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URINARY EXCRETION OF N-HYDROXY DERIVATIVES OF SOME AROMATIC AMINES BY RABBITS, GUINEA PIGS, AND DOGS*

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Abstract—The N-hydroxy derivatives of *p*-ethylaniline, *p*-chloroaniline, *p*- and *m*-aminopropiophenone, 4-aminobiphenyl, and 2-aminofluorene were found in the urine after injection. Rabbits excrete 30 per cent of the *p*-aminopropiophenone and 20 per cent of the 4-aminobiphenyl as N-hydroxy derivative. The N-hydroxy derivatives of the other amines appear in the urine to a much smaller extent. Guinea pigs excrete a smaller proportion of the amines as N-hydroxy derivative than rabbits; 15 per cent of *p*-aminopropiophenone was found in the urine as N-hydroxy derivative. Dogs excrete only 1 per cent or less of the amines tested as N-hydroxy derivative. N-Hydroxy-*p*-aminopropiophenone is excreted to a large extent as a conjugate which is split in acid solution. The fraction of *p*-aminopropiophenone excreted as N-hydroxy derivative is the same over a wide range of doses. The relationship between the concentration of N-hydroxy derivative, and nitroso analogue, in the blood and urine of rabbits is quite different from that observed in dogs.

WHEN nitrosobenzene was found in the blood of dogs injected with aniline or N-methylaniline[13, 14] the urine was also tested for N-hydroxylation products, but none was found. A short time after, Cramer *et al.*[6] reported the appearance of N-hydroxy-2-acetylaminofluorene in the urine of rats fed on a diet containing 2-acetylaminofluorene. After 5 weeks feeding the fraction excreted as N-hydroxy derivative had increased to 15 per cent of the administered amine. With rabbits Irving[10] even found up to 30 per cent of the 2-acetylaminofluorene given orally as the N-hydroxy derivative in the urine. Further data concerning the urinary excretion of N-hydroxy-N-acetylarylamines have been compiled by Kiese.[16] Whether the acetylation of the nitrogen is essential for the urinary excretion of arylhydroxylamines or whether substituents in other positions of the aniline also render possible the urinary excretion of N-hydroxy derivatives was investigated and recently we reported that N-hydroxy-2-aminofluorene had been found in the urine of guinea pigs injected with 2-aminofluorene[18] or 2-acetylaminofluorene.[19]

METHODS

For collecting urine the animals dosed with aromatic amines were housed individually in metabolism cages. The urine was acidified immediately after micturition. Samples which could not be extracted at once were stored at −20° after being adjusted to pH 4·5. In the experiments with guinea pigs the urine of ten animals was pooled.

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Bile was obtained from dogs anaesthetized by intravenous injection of 0·05 g chloralose and 0·5 g urethane, and from rabbits anaesthetized by intraperitoneal injection of 1 g urethane per kg.

Aniline, N-ethylaniline, *p*-ethylaniline, and *p*-chloroaniline were used as hydrochlorides and injected intravenously in aqueous solution. For intravenous injection *m*- and *p*-aminopropiophenone, 4-aminobiphenyl, and 2-aminofluorene were mixed with 1·5 ml Tween 80 and then diluted with 10 ml 0·9% sodium chloride solution; for i.p. injection these amines were suspended in a solution of 0·25% agar agar in 0·9% sodium chloride solution.

N-Hydroxy-*m*-aminopropiophenone and N-hydroxy-*p*-aminopropiophenone had been prepared by Dr. Elli Rauscher.[8, 17] For intravenous injection into non-anaesthetized rabbits and for slow intravenous infusion into rabbits anaesthetized by intraperitoneal injection of 1 g urethane per kg, N-hydroxy-*p*-aminopropiophenone was suspended under nitrogen in a mixture of one volume of Tween 80 and 4 volumes of 0·9% sodium chloride solution.

For identification and determination N-hydroxy derivatives of aromatic amines in urine, blood, and bile were oxidized to the nitroso analogues by means of potassium hexacyanoferrate(III) and extracted according to Herr and Kiese.[9] When the concentration of N-hydroxylation products in urine was very low, 10 ml of undiluted urine were extracted by means of 6 ml carbon tetrachloride. In the experiments with 4-aminobiphenyl the carbon tetrachloride extracts were washed twice with 5 N sulphuric acid and in the experiments with 2-aminofluorene the carbon tetrachloride extracts were washed seven to ten times with 0·5 N sulphuric acid. The u.v.-absorption of the carbon tetrachloride extracts was checked against that of similar extracts taken from urine, blood, or bile before the administration of the amines. Then the extracts were used for thin-layer chromatography or determination of the nitroso compounds by Herr and Kiese's[9] method. The results presented below in the tables are corrected on the basis of recoveries obtained by the addition of known amounts of the substances determined.

Silica gel HF 254 + 366 and a mixture of two volumes of ethylacetate and one volume *n*-hexane as the solvent system were used for thin-layer chromatography.

The yield of N-hydroxy derivatives extracted into carbon tetrachloride as nitroso analogues was found to depend on the pH at which the urine was extracted by means of carbon tetrachloride. Experiments with N-hydroxy-*p*-aminopropiophenone added to urine in which the pH varied from 2 to 8 have shown that the highest yield, namely 80%, of nitroso analogue in carbon tetrachloride is obtained at a pH between 4 and 5; only half as much was recovered from urine at pH 7. With the N-hydroxy derivatives of the other aromatic amines used in this study too, a pH between 4 and 5 was found to yield most nitroso analogue in the carbon tetrachloride extract.

Irving's[10] method was used for determining N-hydroxy-2-acetylaminofluorene in urine samples incubated with β-glucuronidase.

Ferrihemoglobin was determined by the increase in extinction at 550 mμ which is caused by adding cyanide to the solution of a blood sample at pH 6·8.

2-Nitrosofluorene was prepared by reducing 2-nitrofluorene to 2-hydroxylaminofluorene and, without purification, oxidizing the hydroxylamine to the nitroso analogue.

Six grammes of 2-nitrofluorene were dissolved in 3 l. hot ethanol (95%, denatured

with 1% petrol ether) and mixed with 225 ml 12% aqueous ammonium chloride solution. The solution was cooled to 25° and kept at this temperature. Under nitrogen and rapid stirring, 6 g zinc dust were added to the clear yellowish solution. Five hours later the solution, which had been continuously stirred and kept undern itrogen, was filtered under nitrogen into a separatory funnel and mixed with 180 ml of a 25% aqueous potassium hexacyanoferrate (III) solution and 18 ml concentrated hydrochloric acid. Then the mixture was diluted with water until the colour turned green and extracted twice with ether. The ether which contained the 2-nitrosofluorene was washed several times with each 100 ml 1 N hydrochloric acid until the extinction at 280–300 mμ showed the acid to contain no more 2-aminofluorene. After being washed twice with water the ether was dried over calcium chloride and evaporated. The green residue was treated with warm *n*-hexane. From the undissolved residue 2-nitrofluorene and 2,2′-azoxy-bis-fluorene were isolated and identified.* For further purification the hexane was passed through a column of silica gel E_M 0·15–0·30 (Gebr. Hermann, Köln). The green 2-nitrosofluorene moved more rapidly than the impurities and was eluted from the column by means of *n*-hexane. On concentrating and cooling of the turquoise coloured eluate, emerald-green crystals precipitated. After recrystallization from *n*-hexane the crystals were found to melt at 79·5–80°. The elementary analysis fits the formula $C_{13}H_9NO$:

calc.	C 79·98	H 4·64	N 7·17	O 8·19
found	80·20	4·61	7·17	7·93
	79·80	4·68	7·12	

The extinction of 2-nitrosofluorene in carbon tetrachloride in the range from 330 to 390 mμ may be found in the paper by Kiese and Wiedemann.[19] In benzene the extinction maximum was found at 364·5 and in methanol at 363 mμ.

Recently Lotlikar *et al.*[20] have described the preparation of 2-nitrosofluorene from N-hydroxy-2-aminofluorene by oxidation in aqueous dimethylformamide solution with ferric ammonium sulphate. After recrystallization from *n*-hexane the crystals melted at 77–79°. The extinction maximum in 95% ethanol was found at 362 mμ.

4-Nitrosobiphenyl was prepared from 4-nitrobiphenyl by a similar procedure as used for preparing 2-nitrosofluorene. After 30 g 4-nitrobiphenyl had been reduced the mixture was filtered under nitrogen into 90 ml glacial acetic acid and diluted to twice its volume with water. The yellowish precipitate, which contains N-hydroxy-4-aminobiphenyl, was filtered off, dried, and dissolved in 1 l. ether. By shaking with 1 l. of 25% hexacyanoferrate (III) solution the hydroxylamine was oxidized to 4-nitrosobiphenyl. A yellow precipitate was filtered off and, after purification, identified as 4,4′-azoxy-bis-biphenyl, m.p. 215–216°; elementary analysis:

calc.	C 82·26	H 5·18	N 7·99
found	82·50	5·19	7·80

* 2-Nitrofluorene: yellowish crystals from benzene, m.p. 157°.
2,2′-Azoxy-bis-fluorene: orange crystals from xylene, m.p. 279°. Extinction maximum in carbon tetrachloride at 385 mμ, molar extinction coefficient log ε = 4·62.

calculated	C	83·40	H	4·84	N 7·48
found		84·02		4·76	7·58

2-Aminofluorene: colourless crystals from 50% ethanol, m.p. 127·5–128·5°.

The green ether was extracted several times with 100 ml 1 N hydrochloric acid, until by measuring the extinction at 249 mμ, no more 4-aminobiphenyl was found in the acid. The further procedure followed that described above for 2-nitrosofluorene.

With fractional crystallization a green and a yellow crystalline substance precipitated in the eluate obtained from the silica gel column. On recrystallization from ethanol the yellow substance formed small rhombic crystals. They melted at 74·5° to give a green melt. The green solution in carbon tetrachloride showed an extinction maximum at 338·5 mμ (Fig. 1). The elementary analysis fits the formula $C_{12}H_9NO$:

calc.	C 78·68	H 4·95	N 7·65	O 8·73
found	78·74	5·11	7·44	8·64
	78·80	5·13	7·41	8·68

The green crystals were found to be mixed crystals of monomeric 4-nitrosobiphenyl and 4-nitrobiphenyl (Renner[23]).

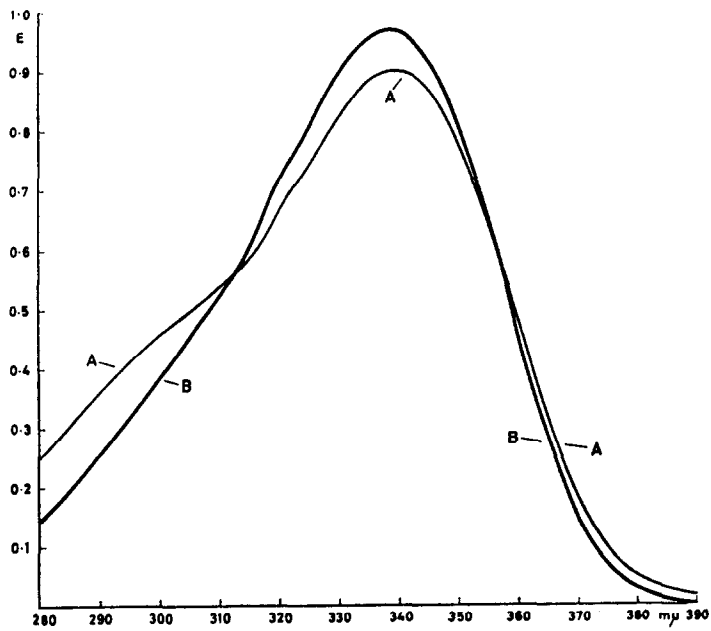

FIG. 1. 4-Nitrosobiphenyl in the carbon tetrachloride extract taken from urine of guinea pigs which was excreted during the 6th–24th hr after the intraperitoneal injection of 100 mg 4-aminobiphenyl per kg.

A. 10 ml of urine acidified to pH 4·5 were oxidized with hexacyanoferrate(III) and extracted by means of 6 ml carbon tetrachloride. After being washed with 0·5 N sulphuric acid the carbon tetrachloride was checked against a similar extract taken from urine before the injection of 4-aminobiphenyl. Optical path 2 mm.

B. 42·8 μg authentic 4-nitrosobiphenyl per ml carbon tetrachloride. Optical path 2 mm.

RESULTS

A. N-*Hydroxy derivatives of several aromatic amines in the urine*

No phenylhydroxylamine was detected in the urine of rabbits intravenously injected with as much as 200 mg aniline per kg. Substituted anilines, however, were found to

TABLE 1. URINARY EXCRETION OF N-HYDROXYLATION PRODUCTS OF SOME AROMATIC AMINES BY DOGS, RABBITS, AND GUINEA PIGS

	Aniline and N-ethylaniline		*p*-Ethylaniline		*p*-Chloroaniline		*p*-Aminopropiophenone		*m*-Aminopropiophenone		2-Aminofluorene		4-Aminobiphenyl	
	Dose mg/kg	−NOH % of dose	Dose mg/kg	−NOH % of dose	Dose mg/kg	−NOH % of dose	Dose mg/kg	−NOH % of dose	Dose mg/kg	−NOH % of dose	Dose mg/kg	−NOH % of dose	Dose mg/kg	−NOH % of dose
Dog	—		50 (i.v.)	0·03(1)	39 (i.v.)	0·04(4)	—		—		100 (i.p.)	0·2(3)	80 (i.v.)	1·1(2)
Rabbit	200 (i.v.)	0	30 (i.v.)	0·7 (3)	78 (i.v.)	0·4 (2)	1–40 (i.v.)	31 (23)	6 (i.v.)	4·0 (4)	100 (i.p.)	2·2 (3)	100 (i.p.)	20 (4)
Guinea pig	—		—		—		1–100 (i.p.)	15 (10)	25 (i.p.)	3·2 (3)	100 (i.p.)	0·5 (3)*	100 (i.p.)	4·7 (3)

* Data reported in Kiese *et al.*[18]

The figures indicate percentage of the amine found as N-hydroxy derivative in the urine; they are the means of the number of experiments shown by the figures in brackets. In the case of *m*- and *p*-aminopropiophenone the data refer to the amount of N-hydroxylation product found after the urine had been incubated for 2 hr at pH 4·5 and 37°. In all other experiments the figures indicate the amount of N-hydroxy derivative determined in the urine acidified to pH 4·5.

be excreted partly as N-hydroxy derivatives by rabbits, dogs, and guinea pigs. This is demonstrated by the extinctions of carbon tetrachloride extracts prepared from urines and the extinctions of the authentic nitroso compounds. Except for 4-nitrosobiphenyl extinction curves of the nitroso compounds may be found in earlier publications.[8, 15, 19] The extinction of 4-nitrosobiphenyl dissolved in carbon tetrachloride is shown in Fig. 1, which also demonstrates the N-hydroxylation of 4-aminobiphenyl by guinea pigs. Table 1 shows the doses of aromatic amines and the fraction of the dose which was determined as N-hydroxy derivative in the urine. Except for the N-hydroxy-aminopropiophenones these fractions refer to the amount of N-hydroxy derivative found in the urine collected as described above, because the N-hydroxy derivatives do not stand the incubation of the urine for 2 hr at 37°. The figures for the N-hydroxy-aminopropiophenones indicate the amount found after 2 hr incubation of the urine under nitrogen at 37°. When these hydroxylamines were added to urine and incubated at pH 4·5 less than 10 per cent was lost.

p-Aminopropiophenone and 4-aminobiphenyl are excreted to a larger fraction as N-hydroxy derivatives than other aromatic amines by the species tested. Apart from N-hydroxy-2-aminofluorene its N-acetyl derivative was found in the urine of rabbits dosed with 2-aminofluorene, after it had been incubated with β-glucuronidase. In the urine of dogs only small fractions of the injected amines were found as N-hydroxy derivatives. *p*-Aminopropiophenone was not studied in dogs because of its high toxicity with this species. Several experiments with *p*-ethylaniline on dogs failed because the animals died too soon after the injection of 50 mg/kg.

Incubation under nitrogen for 2 hr at pH 4·5 and 37° of the urine of rabbits and guinea pigs injected with *p*-aminopropiophenone was found to increase the amount of N-hydroxy-*p*-aminopropiophenone determined by about 200 per cent. No further increase in free N-hydroxy-*p*-aminopropiophenone was observed if the urine was incubated with 2000 units β-glucuronidase Boehringer per ml. Incubation of the urine of rabbits or guinea pigs dosed with *m*-aminopropiophenone increased the amount of N-hydroxy derivative found in the urine by only about 50 per cent.

The large fraction of *p*-aminopropiophenone excreted as the N-hydroxy derivative by rabbits and guinea pigs and the stability of the N-hydroxy derivative during the incubation for splitting conjugates recommended this amine as a model for studying its metabolism and excretion in more detail. The relationship between the dose of *p*-aminopropiophenone injected and the fraction excreted as N-hydroxy derivative was studied with guinea pigs and rabbits using doses from 1 to 100 mg/kg and from 1 to 40 mg/kg respectively. The data listed in Tables 2 and 3 show that with guinea pigs as well as rabbits the fraction of the amine excreted as N-hydroxy derivative is independent of the dose injected for a wide range of doses. The data presented in Tables 2 and 3 also show that most of the N-hydroxy derivative found in the urine was excreted in the 4 hr following the injection of the amine. In this period rabbits excreted 90 per cent and guinea pigs 70 per cent of the N-hydroxy-*p*-aminopropiophenone. A similar ratio was observed with the excretion of N-hydroxy-4-aminobiphenyl and N-hydroxy-2-aminofluorene.

B. *Excretion of* N-*hydroxy-p-aminopropiophenone following its intravenous injection*

The excretion of a large fraction of *p*-aminopropiophenone as the N-hydroxy derivative points to a rapid N-hydroxylation of the amine in rabbits and guinea pigs.

TABLE 2. EXCRETION OF N-HYDROXY-*p*-AMINOPROPIOPHENONE BY GUINEA PIGS DURING VARIOUS PERIODS AFTER THE INTRAPERITONEAL INJECTION OF DOSES FROM 1 TO 100 mg *p*-AMINOPROPIOPHENONE PER kg

Dose (mg/kg)	0–4th hr		4th–6th hr		6th–24th hr		24th–30th hr		Total % of dose
	mg N-OH	% of dose	mg N-OH	% of dose	mg N-OH	% of dose	mg N-OH	% of dose	
1	0·28	7·6	0·16	4·3	0·03	0·8	0·007	0·19	12·9
5	2·4	9·8	0·26	1·1	0·15	0·6	0·03	0·12	11·6
25	14·3	14·3	4·7	4·7	0·53	0·5	0·02	0·02	19·5
50	35·6	14·5	8·0	3·2	2·9	1·1	0·06	0·02	18·8
100	52·4	10·5	12·8	2·6	2·6	0·5	0·06	0·01	13·6
Average		11·3		3·2		0·7		0·07	15·3

The figures show the averages of two experiments each with 10 guinea pigs. The urine samples were incubated for 2 hr at 37° before analysis.

TABLE 3. EXCRETION OF N-HYDROXY-*p*-AMINOPROPIOPHENONE BY RABBITS DURING VARIOUS PERIODS AFTER THE INTRAPERITONEAL INJECTION OF DOSES FROM 1 TO 40 mg *p*-AMINOPROPIOPHENONE PER kg

Dose (mg/kg)	Number of experiments	0–4th hr		4th–24th hr		24th–30th hr		Total % of dose
		mg N-OH	% of dose	mg N-OH	% of dose	mg H-OH	% of dose	
1	5	0·67	24·3	0·02	1·3	0·001	0·03	25·6
6	7	5·2	27·2	0·6	4·1	0·006	0·06	31·4
30	5	31·0	31·9	2·2	2·3	0·03	0·03	34·2
40	6	42·4	31·3	1·9	1·3	0·014	0·01	32·6
Average			28·7		2·3		0·03	30·9

The figures show the averages of the number of experiments indicated in the second column. The urine samples were incubated for 2 hr at 37° before analysis.

Since N-hydroxy arylamines are also reduced to the amines *in vivo* it appeared of interest to compare the amount of N-hydroxy-*p*-aminopropiophenone found in the urine of rabbits after the intravenous injection of this compound and an equimolar dose of *p*-aminopropiophenone. The amine was intravenously injected in about 10 min. In order to avoid high concentrations of N-hydroxy-*p*-aminopropiophenone in the blood, quite different from what was observed after the injection of the amine, the N-hydroxy-*p*-aminopropiophenone was injected more slowly. The dose was divided in four portions. The intravenous injection of each portion took 10 min and was followed by the next one after 10 min intermission.

This procedure caused about the same increase in ferrihaemoglobin concentration in the rabbit's blood as was observed after the injection of the amine. Therefore the concentrations of N-hydroxy derivative and the time they prevailed in the blood were probably similar (see below). As an average of three experiments with 6·7 mg N-hydroxy-*p*-aminopropiophenone per kg and 6 mg *p*-aminopropiophenone 29 per cent and 31 per cent respectively of the dose were found in the urine as N-hydroxy-*p*-aminopropiophenone.

The rate of elimination of N-hydroxy-*p*-aminopropiophenone from the blood of anaesthetized rabbits was determined by continuous intravenous infusion of N-hydroxy-*p*-aminopropiophenone until its concentration remained constant. Figure 2

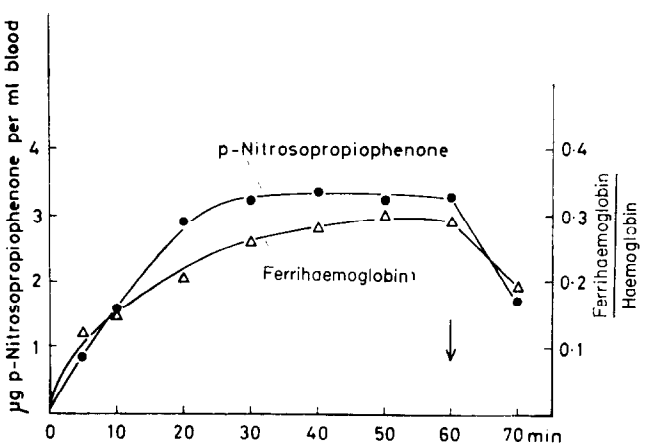

FIG. 2. Concentration of N-hydroxy-*p*-aminopropiophenone and *p*- nitrosopropiophenone (both determined as *p*-nitrosopropiophenone) and of ferrihaemoglobin in the blood of rabbits during the intravenous infusion of 55 µg N-hydroxy-*p*-aminopropiophenone per kg and min. The infusion was ended after 60 min (arrow). The symbols indicate the means of three experiments.

presents the results of these experiments. During an infusion of 55 µg/kg/min the concentration of N-hydroxy-*p*-aminopropiophenone and its nitroso analogue increased in 30 min to 3·3 µg/ml blood and stayed so until the infusion was finished. Then it dropped to half its value in 10 min. The ferrihaemoglobin concentration increased with the N-hydroxy compound and levelled to 30 per cent of the haemoglobin.

C. *Concentrations of* N-*hydroxy arylamines and their nitroso analogues in the blood after the administration of arylamines*

In several experiments with rabbits and dogs the concentration of the N-hydroxy derivative and its nitroso analogue has been determined in the blood as they appear

after the injection of some aromatic amines. The results presented in Figs. 3 and 4 show wide species differences in the accumulation of N-hydroxylation products in the blood. N-hydroxy-*p*-ethylaniline and its nitroso analogue accumulated to much higher concentrations in the blood of dogs than the same derivatives of 2-aminofluorene and 4-aminobiphenyl. But in the rabbit the opposite was true. The derivatives of *p*-ethylaniline could hardly be determined, while those of 2-aminofluorene and 4-aminobiphenyl accumulated to respectable concentration. Furthermore, the experiments

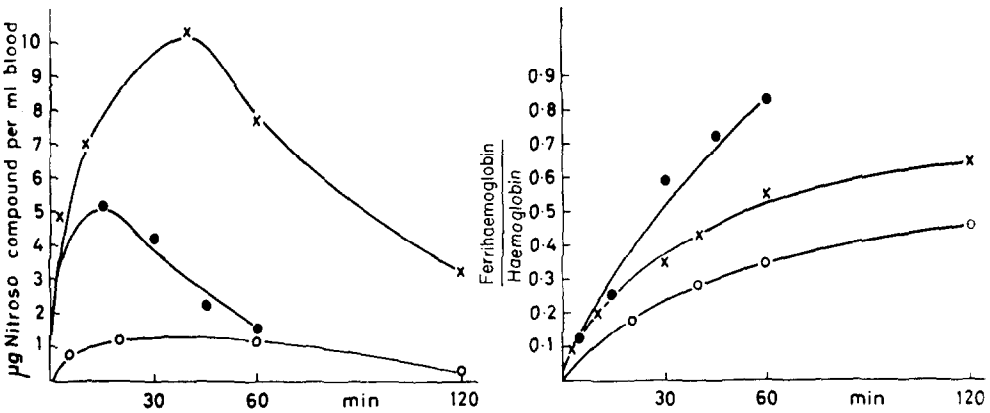

FIG. 3. Concentrations of N-hydroxy derivatives and nitroso analogues (determined as nitroso compounds) and concentration of ferrihaemoglobin in the blood of dogs after the intravenous injection of aromatic amines.

× — *p*-Ethylaniline, 75 mg/kg; five experiments.
○ — 2-Aminofluorene, 100 mg/kg; one experiment.
● — 4-Aminobiphenyl, 80 mg/kg; one experiment.

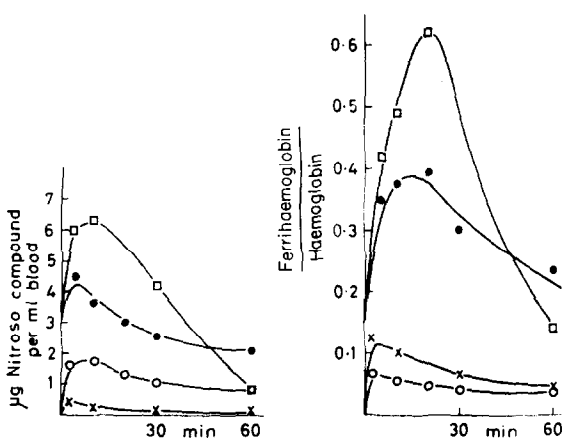

FIG. 4. Concentration of N-hydroxy derivatives and nitroso analogues (determined as nitroso compounds) and concentration of ferrihaemoglobin in the blood of rabbits after the intravenous injection of some aromatic amines.

× — *p*-Ethylaniline 50 mg/kg; one experiment.
○ — 2-Aminofluorene 100 mg/kg; four experiments.
● — 4-Aminobiphenyl 100 mg/kg; two experiments.
□ — *p*-Aminopropiophenone 30 mg/kg; four experiments.

demonstrate that no relationship exists between the tendency for N-hydroxylation products to accumulate in the blood and the rate of urinary excretion. N-Hydroxy-*p*-ethylaniline and its nitroso analogue accumulated in dog's blood to concentrations more than twenty times higher than in rabbit's blood, but rabbit's urine contained more N-hydroxy-*p*-ethylaniline than dog's urine. The intravenous injection of 78 mg *p*-chloroaniline per kg into rabbits caused an average increase in the concentration of the N-hydroxy derivative and nitroso analogue in the blood to 1·9 μg/ml. Ten minutes later the concentration had already dropped to 0·8 μg/ml. Much higher concentrations of N-hydroxy-*p*-chloroaniline and nitroso analogue have been observed n the blood of dogs,[15] but the urinary excretion by dogs is much lower than by rabbits. After the injection of 4-aminobiphenyl similar concentrations of N-hydroxy-4-aminobiphenyl and its nitroso analogue were observed in the blood of dogs and rabbits, but in rabbit's urine almost twenty times more N-hydroxy-4-aminobiphenyl was found than in dog's urine.

Figures 3 and 4 also show the change in ferrihaemoglobin concentration in dog's and rabbit's blood following the injection of various arylamines. In experiments with rabbits, the results of which are not shown in the figures, the intravenous injection of 78 mg *p*-chloroaniline per kg was followed within 5 min by an increase in ferrihaemoglobin concentration to about 30 per cent of the haemoglobin. It may be pointed out that in rabbit's blood changes in ferrihaemoglobin concentration follow changes in the concentration of the respective N-hydroxy and nitroso compound with only a short delay. The curves delineating the concentrations of N-hydroxylation product and ferrihaemoglobin are similar in shape. This does not hold for dogs.

D. *Biliary excretion of* N-*hydroxy derivatives*

After the intravenous injection of 200 mg aniline, 30 mg *p*-ethylaniline or 78 mg *p*-chloroaniline per kg, no N-hydroxy derivative was detected in the bile. N-Hydroxy-*p*-aminopropiophenone appeared in the bile of rabbits after the intravenous injection of 40 mg *p*-aminopropiophenone per kg. As an average of four experiments 0·6 per cent of the dose was found in the bile after it had been incubated with β-glucuronidase at pH 4·5. Only 5 per cent of this amount was determined before incubating the bile. More than 90 per cent of the N-hydroxy-*p*-aminopropiophenone found in the bile was excreted in the 1st hr following the injection of the amine. In the bile collected during the 1st hr the average concentration of N-hydroxy-*p*-aminopropiophenone amounted to 47 μg/ml.

No N-hydroxy derivative was detected in the bile of dogs injected intravenously with 39 mg *p*-chloroaniline or 12 mg *p*-aminopropiophenone per kg.

DISCUSSION

The results of our experiments show that acetylation of the nitrogen is not necessary for urinary excretion of N-hydroxylation products of aromatic amines. Some aromatic amines, like *p*-aminopropiophenone and 4-aminobiphenyl, are excreted to a large extent as N-hydroxy derivatives in the urine. There are only few data in the literature for comparison with the excretion of the acetylated compounds.[16] Except for N-hydroxy-aminopropiophenones the amounts of N-hydroxy derivatives found in the urine in our experiments are probably lower than the amounts actually excreted. The instability of most N-hydroxy derivatives in urine prevented the

determination of the fraction excreted in a conjugated form. The experiments with *p*-aminopropiophenone demonstrate that this fraction can be much larger than the part excreted unconjugated. The nature of the conjugate of N-hydroxy-*p*-aminopropiophenone can only be conjectured. Since no further increase in free N-hydroxy derivative is observed after incubation with glucuronidase, either no O-glucosiduronic acid is excreted or it decays rapidly at 37° in solutions of pH 4·5. It is more likely that the conjugate is an N-glucosiduronic acid or sulphamic acid derivative. These conjugates of aromatic amines are known to be unstable in acid solution,[1, 3-5] whereas O-glucosiduronic acids are fairly stable. Other unknown types of conjugate cannot be excluded.

The state in which the N-hydroxy derivatives of the other aromatic amines are excreted is not known. The urine was acidified immediately after being voided. Conjugates may have been split in the cold before the urine was extracted.

The urinary excretion of N-hydroxy derivatives is favored by *p*-substitution of aniline. The experiments with the aminopropiophenones show that the same group in *m*-position is much less effective than in *p*-position. *p*-Substitution blocks the *p*-hydroxylation, which is the major metabolic change of many aniline derivatives. The lower fraction of 2-aminofluorene found in the urine as N-hydroxy derivative is possibly an illustration of this effect, since this amine is readily hydroxylated in position 5 and 7. Our results do not indicate whether the enhancing effect of *p*-substitution on the urinary excretion of N-hydroxy derivatives is due to the more rapid microsomal N-hydroxylation of these compounds[16] or to differences in the extent of conjugation or behaviour in the kidneys.

The rate of N-hydroxylation is certainly of primary importance, since a rapid reduction of the N-hydroxy derivatives to the amines occurs in various tissues.[16, 20] N-Hydroxylation and dehydroxylation of *p*-aminopropiophenone proceed so rapidly in the rabbit that the same amount of N-hydroxylation product is excreted in the urine when the amine or the N-hydroxy derivative is injected. In unpublished experiments we found that rabbit liver microsomes N-hydroxylate *p*-aminopropiophenone about four times more rapidly than *m*-aminopropiophenone.

The metabolism and urinary excretion of N-hydroxy-2-acetylaminofluorene in the rat has been studied by Miller *et al.*[21] as well as Weisburger *et al.*[25] and Shirasu *et al.*[24] In spite of the complication caused by the deacetylation the results may be assumed to show that N-hydroxylation and dehydroxylation of 2-acetylaminofluorene in the rat proceed more slowly than that of *p*-aminopropiophenone in the rabbit.

The appearance of N-hydroxylation products of some aromatic amines in the blood of rabbits in substantial concentration and the formation of ferrihaemoglobin is worth mentioning. Aniline and N-ethylaniline produce only traces of ferrihaemoglobin in rabbits. Phenylhydroxylamine and nitrosobenzene could not be demonstrated in the blood after the amines had been injected.[2] The formation of ferrihaemoglobin by an aromatic amine in easily recognizable concentrations in rabbits has earlier been observed with *p*-bromoaniline[26] and *p*-chloroaniline.[12] *p*-Aminopropriophenone and 4-aminobiphenyl surpass *p*-chloroaniline in the accumulation of N-hydroxylation product in the blood as well as in formation of ferrihaemoglobin.

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