detoxication mechanisms. Chapman and Hall Ltd., London, 1959.
508. WILLIAMS, R. T.: Detoxication mechanisms. London, 1959.
509. WILLIAMS, C. H. AND KAMIN, H.: Microsomal triphosphopyridine nucleotide-cytochrome *c* reductase of liver. J. biol. Chem. **237**: 587, 1962.
510. WISEMAN, R. AND IRVING, C. C.: Carcinogenicity of 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene in the rabbit. Proc. Amer. Ass. Cancer Res. **5**: 69, 1964.
511. WONG, D. T. AND TERRIERE, L. C.: Epoxidation of aldrin, isodrin, and heptachlor by rat liver microsomes. Biochem. Pharmacol. **14**: 375, 1965.
512. ZIEGLER, C.: Bildung von Hämiglobin unter der Einwirkung N-acylierter Derivate des Phenylhydroxylamins auf Erythrocyten. Diss. Berlin, 1953.
513. ZIEGLER, D. M. AND PETTIT, F. H.: Formation of an intermediate N-oxide in the oxidative demethylation of N,N-dimethylaniline catalyzed by liver microsomes. Biochem. biophys. Res. Commun. **15**: 188, 1964.
514. ZIEGLER, D. M. AND PETTIT, F.: Properties of a liver microsomal N-oxide demethylase. Fed. Proc. **23**: 325, 1964.
515. ZIEGLER, D. M., PETTIT, F. H. AND ORME-JOHNSON, W. H.: Properties of an alkylarylamine oxygenase concentrated from pork liver microsomes. Fed. Proc. **24**: 604, 1965.
516. ZINKHAM, W. H.: An *in-vitro* abnormality of glutathione metabolism in erythrocytes from normal newborns· mechanism and clinical significance. Pediatrics **22**: 18, 1959.