A WORKING HYPOTHESIS FOR THE MODE OF CARCINOGENESIS OF AROMATIC AMINES.

D. B. CLAYSON

Department of Experimental Pathology and Cancer Research, School of Medicine, Leeds, 2.

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A CONSIDERABLE amount is known about the carcinogenic properties of certain aromatic amines and azo-compounds. In this paper a working hypothesis is put forward for the mode of carcinogenic action of these compounds and the evidence to support it is discussed. A working hypothesis would be of value not only as a basis for further systematic work in this field but ultimately if it could be established as having validity in prediction it would be useful in selecting any suspected compounds of industrial importance for full biological investigation. No attempt has been made in this account to review exhaustively the literature of aromatic amine carcinogenesis: only those papers which have a direct bearing on the argument will be quoted.

Those aromatic amines which are known to be carcinogenic can be regarded as derivatives of aniline in which the *para* position is substituted by a large conjugated group. (In the examples quoted the *para* position is marked by an asterisk.) The amino group may carry certain substituents which the body is capable of removing (Williams, 1947). Thus, in addition to the carcinogenic aromatic amines themselves, derived acetamides, diacetylamines, and mono- and dimethylamines have been shown to be carcinogenic. Similarly, some corresponding nitro-compounds are active and it is known that the nitro group may be reduced to an amine *in vivo* (Robinson, Smith and Williams, 1951).

The carcinogenic aromatic amines have been found to induce tumours in a wide variety of sites in different species as is shown in the accompanying tables. The relatively large doses of the aromatic amines required to induce tumours has resulted in the use of feeding in preference to injection for their administration. This may be the reason why carcinomas are more frequent than sarcomas. The amino-stilbenes (Haddow, Harris, Kon and Roe, 1948), for example, gave rise to sarcomas in both rats and mice on subcutaneous injection. There is a tendency for aromatic amine carcinomas to occur in the liver; sites on the various routes of excretion such as the bladder, ureters and intestine; and also, in the case of the rat, the acoustic gland. The widespread distribution of tumours suggests that the active agents circulate and induce tumours where their concentration and other factors are favourable. In support of this Bielschowsky (1944) has shown that stimulation of the thyroid of the rat by allyl thiourea results in the formation of 2-acetamidofluorene tumours in this gland.

Different species respond differently to the same aromatic amine. 2-Naphthylamine (I), for example, induces tumours readily in the dog (Hueper and Wolfe, 1937; Bonser, 1943) and with difficulty in the rat and rabbit (Bonser,

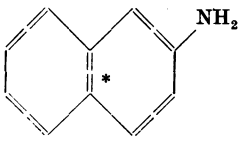
Clayson, Jull and Pyrah, 1952). Benzidine (II) tumours are induced readily in the rat and with difficulty, if at all, in the dog (Spitz, Maguigan, and Dobriner, 1950). These differences in the tumour incidence in different species suggest that (i) it is a metabolite rather than the amine itself which is responsible for the carcinogenicity of the aromatic amines and (ii) that metabolism varies from species to species (as has already been shown to be the case with some amines).

Bonser, Clayson and Jull (1951) have obtained evidence that 2-amino-l-naphthol (III) (or one of its derivatives) is the carcinogen responsible for the tumours induced by 2-naphthylamine in the dog, mouse, rat and rabbit. Recently they (Bonser *et al.*, 1952) have shown that whereas 2-amino-l-naphthol is locally active, 2-naphthylamine is not. In its quantitative aspects, the conversion of 2-naphthylamine to 2-amino-l-naphthol derivatives, offers an explanation of the differences in species susceptibility to 2-naphthylamine and for the localisation of tumours in the bladder of the dog, rat and rabbit, and in the liver of the mouse. Baker (1951) has obtained some evidence to show that (i) the urine of men exposed to benzidine contains a carcinogen and that (ii) 3 : 3′-dihydroxy-benzidine (IV), a probable metabolite of benzidine, is carcinogenic (Baker, 1952). He has published no evidence to show that this compound is a local carcinogen. Walpole, Williams and Roberts (1952) quote a personal communication from Baker that, not only were the intestinal tumours with 3 : 3′-dihydroxy-benzidine obtained earlier than those with benzidine in the parallel experiments of Spitz *et al.* (1950) and Walpole *et al.* (1952), but that he had also obtained papillomas of the forestomach. No inference can safely be drawn from the first observation, as the use of different strains and different means of administration might make considerable differences to the time of appearance of the tumours. The latter observation is of doubtful significance as Bonser *et al.* (1952) found forestomach tumours in 30 per cent of control rats and of those fed 2-naphthylamine in their food. Proof of the contention of Walpole *et al.* (1952) that 3 : 3′-dihydroxy-benzidine is indeed the effective metabolite can only be found by the direct investigation of the local action of 3 : 3′-dihydroxybenzidine on the tissues. Bielschowsky (1945) isolated 7-hydroxy-2-acetamido-fluorene (V) from the acetamidofluorene (VI) urine of the rat. Hoch Ligeti (1947) suggested that this metabolite was a weak carcinogen, but as in her experiment the controls were killed at 500 days and most of the tumours in the experimental group occurred between 500 and 700 days this observation requires confirmation.

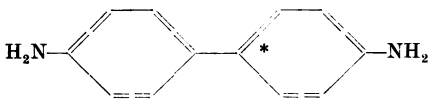
The present hypothesis is an attempt to bring these first results into orderly relationship. It is suggested that :

(i) compounds which contain a hydroxyl and an amino group *ortho* to each other in an aromatic system of two or more rings may be carcinogenic either in their own right or as a result of their further reaction ; (ii) the reason why some aromatic amines induce tumours whereas others do not is that the former are more readily converted in the body to ortho-hydroxy amines than the latter.

Hydroxylation, and acetylation, appear to be the main detoxication mechanisms undergone by the aromatic amines. Aniline, for example, is converted by the rabbit to derivatives of *para*- and to a lesser extent *ortho*-aminophenol (Smith and Williams, 1949). Williams (1947) in his excellent review indicated that other aromatic amines behave similarly. It is now suggested that those aromatic amines which have a blocked *para* position will tend to



(I)



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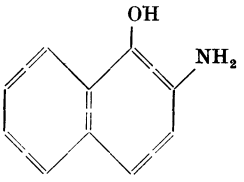

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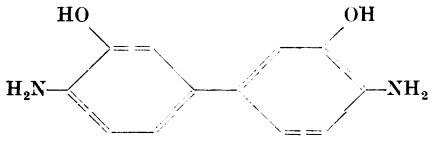

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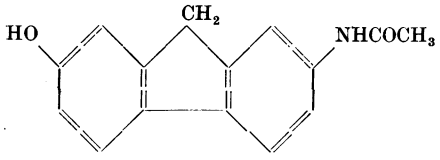

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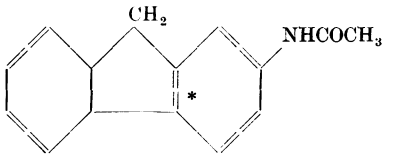
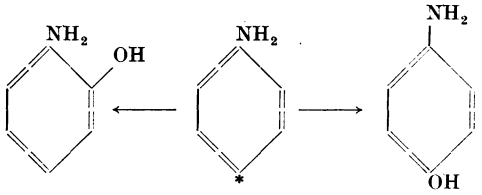
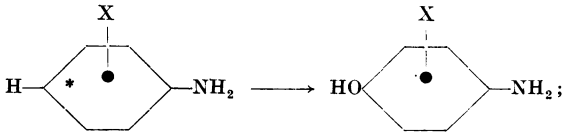


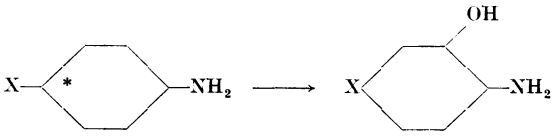
(VI)

hydroxylate to a greater extent in the *ortho* position and will thus lead to the postulated carcinogens, as has been shown to be the case with 2-naphthylamine.



The experimental testing of such a hypothesis requires three lines of approach : (i) proof of the carcinogenicity of the *ortho*-hydroxy amines ; (ii) demonstration that the carcinogenic aromatic amines are, in fact, converted to *ortho*-hydroxy amines *in vivo* ; and (iii) demonstration that the carcinogenic metabolites, if formed, reach the sites of election of the tumours induced by the original aromatic

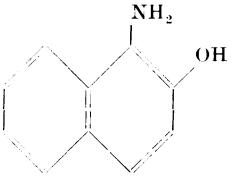




amines. A start has been made in Leeds on the first of these problems in the last eighteen months. A number of *ortho*-hydroxy amines are under test by the bladder implant technique (Jull, 1951) in which a paraffin wax pellet containing the chemical is introduced surgically into the mouse bladder. These substances include : 1-amino-2-naphthol, 3-amino-4-hydroxy-biphenyl, 3-amino-2-naphthol, 1-amino-2-naphthol-4-sulphonic acid, 3 : 3′-dihydroxybenzidine and *ortho*-aminophenol. A commercial sample of 1-amino-2-naphthol (VII) hydrocholride has been found to induce tumours in five of six mice surviving for more than 16 weeks (Bonser and Jull, personal communication).

Detailed consideration of the aromatic amines :

It is difficult to subdivide the known carcinogenic aromatic amines into further classes although they can be regarded as derivatives of 2-naphthylamine (II), 4-aminobiphenyl (VIII) or 4-aminostilbene (IX). The evidence for the mode of action of 2-naphthylamine has already been considered. The first



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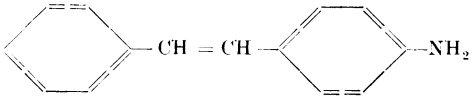
(VIII)

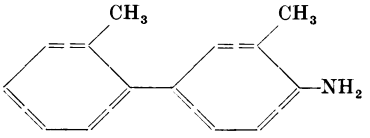


(IX)

published study of the aminostilbenes (Haddow *et al.*, 1948) was more concerned with their tumour inhibitory properties than with their carcinogenic action. In view of the suggested connection between tumour inhibition and carcinogenicity it is interesting to notice that those stilbenes found to inhibit the growth of the test tumour significantly were all *para*-substituted anilines : but it should be noted that by no means all *para*-substituted anilines in this series were tumour inhibiting. Thus it appears that factors other than para-substitution of an amine are of importance.

By far the most extensively investigated is the 4-aminobiphenyl series which includes compounds of the 2-aminofluorene type. With the exception of 2-aminofluorene, however, most of the work has been carried out in the rat only. 4-Aminobiphenyl (VIII) (Walpole *et al.*, 1952), its acetamide (Miller, Miller, Sandin and Rusch, 1952) and 4-dimethylaminobiphenyl (Miller, Miller, Sandin and Brown, 1949) have been shown to induce tumours in the rat. Various methyl homologues have been found to be active including 3 : 2′-dimethyl-, (X), 3 : 3′-dimethyl (XI) and 3-methyl-4-aminobiphenyl (XII). On the other hand 2-methyl- (XIII) and 2′-methyl-4-acetamidobiphenyl (XIV) were found not to induce tumours in the rat. The methylene bridge in 2-amino-fluorene

may be replaced by various other substituents (XV–XVIII) and a fluorine atom may be substituted in the 7-position in this compound (XIX) without loss in activity.



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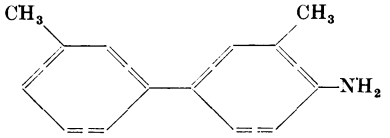

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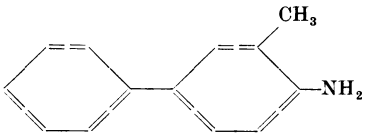

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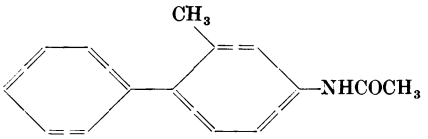

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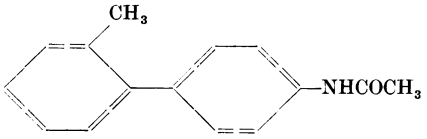

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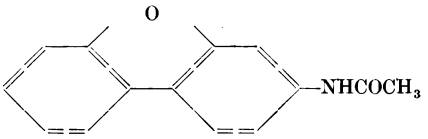

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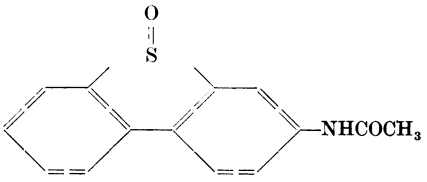

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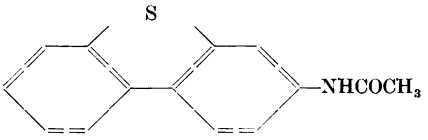

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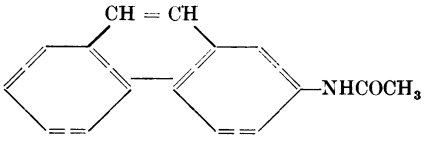

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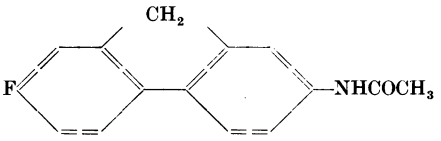


(XIX)

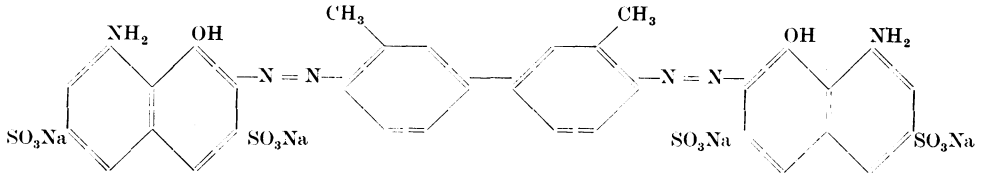
Miller *et al.* (1952) explain their failure to induce tumours in the 2- and 2′-methyl-4-acetamidobiphenyls by suggesting that the substituent destroys the planarity of the molecule, and thus its carcinogenicity. This is clearly inadequate as 2′ : 3-dimethyl-4-aminobiphenyl, which was found to be highly active by Walpole *et al.* (1952), contains a methyl substituent in the 2′-position. The contradiction might be explained by differing metabolism of the compounds. As Walpole *et al.* (1952) have noted, *o*-acetotoluidine is hydroxylated whereas *m*-acetotoluidine is oxidised to meta-acetamido-benzoic acid by the dog, and it seems possible that the corresponding aminobiphenyls behave similarly in the rat.

Walpole *et al.* (1952) make a special point of the enhancing of the activity of the 4-aminobiphenyl derivatives by the 3-methyl substituent. While their view provides a satisfactory explanation of the known carcinogenic activities of the methyl-4-aminobiphenyls, other substitutions can also be made without destroying carcinogenic activity. For example, a methylene bridge in the 2 : 2′- position as in 2-aminofluorene converts 4-aminobiphenyl into one of the most ubiquitous and potent carcinogens known, and according to Miller *et al.* (1952) substitution of the 7- position with fluorine in 2-acetamidofluorene may enhance this activity still further. If, as is now suggested, metabolism is a prerequisite to carcinogenic activity, substituents might have one or more of the following effects : (i) a substituent might alter the reactivity of the molecule and render it more or less easily metabolised ; (ii) a substituent might act as an additional centre for metabolism, as in the oxidation of a methyl to a carboxyl group, and thus reduce the amount of the carcinogenic metabolite formed ; (iii) a substituent might block one of the routes of metabolism of a compound and thus alter the formation of a carcinogenic metabolite ; (iv) a substituent might affect the ease of interaction of a carcinogenic metabolite with the tissue. As only a score of aromatic amines have been adequately tested for carcinogenicity it is not surprising that it is still impossible to predict the effects produced by any particular substituent. Nevertheless, a consideration of the possible effects of substituents helps to account for the apparently random arrangement of carcinogenic molecules within a chemically similar series.

Azo compounds.

The literature on the azo compounds has been fully reviewed recently by Badger and Lewis (1952). Although they discussed the various hypotheses which have been advanced to account for the carcinogenicity of azo compounds in terms of metabolism and *in vitro* reactivities, they were unable to come to any conclusions. To the writer it seems that the azo compounds fall into four classes : (i) the butter yellow group (4-dimethylaminoazobenzenes) ; (ii) amino-azo compounds (like *ortho*-aminoazotoluene) ; (iii) compounds containing an azo-2-phenol group and (iv) other azo compounds without an amino group. The second and third of these classes are of special interest in regard to their possible conversion to *o*-hydroxy amine derivatives.

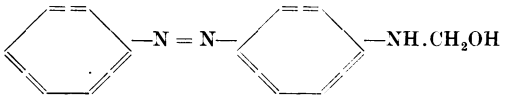
Those azo dyestuffs which have been found to be carcinogenic experimentally are oil soluble, hydrophobic compounds. From this it appears to be concluded that water soluble azo compounds containing sulphonic and carboxylic acid groups are eliminated from the body before they have an opportunity to induce tumours. The negative results obtained in the biological testing of these compounds, quoted by Hartwell (1951) supports the conclusion. Recent work by



(XX)

Gillman, Gillman and Gilbert (1949) and by Simpson (1952) on trypan blue (XX) in the rat suggests that the introduction of a sulphonic acid group may not invariably render the substance harmless.

(i) *Butter yellow and its analogues*: these compounds, in which an N-methyl group appears to be essential for carcinogenicity, induce tumours almost exclusively in the liver of the rat. Thus they differ from the carcinogenic aromatic amines not only in their chemical structure but also in their restricted biological activity. Mueller and Miller (1951) have shown that a substance which produces formaldehyde is formed by the action of rat liver homogenates on 4-methylaminoazobenzene. Hendry, Homer, Rose and Walpole (1951) suggest that this substance may be 4-hydroxylmethylaminoazobenzene (XXI) which is analogous to the cytotoxic methanolamines.

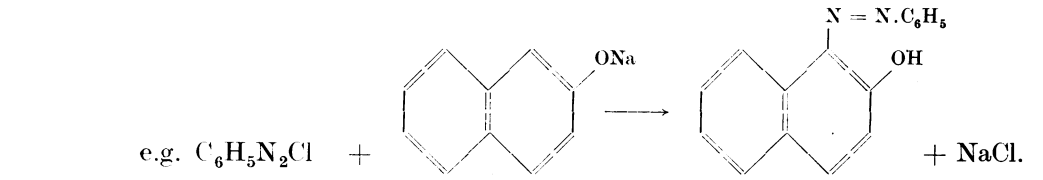


(XXI)

The production *in vivo* of a cytotoxic agent could explain the cirrhosis and subsequent regeneration observed in the rat liver after administration of 'butter yellow' and its analogues. If it should be established that *o*-hydroxyamino-derivatives are carcinogenic as a class it will be necessary to determine whether the cytotoxic methanolamines or the hydroxylated metabolites are responsible for the initiation of the butter-yellow tumours.

(ii) *Aminoazo compounds*: *o*-aminoazotoluene is the outstanding example of this class. Crabtree (1949) examined this compound and five of its isomers for carcinogenic activity in rats and mice. He found, that whereas *o*-aminoazotoluene was carcinogenic in both rats and mice, 2 : 4′-dimethyl-4-aminoazobenzene and 2′: 5-dimethyl-2-aminoazobenzene were only carcinogenic to the mouse liver. Crabtree (1949) considered that the 3 : 2′- and 4′ : 3-dimethyl-4-aminoazobenzenes were also carcinogenic in the mouse, but the evidence on which this contention is based is very meagre as only one mouse in each case showed microscopic hepatomas. It is now suggested that as the active compounds are para- substituted anilines their mode of action may be explained by the hypothesis under discussion.

(iii) *Azo-2-phenols*: azo compounds are often made by coupling a phenol and a diazonium compound and on occasion may lead to an azo-2-phenol:



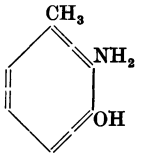
Benzene-azo-2-naphthol (Peacock, 1948) is not only known to induce tumours in mice but also would be expected to be metabolised to 1-amino-2-naphthol. There is evidence to suggest that the latter is carcinogenic (Bonser and Jull,

personal communication). Previously, other similar azo compounds have been tested and found inactive (Badger and Lewis, 1952). If the ideas expressed here are valid it should be possible to find other carcinogenic azo- compounds capable of reduction to the suspected carcinogenic *ortho*-hydroxy amines.

(iv) 2 : 2′-*Azonaphthalene* and 2 : 3′-azotoluene are examples of other azo compounds which have been found to be carcinogenic but do not fit into any of the above categories. It is not possible to suggest the nature of their mode of action but the author was unable to detect any 2-amino-1-naphthol derivatives in the urine of dogs fed with 2 : 2′-azonaphthalene (unpublished observation).

The tendency, hitherto, has been to regard the azo group as the part of the molecule conferring carcinogenicity. It is now suggested that this group is only of importance insofar as it helps to form a molecule of a suitable structure to carry functional groups which are themselves responsible for the biological activity. If this is so, the similarities in both chemical configuration, and in the induction of distant tumours, by the azo compounds, aminostilbenes and amino-biphenyls are more readily comprehensible.

There seems to be no convincing evidence that aromatic amines containing only a single ring are carcinogenic and therefore the present hypothesis has been put forward in such a manner as to apply to compounds containing two rings or more. On the other hand Walpole *et al.* (1952) have applied similar ideas, without any additional experimental evidence, to explain the carcinogenicity of methylated aminobiphenyls and have suggested that single ring *ortho*-hydroxyamines may be carcinogenic. Their reasons for so doing are as follows: firstly, they quote an industrial report of tumours of the bladder amongst workpeople handling crude *ortho*-toluidine; secondly, they suggest that Crabtree's (1949) distribution of carcinogenicity amongst the aminoazotoluenes is best accounted for by assuming metabolism of the active compounds to 2-amino-3-hydroxytoluene (XXII), (they overlook the possibility of direct hydroxylation to give phenylazo substituted *ortho*-aminophenols;) and thirdly, they suggest that acceptance of their postulate would give a possible mechanism for the mode of carcinogenesis of 2 : 3′-azotoluene. However, it should be pointed out that the main experimental evidence for the carcinogenicity of *o*-toluidine (Hartwell, 1951) is based on unpublished experimental work. The lack of reliable information in regard to the carcinogenic action of single ring compounds is due to inadequate testing and until good experimental evidence is available an open mind must be kept on this point.



(XXII)

SUMMARY.

1. It has been suggested that aromatic amines are carcinogenic because of their conversion to *ortho*-hydroxy amines. This conversion is facilitated if the position *para* to the aromatic amino group is blocked to biological hydroxylation.

TABLE 1.—*Sites of Election of Tumours induced by Aromatic Amines in the Rat.*

Compound.	Administration.*	Adequacy of testing.†	Site of Tumours: Local.	Liver.	Intestine.	Renal pelvis and kidney.	Bladder.	Acoustic duct.	Salivary gland.	Thyroid.	Breast.	Others.	References.
2-naphthylamine	Oral	A	—	—	—	—	+	—	—	—	—	—	(*a*), (*b*)
2-anthramine	P.	S	+	—	—	—	—	+	—	—	—	—	(*a*)
4-aminobiphenyl	S.C.	S	—	+	+	—	—	—	—	—	+	—	(*c*)
benzidine	S.C.	A	—	+	+	—	—	+	—	—	—	—	(*c*), (*h*)
4-acetamidobiphenyl	Oral	S*	—	—	?	—	—	?	—	—	+	—	(*d*)
4-dimethylaminobiphenyl	Oral	S	—	? —	—	—	—	+	—	—	+	+	(*e*)
3-methyl-4-aminobiphenyl	S.C.	S*	—	—	+	—	—	—	—	—	—	—	(*c*)
3 : 3′ - dimethyl - 4 - amino - biphenyl	S.C.	S*	—	—	+	—	—	—	—	—	—	—	(*c*)
2′ : 3 - dimethyl - 4 - amino - biphenyl	S.C.	S	—	—	+	—	? —	+	+	—	? —	—	(*c*)
2-aminofluorene	P. S.C. Oral	A	+	+	—	—	+	+	—	—	+	+	(*a*), (*f*)
2-acetamidofluorene	P. S.C. Oral	F	—	+	+	+	+	+	+	+	+	+	(*a*)
2-diacetylaminofluorene	P. Oral	S	—	+	—	—	—	+	—	—	+	+	(*f*)
2-monomethylamino-fluorene	Oral	S	—	+	+	—	—	+	—	—	+	+	(*g*)
2-dimethylaminofluorene	Oral	S	—	+	+	—	—	+	—	—	+	+	(*g*)
7-fluoro-2-acetamido-fluorene	Oral	S*	—	+	?	—	—	?	—	—	+	—	(*d*)
2-acetamidophenanthrene	Oral	S*	—	—	?	—	—	?	—	—	+	+	(*d*)
3-acetamidodibenzfurane	Oral	S	—	—	?	—	—	+	—	—	+	—	(*e*), (*d*)
3-acetamidodibenzthio-phene	Oral	S	—	—	?	—	—	+	—	—	+	—	(*e*), (*d*)
3-acetamidodibenzthio-phene oxide	Oral	S	—	—	?	—	—	+	—	—	+	—	(*e*), (*d*)
4-aminostilbene	S.C.	S	+	+	—	—	—	+	—	—	+	—	(*a*)
4-acetamidostilbene	S.C.	S	+	—	—	—	—	+	—	—	—	—	(*a*)
4-dimethylaminostilbene	I.P. S.C. Oral	A	+	+	+	+	—	+	—	—	+	+	(*a*), (*i*)
4-diethylaminostilbene	S.C.	S	+	+	--	—	—	+	—	—	—	—	(*a*)
2′-methyl-4-dimethylamino-stilbene	S.C.	S	+	+	--	—	—	+	—	—	—	—	(*a*)
1-(4′-dimethylaminophenyl) 2-(1′-naphthyl)-ethylene	S.C.	S	+	—	—	—	—	—	—	—	—	—	(*a*)

2′-choro-4-dimethylamino-stilbene	I.P.	S	−	+	+	+	−	−	−	−	+	+	.	(*i*)
4-aminoazobenzene	Oral	S	−	+	−	−	−	−	−	−	−	−	.	(*a*)
4-dimethylaminoazobenzene	Oral S.C.	F	−	+	−	−	−	−	−	−	−	−	.	(*a*)
2′ : 3-dimethyl-4-aminoazobenzene	Oral	F	+	+	−	−	−	−	−	−	−	−	.	(*a*)

* P = Painting. S.C. = Subcutaneous injection. I.P. = Intraperitoneal injection.
† A = Compound tested on two or more occasions. S = Single publication from one laboratory only. S* = Preliminary communication. F = Compound fully tested.

References.—(*a*) Hartwell, 1951 ; (*b*) Bonser, *et al.*, 1952 ; (*c*) Walpole, *et al.*, 1952 ; (*d*) Miller, *et al.*, 1952 ; (*e*) Miller, *et al.*, 1949 ; (*f*) Morris, Dubnik and Johnson, 1950 ; (*g*) Bielschowsky and Bielschowsky, 1952 ; (*h*) Spitz, *et al.*, 1950. (*i*) Elson, 1952.

TABLE 2.—*Sites of Election of Tumours induced by Aromatic Amines in the Mouse.*

Compound.	Administration.*	Adequacy of testing.†	Site of Tumours: Local.	Liver.	Intestine.	Renal pelvis or kidney.	Bladder.	Thyroid.	Breast.	Female generative tract.	Others.	References.
2-naphthylamine	S.C. Oral B.I.	S	+*	+	−	−	−	−	−	−	−	(*a*), (*d*)
2-anthramine	S.C.	S	−	+	−	−	−	−	−	−	−	(*b*)
2-aminofluorene	Oral	S	−	+	−	+	+	−	−	−	−	(*b*)
2-acetamidofluorene	Oral	F	−	+	−	+	+	+	+	+	+	(*b*)
4-dimethylaminostilbene	S.C.	S	+	−	−	−	−	−	−	−	−	(*b*)
1-(4′-dimethylaminophenyl)-2-(1′-naphthyl) ethylene	S.C.	S	?	−	−	−	−	−	−	? −	−	(*b*)
4-amino-2′ : 3-azotoluene	Oral S.C.	F	+	+	−	−	−	−	−	−	Lung	(*b*), (*c*)
4-amino-2 : 4′-azotoluene	Oral	S	−	+	−	−	−	−	−	−	−	(*c*)
2-amino-2′ : 5′-azotoluene.	Oral	S	−	+	−	−	−	−	−	−	−	(*c*)
4-dimethylaminoazobenzene	Oral	F	?	+	−	−	−	−	−	−	−	(*b*)

* S.C. = Subcutaneous injection. B.I. = Bladder implantation.
† S = Single publication from one laboratory only. F = Compound fully tested.

References.—(*a*) Bonser *et al.*, 1952 ; (*b*) Hartwell, 1951 ; (*c*) Crabtree, 1949 ; (*d*) Hackmann, 1951.
* Two tumours obtained after 2 mg. technical 2-naphthylamine injected in oil.

TABLE 3.—*Sites of Tumours induced by Aromatic Amines in Various Species.*

Compound.	Species.	Administration.	Adequacy of testing.†	Site of Tumours. Local.	Liver.	Bladder.	Ureteric.	Renal cortex and Kidney.	Lung.	Others.	References.
2-naphthylamine	Dog	Oral	F	—	—	+	—	—	—	—	(*a*)
	Rabbit	Oral	S	—	—	+	—	—	—	—	(*b*)
2-acetamidofluorene	Dog	Oral	A	—	? *	—	—	—	—	—	(*c*)
				—	+	+	—	—	—	—	(*f*)
	Cat	Oral	S	—	+	—	—	+	+	—	(*a*)
	Rabbit	Oral	A	—	—	+	+	—	—	—	(*d*)
	Fowl	Oral	S	—	—	—	—	+	—	—	(*a*)
benzidine	Dog	Oral	S	—	—	? —	—	—	—	—	(*e*)
4-dimethylaminoazobenzene	Dog	Oral	S	—	—	+	—	—	—	—	(*g*)
o-aminoazotoluene	Dog	Oral	S	—	+	+	—	—	—	Gall bladder	(*g*)

† F = Compound fully tested. S = Single publication from one laboratory only. A = Compound tested on two or more occasions.

References.—(*a*) Hartwell, 1951; (*b*) Bonser *et al.*, 1952; (*c*) Allison *et al.*, 1950; (*d*) Bonser and Green, 1950; (*e*) Spitz, *et al.*, 1950; (*f*) Morris and Eyestone, 1953; (*g*) Nelson and Woodard, 1953.

* Changes may have been due to diet as histology of controls not reported.

2. The similarities in chemical structure and biological activity of those carcinogens which contain an aromatic amino group have been reviewed.

3. The need for further experimental work, both biological and biochemical, has been emphasised.

REFERENCES.

ALLISON, J. B., WASE, A. W., LEATHEM, J. H., AND WAINIO, W. W.—(1950) *Cancer Res.*, **10**, 266.

BADGER, G. M., AND LEWIS, G. E.—(1951) *Brit. J. Cancer*, **6**, 270.

BAKER, K. W.—(1951) *Acta Un. int. Cancr.*, **7**, *cif.* 46.

BAKER, K. W.—(1952) *II^me int. Congr. Biochim.*, Lons-le-Saunier (M. Declume), p. 459.

BIELSCHOWSKY, F.—(1944) *Brit. J. exp. Path.*, **25**, 90.—(1945) *Biochem. J.*, **39**, 287.

Idem AND BIELSCHOWSKY, M.—(1952) *Brit. J. Cancer*, **6**, 89.

BONSER, G. M.—(1943) *J. Path. Bact.*, **55**, 1.

Idem, CLAYSON, D. B., AND JULL, J. W.—(1951) *Lancet*, ii, 286.

Iidem, AND PYRAH, L. N.—(1952) *Brit. J. Cancer*, **6**, 412.

Idem, AND GREEN, H. N.—(1950) *J. Path. Bact.*, **62**, 531.

CRABTREE, H. G.—(1949) *Brit. J. Cancer*, **3**, 387.

ELSON, L. A.—(1952) *Ibid.*, **6**, 392.

GILLMAN, J., GILLMAN, T., AND GILBERT, C.—(1949) *S. Afr. J. med. Sci.*, **14**, 21.

HACKMANN, C. W.—(1951) *Z. Krebsforsch.*, **58**, 56.

HADDOW, A., HARRIS, R. J. C., KON, G. A. R., AND ROE, E. M. F.—(1948) *Phil. Trans.*, **241A**, 147.

HARTWELL, J. L.—(1951) 'Survey of Compounds which have been Tested for Carcinogenic Activity.' 2nd ed., Washington, D.C. (U.S. Publ. Hlth Serv.).

HENDRY, J. A., HOMER, R. F., ROSE, F. L., AND WALPOLE, A. L.—(1951) *Brit. J. Pharmacol.*, **6**, 357.

HOCH-LIGETI, C.—(1947) *Brit. J. Cancer*, **1**, 391.

HUEPER, W. C., AND WOLFE, H. D.—(1937) *Amer. J. Path.*, **13**, 656.

JULL, J. W.—(1951) *Brit. J. Cancer*, **5**, 328.

MILLER, J. A., MILLER, E. C., SANDIN, R. B., AND BROWN, R. K.—(1949) *Cancer Res.*, **9**, 504.

Idem, MILLER, E. C., SANDIN, R. B., AND RUSCH, H. P.—(1952) *Ibid.*, **12**, 283.

MORRIS, H. P., DUBNIK, C. S., AND JOHNSON, J. M.—(1950) *J. nat. Cancer Inst.*, **10**, 1205.

Idem, AND EYESTONE, W. H.—(1953) *Ibid.*, **13**, 1139.

MUELLER, G. C., AND MILLER, J. A.—(1951) *Cancer Res.*, **11**, 271.

NELSON, A. A., AND WOODARD, G.—(1953) *J. nat. Cancer Inst.*, **13**, 1497.

PEACOCK, P. R.—(1948) *Ann. Rep. Brit. Emp. Cancer Campgn.*, **26**, 198.

ROBINSON, D., SMITH, J. N., AND WILLIAMS, R. T.—(1951) *Biochem. J.*, **50**, 221, 228.

SIMPSON, C. L.—(1952) *Brit. J. exp. Path.*, **33**, 524.

SMITH, J. N., AND WILLIAMS, R. T.—(1949) *Biochem. J.*, **44**, 242.

SPITZ, S., MAGUIGAN, W. H., AND DOBRINER, K.—(1950) *Cancer*, **3**, 789.

WALPOLE, A. L., WILLIAMS, M. H. C., AND ROBERTS, D. C.—(1952) *Brit. J. industr. Med.*, **9**, 255.

WILLIAMS, R. T.—(1947) 'Detoxication Mechanisms.' London (Chapman Hall), p. 138.