THE RELATIVE CARCINOGENIC ACTIVITY OF SIX ISOMERIC AMINOAZOTOLUENES.

H. G. CRABTREE.

From the Laboratories of the Imperial Cancer Research Fund, London, N.W.7.

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IT has been suggested from studies of the action of inhibitors of S-metabolism on tumour induction by carcinogenic hydrocarbons that S-metabolism and the process of carcinogenesis are closely linked (Crabtree, 1947). This association is emphasized when the properties of azo-carcinogens are considered. The products of their metabolism have been shown to inhibit the activities of urease (Potter, 1942) and succinoxidase (Elson and Hoch-Ligeti, 1944), two enzymes dependent upon intact SH-groups for their proper functioning. Kensler, Dexter and Rhoads (1942) studied the relative inhibitory action of a series of " split-products " on a standard diphospho-pyridine-nucleotide system, and sought to correlate this activity with the carcinogenic power of the parent azo-compound. Suggestive parallelisms were found, but a clear-cut relationship did not emerge.

Much of this work has been based on the conception (experimentally verified for p-dimethylaminoazobenzene by Stevenson, Dobriner and Rhoads (1942), and for azo-benzene by Elson and Warren (1944)) that an azo-carcinogen is initially metabolized by the process of reductive fission with the liberation of an amine and a diamine, and that the latter is the effective carcinogen by virtue of its capacity to interfere with normal enzymic activities.

It is not clear why the diamine moiety alone should have been conceived as having special significance for the process of cancer induction. A survey of the azo-compounds so far tested for carcinogenic activity makes it evident that in some cases the amine moiety of the reduced molecule plays a significant role in determining potency, e.g. on comparing p-dimethylaminoazobenzene with its p'-methyl derivative, the introduction of the p'-methyl group causes a change from a very strong to a very weak carcinogen.

The present work was undertaken to probe this problem further. Isomeric aminoazotoluenes were chosen as suitable for a study of the relative contributions made by the amines and diamines which would arise from their reduction. The well-known carcinogen 4'-amino-2 : 3'-azotoluene (o : o) and the non-carcinogen 2'-amino-4 : 5'-azotoluene (p : p) served as bases of reference, and their behaviour was compared with that of the hybrid compounds o : p and p : o. Two other isomers—m : m and p : m—were also tested to provide further comparative data.

Note on abbreviations used.—All the isomeric aminoazotoluenes are prepared by coupling diazotized o-, m-, or p-toluidine with o-, m-, or p-toluidine, and the abbreviations reflect their molecular structure, e.g. p : m signifies that diazotized p-toluidine is coupled with m-toluidine, forming 4'-amino-4 : 2'-azotoluene, and so on.

MATERIAL AND METHODS.

Animals.—The experiments were carried out with rats of strain 52, descendants of a group originally selected for high susceptibility to transplantation of Jensen's rat sarcoma, and mice of the Simpson strain. A total of 210 rats weighing from 80 to 110 g., and 280 mice, 3–4 months old, were used, each divided into 7 groups of 30 rats or 40 mice. Six groups of each species were fed on the basal diet supplemented by 0·06 per cent of one of the six aminoazotoluenes, and the seventh group, fed on the basal diet alone, acted as controls.

In order that the possible influence of sex could be observed, each group of rats was divided into sub-groups of 10 males, 10 females, and 10 with members of both sexes. A similar separation was made with the mice into corresponding sub-groups of 10, 10 and 20.

Each cage or box contained 5 animals, and excess of the diets was always present, together with water *ad libitum*.

Diet.—The semi-synthetic diet was based on the one named R.D. 3 by Kirby (1947) which, in turn, closely resembled that developed by Miller, Miner, Rusch and Baumann (1941) as being suitable for promoting a high incidence of liver tumours with p-dimethylaminoazobenzene and related compounds. The latter was low in protein content, inadequate in some members of the B group of vitamins, and incapable of promoting normal growth in rats. The single source of carbohydrate was glucose monohydrate, for which Kirby substituted starch in his R.D. 3 diet. The carbohydrate used in the experiments described here was commercial dextrin, with 5 per cent sucrose added to increase palatability.

Basal diet.

	Per cent.
Casein (B.D.H. soluble, white, light)	12
Dextrin (B.D.H. technical yellow)	71
Sucrose	5
Salts (Harris, Krahl and Clowes, 1947)	4
Arachis oil	5
Cod-liver oil	1
Dried yeast	2

The dry constituents were mechanically mixed and the two oils then added with further mixing. The azo-dye was dissolved in the oils in an amount representing 0·06 per cent of the complete diet.

N.B.—When 0·06 per cent of p-dimethylaminoazobenzene was incorporated in this basal diet and fed to rats, liver tumours were produced in 90 per cent of the animals during 3–5 months.

Isomers of aminoazotoluene used.

Name of isomer.	Abbreviation.
2′-amino-4 : 5′-azotoluene	p : p
4′-amino-4 : 2′-azotoluene	p : m
4′-amino-3 : 2′-azotoluene	m : m
4′-amino-4 : 3′-azotoluene	p : o
2′-amino-2 : 5′-azotoluene	o : p
4′-amino-2 : 3′-azotoluene	o : o

Preparation of the aminoazotoluenes.

o : o.—Obtained commercially. Purified by crystallization from aqueous EtOH. Long, red-brown prisms with blue sheen. M.P. 100°.

m : m.—A solution of 13·8 g. (1 mole) $NaNO_2$ was added slowly to 57·4 g. (2 moles) m-toluidine hydrochloride dissolved in 200 ml. water, at room temperature (Mehner, 1902). The crystalline hydrochloride suspended in EtOH was neutralized with ammonia, and an equal volume of boiling water added. The free base was re-crystallized from aqueous EtOH. Golden yellow needles. M.P. 81°.

p : p (*a*) 4 : 4′-*diazoaminotoluene.*—Prepared by a modification of the method of Nietzki (1877). 96·6 g. (2 moles) p-toluidine hydrochloride were dissolved in 350 ml. H_2O and cooled to 5–7°. A solution of 23·4 g.(1 mole) of $NaNo_2$ was slowly run in over 2 hours with stirring. The pale yellow, microcrystalline diazoamino-compound was used for stage (*b*) without further purification. M.P. 114–115°. Crystallization from aqueous EtOH gave large prisms. M.P. 116°.

(*b*) 2′-*amino*-4 : 5′-*azotoluene.*—(p : p) 1 mole (*a*) + 1 mole p-toluidine hydrochloride + 6 moles p-toluidine were heated at 65° for 10 hours (Zincke and Lawson, 1886). The molten mass was neutralized with dilute NaOH, p-toluidine removed by steam distillation, and the brown residue crystallized from aqueous EtOH. Orange red needles. M.P. 119°.

p : m.—67·5 g. 4 : 4′-diazoaminotoluene (1 mole) + 43·05 g. m-toluidine hydrochloride (1 mole) + 450 ml. EtOH were kept at 2° for 3 days, with occasional shaking (Mehner, 1902). The crystalline dye-hydrochloride was filtered (35 g.) and a second crop (19 g.) was obtained after addition of 500 ml. N HCl and standing overnight at 2°. Recrystallization from aqueous EtOH gave orange-red needles with metallic sheen. The base was obtained as described for m : m. Large rectangular golden-yellow plates. M.P. 128°.

p : o.—Prepared by method used for p : m (Mehner, 1902). After 5 days at 2° the red-brown solution was treated with an equal volume of N HCl, and EtOH largely removed on the water bath. The dye-hydrochloride crystallized after standing overnight at 2°. Yield = 66 per cent theory. The base was obtained as described for m : m. Orange-red needles. M.P. 128°.

o : p (*a*) 2 : 2′-*diazoaminotoluene.*—Based on the method of Fischer and Wimmer (1887). 28·7 g. o-toluidine hydrochloride (2 moles) + 8·7 ml. HCl (S.G. 1·18 — 1 mole) + 100 ml. H_2O were cooled to − 5°, and a solution of 6·9 g. $NaNO_2$ (1 mole) added during 5 minutes. A solution of 25 g. sodium acetate was slowly run in, and, after stirring for 4 hours, the pale yellow, micro-crystalline diazoamino-compound was collected. Yield = 70 per cent theory. M.P. 47°. This was used for (*b*) without further purification.

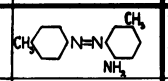
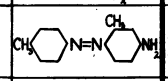
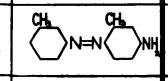
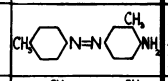
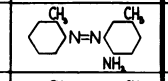
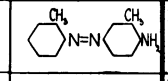
(*b*) 45 g. (*a*) (1 mole) + 28·7 g. p-toluidine hydrochloride (1·1 moles) + 380 ml. EtOH were kept at 2° overnight. 300 ml. N HCl were added and EtOH largely removed on the water bath. Yield of dye hydrochloride was 47 g. (90 per cent theory). Recrystallization from aqueous EtOH gave orange-red needle clusters. The base was obtained as described for m : m. Orange-red rectangular plates. M.P. 95°.

EXPERIMENTAL RESULTS.

The livers, and sometimes other organs, of all animals were examined at death, or whenever possible when death was imminent.

Many rats were lost through cannibalism and intercurrent infection, principally endemic bronchopneumonia. Mice responded badly to the basal diet, with or without the addition of dye. Many were lost in the first 6 months of the experiment, but the death-rate was lowered in the later stages by feeding them with standard laboratory food and the synthetic diet alternately for periods of two weeks.

TABLE I.—*Changes Produced by Six Isomeric Aminoazotoluenes in the Livers of Rats and Mice, surviving* 300–595 *days. The histological classification is based on a succession of changes reflecting increasing degrees of liver damage, and each* × *refers to the liver of one animal.*

		RAT LIVERS					MOUSE LIVERS				
	AMINOAZOTOLUENE ADDED TO BASIC DIET	NORMAL	MULTIPLE AREAS OF PERILOBULAR NECROSIS	REGENERATION OF LIVER CELLS IN NECROTIC AREAS	MICROSCOPIC NODULES OF HEPATOMA IN SOME AREAS OF REGENERATION	FULLY DEVELOPED HEPATOMA (ONE CHOLANGIOMA)	NORMAL	IRREGULARITY OF PATTERN AND SIZE OF NUCLEI	MORE SEVERE CHANGES WITH GIANT VACUOLATED NUCLEI	MICROSCOPIC NODULES OF HEPATOMA OR BEGINNING OF CHOLANGIOMA	MALIGNANT HEPATOMA (OCCASIONAL CHOLANGIOMA)
p:*p*		XXXXX XXXXX XXXXX	XXXXX X				XXXXX XXX	XXXX			
p:*m*		XXXXX XXXXX XXXXX XX	XXX					XXX	XXX	X	XXXXX
m:*m*		XXXXX XXXXX	XXXXX XXXX				XXXX		XXXXX XXXXX	X	
p:*o*		XXXXX XXXXX XXXXX	XXXXX XXX	X			XXXXX	XXXXX XXX	X	X	
o:*p*			XXX	XXXXX XXXXX X	XXX				XX	XXXXX X	XXX
o:*o*		XXXX	XXXXX XXX	XXXXX XX	XXXXX	XXX			XX	XXX	XXXXX XX
	BASIC DIET ALONE	XXXXX XXXXX XXXXX X	XXXXX XXX				XXXXX XXXXX X	XXXXX XX			

Under these circumstances quantitative estimates of the relative tumour-inducing properties of the various aminoazotoluenes were not easy to make. However, since the survival rates in the different groups of animals were not widely different, a qualitative picture of the broad features of the results emerged, and some significant differences in the action of the six isomeric compounds were demonstrated.

(a) *Histological observations.*

Omitting animals which died before 250 days, the average time of survival of both rats and mice in the various groups was 400–435 days from the beginning of the experiment. A few were alive after 595 days and were killed.

The whole liver was removed, fixed in Bouin and sections made from several or all of the lobes, and stained with eosin and haematoxylin. This was necessary, since macroscopically visible tumours were rare.

The histological findings are collected in Table I, where each × refers to the liver of one animal. The succession of changes used in this classification reflect increasing degrees of liver damage. The first characteristic abnormalities visible in the livers of rats differed from those commonly observed in the livers of mice, though the final pictures of tumour emergence were similar in both species. In building up the general picture four grades of cellular change have been used.

In rat livers perilobular necrosis constituted the primary change, and this was followed by regeneration of liver cells in the necrotic areas. In some regions of regeneration microscopic nodules of hepatoma made their appearance, and later these became visible to the eye as dull grey patches on the surface of the lobes. Often a simultaneous intense proliferation of bile ducts was manifest in one or more lobes, and the early phases of cholangioma formation would coincide with the development of small hepatomas. No metastases were ever found.

In mouse livers no preliminary necrosis was observed, but the cellular pattern became abnormal with great irregularity in the disposition and size of the nuclei. Further distortion was accompanied by the formation of cells with giant vacuolated nuclei, which preceded the emergence of microscopic hepatomas. The fully developed hepatomas of mice were more malignant than those of rats as judged by the degree of atypical growth, though again no metastases were ever found.

It will be noted that the earliest histological changes mentioned sometimes occurred in both rats and mice consuming the basal diet without dye-addition. This response of the liver to diets inadequate in several essential food factors may well reflect the basic disturbance of liver function which is a necessary precursor to the carcinogenic action of azo-compounds.

(b) *Relative carcinogenic activity of the six isomeric aminoazotoluenes.*

Considering the data summarized in Table I from the point of view of the relative carcinogenic potency of these isomers, three points emerge:

1. Mice were more susceptible than rats to the action of these azo-compounds. With the exception of p : p, which proved innocuous in both species, all the isomers were capable of inducing liver tumours in mice, but varied in potency among themselves. In three cases, o : o, o : p and p : m, malignant hepatomas were produced, and severe lesions, with one microscopic hepatoma, were caused by m : m.

2. Only two isomers—o : p and o : o—induced tumours in rats. In all other cases, except for one rat receiving p : o, perilobular necrosis represented the utmost limit of damage, and this lesion was found, though in lesser degree, in the livers of rats fed on the basic diet alone.

3. Except in the case of p : m, which induced a high percentage of tumours in mice surviving 400 days and was innocuous for rats, the relative potency of the isomers was similar in both species.

(c) *Growth rate of rats on basal diet with azo-dye addition.*

All rats (but not mice) were weighed at fortnightly intervals throughout the experiment. Small differences of growth-rate within the various groups became noticeable after 3–4 months, and later they became more accentuated. Since all other environmental factors were similar for all the groups, these differential growth rates could only be attributed to the azo-dyes themselves.

The possible influence of sex on the growth rate was assessed by keeping the rats of each series separated into three sub-groups containing respectively 10 males, 10 females and 5 males + 5 females. Though the average female rat weighed 15–20 g. less than the average male of the same age, the differential growth-rates due to the added azo-dyes were evident in both sexes, and were unaffected by pregnancies, since breeding did not occur after the first few months of the experimental period.

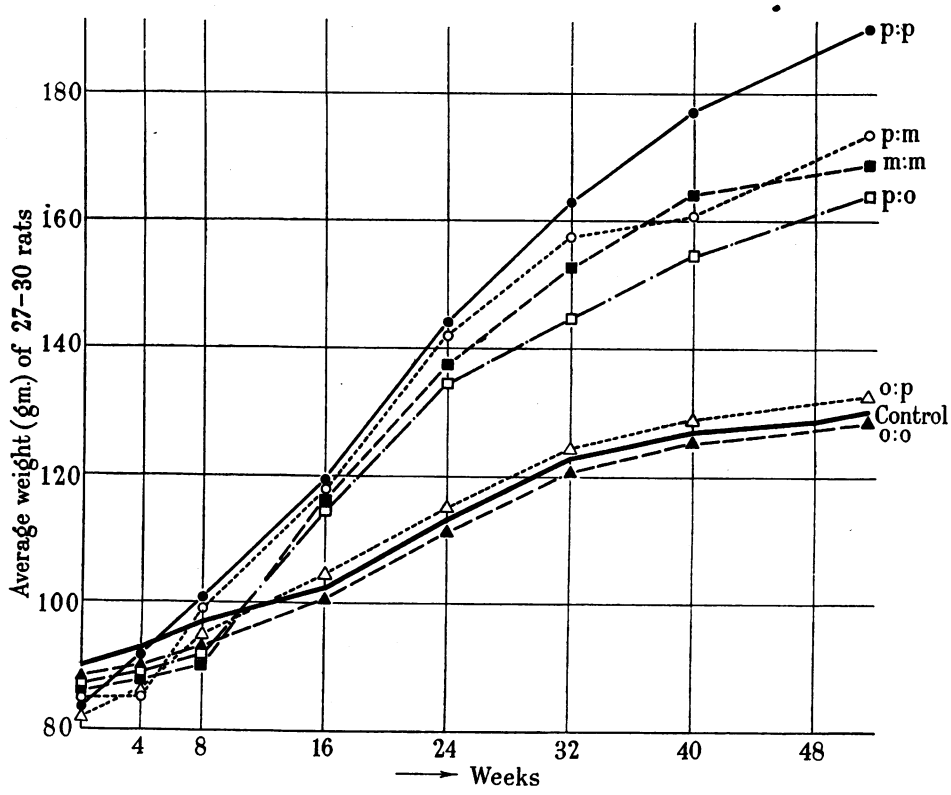


FIG. 1.—Growth curves of rats fed on the basal diet, with addition of 0·06 per cent of an aminoazotoluene. The differential effects on growth of the six isomers are shown.

The points shown in the curves in Fig. 1 are therefore based on the average weights of each group of rats, regardless of sex. The following features of these growth-curves may be emphasized :

1. The basal diet was inadequate for normal growth, but permitted limited growth and long survival.

2. The two isomers o : o and o : p had no inhibitory action on body growth, or more exactly, they had no additional effect on growth already inhibited by the basal diet.

3. The four remaining isomers—p : p, p : m, m : m and p : o—caused a stimulation of growth-rate in varying degrees, the most effective being the p : p isomer.

On comparing these effects on rat-growth with the histological data in Table I a suggestive correlation is obvious ; all the isomers which caused growth-stimulation were non-carcinogenic, whereas those lacking this property were carcinogenic, for rats.

(d) *Growth rate of rats on basal diet, with addition of toluidines.*

Two investigations have shown that azo-compounds are metabolized in the rat by initial reduction at the azo-group, yielding the corresponding amines (Stevenson, Dobriner and Rhoads, 1942 ; Elson and Warren, 1944). On the assumption that this splitting process occurred with the aminoazotoluenes used

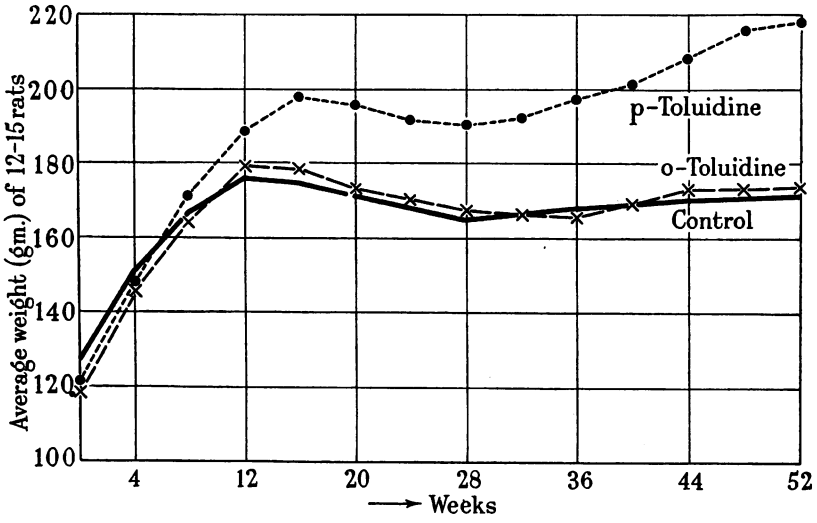


FIG. 2.—Growth curves of rats fed on the basal diet, with addition of 0·06 per cent o- or p-toluidine.

in the work reported here, the two carcinogenic isomers would yield o-toluidine, while 3 of the 4 growth-stimulating isomers would yield p-toluidine as the primary products of reductive fission.

The possible effects on growth of these two toluidines were therefore tested.

Forty-five rats of the 52 strain, each weighing 110–130 g., were divided into three groups of 15. Two groups were fed on the basal diet containing 0·06 per cent of either o-toluidine or p-toluidine, and the third group received the basal diet alone. Each group of 15 rats was subdivided into three separated groups of 5 males, 5 females, and 5 containing males and females. In the latter group two litters were born during the first 3 months, but none subsequently. They were weighed at fortnightly intervals over a year.

The growth curves are shown in Fig. 2. Since no difference of response due to sex was noted, the average weights refer to all surviving animals. At least 12 animals survived in each group for a year, but the general condition of those receiving p-toluidine was significantly better than that of the other groups.

The growth-response caused by o-toluidine was negligible, but p-toluidine acted as a growth-stimulator, particularly in the later stages of the experiment. The sag in the middle range of the curves is an odd feature for which no explanation can be offered, but it does not detract from the main result of this experiment.

Observations on these results.

This commentary will be restricted to the experiments in which rats were used. It makes comparison with previous work easier, since most investigations have been carried out on rats living under a standardized dietary regime similar to the one used here, and current hypotheses as to the mode of action of azo-carcinogens are largely based on such findings.

Though the degree of liver damage and the incidence of liver tumours was higher in mice than in rats, there is a broad parallelism between the relative potency of this series of aminoazotoluenes in the two species.

The variable response of different species to a given carcinogen remains a fundamental problem which cannot, as yet, be expressed in biochemical terms. Kirby (1945), considering the mechanisms by which the azo-compounds are degraded in the organism, has suggested that the known alternative paths of metabolism may vary quantitatively in rats and mice, with formation of benzidine derivatives (postulated by Cook, Hewitt, Kennaway and Kennaway, 1940) as the principal reaction occurring in mice, and reductive fission predominating in rats. On the other hand, Miller and Miller (1947) emphasize the importance of the protein constitution of the host, and associate the likelihood of tumours developing with the degree to which an azo-compound is " bound " to the proteins of rat livers. In the case of rats consuming p-dimethylaminoazobenzene this fixation is prominent, but does not occur in species resistant to the carcinogenic action of the substance. These differences can only be attributed to the special properties of the proteins characteristic for each species.

The " split-product " hypothesis.

Kirby (1945) has reviewed the work bearing on this hypothesis of the mechanism of carcinogenesis by azo-compounds, and throws doubt on its validity by citing several examples which fail to conform with its main premises. He emphasized in particular the low carcinogenic power of p'-methyl-p-dimethyl-aminoazobenzene when compared with the high activity of the parent p-dimethyl-aminoazobenzene, since both compounds should yield the same postulated active diamine split-product. This example, and others to be mentioned, makes it clear that the other half of the reduced molecule—the amine moiety—plays an important role in determining carcinogenic activity if the primary assumption be accepted that reductive fission is an essential preliminary to this process.

This consideration is emphasized by the results presented here. In fact the possible significance of the " second half " of the reduced azo-molecule, almost entirely ignored by previous workers, prompted these experiments. o : o was known to be carcinogenic, and p : p non-carcinogenic for rats and mice, or translated in terms of the " split-product " hypothesis, 2 : 5-toluylene diamine is active while 3 : 4-toluylene diamine is innocuous.

To throw light on the relative contributions made by the amine to diamine parts of the reduced molecule, the hybrid isomers o : p and p : o were prepared and their action compared with that of the related o : o and p : p isomers. The experiments demonstrated that o : o and o : p were carcinogenic, while p : p and p : o were non-carcinogenic.

Reviewing these results in the light of the " split-product " hypothesis, no correlation between carcinogenic activity and the potential diamine fission products is found. p : p and o : p should yield the same " innocuous " o-toluylene diamine, while o : o and o : p should yield the same " active " p-toluylene diamine, but the facts are otherwise, since each of these pairs contains one carcinogen and one non-carcinogen.

By contrast, when the amine halves of the reduced molecule are considered, the results show that the two isomers which should yield p-toluidine on reductive fission—p : p and p : o—are non-carcinogenic, while the pair yielding o-toluidine —o : o and o : p—are carcinogenic. In brief, if carcinogenic activity is indeed a consequence of the action of the " split-products," it is the amine, and not the diamine, which determines the subsequent biological response.

The literature contains several examples of the controlling influence which a methyl group exerts on carcinogenesis, when attached at a para position with respect to the azo-group. Miller and Baumann (1945) compared the activities of the o′, m′, and p′ methyl derivatives of p-dimethylaminoazobenzene with that of the parent compound. The m′-compound was extremely active (7 rats out of 8 bearing liver tumours in 4 months), the o′-compound moderately active (4 rats out of 9 bearing tumours in 8 months), and the p′-compound was very weakly carcinogenic (1 rat out of 10 with a tumour in 10 months). Nagao (1941) also found that p′-methyl-p-dimethylaminoazobenzene was only a weak carcinogen, and that the substances formed by placing a further methyl group in the ring carrying the dimethylamino-group were entirely innocuous. Sugiura (1948) tested a series of derivatives of p-methylaminoazobenzene containing an additional methyl group in the o′, m′ or p′ positions. The order of potency—m′>o′>p′—was similar to that mentioned above.

All these observations seem relevant to the present work. The common feature is the great loss of tumour-producing activity which results when a methyl group is added in the p′ position to the parent carcinogenic molecule, forming derivatives with the potentiality of liberating p-toluidine on reductive fission.

A working hypothesis is put forward tentatively in an attempt to co-ordinate the data outlined above. When the structural features and the biological action of these six isomeric azotoluenes are considered, a correlation is evident between their growth-stimulating properties, their lack of carcinogenic power, and the presence of an additional methyl group in the position described. The two latter characteristics are common to all the substances used by Miller and Baumann, Nagao and Sugiura, but these workers do not mention any differential growth responses in the rats used in their studies.

The amines emerging from reductive fission may undergo further metabolic changes (Stevenson, Dobriner and Rhoads, 1942 ; Elson and Warren, 1944). They are detoxicated and excreted, in the examples given, as acetylated diamines or as aminophenols. The breakdown products of the aminoazotoluenes have not yet been investigated, but presumably the toluidines are initially formed. Jaffe

and Hilbert (1888) fed the three isomers of acetotoluidine to dogs. o-Acetotoluidine was oxidized to an aminophenol and isolated as methylbenzoxalone, while m- and p-acetotoluidines were excreted as acetylated amino-benzoic acids. It may therefore be conjectured that all azo-compounds containing a methyl group at a position para to the azo-group will yield p-aminobenzoic acid as a final product of their metabolic degradation within the rat.

The experiment pictured in Fig. 2 shows that p-toluidine can stimulate growth in rats fed on a diet permitting only limited growth and maintenance. If this is due to the liberation of p-aminobenzoic acid, the question arises : Is the synthesis of folic acid indirectly influenced by feeding appropriate azo-compounds *via* the succession of changes

azo-compound ⟶ p-toluidine ⟶ p-aminobenzoic acid ⟶ folic acid ?

And further, what is the relation of folic acid to the induction of tumours ?

Folic acid, in its relation to the cancer problem, has been widely investigated in recent years, but its possible association with tumour induction is uncertain, since the major effort has been made in assessing its therapeutic possibilities. The early work of Lewisohn, Leuchtenberger, Leuchtenberger and Keresztesy (1946), who reported regression in a high percentage of transplanted mammary tumours when pteroyl triglutamic acid was injected intravenously, has been followed by several clinical trials (Leading Article, 1948). It has also been shown that the development of Rous sarcoma, after injection of the virus in baby chicks, was completely prevented by maintaining the birds on a folic-acid-free diet. Tumour growth was prevented in 40 per cent of birds up to 6 weeks of age. Certain folic acid antagonists were equally effective for this purpose, and were also able to cause cessation of growth in Rous sarcomas already established (Little, Sampath, Paganelli, Locke, and Subbarow, 1948).

In the field of bacterial metabolism there is strong evidence that p-aminobenzoic acid is concerned with the synthesis of folic acid, purines, thymine and methionine, and that the sulphonamides may inhibit reactions leading to the synthesis of proteins and nucleic acids (Woods, 1947).

The folic acid requirements of rats have not been studied so widely as those of chicks, since the symptoms of deficiency are not so easily recognized. Woolley (1947) states that " experimental tricks such as the administration of drugs are necessary to produce a dietary need for the vitamin." This is exemplified by the haematological response following the administration of sulphonamides to rats which is reversed by injection of folic acid (Endicott, Daft and Ott, 1945), or the similar changes produced by protein depletion or inadequate riboflavine (Kornberg, Daft and Sebrell, 1945).

It is tempting to interpret the experimental results described in this paper in terms of folic acid balance. The diamines resulting from the reductive fission of azo-carcinogens have been shown to inhibit many enzyme reactions under oxidative conditions, and it is conceivable that they interfere with the function of enzymes concerned in the synthesis of folic acid, producing adverse effects comparable to those produced by sulphonamides, though by a different mechanism. Conversely the non-carcinogenic, growth-stimulating isomers of amino-azotoluene could be visualized as precursors of p-aminobenzoic acid, the liberation of which could counteract the damage produced by depletion of folic acid.

The limited growth conditioned by a diet low in proteins and inadequate in its complement of B-vitamins reinforced by the postulated inhibition of folic acid synthesis would then constitute the metabolic disturbances favourable for the emergence of tumours.

SUMMARY.

1. Six isomeric aminoazotoluenes have been tested for their carcinogenic activity in the livers of mice and rats, when added at 0·06 per cent concentration to a standard, semi-synthetic diet. They are labelled o : o, m : m, p : p, o : p, p : o and p : m respectively to denote the two toluidines used in their preparation.

2. A series of characteristic histological changes, reflecting increasing degrees of liver damage, has been used in classifying the results, which serve to indicate the relative potency of the six isomers.

3. Mice were more susceptible than rats, though the order of potency, with one exception, was the same in both species. All the isomers, except p : p, proved carcinogenic for mice, but only two of them—o : o and o : p—were carcinogenic for rats.

4. The growth-curves of each group of rats consuming the different amino-azotoluenes are shown. The two carcinogens, o : o and o : p, did not influence the growth rate, but the four non-carcinogens—p : o, p : p, m : m and p : m—stimulated growth in varying degrees the p : p isomer being the most effective.

5. Rats were fed on the same basal diet containing 0·06 per cent of either o- or p-toluidine. o-Toluidine produced no effect on growth, but p-toluidine acted as a growth stimulant.

6. These results are discussed in the light of the " split-product " hypothesis, which they fail to support. Also, on the assumption that carcinogenic properties and growth effects are conditioned by their " split-products," a correlation had been established between growth stimulation, non-carcinogenicity and the release of p-toluidine.

7. Since the detoxication of p-toluidine can produce p-aminobenzoic acid, the possible relation between folic acid metabolism and the mechanism of cancer induction is considered.

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PREVENTION OF ABDOMINAL FIBROIDS INDUCED WITH ARTIFICIAL OESTROGENS.

S. BRUZZONE.

From the Department of Experimental Medicine, National Health Service of Chile, Santiago.

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ABDOMINAL fibroids induced in the guinea-pig by α-oestradiol (Nelson, 1937 ; Lipschutz and Iglesias, 1939) are prevented when progesterone or other 3-keto-steroids are administered simultaneously with the oestrogen (Lipschutz and Vargas, 1941 ; Lipschutz, 1944 ; Lipschutz, Iglesias, Bruzzone, Fuenzalida and Riesco, 1948). The antifibromatogenic action of progesterone becomes manifest with as little as 13 to 24 μg. per day absorbed from a subcutaneously implanted tablet (Lipschutz, Bruzzone and Fuenzalida, 1944). Since similar abdominal fibroids have been elicited also with diethylstilboestrol and hexoestrol (Lipschutz and Vargas, 1940 ; Lipschutz, Vargas, Egaña and Bruzzone, 1941) the question arose whether progesterone will be as active against these artificial oestrogens as against α-oestradiol. This question is of considerable interest. Nothing definite is so far known on the mechanism by which the antifibromatogenic action of steroids is effected. Will the greater resistance of these artificial oestrogens against intrahepatic inactivation (Zondek, Sulman and Sklow, 1943 ; Segaloff, 1944 ; Lipschutz, Quintana and Bruzzone, 1944) be associated with a quantitatively different behaviour towards progesterone ?