THE CARCINOGENIC ACTION OF 4-AMINODIPHENYL AND 3:2'-DIMETHYL-4-AMINODIPHENYL

BY

A. L. WALPOLE, M. H. C. WILLIAMS, and D. C. ROBERTS

*From Imperial Chemical Industries Ltd. Medical Department and
Biological Laboratories, Hexagon House, Manchester*

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Tumours of the bladder in dyestuff workers have been recorded with increasing frequency during the last 50 years. A high proportion of the recorded cases are attributable to exposure to b-naphthylamine or benzidine; experimental evidence of their carcinogenicity has accumulated during recent years. Hueper and Wolfe (1937), Hueper, Wiley, and Wolfe (1938), and Bonser (1943) have produced malignant bladder tumours in dogs with b-naphthylamine. With benzidine, Spitz, Maguigan, and Dobriner (1950) have obtained tumours of the acoustic gland, liver, and large bowel in rats as well as one bladder tumour in a dog, and we have confirmed their results in rats.

The term "aniline cancer" was applied to this hazard in the dyestuffs industry because a number of the early cases of bladder tumours were associated with the manufacture of aniline (Wendel, 1900; Rehn, 1905; Seyberth, 1907), and with dyestuffs from aniline (Rehn, 1895; Bardenheuer, 1904; Seyberth, 1907; Cesa-Bianchi, 1911; Leuenberger, 1912). Owing to the poor fractionation of crude benzol in these early days "anilines" at that time contained considerable amounts of toluidines and xylydines. It was in fact the presence of p-toluidine as an impurity that enabled Perkin in 1856 to discover mauveine, the first synthetic dyestuff. Recent reports tend to discount aniline as a cause of tumours arising during the last 20 years, although a variety of other aromatic compounds have been suspected (Gehrmann, Foulger, and Fleming, 1949; Goldblatt, 1949; Barsotti and Vigliani, 1949; Müller, 1951; Scott, 1952).

Hergt (personal communication), who has records of a number of early cases in Germany, finds that they were rare in aniline makers but not uncommon in workers making toluidines and xylydines. He believes that these early "aniline tumours" are attri-

butable to toluidines and that their complete absence in men starting work in Germany during the last 40 years is due to improved working conditions, resulting in considerably reduced exposure. The first tumours described by Rehn (1895) were in fuchsine workers, who would be heavily exposed to aniline, toluidines, and xylydines in the course of their work. There is, however, no reliable experimental evidence that either aniline, toluidines, or xylydines are carcinogenic. In an attempt to find an explanation for the early tumours attributed to these bases, a survey was made of the literature relating to the manufacture of aniline in Germany before 1900. This revealed that in 1862 Hofmann isolated a new based, 4-aminodiphenyl (xenylamine), from the distillation residues of "heavy aniline" (*anilin für rot*). Loring Jackson, a collaborator of Hofmann, continued this work, and apart from isolating naphthylamines from similar residues, found therein a monomethyl homologue of aminodiphenyl melting at 46.5°–47.5°C. (acetyl derivative, m.p. 114.2°C.) (Loring Jackson, 1875). This latter amine has not been identified but it would not appear to be any of the known isomers.*

Hofmann (1862) thought that the 4-aminodiphenyl in the residues arose from the cracking of aniline, but Loring Jackson's finding of naphthylamines suggests that these bases were formed by the nitration and reduction of naphthalene, diphenyl, and methyl diphenyl (as well as of toluene and xylenes) all occurring as impurities in the benzene used for making aniline. Kruber (1932) has in fact isolated 2-methyldiphenyl and 3-methyldiphenyl from coal tar and Loring Jackson's amine is possibly a derivative of one of these hydrocarbons. Kruber isolated 4:4'-dimethyl and 3:4'-

* The known monomethyl homologues of aminodiphenyl are 3-methyl-2-aminodiphenyl, m.p. 62–63°C., 4'-methyl-4-aminodiphenyl, m.p. 97°C., 3-methyl-4-aminodiphenyl, m.p. 41–42°C., or 2'-methyl-4-aminodiphenyl, m.p. not given (acetyl derivative m.p. 146–147°C.).

dimethyl-diphenyl at the same time and we see no reason to doubt that other isomers also were present.

As methods of distillation have improved greatly it is not surprising that examination of present-day residues from the distillation of aniline has failed to reveal the presence of naphthylamines, 4-aminodiphenyl, or its homologues.

If the naphthylamine present in Loring Jackson's aniline residues resulted from the nitration and reduction of naphthalene contaminating his benzene, it would be equivalent to commercial α -naphthylamine, which is of low carcinogenic power. We therefore thought it worth while to determine whether 4-aminodiphenyl and its methyl homologues were carcinogenic and, if so, whether they were sufficiently active to have played a significant part in the genesis of early "aniline" tumours. We were encouraged to believe that 4-aminodiphenyl might prove to be carcinogenic by the recent demonstration by Miller, Miller, Sandin, and Brown (1949) that *N*:*N*-dimethyl-4-aminodiphenyl produces tumours in the rat and by our own findings and those of Spitz, Maguigan, and Dobriner (*loc. cit*) that benzidine (4:4'-diaminodiphenyl) has a

similar action. In selecting a methyl homologue for first test in comparison with 4-aminodiphenyl itself we chose 3:2'-dimethyl-4-aminodiphenyl.

This choice was made because, in the azo series of liver carcinogens, 4-amino-3:2'-azotoluene is known to be more active than aminoazobenzene itself. As will be seen below, the disposition of methyl and amino groups in 3:2'-dimethyl-4-aminodiphenyl is analogous to that in 4-amino-3:2'-azotoluene.

Preparation of Compounds

4-Aminodiphenyl.—4-Nitrodiphenyl was prepared by the Gomberg reaction (Elks, Haworth, and Hey, 1940) and purified by chromatography. This was reduced with iron and ferrous chloride in aqueous xylene and the final product was fractionated through a gauze packed column (b.p. 302°C.) at atmospheric pressure.

3:2'-Dimethyl-4-aminodiphenyl.—This compound was prepared by Dr. R. H. Wilson especially for this experiment (Wilson, *in press*).

Animal Experiments

The animals were albino rats from a closed but random mated colony which has been maintained for some years in these laboratories. They were housed upon sawdust in galvanized wire mesh cages and were allowed to feed at will upon a composite diet in pellet form, with tap water available at all times. The diet was supplied by Scottish Agricultural Industries Ltd., and had the following percentage composition by weight.

Ground barley	..	26.5	Dried skimmed milk	..	13.0
Ground oats	..	18.5	Meat and bone meal	..	8.6
Bran	..	18.3	White fish meal	..	4.4
Maize meal	..	8.6	Dried yeast	..	1.3
Salt	..	0.4	"Nuclio"*	..	0.4

At the start of the experiment the rats were 3 months old and each weighed about 100 g. They were weighed and dosed on a body weight basis five days a week throughout the dosing period. The dosing of individual animals was temporarily stopped if they lost weight and the length of the dosing period varied between groups according to the response observed.

The compounds were dissolved in arachis oil (B.P.) and the solutions given by subcutaneous injection alternately in the right and left pectoral region. Twelve male and 12 female rats were given 4-aminodiphenyl and like numbers arachis oil alone as a control: 24 male rats were treated with 3:2'-dimethyl-4-aminodiphenyl. Sub-divisions of the groups receiving the two chemicals were dosed at different levels in order to gain information upon the effect of dosage on response. The details of the dosage are shown in Table 1.

Results

The results given in Table 2 show that a large proportion of the treated animals developed tumours in a variety of sites. Whereas the rats given 3:2'-dimethyl-4-aminodiphenyl were all killed by the 259th day, the 4-aminodiphenyl animals survived much longer. All except two of the 23 control rats survived considerably longer than the 260 days. Twelve out of these control animals survived 500 days or over as against 14 in the group given 4-aminodiphenyl. The incidence of tumours at various sites in the different groups will now be compared.

* "Nuclio" is a proprietary vitamin concentrate containing 800 i.u. of vitamin A and 100 B.S.I. units of vitamin D₃ per g.

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TABLE 1
DOSAGES OF EXPERIMENTAL RATS

Compound	Group	No. of Rats	Sex	Mean Duration of Dosing (days)	Mean Total Dose (g./kg.)	Mean Total Volume of Oil (ml./kg.)
4-aminodiphenyl	A	5	Male	376	5.8	280
	B	6	Female	286	4.2	235
	C	6	Male	291	4.4	236
	D	6	Female	250	3.6	203
3:2'-dimethyl-4-aminodiphenyl	E	12	Male	141	2.8	70
	F	11†	Male	131	3.5	69
Arachis oil	G	12	Male	274		331
	H	11‡	Female	296		347

* One died on the 37th day. † One died on the 45th day. ‡ One died on the 30th day.

Tumours at the Site of Injection.—These tumours were observed only in rats which lived more than 400 days. They occurred in five control rats and in four of those given 4-aminodiphenyl. It will be seen that none developed in the animals receiving 3:2'-dimethyl-4-aminodiphenyl, all of whom died or were killed within 260 days. The occurrence

of these tumours in both the control group given arachis oil and those given 4-aminodiphenyl in arachis oil leads us to believe that they were due to the oil.

Compound	Times of Appearance of Tumours (days)
4-aminodiphenyl	418, 498, 500, 502
Controls (arachis oil)	421, 537, 544, 575, 576
3:2'-dimethyl-4-aminodiphenyl	Nil

Sarcomata in bold type, the remaining two being fibromata.

The local tumours were all at least 2 cm. in diameter. Two tumours consisted of well-differentiated coarse connective tissue with few cells and were considered to be fibromata. The remainder had the histological appearance of sarcomata of either spindle cell or mixed cell type. No metastases had occurred, but in the sections examined local infiltration of surrounding tissue was present in one tumour of the arachis oil group and in one where 4-aminodiphenyl had been given.

Mammary Tumours.—These are not unknown in untreated animals of our stock, although none appeared in the control group of 12 males and 11 females in this experiment given arachis oil alone. The appearance of two adenocarcinomata

TABLE 2
INCIDENCE* AND CLASSIFICATION OF TUMOURS

Compound	Group	Number of animals	Sex	Benign hepatoma	Benign cholangioma	Reticulosis	Sarcoma of liver	Intestinal tumours	Mammary tumours	Fibroma at injection site	Sarcoma at injection site	Tumours of salivary glands	Tumours of acoustic sebaceous glands	Papilloma of bladder	Adenoma of kidney	Carcinoma of uterus	Subcutaneous carcinoma of unknown origin	Number of normal animals	Times (days) when Normal Animals Died or Were Killed
4-aminodiphenyl	A	5	Male	—	1	—	1	1	—	—	—	—	—	—	—	—	—	2	(346 and 537)
	B	6	Female	—	—	—	—	2	—	—	—	—	—	—	—	—	—	2	(364 and 398)
	C	6	Male	1	—	1	—	3	—	2	—	—	—	—	—	—	—	—	(105 and 523)
	D	6	Female	—	—	—	—	1	2	1	—	—	—	—	—	1	1	2
3:2'-dimethyl-4-aminodiphenyl	E	12	Male	—	—	—	—	12	1	—	—	4	4	—	—	—	—	—	
	F	11	Male	—	—	—	—	9	—	—	—	1	—	1	—	—	—	2	(69 and 119)
Controls	G	12	Male	—	—	—	—	—	—	—	1	—	—	—	—	—	—	11	(73, 253, 322, 329, 383, 435, 572, 572, 575 and 575)
	H	11	Female	—	—	—	—	1	—	—	4	—	—	—	—	—	—	7	(262, 434, 492, 501, 575, 582 and 582)

* The figures refer to the number of rats bearing the types of tumour listed.

and one adenofibroma with areas of exuberant growth amongst 12 females given 4-aminodiphenyl and one adenocarcinoma in a male given 3 : 2'-dimethyl-4-aminodiphenyl must be regarded as abnormal.

Tumours Involving the Salivary Glands.—These tumours were observed only in the group given 3 : 2'-dimethyl-4-aminodiphenyl. They occurred at 193, 193, 216, 224, and 252 days respectively and were sarcomata infiltrating and strangling the salivary glands. They were not associated with oil collected at the injection site as were the fibromata and sarcomata observed at a much later date in the other two groups. Tumours involving these glands have been described by Heimann and Meisel (1946) in animals given acetaminofluorene by mouth.

Tumours of the Acoustic Sebaceous Gland.—Tumours of this gland occurred in four rats given 3 : 2'-dimethyl-4-aminodiphenyl. They were first observed at 153, 156, 173, and 176 days respectively. In one rat a squamous cell carcinoma developed, whereas bilateral tumours of the same type occurred in another (Fig. 1). A third developed a sebaceous cell carcinoma, and the fourth gross cystic enlargement of the gland with thick irregular squamous stratified epithelium lining the cysts.

Lesions of the Liver.—No liver tumours were observed in the control group but some of these older animals did show a mild proliferation of the bile ducts. In the 4-aminodiphenyl group one animal developed a large benign cholangiomatous cyst with no invasion of the liver parenchyma. Another developed a hepatoma whilst a third had an extensive sarcoma in the liver which infiltrated the pancreas as well. No primary sarcoma was found elsewhere in this rat. Yet another animal in this group developed a reticulosis, with gross infiltration of the whole liver. Cells were escaping into the circulation and were found in the lung vessels. They were not, however, forming secondary infiltrations.

No gross changes were observed in the livers of the animals given 3 : 2'-dimethyl-4-aminodiphenyl.

Tumours of the Intestinal Tract.—One tumour of the caecum was observed in a control rat killed at 570 days. This lesion was raised on the internal surface of the bowel, and atypical disorganized glands penetrated the muscularis mucosae. No other tumours of the intestine occurred in the control group.

Tumours of the intestines occurred in all but two of the animals treated with 3 : 2'-dimethyl-4-aminodiphenyl. In almost every case they were multiple and appeared along the whole length

FIG. 1.—Transverse section of rat's head (3 : 2'-dimethyl-4-aminodiphenyl) showing bilateral carcinoma of sebaceous acoustic gland. Note invasion of external auditory meatus on one side. ($\times 4$)

FIG. 2.—Abdominal contents of rat (3 : 2'-dimethyl-4-aminodiphenyl) showing multiple lesions of the large and small intestines.

of the large and small intestine (Fig. 2). In only one rat did a tumour occur in the small intestine when there were none in the large bowel.

In the group given 4-aminodiphenyl tumours occurred only in the large intestine. They were found in seven out of the 14 rats which survived over 500 days.

In the 3 : 2'-dimethyl-4-aminodiphenyl series the animals were killed because of haemorrhage, diarrhoea, or intestinal obstruction often associated with intussusception. Thus the relative times of discovery of the tumours in the three groups have some significance, although the majority of 4-aminodiphenyl tumours occurred in the caecum where obstruction was less likely to occur. No tumours of the forestomach were observed in any of the animals.

Compound	Times of Appearance of Intestinal Tumours (days)
3 : 2'-dimethyl-4-aminodiphenyl	80, 104, 138, 146, 149, 160, 167, 189, 190, 191, 191, 193, 195, 205, 209, 216, 248, 259, 259, 259 and 259
4-aminodiphenyl	570, 570, 570, 570, 576, 577 and 577
Controls	570

It is clear that all the tumours in the group given 3 : 2'-dimethyl-4-aminodiphenyl developed very much earlier than those in the other groups. This fact, coupled with the incidence of tumours of the small intestine in this group, is taken as

an indication of the greater carcinogenic activity of 3 : 2'-dimethyl-4-aminodiphenyl as against 4-aminodiphenyl itself.

The tumours of the large intestine varied considerably in type and extent. Those involving several centimetres of the intestine showed generalized thickening of the gut wall associated with multiple hard nodules on the peritoneal surface. The early tumours tended to be of this type and sometimes the mucosal surface was ulcerated whereas later lesions appeared as either sessile reddened adenomata or pedunculated tumours. The latter were particularly prone to be carried down the lumen with resultant intussusception.

The histological picture of tumours of the large intestine may be divided into the following classes for descriptive purposes but, in fact, a variety of intermediate lesions was observed. The smallest tumours were characterized by a thickening of the submucosa associated with disorganization and hypertrophy of the glands (Fig. 3). The

FIG. 3.—Early lesion of large intestine of rat (4-aminodiphenyl). ($\times 12$)

larger, sessile adenomatous type showed an increase of this process and thickening of the bowel wall with or without breaching of the muscularis mucosae, which was often drawn up on either side of the tumour. The glands extended down into the submucosa, sometimes with cyst formation, infiltration with leucocytes and eosinophils, and associated thickening of the peritoneum. Alternatively, the tumour took on a pedunculated form with considerable hypertrophy of the glands, the muscularis mucosa being drawn up into the

FIG. 4.—Adenomatous polyp of large intestine of rat (4-aminodiphenyl). ($\times 6$)

connective tissue core (Fig. 4). In the most marked lesions, disorganized and atypical glands, usually hyperchromatic and containing a diminished number of goblet cells, repeatedly penetrated the submucosa and invaded or penetrated the muscularis externa forming cystic projections on the bowel surface covered with thickened peritoneum (Fig. 5). In these latter cases the circular muscle often showed local thickening and loss of polarity as described by Stewart and Lorenz (1946-47).

The tumours of the small bowel followed a similar pattern with local thickening of the bowel and protrusion of hyperplastic glandular tissue into the lumen. In some other cases the muscle layers were breached and cystic projections covered with thickened peritoneum appeared on the surface of the small bowel.

In our lesions of the large and small intestines there was no infiltration of surrounding tissues as occurred in the adenocarcinomata of the colon described by Spitz, Maguigan, and Dobriner (1950). Our material shows a marked similarity to the lesions of the bowel of mice following oral administration of 20-methylcholanthrene and 1, 2, 5, 6-dibenz-anthracene described by Stewart and Lorenz (1946-47). Indeed, following Stewart's classification, our most advanced tumours could be considered as adenocarcinomata. On the other hand, Lushbaugh and Hackett (1948-49), who obtained infiltrating adenomatous lesions of the colon in rats given lubricating oil by mouth, inclined towards a diagnosis of reactive hyperplasia for lesions which again closely resembled those described here.

Further work which is proceeding on the characterization of these intestinal lesions will be described in a later communication.

Discussion

Our results demonstrate that 4-aminodiphenyl produces tumours in the rat at various sites, including the intestinal tract. Benzidine also produces tumours of the intestine in the rat and causes bladder tumours in man. By analogy 4-aminodiphenyl also may be capable of causing human bladder tumours. In view of this possibility, we suggest that the manufacture of 4-aminodiphenyl for industrial use should be carried out only with the most stringent precautions.

Despite our belief that 4-aminodiphenyl and commercial α -naphthylamine are capable of producing bladder tumours in man, we do not believe that the small amounts of these high-boiling amines present in distillation residues could have accounted fully for the early tumours amongst "aniline" makers. It appears to be even less likely that significant quantities of these amines would have been present in the distillate used subsequently for dyestuff manufacture. Similar conclusions can be reached regarding compounds as active as 3:2'-dimethyl-4-aminodiphenyl. Nevertheless our results are of intrinsic interest and have led us to develop a hypothesis concerning the carcinogenic action of aromatic amines.

FIG. 5.—Adenocarcinoma of the large intestine of rat (3:2'-dimethyl-4-aminodiphenyl) showing glands infiltrating through muscularis externa and forming cyst-like projections under peritoneal surface. ($\times 23$).

Our experiments show that 3:2'-dimethyl-4-aminodiphenyl produces distant tumours earlier and more frequently than 4-aminodiphenyl itself. The tumours obtained by Miller and others (1949) with N:N-dimethyl-4-aminodiphenyl also appeared earlier and in a higher proportion of animals than we observed with 4-aminodiphenyl. It will be recalled that 4-amino-3:2'-azotoluene and N:N-dimethylaminoazobenzene are much more active liver carcinogens than aminoazobenzene itself. It thus appears that 3:2'-dimethyl or N:N-dimethyl substitution augments carcinogenic activity in both the 4-aminodiphenyl and aminoazobenzene series.

In experiments as yet uncompleted tumours of the intestine have appeared at 106 days with 3:3'-dimethyl-4-aminodiphenyl and at 120 days with 3-methyl-4-aminodiphenyl. These times correspond very closely with the earliest appearance of tumours in our animals treated with 3:2'-dimethyl-4-aminodiphenyl. These latter results lead us to believe that it is the methyl group ortho to the amino rather than the methyl group in other ring positions which is responsible for the increase in activity in this series.

Since the incidence of tumours at the site of injection of 4-aminodiphenyl and its homologues in no cases exceeded that in the controls, it appears that the compounds do not exert a direct and local carcinogenic action, such as is observed with polycyclic hydrocarbons of the type of dibenzanthracene or methyl cholanthrene. We conclude that the effective carcinogen is in all cases a metabolite of the amine given and that the appearance of tumours throughout the intestinal tract (excluding the forestomach) in the rat suggests that these metabolites are excreted in this species in the bile.

Recently published work upon two aromatic amines which similarly produce only distant tumours suggests that the effective metabolite may be an ortho-hydroxy amine. Engel (1920) showed that b-naphthylamine was metabolized by the dog to 2-amino-1-naphthol, and Hueper (1938) obtained evidence suggestive of carcinogenicity by injecting the latter compound intraperitoneally into mice. Bonser, Clayson, and Jull (1951) have shown that 2-amino-1-naphthol has a local carcinogenic action on the bladder epithelium of mice, and

have obtained (personal communication) further evidence that 2-amino-1-naphthol is locally carcinogenic although control tests were negative. Further, Clayson has been preparing o-hydroxy amines related to other carcinogenic aromatic amines for biological testing.

Adler (1908) showed that benzidine was metabolized by the rabbit to a dihydroxy derivative of melting point 130–138°C. Baker (1950) isolated a compound from human urine subsequently identified as 3:3'-dihydroxybenzidine and has shown (personal communication) that when fed to rats it rapidly produces intestinal tumours. These intestinal tumours appeared considerably earlier than any obtained by Spitz, Maguigan, and Dobriner (1950) or by ourselves by injecting benzidine itself subcutaneously. This and the fact that Baker obtained tumours of the forestomach suggests that 3:3'-dihydroxybenzidine may be the effective metabolite.

A further example of an o-hydroxy amine acting as a carcinogen may be deduced from the finding of Kirby and Peacock (1949) that benzeneazo-b-naphthol is a liver carcinogen in the mouse. Reductive fission of the azo linkage is well known to occur in this animal, and would yield 1-amino-2-naphthol in this case.

Although direct evidence is not as yet available regarding the metabolism of compounds of the 4-aminodiphenyl series, results obtained with aniline and its derivatives in the dog indicate that o-methyl or N:N-dimethyl substitution results in the "steering" of metabolic hydroxylation from the para into the ortho position relative to the amino group.

Thus aniline is mainly metabolized to para-aminophenol by the dog, whereas o-toluidine is excreted largely as o-amino-m-cresol. This picture is made complete by the fact that the dog demethylates dimethylaniline and excretes o-aminophenol (Williams, 1947).

We suggest that the metabolism of the corresponding derivatives in the 4-aminodiphenyl series follows the same pattern as shown below, although it is again pointed out that we have no direct evidence of this.

If in this case also the o-hydroxyamines are the effective carcinogenic metabolites, this would explain the increased carcinogenic activity of the methyl substituted amines as compared with 4-aminodiphenyl itself.

If the suggestion that o-hydroxyamines are direct-acting carcinogens is valid for such simple compounds as o-aminophenol, we should expect o-toluidine and dimethylaniline, but not aniline, to produce bladder tumours in the dog. Gehrman and others (1949) in fact state that they have been unable to produce tumours in the dog with aniline. In order to obtain evidence to substantiate these hypotheses, experiments have been started in which o-toluidine and dimethylaniline will be given for long periods in high dosage to dogs.

Indirect support for the suggestions, first, that simple o-hydroxyamines are direct acting

carcinogens and, secondly, that the metabolic yield of them from an amine is increased by o-methylation or N:N-dimethylation, may be deduced from the observation that 2:3'-azotoluene yields bladder papillomata in rats (Otsuka and Nagao, 1936) whereas azobenzene itself is inactive as a carcinogen (Spitz, Maguigan, and Dobriner, 1950). Reductive fission of the azo linkage would yield o- and m-toluidine in case of 2:3'-azotoluene, whereas azobenzene would only yield aniline.

The relationship between structure and carcinogenic activity in other azo compounds can be explained in the same way, although clearly the reducibility of the azo linkage will exert a considerable influence on the result. Thus, of the six following isomers of aminoazotoluene fed by Crabtree (1949) to mice, only one, namely 2'-amino-4:5'-azotoluene, failed to show any activity, and this is the only one of these which on reductive fission would not form an o-methyl amine. Furthermore, activity was highest in 4'-amino-2:3'-azotoluene which would divide to form two molecules each containing an o-methyl amine.

Finally this hypothesis provides a theoretical explanation for Hergt's view that the early "aniline tumours" amongst dyestuffs workers were due to o-toluidine, absorbed by workmen in considerable

quantities during the early days of the chemical industry. The fact that such tumours, unassociated with contact with b-naphthylamine or benzidine, are very rare today suggests that modern conditions have removed the heavy contamination necessary for the production of such tumours in man.

Summary

4-Aminodiphenyl has been shown to be carcinogenic in the rat and it is suggested that it may be capable of causing bladder tumours in man. The presence of this base, together with naphthylamines, in the distillation residues of "heavy aniline" is thought to have been partly responsible for early "aniline cancers".

3:2'-Dimethyl-4-aminodiphenyl has been shown to be more potent than 4-aminodiphenyl itself as a carcinogen in the rat. It has been suggested that in each case the effective carcinogen is an o-hydroxyamine, formed in the body by metabolic hydroxylation, and that the higher activity of the 3:2'-dimethyl homologue of 4-aminodiphenyl is due to the preferential direction of the hydroxy group into the position ortho to the amino group under the influence of the 3-methyl group.

It is suggested that o-hydroxy amines as simple as o-aminophenol may be direct-acting carcinogens. On this basis it is possible to explain the relationship between structure and the capacity of azo compounds to induce tumours of the liver in experimental animals. On this basis also it appears likely that o-toluidine may be capable of producing bladder tumours in man and the dog.

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Since this paper was submitted Bonser, Clayson, and Jull have shown that commercial 1-amino-2-naphthol is also a local carcinogen.