The Formation of Hydrogen Carriers by Haematin-Catalyzed Peroxidations

1. HYDROGEN CARRIERS FROM CERTAIN ACRIDINE AND QUINOLINE COMPOUNDS

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(Received 3 June 1948)

During studies of the inhibition of enzymes by anti-bacterial acridines, it was found that certain of these drugs caused a large stimulation of oxygen uptake in solutions containing ascorbic acid, cytochrome *c* and cytochrome oxidase. The same oxygen uptake occurred when the oxidase was omitted, and hence this enzyme played no part in the reaction. The studies which follow were, therefore, made in the absence of cytochrome oxidase.

Because the new effect interfered with the desired measurement of enzyme inhibitions, and, because it had possible importance in regard to the biological action of acridine compounds, its mechanism was studied in some detail. The effect occurred whenever certain amino- or hydroxy-acridines or analogous quinolines were shaken with ascorbic acid, cytochrome *c* and air. Most of the detailed studies which follow were made with 3-aminoacridine.

A brief outline of the apparent mechanism is given here. The new effect is characterized by a primary phase during which the rate of oxygen uptake increases to a maximum, and a secondary phase during which this maximum rate is maintained until the substrate is exhausted, whereupon the uptake of oxygen ceases. The initiation of the effect depends on a trace of hydrogen peroxide (the origin of which is discussed below) which, in the presence of cytochrome *c*, brings about a catalyzed peroxidative oxidation. For example, 3-aminoacridine is oxidized to an *o*-hydroxyaminoacridine which is, in turn, autooxidizable to an *o*-quinonimine with production of hydrogen peroxide. By utilization of this hydrogen peroxide in the peroxidative reaction the effect becomes autocatalytic. The *o*-quinonimine acts as a hydrogen carrier in the oxidation of the substrate and oxygen is the ultimate hydrogen acceptor.

A similar series of reactions was found to occur when cytochrome *c* was replaced by methaemoglobin, so that the initial reaction is evidently a nonspecific haematin peroxidative effect. Similar effects were found with cysteine as substrate but not with hydroquinone. There was no relation between the antibacterial activity of the acridine and quinoline compounds and their ability to give the effect.

EXPERIMENTAL

Materials. Ascorbic acid was used as the pure substance, kindly supplied by the Colonial Sugar Refining Co., Sydney; it was determined by titration with 2:6-dichlorophenolindophenol (cf. Lemberg, Cortis-Jones & Norrie, 1938). Cysteine was the Schering-Kahlbaum copper-free preparation. Hydrogen peroxide (100 vol.) was freshly diluted before use and standardized with 0.01N-KMnO₄. Water for all purposes was double distilled from an all glass still. The catalase was a preparation from horse liver, with a *Kat.-f.* of 25,000. The cytochrome *c* contained 0.33% Fe and was obtained from ox heart by method (a) of Keilin & Hartree (1945). It was dialyzed against distilled water in Tee-Pak synthetic sausage casings (Harvey Little and Co., Sydney) which adsorbed very little cytochrome. The catalase-free methaemoglobin solution was prepared as follows. A solution of pure crystalline haemin in dilute Na₂CO₃ was accurately determined spectrophotometrically as pyridine haemochromogen. A solution of native human globin was prepared by the method of Anson & Mirsky (1925) and its protein content determined. The solutions of haemin and globin were mixed so that there was 10% excess of globin. For the manometric experiments a volume of this solution equivalent to 1.0 mg. of methaemoglobin was used. In terms of Fe content, this was equivalent to 1 mg. of cytochrome *c*.

Aniline (b.p. 184°) and phenol (b.p. 182°) were freshly distilled. The m.p. of the *o*-phenylenediamine was 103°; *p*-phenylenediamine, 139°; catechol, 104°; *o*-aminophenol, 170° and *p*-aminophenol, 184°. The preparations and m.p.'s of the acridine compounds listed in Table 3 were as given by Albert, Rubbo, Goldacre, Davey & Stone (1945), and of the quinoline compounds as given by Albert & Magrath (1947) with the following additions: 3:5-dihydroxyacridine (m.p. 347–348°) was prepared according to Ullmann & Kipper (1907); 5-aminoquinoline (m.p. 110°) by the hydrogenation of 5-nitroquinoline over Raney nickel; 5-hydroxyquinoline (m.p. 223°) by the method of Claus & Howitz (1893) and 5-amino-6-hydroxyquinoline (m.p. 195°, decomp.) by the method of Mathéus (1888). Phenol blue, *p*-dimethylaminoaniline hydrochloride and *p*-diethylaminoaniline hydrochloride were obtained from British Drug Houses Ltd.

Methods. The measurements of O₂ uptake were made in Warburg respirometers at pH 7.0 and 25 or 37.5°. The shaking rate was 120/min., the gas phase usually air, and, in the total liquid volume of 2.0 ml., the phosphate buffer was 0.12M; changing the buffer concentration did not affect the results. Experimental pH values were checked with a glass

electrode. All solutions were prepared just prior to use. The basic compounds were dissolved by the aid of 1-1.5 equiv. of HCl, and the hydroxy compounds, when necessary, with 1 equiv. of NaOH. The ascorbic acid was dissolved in water, with the addition of 1 equiv. of 0.1N-NaOH, just before pipetting.

RESULTS

The effect

The nature of the effect is summarized in Fig. 1, curve 1 showing the typical autocatalytic reaction. When the ascorbic-acid concentration was halved, the total oxygen uptake was also approximately halved (curve 2).

Fig. 1. The effect obtained with 3-aminoacridine, sodium ascorbate and cytochrome *c* at pH 7.0 and 25°. Curve 1: 3-aminoacridine, ascorbate and cytochrome *c* (concentrations in Table 1). Curve 1a: uptake on addition of a second lot of ascorbate after reaction 1. Curve 2: as curve 1 with ascorbate concentration halved. Curve 3: the rate of utilization of ascorbate determined by titration (see text). Curve 4: 80% of the precipitate from reaction 1, isolated as described in text, and allowed to react with ascorbate only; addition of cytochrome *c* made no difference. Curve 5: autooxidation controls, with ascorbate. Curve 6: autooxidation controls without ascorbate.

When the reaction was stopped at successive time intervals by the addition of trichloroacetic acid, and the amount of ascorbic acid used determined by titration, the rate of its utilization was parallel to the rate of oxygen uptake; in curve 3 this rate is plotted as the calculated amount of oxygen required to oxidize (to dehydroascorbic acid) the ascorbic acid used up in the given times. The calculated amount of oxygen required to oxidize all the ascorbic acid to dehydroascorbic acid was 125 μ l., and the total uptake in curve 1 shows that it was not oxidized past this stage. In a small number of experiments with cysteine as substrate, a similar effect was found, the total oxygen uptake then being only slightly

more than that required to produce cystine. Hydroquinone gave no oxygen uptake at all, although it is a substrate for the full cytochrome *c*-cytochrome oxidase system.

When the effect occurred, a dark-coloured precipitate was usually observed at the end of the reaction. This precipitate dissolved, with loss of the dark colour, on addition of further ascorbic acid, and reappeared when this had been oxidized. It was similarly dissolved by the addition of cysteine or sodium dithionite (hydrosulphite). The precipitate, isolated by centrifuging and washed well at the centrifuge with cold phosphate buffer, pH 7.0, catalyzed the oxidation of ascorbic acid by oxygen (curve 4). Addition of cytochrome *c* made no difference to the rate, unless some unchanged test substance was added with an incompletely washed precipitate, in which case cytochrome *c* caused a significant increase. The precipitate lost its catalytic activity entirely when kept in the buffer for 2 days at 0°. Its colour, its behaviour on reduction and re-oxidation and its instability strongly suggested a substance of quinonoid type.

Table 1. The effect of varying the concentration of 3-aminoacridine and of cytochrome *c*, at pH 7.0 and 25°

(Each flask contained hydrogen peroxide, 10^{-3} M, sodium diethyldithiocarbamate 6.4×10^{-3} M, and sodium ascorbate 5.6×10^{-3} M, as well as, in experiment (a), cytochrome *c* 2×10^{-5} M and, in experiment (b), 3-aminoacridine 10^{-3} M.)

(a)	
3-Aminoacridine M	O ₂ uptake in 30 min. (μ l.)
0	0
10^{-5}	12
10^{-4}	52
10^{-3}	98
(b)	
Cytochrome <i>c</i> M $\times 10^{-5}$	
0	0
0.18	14
0.54	47
0.90	79
1.80	105

It was important to determine whether the precipitate contained cytochrome *c* or a derivative, e.g. an autoxidizable haem compound. No absorption band whatever could be seen in a suspension of well washed, active precipitate, or in a solution made by reducing it with sodium dithionite. Nor could iron be detected in the precipitate after digestion with concentrated sulphuric acid and hydrogen peroxide. If the precipitate were a compound or mixture of 3-aminoacridine and cytochrome *c* in molecular ratios 100:1, iron could readily have been detected under the conditions of the test. The precipitate must, therefore, be an iron-free derivative of 3-aminoacridine.

Studies of the mechanism

In the presence of either cytochrome *c* or met-haemoglobin, the effect was inhibited by catalase on the one hand (Table 2), and stimulated by hydrogen peroxide on the other (Fig. 2). In the absence of a haematin compound the effect did not occur, and was not induced by hydrogen peroxide (Table 2). There was thus strong evidence for the occurrence of a haematin-catalyzed peroxidative reaction.

Under certain conditions, either the 3-aminoacridine concentration or the cytochrome *c* concentration could limit the rate of reaction (Table 1). In these experiments hydrogen peroxide was added so that there was no lag, and the initial rates were comparable.

Cytochrome *c* does not combine with carbon monoxide, but haemoglobin formed by reduction of methaemoglobin by ascorbic acid, combines with carbon monoxide to form carboxyhaemoglobin in which the iron is fixed in the reduced state. In a gas phase of 80 % CO + 20 % O₂, the rates were only slightly lower than when the gas phase was air. A similar slight CO inhibition was found when cytochrome *c* was the catalyst (Table 2). Carbon monoxide is known to inhibit the copper-catalyzed autoxidation of ascorbic acid (Barron, DeMeio & Klemperer, 1936), and in our control experiments autoxidation was inhibited by carbon monoxide as well as by diethyldithiocarbamate. The latter reagent also slightly depressed the effect in the presence of cytochrome *c* (Table 2). The autoxidation of ascorbic acid produces hydrogen peroxide, and the CO and diethyldithiocarbamate inhibitions of the effect may be due to their prevention of the formation

of this hydrogen peroxide. If this was a limiting factor, the rate during the autocatalytic stage would be depressed and the lag lengthened. This was

Fig. 2. The effect obtained with 3-aminoacridine and different concentrations of hydrogen peroxide at pH 7.0 and 25°. Each flask contained 3-aminoacridine, cytochrome *c* and ascorbate with concentrations as in Table 1. Curves 1-7: hydrogen peroxide: nil, 0.01, 0.1, 0.25, 0.5, 1.0 and 10.0 $\times 10^{-4}$ M respectively.

Table 2. *The effect of various reagents on the rate of oxygen uptake of ascorbic acid with 3-aminoacridine at pH 7 and 37.5°*

(Each flask contained, in separate side arms, sodium ascorbate (final molarity 5.6×10^{-3}) and 3-aminoacridine (final molarity 2×10^{-3}). These were tipped on to the reagents indicated in column 1 after equilibration. Molarity of cytochrome *c*, 0.9×10^{-5} ; of methaemoglobin, 0.22×10^{-5} ; of H₂O₂, 1.0×10^{-3} ; of CuSO₄, 2.0×10^{-5} ; of sodium diethyldithiocarbamate 6.4×10^{-3} . Values of *t* (time to reach maximum rate) were accurately reproduced to ± 3 min., and of *v* (the maximum rate) to at least $\pm 0.5 \mu\text{l.}/\text{min.}$, accuracy increasing as *v* became smaller.)

Added to main bulb	Gas phase	<i>t</i> (min.)	<i>v</i> ($\mu\text{l. O}_2/\text{min.}$)
Nil	Air	0	0.5
Nil	CO-O ₂	0	0
Diethyldithiocarbamate	Air	0	0
Cytochrome <i>c</i>	Air	35	5.5
Cytochrome <i>c</i>	CO-O ₂	55	3.0
Cytochrome <i>c</i> + H ₂ O ₂	Air	0	18.3
Cytochrome <i>c</i> + catalase	Air	65	2.0
Cytochrome <i>c</i> + diethyldithiocarbamate	Air	43	5.0
Cytochrome <i>c</i> + CuSO ₄	Air	0	5.0
Cytochrome <i>c</i> + CuSO ₄	CO-O ₂	0	4.0
Methaemoglobin	Air	45	3.6
Methaemoglobin	CO-O ₂	60	2.9
Methaemoglobin + H ₂ O ₂	Air	0	18.0
Methaemoglobin + catalase	Air	0	0.6

indirectly confirmed by addition of small amounts of copper sulphate. As shown in Table 2, the lag was abolished; in the presence of copper the reaction was again inhibited to some extent by carbon monoxide.

Although hydrogen peroxide could arise from autoxidation of the ascorbic acid, it was realized that this was not necessarily the source of the peroxide which initiated the effect. The effect still occurred in the presence of carbon monoxide or diethyldithiocarbamate, although autoxidation was then negligible. In addition, when the 3-aminoacridine was shaken with the cytochrome *c* for 30 min. before adding the ascorbic acid, considerable darkening in colour had occurred, and the lag was shorter than when the reagents were mixed immediately after equilibration as was usual. This darkening was inhibited by catalase.

This formation of carrier in the absence of ascorbic acid suggested that a trace of hydrogen peroxide was introduced from another source. It was found that the cytochrome *c* solutions, prepared as above, gave an output of oxygen gas on the addition of catalase. It is not yet known at what stage of the preparation this peroxide arose, and, in view of the importance of the presence of this unsuspected contaminant in preparations of cytochrome *c* made in accordance with published instructions, this question is now under investigation.

The chemical change in the peroxidative reaction

The following (I, II, III) are the reversible hydrogen-carrying systems which appear most likely to be formed by oxidation of 3-aminoacridine.

In order to explore these possibilities, it was decided to examine the series of acridine and quinoline compounds given in Table 3. The ionization (as bases) under the conditions of the experiment have been included in the table as this was found to be relevant. The antibacterial activities of these compounds are also recorded to show that the well-known antibacterial properties of some members of these series do not run parallel to their ability to become carriers.

The results obtained from the acridine compounds are given in Table 4 and those from the quinoline compounds in Table 5.

Compounds of the types (I–III) could be formed from any of the acridine compounds which gave the effect (Table 4); quinones would be the corresponding oxidized forms of the carriers derived from hydroxyacridines.

Carriers of type (I) were excluded, however, since neither 3-amino-5-hydroxyacridine nor 3:5-dihydroxyacridine (nos. 15 and 16) had any carrier activity. Oxidation in the position ortho to the existing amino or hydroxyl group thus seemed more likely.

Since acridine compounds of types (II) and (III) are unknown and likely to be extremely difficult of access, attention was concentrated on the quinoline

compounds (Table 5). From 5-aminoquinoline, which gave an effect similar to 3-aminoacridine, only one ortho-substituted compound (IV) is possible.

This compound, 5-amino-6-hydroxyquinoline, was synthesized, and was found to act directly as a reversible hydrogen carrier in the oxidation of ascorbic acid; 7-amino-8-hydroxyquinoline acted similarly, as also did the indamine dye, phenol blue.

There is thus good reason to believe that the carrier formed from 5-aminoquinoline is the readily autoxidizable substance, 5-amino-6-hydroxyquinoline. It became important to determine whether, as assumed above, autoxidation of compounds of this type gives rise to hydrogen peroxide (as in V).

When solutions in phosphate buffer, pH 7, of 5-amino-6-hydroxyquinoline and 7-amino-8-hydroxyquinoline were shaken with air at 25 or 37.5°, there was with the latter compound a fast uptake of oxygen, the full theoretical amount being consumed in about 1 hr. The former consumed oxygen more slowly, and, in 1 hr., only 75% of the theoretical amount had been taken up. When catalase was added after 1 hr., there was in each case an oxygen output

Table 3. *The acridine and quinoline compounds used, their strength as bases and their antibacterial activity (where known)*

No.	Compound	pK _a , basic, 25° in water	Percentage ionized as base, 25° pH 7 (g)	B.I. (h)
1	Acridine	4.52 (a)	0.34	6
2	1-Aminoacridine	4.33 (a)	0.22	4
3	2-Aminoacridine	7.94 (a)	89.70	21
4	3-Aminoacridine	5.80 (a)	5.94	8
5	4-Aminoacridine	5.96 (a)	8.37	9
6	5-Aminoacridine	9.88 (a)	99.86	25
7	2:7-Diaminoacridine	8.02 (b)	91.2	26
8	2:8-Diaminoacridine	9.54 (b)	99.4	22
9	2:8-Diamino-1:9-dimethylacridine	9.05 (c)	99.11	30
10	2:8-Diamino-3:7-dimethylacridine	10.23 (c)	99.94	21
11	2:8-Bisdimethylaminoacridine	10.57 (c)	99.97	17
12	3-Amino-5-methylacridine	6.14 (c)	12.23	10
13	3-Hydroxyacridine	4.63 (d)	0.43	0
14	5-Hydroxyacridine (acridone)	—	<0.01 (d)	0
15	3:5-Dihydroxyacridine	—	<0.01	—
16	3-Amino-5-hydroxyacridine	—	—	—
17	5-Aminoquinoline	5.43 (e)	2.35	0
18	6-Aminoquinoline	5.54 (e)	3.36	0
19	5-Hydroxyquinoline	—	—	0
20	8-Hydroxyquinoline	4.95 (f)	0.95	26
21	5-Amino-6-hydroxyquinoline	—	—	—
22	7-Amino-8-hydroxyquinoline	—	—	—

(a) Calculated from values in water at 20° (Albert & Goldacre, 1946), by a temperature correction found by interpolation in a curve based on the data of Hall & Sprinkle (1932).

(b) Kindly determined for this work by Mr J. N. Phillips.

(c) Calculated from values in 50% ethanol (Albert & Goldacre, 1946), by adding 0.64, the mean depression by ethanol for 11 compounds (Albert & Goldacre, 1946). Temperature correction as in (a) then applied.

(d) Calculated from data of Albert & Goldacre (1943).

(e) Calculated from data of Albert & Goldacre (1944).

(f) Calculated from data of Albert & Magrath (1947).

(g) Calculated from the equation: % ionized = $\frac{100}{1 + \text{antilog}(\text{pH} - \text{pK}_a)}$.

(h) The Bacteriostatic Index (from Albert *et al.* 1945; Albert, Rubbo, Goldacre & Balfour, 1947). A B.I. of 15 indicates significant antibacterial activity, and a B.I. of 20 or higher, marked activity.

Table 4. *The results of testing various acridine compounds*

(The compounds were tested with ascorbic acid and cytochrome *c*, with air as gas phase, with concentrations and conditions as in Table 1. Where the effect was found, the reactions when hydrogen peroxide or catalase was added were similar to those shown in Table 2. Hydrogen peroxide did not induce the effect in the compounds in column 2.)

(1) Compounds which gave the effect at pH 7		(2) Compounds which were completely inactive	
No.		No.	
4	3-Aminoacridine	1	Acridine
5	4-Aminoacridine	2	1-Aminoacridine
7	2:7-Diaminoacridine	6	5-Aminoacridine
8	2:8-Diaminoacridine	10	2:8-Diamino-3:7-dimethylacridine
9	2:8-Diamino-1:9-dimethylacridine	11	2:8-Bisdimethylaminoacridine
13	3-Hydroxyacridine	12	3-Amino-5-methylacridine
3	2-Aminoacridine (at pH 8.5)	14	5-Hydroxyacridine (acridone)
		15	3:5-Dihydroxyacridine
		16	3-Amino-5-hydroxyacridine

Table 5. *The results of testing various quinoline compounds*

(The compounds in column 2 were directly active as carriers in the oxidation of ascorbic acid; the *v* values were for the O₂ uptake of 5.6 × 10⁻³ M ascorbic acid with the carrier 2 × 10⁻³ M, at pH 7.0 and 25°; there was no lag, and addition of cytochrome *c* made no difference. Of these compounds, only nos. 18 and 22 gave a precipitate (see text).)

(1) Compounds which gave the effect		(2) Compounds which were active even in the absence of cytochrome <i>c</i>		<i>v</i> (μl. O ₂ /min.)
No.		No.		
17	5-Aminoquinoline	21	5-Amino-6-hydroxyquinoline	17.2
18	6-Aminoquinoline	22	7-Amino-8-hydroxyquinoline	18.0
19	5-Hydroxyquinoline		Phenol blue (for comparison)	
20	8-Hydroxyquinoline			

corresponding to half the oxygen consumed as required by (V) and the equation,

That hydrogen peroxide is formed on autooxidation of these compounds at pH 7 was thus established. When *o*- and *p*-aminophenol were tested in the same way, however, no oxygen uptake or output was found with either.

The failure of some of the acridine compounds to give the effect must now be considered. The failure of 2-aminoacridine to yield any carrier in the usual test at pH 7 was surprising, in view of the activity of its derivatives, nos. 7 and 8 (Table 4). 3-Aminoacridine at pH 7 is mainly un-ionized, while 2-aminoacridine is ionized to the extent of 89.7%. Although 2:8-diaminoacridine exists almost entirely in the cationic form at pH 7, it is likely that a significant proportion of one amino group remains un-ionized. In 2:7-diaminoacridine, the 7-amino group does not enter into the ionic resonance and is entirely un-ionized (Albert & Goldacre, 1946). It thus seemed possible that 2-aminoacridine might give the effect if it could be tested with a higher proportion of its amino group in the un-ionized form. This proved to be the case (Table 6). At pH 8.5, when it was 78% un-ionized, it gave a carrier effect similar to that of 3-aminoacridine.

Although, in this group of substances, the presence of an un-ionized amino group appears to be one of the prerequisites for carrier formation, other factors also operate. For example, at pH 7 no. 2 is almost completely un-ionized but gives no carrier (Table 4).

A few benzenoid compounds were tested to find whether the effect would also occur in this series. The result was negative. Aniline, phenol, catechol, hydroquinone and *o*-phenylenediamine gave no carriers under any conditions. Phenol blue and 2:6-dichlorophenolindophenol acted directly as carriers in the well known manner, no cytochrome *c* being required. *p*-Phenylenediamine, *p*-dimethylaminoaniline, *p*-diethylaminoaniline and *p*-aminophenol all gave carriers which acted similarly to phenol blue in Table 5, provided that they were shaken with cytochrome *c* at 25° and pH 7 for 0.5 hr. before adding the ascorbic acid. Under these conditions they were oxidized to coloured compounds; on

adding the ascorbic acid the colour disappeared, but returned when the ascorbic acid had been oxidized. In the case of the first three compounds it was probable that the carriers were the Wurster's salts which they are known to yield on oxidation. It seemed that ascorbic acid, if added too soon, protected them from oxidation to this form.

DISCUSSION

For the effect to occur, it is evidently necessary that: (a) A peroxidative oxidation should take place in such a way that a hydroxyl group enters the acridine or quinoline nucleus ortho (or, conceivably, para) to an existing amino or hydroxyl group. (b) The product of this reaction should be of suitable oxidation-reduction potential to act as a reversible hydrogen carrier in the oxidation of ascorbic acid by oxygen.

In Table 4, a number of compounds which did not give the effect are shown. Comparison of nos. 4 and 12 is interesting; the 5-methyl group in no. 12 should make little difference to its oxidizability ortho to the amino group (i.e. in the 2- or 4-positions), or to the oxidation-reduction potential of the oxidized compound, but it is possible that the methyl group sterically hindered the peroxidative reaction. Similar considerations apply to no. 10 (compare no. 9) and to nos. 15 and 16 which have been discussed above. No. 6 has no oxidizable position ortho to its amino group.

The 3-aminoacridine effect appears to depend on the mechanism shown in the scheme on p. 135.

In the case of the hydroxy compounds (e.g. 3-hydroxyacridine) the reduced form of the reversible system is probably an *o*-dihydric phenol, and the oxidized form an *o*-quinone.

Evidence has been found that solutions of cytochrome *c*, prepared by the method of Keilin & Hartree (1945), may contain small amounts of hydrogen peroxide. This could make a considerable difference to *in vitro* studies, for example by initiating coupled oxidations, such as have been described above. Work which is proceeding on this subject will be reported separately.

Some examples will now be mentioned in which previous workers seem to have met the effect reported here without having recognized its true nature.

Table 6. *The effect with 2-aminoacridine at pH 8.5 and 25°*

(Each flask contained test substance, ascorbate and cytochrome *c* in the concentrations shown in Table 2. Borate buffer, pH 8.5 (0.035M) was used.)

	<i>t</i> (min.)	<i>v</i> (μl. O ₂ /min.)	Percentage un-ionized at	
			pH 8.5	pH 7.0
2-Aminoacridine	120	0.8	78.0	10.3
2-Aminoacridine with H ₂ O ₂ , 10 ⁻³ M	0	5.0	—	—
3-Aminoacridine	60	1.6	99.8	94.1
3-Aminoacridine with H ₂ O ₂ , 10 ⁻³ M	0	10.5	—	—

Ames, Ziegenhagen & Elvehjem (1946), using 8-hydroxyquinoline as a copper-removing reagent in systems containing cytochrome oxidase, cytochrome *c* and ascorbic acid, reported a 'non-enzymic

hydrogen peroxide only if 'substances capable of forming quinones are present', his examples of the latter being vanillin, *p*-phenylenediamine and fresh adrenal extract (but not adrenaline).

reaction in which a rapid oxygen uptake masked the enzyme reaction'. This was without doubt due to the reaction of 8-hydroxyquinoline as described above (cf. Table 5). A similar stimulation of oxygen uptake was found by Stotz, Harrer, Schultze & King (1937) when 8-hydroxyquinoline was added to washed liver brei to which ascorbate and haemolyzed blood had been added. Washing the tissue removed blood pigments and other haematin compounds, and in the absence of haemolyzed blood no stimulation was found. From the work of Lemberg, Legge & Lockwood (1939), the oxygen uptake on incubation of haemolyzed blood with ascorbate at pH 7 is known to be due to a coupled oxidation resulting in choleglobin formation; this process is stimulated by hydrogen peroxide and inhibited by catalase. Stotz *et al.* (1937) observed green pigment formation when 8-hydroxyquinoline was present, and it has since been found (Mr E. C. Foulkes, private communication) that choleglobin formation is markedly accelerated on addition of the carrier 7-amino-8-hydroxyquinoline (Table 5) to haemolyzed blood incubated with ascorbate at pH 7 and 37°. The influence of 8-hydroxyquinoline may be explained by assuming preliminary peroxidative oxidation of the 8-hydroxyquinoline to a hydrogen carrier, which catalyzes the oxidation of the ascorbate with increased rate of oxygen uptake as described by Stotz *et al.* The peroxide produced in this carrier-catalyzed oxidation should accelerate choleglobin formation, and the same explanation would apply to the experiment with pre-formed carrier.

Tauber (1936) showed that horseradish peroxidase catalyzes the oxidation of ascorbic acid by

The possibility must be kept in mind that carriers of these types may also be formed *in vivo*, the introduction of hydroxyl groups into benzenoid rings being a well-known biological mechanism. Specifically, Fühner (1904) found that acridine fed to rabbits was excreted in the urine as 3:5-dihydroxy-acridine and later Fühner (1906) showed that when quinoline was given to man a substance appeared in the urine which oxidized in air to the *o*-quinone (IX):

Schönhöfer (1942) found an interesting relationship among aminoquinoline compounds, in that gametocidal antimalarial activity was linked with the possibility of their biological conversion to quinonoid compounds.

SUMMARY

1. Cytochrome *c* and methaemoglobin catalyze (*in vitro*) the oxidation, by hydrogen peroxide, of certain amino- and hydroxy-acridines and -quinolines.

2. The oxidized products are hydrogen carriers which efficiently promote the autoxidation of ascorbic acid or cysteine.

3. The oxidized forms of the carriers appear, in the case of the amino compounds, to be *o*-quinonimines and, in the case of hydroxy compounds, *o*-quinones.

4. The mechanism of the formation and action of the carriers is discussed.

Dr R. Lemberg is thanked for his guidance on several important points, and for providing facilities and accom-

modation in the Institute of Medical Research, Royal North Shore Hospital, Sydney; Dr R. N. Robertson for many helpful discussions, and Prof. J. C. Earl, Prof. E. Ashby and Prof. A. Burges for providing facilities and accommodation in the University of Sydney. The work was financed by a grant from the Wellcome Foundation.

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Metabolism of Derivatives of Toluene

2. NUCLEAR METHYL-SUBSTITUTED DERIVATIVES OF N-PHENYLUREA

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(Received 4 June 1948)

The compounds included in this study were *N*-phenylurea and *N*-(*o*-, *m*- and *p*-tolyl)ureas. The metabolism of these substances does not appear to have been investigated previously, although two drugs which may be regarded as derivatives of *N*-phenylurea have received attention. 'Elbon' (I, $R = C_6H_5$) was shown by Morinaka (1922) to be

hydrolyzed in the rabbit to give benzoic acid and *N*-(*p*-hydroxyphenyl)urea, the former being excreted as hippuric acid and the latter as ether glucuronide, which was obtained as a crystalline potassium salt. Tsunoo (1935) showed that 'Ethynal', the corresponding 2-furyl derivative (I, $R = C_4H_3O$) gave rise in the dog to 2-(2'-furyl)-acrylic acid (excreted as such, and as its glycine conjugate) and the glucuronide of *N*-(*p*-hydroxyphenyl)urea; in the rabbit its fate was believed to be similar, although only the glucuronide was isolated. In man the only metabolite identified was furan-2-carboxylic acid (α -furoic acid).

There appears to be no account in the literature of the behaviour *in vivo* of the ureido group ($-NH.CO.NH_2$) attached to an aromatic ring, except for the two papers mentioned above, although compounds containing it were often claimed as metabolites of amino compounds by earlier workers. Such 'uramino' compounds include those stated to be derived from sulphanilic acid (Ville, 1892), anthranilic acid (Mitsuba & Ichihara, 1927), *m*-aminobenzoic acid (Salkowski, 1882) and various aminohydroxybenzoic acids (Pruszyński, 1893). All these compounds were believed to have been formed by the conjugation of urea with the amino group of the substance administered, ammonia being eliminated. These observations have, however, not been repeated in more recent investigations and it now appears likely that the compounds were artefacts, formed during isolation, which, in most early investigations, involved evaporation of the urine to dryness (cf. Schmidt & Allen (1920) on Salkowski's (1873) uramino compound from taurine; Friedmann (1931) on excretion products of α -naphthylalanine and Bray, Lake, Neale, Thorpe & Wood