Final Report

Grant OH-00611

Title: "Mechanism of Action: Carcinogenic o-Methylarylamines"

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Introduction

Studies by Walpole, in the early 1950s, showed that the introduction of a methyl group ortho to the amine function of 4-aminobiphenyl (AB) increased the carcinogenicity of the compound towards the large intestine of rats. Furthermore, a second methyl group substitution at the 2' position to give 3,2'-dimethyl-4-aminobiphenyl produced an even more potent carcinogen toward the colon in male and female rats and the mammary gland in female rats. It will be noted that the parent compound, AB, is a potent carcinogen for the urinary bladder in the dog and in man, although bladder tumors in rats are not normally seen.

The effect of ortho methyl substitution in increasing the carcinogenicity of aromatic amines was further investigated extensively by the Weisburgers. In a series of bioassays using both rats and mice, these workers showed that ortho methyl or methoxy substitution has a dramatic enhancing effect on the carcinogenicity of not only dual ring aromatic amines (for instance 2-naphthylamine) but also of single ring aromatic amines (for instance, o-toluidine and o-anisidine are much more potent carcinogens than aniline, and the corresponding m- and p-isomers are either inactive or marginally active).

This program was initiated in order to elucidate some of the mechanisms whereby ortho-methyl substitution of AB and 2-naphthylamine leads to enhanced carcinogenicity and modified organotropism. As previously mentioned, AB and 2-naphthylamine are known human urinary bladder carcinogens, while DMAB and 3-methyl-2-naphthylamine (MNA) produce either bladder tumors or colon and mammary tumors, depending on the species and sex of the experimental animal. Moreover, as will be discussed later, DMAB also produces prostatic carcinoma in male F344 rats. Thus these compounds represent excellent experimental animal models for the induction of three of the most important human cancers. Also significant is the occurrence of closely related aromatic and heterocyclic amines in occupational and non-occupational environments including dye manufacturing processes, tobacco smoke, polluted air, broiled meats and hair dyes. Thus an understanding of the mechanisms of action of these compounds can provide leads to rational preventive measures as well as enhanced ability to extrapolate animal bioassay data to man. To achieve such understanding, several different approaches were used. These included a) animal bioassay studies; b) structure-activity studies and c) metabolism studies. Each is described in turn below.

Summary of work performed

a) Animal bioassay studies

It had been noted by So and Wynder (1) that DMAB produced

urinary bladder tumors as well as intestinal cancers in hamsters, although extensive experience with this compound in rats (2-5) showed an absence of bladder tumors. This curious species difference was therefore re-investigated and extended to MNA, since such species differences can often be meaningful in corroborating information as to mechanisms obtained from metabolism studies.

In this bioassay, as more fully described in reference 6, seventy-two male non-inbred Syrian golden hamster weanlings were obtained from Charles River Breeding Laboratories, North Wilmington, Mass. The hamsters were housed 5/polycarbonate cage and fed the basal diet Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.). The average initial weight of each hamster was 150 g. All the hamsters were found to have pinworms during the initial quarantine period and were treated with 25 mg dichlorvos before starting the experiment.

DMAB was obtained from the National Cancer Institute Chemical Repository through the assistance of Dr. D. Longfellow and Dr. E.K. Weisburger. MNA was provided by Dr. E.K. Weisburger. Both chemicals were homogeneous by gas-liquid chromatography.

The hamsters were divided into 3 groups. Two groups were given repeated sc injections of peanut oil (U.S.P.) solutions of the test compounds, whereas the controls received the vehicle alone.

In group 1, 22 hamsters served as controls and received 0.3 ml of peanut oil injected sc once a week for 8 weeks and then once every 2 weeks up to 22 months, for a maximum total number of 38 injections.

Twenty-five hamsters in group 2 were given sc injections of 100 mg/kg body weight of MNA in peanut oil on the same schedule as group 1, for a maximum total of 555 mg (3.5 mmol) given in 38 injections over 22 months.

Twenty-five hamsters in group 3 were also given sc injections of 100 mg DMAB/kg body weight in 0.3 ml of peanut oil base as in group 1, for a maximum total dose of 555 mg (2.8 mmol) given in 38 injections over 18 months.

All hamsters were killed with CO_2 and necropsied either during the experiment if they appeared moribund or at the end of the experimental period at 21-22 months. All organs were examined for gross abnormalities, and slices were taken from liver, lung, spleen, kidney, urinary bladder, stomach, random normal, and grossly abnormal portions of small and large bowel, pancreas, lymph nodes (if enlarged), and injection site. Slices were submitted for histologic examination in 10% buffered Formalin. The Formalin-fixed tissues were embedded in paraffin from which 5-µm-thick sections were cut. The slides were stained with H & E by

standard procedures. Whenever necessary, additional sections were cut and stained with trichrome (Mallory's), periodic acid-Schiff and mucicarmine stains.

The tumor yields are reported in Table 1.

TABLE 1.—*Tumors produced in Syrian golden hamsters by MeNA and DMAB*[a]

Organ and pathologic lesion	Group 1, control				Group 2, MeNA				Group 3, DMAB			
	0–6 (2)	7–12 (1)	13–18 (7)	19–22 (12)	0–6 (1)	7–12 (9)	13–18 (12)	19–21 (3)	0–6 (2)	7–12 (15)	13–18 (8)	19–21 (0)
Urinary bladder												
Noninvasive carcinoma	0	0	0	0	0	1	2	1	1	2	0	0
Invasive carcinoma	0	0	0	0	0	0	1	0	0	10	7	0
Sarcoma	0	0	0	0	0	0	0	0	0	0	1	0
Stomach												
Squamous papillomas	0	0	0	0	0	2	1	1	0	5	7	0
Small intestine												
Adenocarcinoma	0	0	0	0	0	0	0	0	0	5	2	0
Atypical proliferative enteritis	2	0	4	10	0	3	2	0	1	6	4	0
Colon												
Adenocarcinoma	0	0	0	0	0	0	0	0	0	4	2	0
Atypical proliferative enteritis	2	1	6	10	0	5	5	1	1	10	7	0
Lymph nodes												
Lymphoma and leukemia	0	0	0	0	0	1	2	0	0	5	1	0
Subcutaneous tissue sarcoma	0	0	0	0	1	0	6	1	0	0	0	0

[a] Ranges are experimental intervals in mo. *Numbers in parentheses* represent No. of hamsters found dead or killed. Values represent the numbers of animals affected.

These results confirm that DMAB produces mainly urinary bladder tumors in hamsters (1) and demonstrate that another aromatic amine, MNA, also has the bladder as one of its principal target organs in the hamster. These findings differ from the organotropism of the two carcinogens in rats, and reflect species differences in metabolism and distribution as discussed in section c.

Under exactly comparable conditions of application, MNA was definitely less active than DMAB. In addition to the absence of intestinal carcinogenicity by MNA, other key differences occurred in the organs affected. MNA but not DMAB caused local sarcoma formation at the injection site. The production of subcutaneous sarcomas by MNA corresponds to a similar action in mice and rats and adds to the list of chemicals known to cause such tumors in hamsters. The fact that MNA produced local sarcomas, but failed to induce intestinal neoplasms whereas DMAB did the opposite, relates to distinct metabolic pathways of these two compounds. Local sarcomas appear to result with arylamines with relatively low carcinogenic activity, such as o-toluidine. Yet even with more powerful carcinogens, which normally would kill the animals as a result of cancer development in organs such as liver, ear duct, or breast, the subcutaneous tissue contains initiated cells

The production of tumors in the forestomach by both MNA and DMAB suggests that the chemicals or metabolites after absorption from the subcutaneous site of injection may be secreted either through the salivary gland or the stomach to account for action at this site.

As a part of the same experiment, male F344 rats were compared to the hamsters in terms of their susceptibility to DMAB carcinogenesis. Also included in these studies was an examination of the possible influence of disulfiram to modify the carcinogenicity of DMAB in both species. Disulfiram had previously been found to completely inhibit the carcinogenicity of 1,2-dimethylhydrazine for the colon in mice (7), an effect which is due to the inhibition of the enzymes responsible for the N-oxidation of azomethane to azoxymethane, as elucidated by our laboratory (8). Since N-oxidation is also an activation pathway for aromatic amines, we expected that disulfiram might also decrease the carcinogenicity of DMAB.

The protocols and results are extensively described in reference 9. Briefly, male Syrian golden hamster weanlings, were maintained as described above. The animals were divided into three groups: Group I, the control group, consisted of 22 hamsters and received 0.3 ml of peanut oil, s.c., once a week for 8 weeks, then once every 2 weeks for up to 22 months (38 injections total). Group II, comprising 25 hamsters, was injected with 100 mg/kg of DMAB in peanut oil, s.c., once a week at the same dose schedule as Group I (total average cumulative dose of DMAB was 555 mg). Group III, consisting of 25 hamsters, was given disulfiram in the diet and DMAB at the same dose schedule as Group II. The concentration of disulfiram (Aldrich Chemical Co.) was 5 mg/kg at the beginning of the experiment, but due to a high (25%) mortality at that level within the first month, the concentration as reduced to 2.5 mg/kg for the remainder of the experimental period.

Seventy five Charles River CDF male rats were divided into three groups and maintained on Purina Lab chow. The first two groups of 30 rats each were injected with 20 mg DMAB/kg body weight in corn oil (approximately 0.1 ml/100 g body weight), s.c., once a week for 37 weeks. The total average cumulative dose per rat in Group I was 289 mg and in Group II, 241 mg. Simultaneously to the carcinogen administration, Group II was fed disulfiram initially at a concentration of 2.5 mg/g of diet. The third group of 15 rats was injected with 0.1 ml corn oil/100 g body weight, s.c., once a week for 37 weeks and fed 2.5 mg/g disulfiram in the diet. Due to poor weight gain on the disulfiram diet, at week 9 the concentration was reduced to 1 mg/g diet for both rat groups receiving the compound.

At the end of the experimental period, all the surviving animals were killed with CO_2 and necropsied. During the experiment, animals that appeared moribund were killed and autopsied as

were those that died spontaneously. Major organs and all tissues with tumors or other lesions were submitted for histopathological evaluation.

The results comparing the carcinogenicity of DMAB and the effects of disulfiram are shown in Tables 2 and 3.

Table 2

Incidence of pathologic lesions in Syrian golden hamsters.

Pathological lesion	Group I Control				Group II DMAB				Group III DMAB + Disulfiram			
	0–6[a] (2)	7–12 (1)	13–18 (7)	19–22 (12)	0–6 (2)	7–12 (15)	13–18 (8)	19–21 (0)	0–6 (8)	7–12 (5)	13–18 (12)	19–21 (0)
Stomach												
Squamous papillomas	0	0	0	0	0	5	7	0	0	2	9	0
Small intestine												
Adenocarcinoma	0	0	0	0	0	5	2	0	0	0	1	0
Atypical proliferative enteritis	2	0	4	10	1	6	4	0	0	2	7	0
Colon												
Adenocarcinoma	0	0	0	0	0	4	2	0	0	1	9	0
Atypical proliferative enteritis	2	1	6	10	1	10	7	0	–[b]	2	9	0
Urinary bladder												
Non-invasive carcinoma	0	0	0	0	1	2	0	0	1	0	0	0
Invasive carcinoma	0	0	0	0	0	10	7	0	0	2	12	0
Sarcoma	0	0	0	0	0	0	1	0	0	1	0	0

[a]Experimental interval in months; number in parenthesis represents hamsters found dead or killed.
[b]Autopsy material inadequate for histologic diagnosis.

Table 3

Incidence of pathologic lesions in rats.

Pathologic lesion	Group I DMAB				Group II DMAB + Disulfiram				Group III Disulfiram	
	0–40[a] (0)	41–50 (1)	51–60 (3)	61–68 (26)	0–40 (0)	41–50 (4)	51–60 (2)	61–68 (24)	0–50 (0)	51–68 (15)
Small intestine										
Adenoma	0	0	0	1	0	0	0	0	0	0
Adenocarcinoma	0	0	0	8	0	0	0	2	0	0
Colon										
Adenoma	0	0	0	7	0	0	0	6	0	0
Adenocarcinoma	0	0	0	1	0	0	0	3	0	0
Urinary bladder										
Non-invasive carcinoma	0	0	0	0	0	0	0	3	0	0
Invasive carcinoma	0	0	0	0	0	1	0	0	0	0
Prostate										
Carcinoma in situ	0	0	0	5	0	0	0	3	0	0
Testis										
Leydig cell tumor	0	0	0	10 (2)[b]	0	0	0	2 (2)	0	2
Peritesticular mesothelioma	0	0	0	2	0	0	0	0	0	0
Lung	0	0	0	5	0	0	0	4	0	1
Skin[d]	0	0	0	14 (4)	0	0	0	6 (1)	0	0
Subcutaneous tissue[e]	0	0	3 (2)	3 (1)	0	0	1	1	0	0
Ear duct	0	0	0	2	0	1	1	2 (2)	0	0
Others	0	0	0	1	0	0	0	1[c]	0	0

[a]Experimental interval in months; number in parenthesis represents rats found dead or killed.
[b]Number in parenthesis represents rats for which histologic material was not adequate.
[c]Group I, one metastatic adenocarcinoma in liver (origin unknown); Group II, one neurofibroma of heart.
[d]Eight tumors in Group I and 3 in Group II were found at or around the injection site.
[e]Five tumors in Group I and one in Group II were found at the ventral part of the neck.

Several features of this experiment are of interest. In both rats and hamsters, disulfiram treatment decreased the carcinogenicity of DMAB toward the small intestine, while little or no effect on carcinogenicity toward the colon was observed. In rats, administration of disulfiram together with DMAB led to the appearance of bladder tumors, which are not observed in rats given DMAB alone. In contrast, disulfiram did not increase the incidence of urinary bladder tumors in hamsters. A potentially very significant finding was the observation that DMAB induced prostatic carcinoma in situ in male rats but not in hamsters. This type of lesion had not previously been reported by other laboratories utilizing DMAB (2-5).

Prostatic tumors in animals have been induced by hormones or radiation. However, reliable models for chemically-induced prostatic cancer in animals are still not available. Our finding should contribute to the development of such models and may provide information as to the structural features necessary in a chemical carcinogen for organotropism toward the prostate.

Because of the importance of the finding that DMAB induced prostate tumors in F344 rats, a more thorough examination was made of all of the experiments carried out at this Institute in which DMAB was used. These results are present in reference 9, and are summarized in Table 4.

TABLE 4.—*Carcinogenicity of DMAB in rat prostate gland*[a]

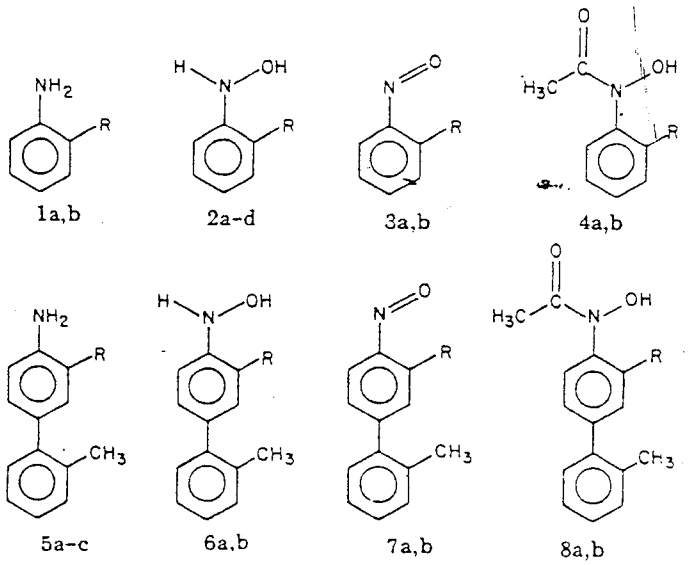
Experiment (reference)	No. of animals examined	No. of animals with change			Single DMAB dose, mg/kg body wt/wk (No. of wk)	Average cumulative dose DMAB/animal, mg	Total experiment period, No. of wk
		Early (small) carcinoma in situ (%)	Carcinoma in situ (%)	Invasive adenocarcinoma			
Expt I (*29*)							
DMAB alone	21	3	3	0	20 (42)	289	68
DMAB+disulfiram	25	2	5	0	20 (42)	241	68
Expt II[b] (*27*)							
DMAB+low fat	23	1	0	1	50 (20)	292	42
DMAB+high fat	21	4	0	0	50 (20)	296	42
Expt III[b,c] (*26, 27*)							
DMAB+low fat	37	3	1	0	50 (20)	259	50
DMAB+high fat	31	3	1	0	50 (20)	260	50
Expt IV (*28*)							
DMAB alone	44	17	3	0	50 (20)	275	60
DMAB+wheat bran	45	16	9	0	50 (20)	272	60
DMAB+citrus pulp	46	17	6	0	50 (20)	266	60
Total	293	66 (23)	28 (10)	1			
Expt V[d] (unpublished data)							
DMAB+low fat	21	0	0	0	20 (40)	256	86
DMAB+high fat	21	0	0	0	20 (40)	260	86
Control[e]	144	0	0	0	0	0	42-86
Control[f]	30	1	3	0	0	0	82

[a] Data from 5 experiments, with diverse-specific aims, that were performed at our institute, March 1976 through April 1980.
[b] Low fat diet: 5% beef tallow; high fat diet: 20% beef tallow.
[c] Germfree F344 strain maintained at our institute were used. (For details, *see* text.)
[d] All animals were castrated at 90 days of age, according to procedure of Fingerhut and Veenema (*5*). Low fat diet: 5% lard; high fat diet: 33% lard.
[e] Vehicle controls and untreated animals of all experiments. The longest experiment with normal rats lasted 68 wk.
[f] Control group of experiment performed in 1980-81, extending over 82 wk.

These results corroborate our previous findings and indicate that DMAB is the first available systemically acting chemical that reliably induces prostate adenocarcinoma. Modification of the experiment protocols may lead to improved yields, especially of the more advanced lesions. Such studies are currently planned and in part have been initiated at this Institute, particularly since DMAB has a structure similar to certain naturally occurring dietary compounds suspected of being associated etiologically with the development of cancer in the colon, breast, and prostate gland. It will be important to study the underlying endocrine situation, inasmuch as prostate cancer is likely under endocrine control, as is breast cancer and, therefore, possibly is subject to dietary modulation. The model described should be useful to study systematically the modifying factors, such as diet and the endocrine elements, under highly controlled conditions. Thus, as in the case of breast cancer such model studies can provide background information on the pathogenesis and etiology of prostate carcinoma in humans.

b. Structure-activity relationship studies

For the purposes of corroborating the ortho-methyl effect, a series of N-oxidixed derivatives of aniline (1a), 2'-methyl-4-aminobiphenyl (5a), and the carcinogens o-toluidine (1b) and 3,2'-dimethyl-4-aminobiphenyl (5b) were prepared and tested for mutagenic activity toward S. typhimurium. The compounds tested were the hydroxylamines 2a, b and 6a, b; C-nitroso compounds 3a, b and 7a, b; and the hydroxamic acids 4a, b and 8a, b derived from the amines 1a, b and 5a, b as well as the parent amines and N-[2-(hydroxymethyl)phenyl]hydroxylamine (2d), N-(2-methylphenyl)hydroxylamine-methyl-d_3 (2c), and 3,2'-dimethyl-4-aminobiphenyl-3-methyl-d_3 (5c). The structures of the compounds are shown below.



a, R = H; b, R = CH_3; c, R = CD_3; d, R = CH_2OH

Compounds 2a, b and 2d-4b were obtained commercially and purified or were prepared according to previously described procedures, while 2c was synthesized by nitration of toluene-d_3 separation of the isomers, and reduction. Compounds 5a, b and 6a-8b were prepared by standard procedures from 2'-methyl-4-nitrobiphenyl(9)and 3,2'-dimethyl-4-nitrobiphenyl(10); 5c was prepared by treatment of 10 with $NaOCD_3$in CD_3OD to give 3,2'-dimethyl-4-nitrobiphenyl-3-methyl-d_3(11), followed by reduction.

Aniline (1a) and o-toluidine (1b) were inactive in strains TA 100, TA 1535, and TA 1538, both with or without activation by rat liver S9. The hydroxylamine 2b and C-nitroso compound 3b were mutagenic toward TA 100 and TA 1535 with activation but not toward TA 1538.Hydroxylamine 2d was less active than 2b toward TA 100. Hydroxylamine 2a and C-nitroso compound 3a were not mutagenic toward TA 100, TA 1535, or TA 1538 with activation. When assayed without activation, 2a, b and 3a, b were either toxic or nonmutagenic. The hydroxamic acid 4b was mutagenic toward TA 1535 without activation; 4a, and 4b did not show activity in TA 100 or TA 1535 with activation. The aminobiphenyls 5a, b were highly mutagenic toward TA 1538 and TA 100 with activation but not toward TA 1535. Neither 5a nor 5b was active in the absence of rat liver S9. Hydroxylamines 6a, b, C-nitroso compounds 7a, b and hydroxamic acids 8a, b were mutagenic toward strains TA 1538 and TA 100 with activation; 6b and 7b were highly mutagenic toward TA 1538 without activation. The biphenylamine derivatives were more mutagenic than the single ring compounds; the former were frameshift mutagens, while the latter were base-pair mutagens. Among the compounds showing mutagenic activity, the derivatives having a methyl group ortho to the amine functionality were generally more mutagenic, which parallels their carcinogenic activities. Substitution of deuterium for hydrogen in the o-methyl groups of 2b, and 5b resulted in no significant loss of mutagenicity.

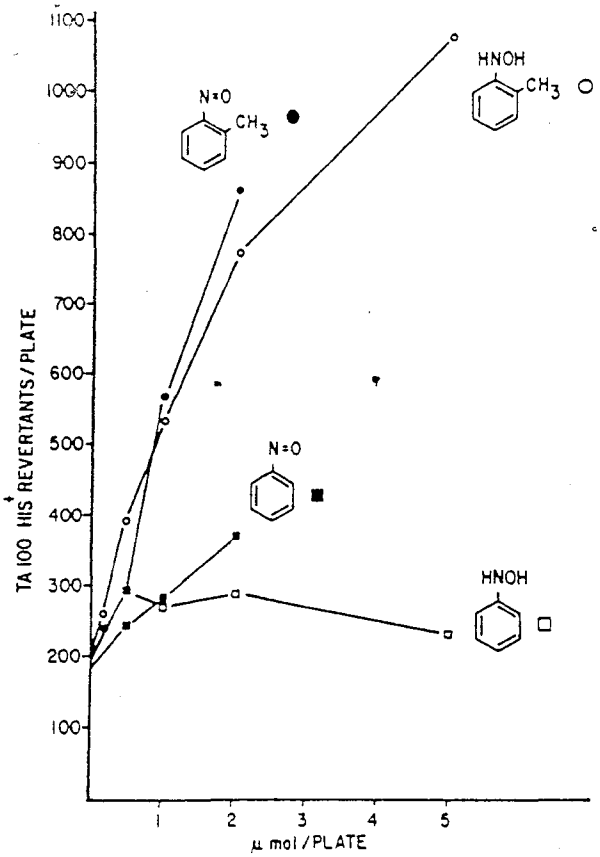


Figure 1. Mutagenicity toward *S. typhimurium* TA 100 of 2a (□–□), 2b (○–○), 3a (■–■), and 3b (●–●) in the presence of rat liver homogenate. 3a,b exhibited only toxicity at doses greater than 2 μmol/plate. At the doses shown, the toxicities of 3a and 3b were similar, as were 2a and 2b.

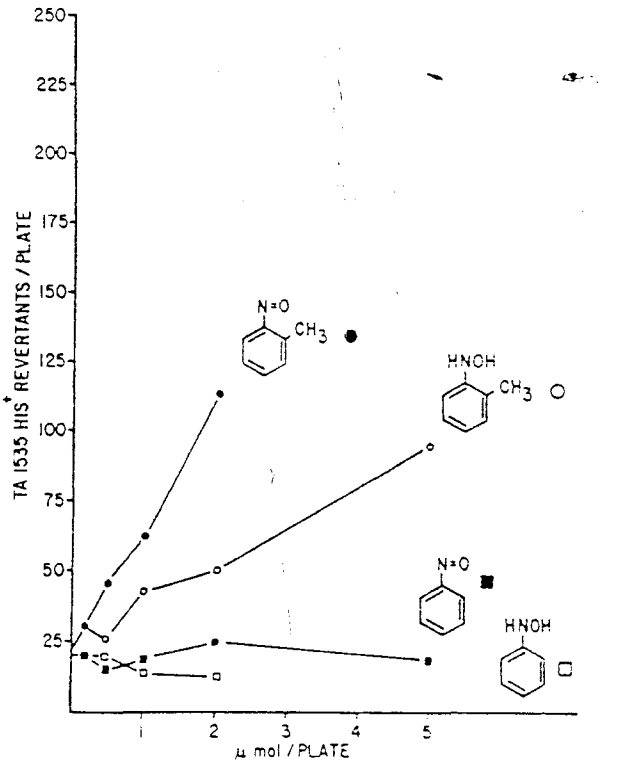


Figure 2. Mutagenicity toward *S. typhimurium* TA 1535 of 2a (□–□), 2b (○–○), 3a (■–■), and 3b (●–●) in the presence of rat liver homogenate. At doses higher than those shown, only toxicity was observed. At the doses shown, the toxicities of 3a and 3b were similar, as were 2a and 2b.

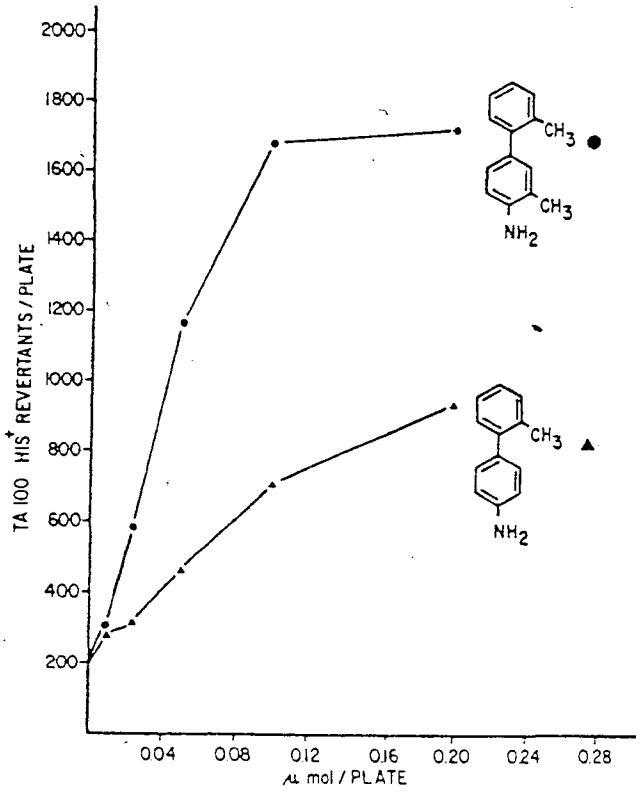


Figure 3. Mutagenicity toward *S. typhimurium* TA 100 of 5a (▲–▲) and 5b (●–●) in the presence of rat liver homogenate. No significant toxic effects were observed.

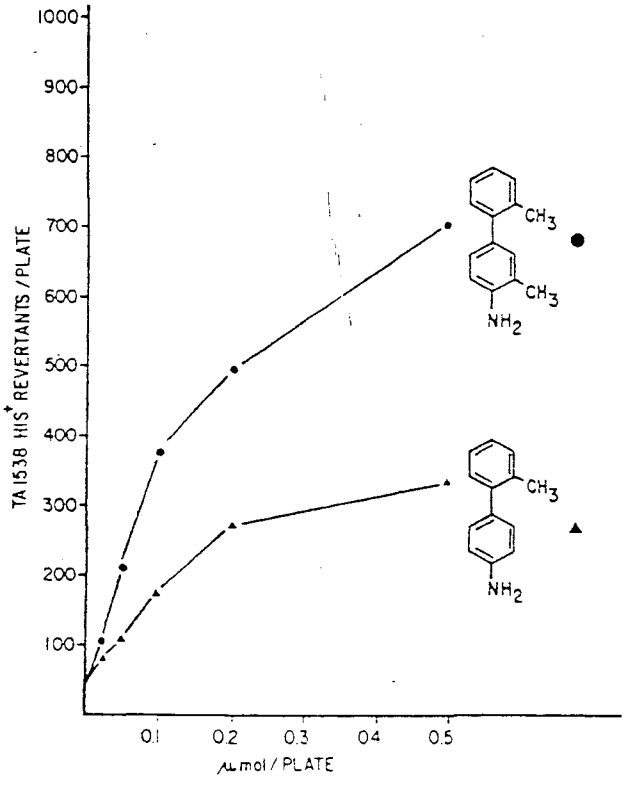


Figure 4. Mutagenicity toward *S. typhimurium* TA 1538 of 5a (▲–▲) and 5b (●–●) in the presence of rat liver homogenate.

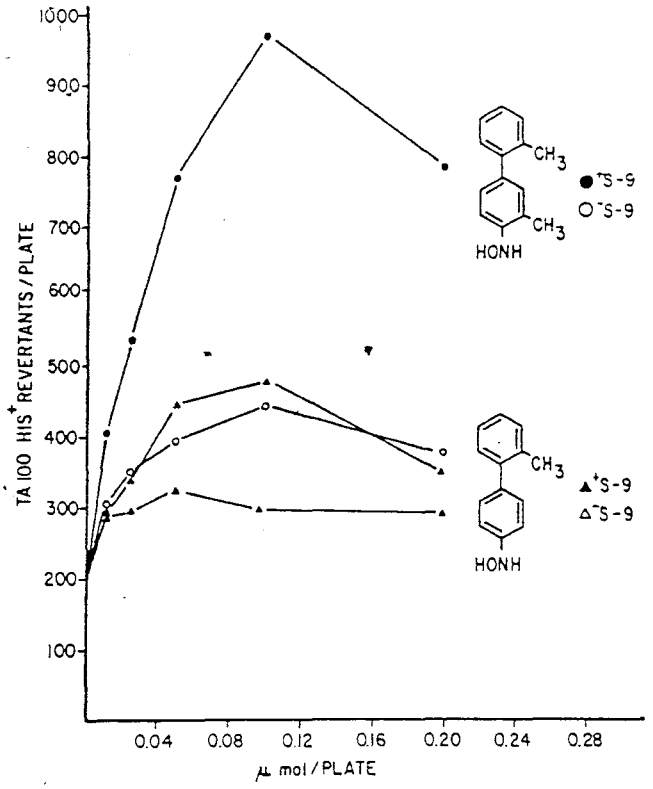


Figure 5. Mutagenicity toward *S. typhimurium* TA 100 of **6a** in the presence (▲–▲) or absence (△–△) of rat liver homogenate and of **6b** in the presence (●–●) or absence (○–○) of rat liver homogenate. No toxicity was observed for **6a,b** in the presence of liver homogenate, but **6a** was toxic in the absence of liver homogenate.

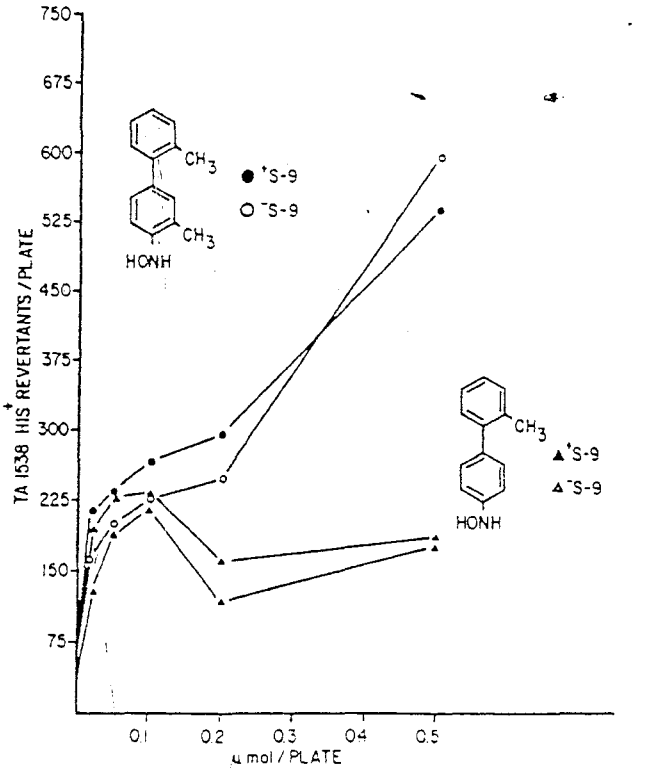


Figure 6. Mutagenicity toward *S. typhimurium* TA 1538 of **6a** in the presence (▲–▲) or absence (△–△) of rat liver homogenate and of **6b** in the presence (●–●) or absence (○–○) of rat liver homogenate.

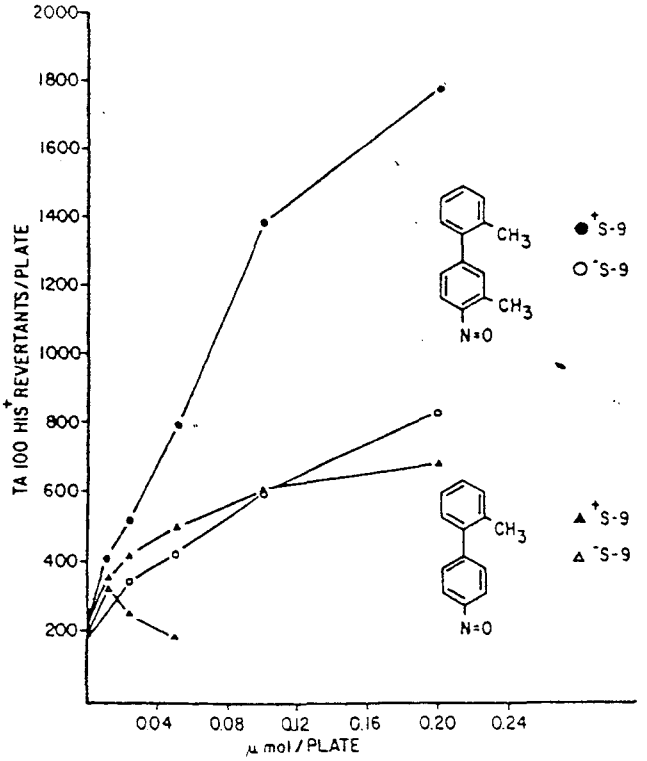


Figure 7. Mutagenicity toward *S. typhimurium* TA 100 of **7a** in the presence (▲–▲) or absence (△–△) of rat liver homogenate and of **7b** in the presence (●–●) or absence (○–○) of rat liver homogenate. No toxicity was observed for **7a,b** in the presence of liver homogenate, but **7a** was toxic in the absence of liver homogenate.

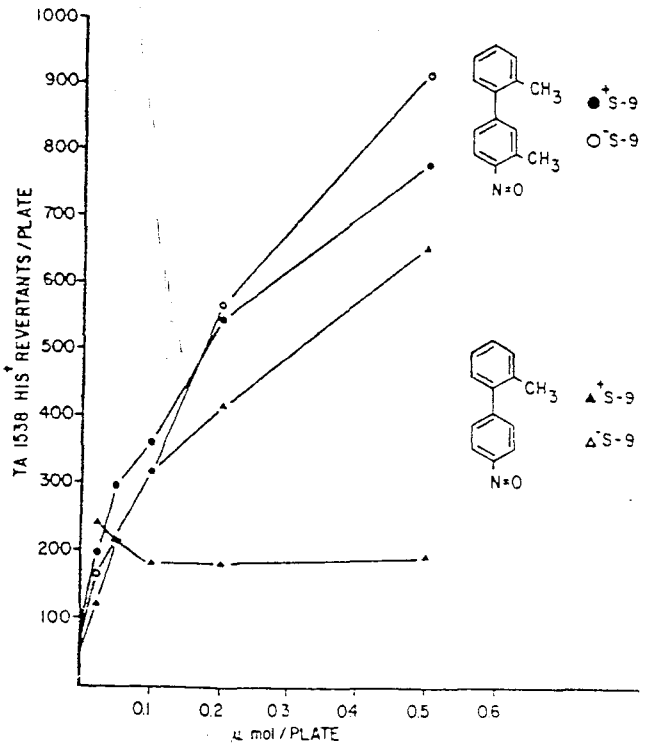


Figure 8. Mutagenicity toward *S. typhimurium* TA 1538 of **7a** in the presence (▲–▲) or absence (△–△) of rat liver homogenate and of **7b** in the presence (●–●) or absence (○–○) of rat liver homogenate.

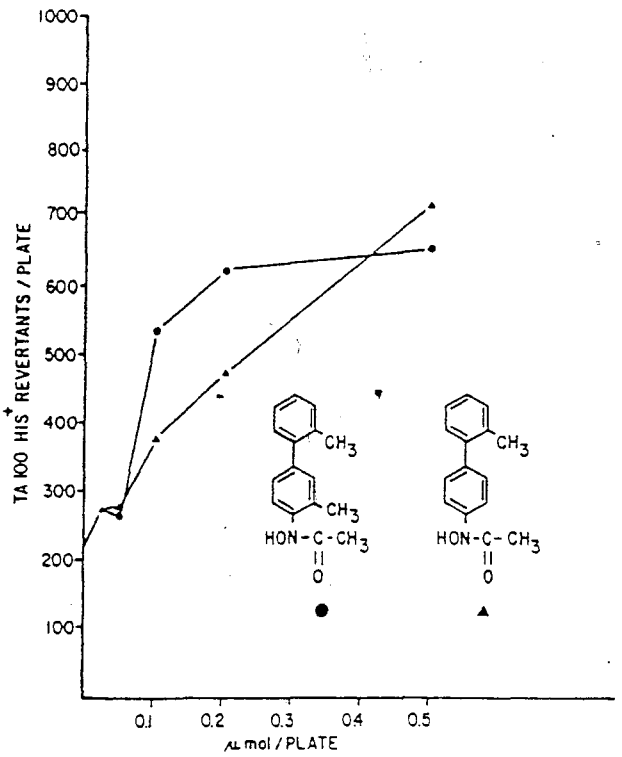


Figure 9. Mutagenicity toward *S. typhimurium* TA 100 of 8a (▲–▲) and 8b (●–●) in the presence of rat liver homogenate. No significant toxicity was observed.

The results, shown in Figs. 1-9, and presented in more detail in reference 10, clearly indicate that the o-methyl effect, first observed in animal models, is also demonstrable in the Ames system. Moreover, since the effect is seen not only with the parent amines but also with the N-oxidixed derivatives, this implies that biological events following, rather than preceeding or involving enzymatic N-hydroxylation are involved in the o-methyl effect (i.e., the function of the o-methyl group is not to make these compounds better substrates for N-hydroxylating enzymes). It is of interest that similar o-methyl substitution effects on increases in mutagenicity were observed recently by Sugimura's laboratory (11) with the imidazoquinoline mutagens isolated from broiled foods.

c. Metabolism studies

During comparative carcinogenicity studies with various derivatives of AB, Walpole et al. observed that the introduction of a methyl group ortho to the amine function (3-methyl-4-aminobiphenyl) resulted in increased carcinogenicity towards the intestinal tract of rats. On the other hand methyl substitution meta to the amine (2-methyl-4-aminobiphenyl) decreased the carcinogenicity and changed the organotropism, with the appearance of liver rather than intestinal tumors. The introduction of a second methyl group at the 2' position to give DMAB, enhanced the carcinogenicity toward the intestinal tract even further; moreover significant carcinogenicity appeared toward the ear duct, the salivary gland and other organs. Interestingly a methoxy group ortho to the amine produced a carcinogen with organotropism toward the urinary bladder. These relationships are summarized in Table 5. To explain the carcinogenicity of 4-aminobiphenyl, 3-methyl-4-aminobiphenyl and DMAB toward the small and large intestines, Walpole speculated that the effective carcinogen of the amines was a metabolite excreted in the bile.

Because of the great interest in animal models for colorectal cancer which would accurately reflect the disease in man, DMAB was utilized by Spjut et al. (2-4) by So and Wynder (1) and others (5) to study the induction and development of colon cancer in rodents. In experiments designed to test Walpole's suggestion that metabolites of DMAB acted topically on the intestine, Navarrette-Reyna and Spjutt (12) performed colostomies 4 cm above the rectum in rats. Following the s.c. administration of DMAB, they found that tumors were found exclusively in the intestine proximal to the colostomy. Other experiments by Cleveland et al. (13), involving the s.c. administration of DMAB to rats with surgically defunctionalized colon segments, similarly indicated that tumors appeared only in those segments which were in actual contact with the fecal stream. These experiments provided strong evidence that the induction of tumors in the intestine was related to the transport of some form of the carcinogen via the bile and intestinal contents rather than by the blood stream.

In the experiments of So and Wynder (1) an interesting species difference in the response to DMAB was observed - whereas in the rat, the predominant sites of tumors were the small and large intestine, in the hamster DMAB caused a high incidence of tumors of the urinary bladder. This species difference was confirmed later by our work (9) and is summarized in Table 6.

To elucidate the mechanisms of the contrasting organotropisms of DMAB in the rat and hamster, we began studies on the in vivo metabolism of the carcinogen (13). The results are presented here.

Male outbred Syrian golden hamsters and male F344 rats were obtained from Charles River Breeding Laboratories. The animals

Organotropism of 3,2'-Dimethyl-4-aminobiphenyl in Male Syrian Golden Hamsters and F344 Rats

Organ	% Incidence	
	Hamsters (25)	Rats (26)
Stomach, sq. papillomas	48	0
Urinary bladder, carcinoma	64	0
Small intestine, adenocarcinoma	28	35
Colon, adenocarcinoma	24	31
Ear duct	0	8
Lung	0	19
Skin	0	54
Subcutaneous tissue	0	23
Prostate, carcinoma *in situ*	0	19

E.S. Fiala *et al.*, Carcinogenesis 2:965 (1981).

Table 6

were maintained on NIH-07 diet and water ad libitum. The approximate mean weight of the hamsters used for these studies was 175 g; that of the rats was 225 g.

DMAB HCl was custom tritiated by New England Nuclear Corp. For purification, 25-30 mCi of the tritiated DMAB was mixed with 0.5-1.0 g of unlabeled DMAB HCl in a small volume of ether. After conversion of the HCl salt to the free base by addition of an excess of saturated $NaHCO_3$ solution with stirring, the mixture was extracted several times with ether. The ether extracts were combined and passed through ClinElut columns (Fisher Scientific) to remove water and concentrated by rotary evaporation to yield a viscous oil. This was diluted with an equal amount of ether and applied to an E.M. Merck size "C" Lobar silica gel column equilibrated with n-hexane-ethanol, 96:4, v/v. The column was eluted with the same solvent at a rate of 0.5 ml/min. After determination of radioactivity, fractions containing DMAB were identified by serially spotting aliquots onto a TLC plate and spraying with Ehrlich reagent. The fractions showing a yellow color on the plate with the reagent were pooled and concentrated to yield a clear viscous oil which turned into a white solid on storage at 4°. The radiochemical purity was > 97% by TLC on silica gel (benzene-$CHCl_3$-ethyl acetate-methanol, 70:15:15:3, v/v; R_f = 0.63) and by HPLC (2 Waters μBondapak C_{18} columns in series eluted with methanol-H_2O, 75:25, v/v; elution volume, 11.5 ml).

For use as chromatographic standards, the N-hydroxy and C-nitroso derivatives were synthesized from DMAB by methods developed under this grant (10).

For enzymatic hydrolyses, E. coli β-glucuronidase (Sigma type IX) and Helix pomatia aryl sulfatase (Sigma type H-1) were used. All other chemicals and solvents were reagent or HPLC grade and distilled deionized H_2O was used in all phases of these experiments.

Rats or hamsters were anesthetized and the common bile duct was cannulated with polyethylene tubing, size No. 1. In early experiments ether was used as the anesthetic. In later experiments, ether was used in combination with halothane for more uniform anesthesia. No differences in biliary excretion which could be ascribed to the method of anesthesia were noted. In the rat, the cannula emerged from the animal by way of the posterior aspect of the hind leg. In the hamster, the gall bladder was ligated before cannulation of the bile duct and the cannula was channeled subcutaneously to the interscapular area and exteriorized at that point. Tritiated DMAB was suspended in corn oil and was injected s.c. at a dose level of 75 mg/kg immediately after the cannulation of the bile duct. Approximately 20 μCi were given to each animal. The animals were placed in plastic restrainers and bile was collected in test tubes cooled to 0-4°.

Non-operated animals were treated with tritiated DMAB as above and placed in stainless steel metabolism cages with provisions for the separation of urine and feces. Urine was collected at 0-4° in containers to which 0.5 ml of 0.5 M triethylamine-CO_2 buffer, pH 8.0, was added to maintain alkaline pH. All animals had free access to food and water during either bile or urine collection.

For separation of conjugated DMAB metabolites, a wet-packed 1.5 X 110 cm column of Sephadex LH-20 (25-100μ) equilibrated with methanol-H_2O-0.5 M triethylamine-CO_2 buffer, pH 8.0, 100:100:2, v/v, was used. The column was eluted at a rate of 12 ml/hr and 6 ml fractions were collected. The inclusion of the triethylamine buffer in the eluant served not only to maintain moderately alkaline pH, but also appeared to significantly increase resolution, an effect which might be due to in part to ion-pairing with the acid conjugated metabolites. The separation of conjugated urinary o-toluidine metabolites using this technique was reported by us earlier (14).

Silica gel G or GF plates, 250 μm, were obtained from Analtech, Newark, Del. For the separation of glucuronic and sulfuric acid conjugates, the plates were developed with n-butanol-acetic acid-water, 3:1:1, v/v. We refer to this as system A. For the separation of DMAB, 3,2'-dimethyl-4-nitrosobiphenyl, and various aglycones from enzymatic hydrolyses, the plates were developed with system B: benzene-chloroform-ethyl acetate-methanol (70:15:15:3, v/v).

A comparison of the total amount of radioactivity excreted in a 24 hour period after dosing with 75 mg/kg of tritiated DMAB by the rat and hamster via the bile and urine is shown in Fig. 10. While the rat excreted approximately 6% of the dose in the urine and 12% in the bile, the hamster excreted approximately 17% in the urine and 5% in the bile. In those cases where collections were extended to 48 hrs, the rats excreted an additional 5.6 $\pm$ 2.6% of the dose in the urine, 10.9 $\pm$ 3.1% in the bile, and the hamsters excreted an additional 3.9 $\pm$ 1.6% in the urine and 3.1% (only one hamster with a bile fistula survived 48 hrs) in the bile.

Submission of the 0-24 hour samples of bile and urine of the two species to Sephadex LH-20 chromatography yielded the metabolite profiles shown in Figs. 11 and 12. While only three major metabolites, designated α, β and γ were resolved from rat bile (Fig. 11A), at least seven metabolite peaks were detected in rat urine (Fig. 11B). Peaks β and γ were present in both bile and urine, peak α, a major component was present only in the bile while peaks and and two other late eluting minor peaks were present in significant amounts only in the urine.

In the hamster (Fig. 12), peaks corresponding to the elution volumes of rat metabolites α, β and γ were present in both bile and urine, and peak α was the major metabolite in both fluids.

Sephadex LH-20 peaks α and β from either rat bile, hamster bile or rat and hamster urine were positive for glucuronic acid by the naphthoresorcinol reaction, indicating glucuronic acid conjugates.

TLC of combined concentrated fractions comprising peak α on silica gel plates eluted with system A showed one radioactive peak near the solvent front which developed yellow color immediately with Ehrlich reagent but was negative to naphthoresorcinol. However the latter reagent showed a distinct blue zone on the plate at R_f = 0.42 which was devoid of radioactivity and which corresponded to free glucuronic acid. This suggested that the glucuronic acid conjugate in peak α was hydrolyzed by the acid TLC solvent.

To determine whether this was the case, a portion of the combined, concentrated α peak from rat bile was adjusted to pH 5.0 with sodium acetate buffer and incubated at 37°. Aliquots were taken periodically and submitted to reverse phase HPLC using methanol-water, 75:25, v/v as eluant. With increasing time of incubation, radioactivity in the most polar peak gradually decreased with corresponding increases in radioactivity in peaks at elution volumes of 8.5 and 16.5 ml (Fig. 13). The elution volumes of these peaks corresponded exactly to those of DMAB and 3,2'-dimethyl-4-nitrosobiphenyl standards, respectively. At short times of incubation, e.g., 30-60 minutes, a radioactive peak appeared at an elution volume of 7.0 ml which corresponded to N-hydroxy-DMAB. This peak disappeared after longer times of incubation, presumably due to further oxidation to the nitroso form. After 3 hours of incubation, the mixture was extracted with ether and the ether extract submitted to TLC using solvent system B. Two major radioactive u.v. absorbing bands were observed at R_f = 0.63, corresponding to DMAB, and at R_f = 0.79, corresponding to 3,2'-dimethyl-4-nitrosobiphenyl. These bands were eluted and submitted to mass spectrometry (Fig. 14A and 14B) whereby their identity was confirmed.

The time course of hydrolysis of the α peak from rat bile at pH values of 5.0, 6.2 and 7.8 is shown in Fig. 15. At pH 7.8 very little hydrolysis occurred over the 3 hour period, but hydrolysis was rapid at pH 6.2 and more so at pH 5.0. At the latter pH approximately 80% of the α peak was hydrolyzed within 3 hours.

Two alternatives concerning the origin of the DMAB and the nitroso derivative were considered. It was possible that the α peak obtained from the Sephadex LH-20 chromatography was not homogeneous and contained the N-glucuronide of DMAB as well as the N-glucuronide of N-hydroxy-DMAB. Such N-glucuronides are known to be hydrolyzed under mildly acidic conditions. In the presence of oxygen, the N-hydroxy-DMAB aglycone would be rapidly oxidized to the nitroso form. On the other hand, it was possible that the α peak consisted entirely of the N-hydroxy-N-glucuronide of DMAB. After hydrolysis, the N-hydroxy aglycone could conceivably disproportionate to DMAB and the nitroso product.

To determine which was the case, the α peaks obtained from rat bile and hamster bile and urine were submitted to HPLC using a μBondapak C_{18} column eluted with 30:70 methanol-water, 0.005 M in sodium phosphate buffer, pH 8.0. In both cases, two radioactive peaks, designated as α_1, and α_2, eluting at approximately 15 and 18 ml, respectively, were obtained, which were positive for glucuronic acid. The HPLC resolution of the α peak obtained from rat bile is shown in Fig. 16. Upon mild acid hydrolysis (pH 5) under aerobic conditions, the α_1 peak yielded DMAB as the only component (Fig. 17, upper trace) whereas hydrolysis and HPLC of α_2 yielded 3,2'-dimethyl-4-nitrosobiphenyl as the major component and a small amount of DMAB (Fig. 17, upper trace). This indicates that the Sephadex LH-20 α peak was in fact composed of two glucuronic acid conjugates: the N-glucuronide of DMAB (α_1) and the N-hydroxy-N-glucuronide of DMAB (α_2). The approximate ratio of α_2 to α_1 was 5:1 in rat bile, 1:3 in hamster bile and 1:2 in hamster urine, as determined by HPLC.

Sephadex LH-20 peak β was present in the urines and biles of both hamsters and rats (Figs. 11 and 12). On TLC using solvent system A, a naphthoresorcinol-positive, radioactive band with an R_f of 0.74 was noted in all four cases. A strong immediate reaction with Ehrlich reagent spray indicated the presence of a free amine group. Following β-glucuronidase hydrolysis the aglycone was recovered by ether extraction and purified by TLC using solvent system B (R_f = 0.46). The purified aglycone yielded a mass spectrum (Fig. 18) which is compatible with that of a ring hydroxylated metabolite of DMAB.

Sephadex LH-20 peak γ, present in both rat urine and bile and in hamster urine was positive for glucuronic acid. After β-glucuronidase hydrolysis of γ obtained from rat urine, TLC in system B gave a major radioactive zone with an R_f of 0.27. Mass spectral analysis of the aglycone yielded distinct peaks at m/e = 255 (M^+), 213, 198, 181 and 163. We infer that peak γ represents a glucuronide of N-acetyl ring hydroxylated DMAB.

Sephadex LH-20 peak δ, a major metabolite in rat urine and possibly a minor metabolite in rat bile and hamster urine gave an R_f of 0.60 on TLC using solvent system A. The metabolite was negative for glucuronic acid and gave a yellow color with Ehrlich reagent which developed only after a period of time. The greater elution volume of δ compared to the glucuronides α and β on Sephadex LH-20, which effects separations mainly by molecular sieving, suggested that peak δ might be a sulfuric acid conjugate. In fact after incubation with aryl sulfatase at 37° for 4 hrs at 37°, more than 80% of the total radioactivity was extractable into ether. The concentrated ether extract gave a single radioactive band on TLC with system B with an R_f of 0.27. Submission of the eluted aglycone to mass spectrometry gave a spectrum essentially identical to that obtained using the aglycone of peak γ. Thus peak δ represents the sulfate ester of N-acetyl-C-hydroxy DMAB.

Among the arylamine carcinogens, DMAB is of interest because it induces colon tumors in rats and hamsters, mammary tumors in female rats and urinary bladder tumors in hamsters. Thus, depending on the animal, DMAB provides a good experimental model for three major sites of human cancers. Since it contrasts with other colon carcinogens such as 1,2-dimethylhydrazine whose activated metabolites reach the colon mucosa via the blood rather than the fecal stream, as is the case with DMAB, clarification of the mode of action of these carcinogens can yield mutually complementary information that can be relevant to the as yet unknown etiology of the disease in man.

The distinct differences (excepting the overlap in the small and large intestines) in the organotropism of DMAB in the rat and hamster presumably result from differences in the metabolism and disposition of the carcinogen. In this respect it is of interest that the rat, in which intestinal tumors are the major lesions excretes a greater proportion of DMAB metabolites in the bile than in the urine (Fig. 10). In contrast, the hamster which is sensitive to the development of urinary bladder tumors in response to DMAB, excretes a greater portion of the metabolites in the urine than in the bile.

A still better correlation exists between the presence of the metabolite which we have identified as the N-hydroxy-N-glucuronide of DMAB in the two physiological fluids and the sites of tumor formation in the twospecies. Thus this metabolite is present in both the bile and urine of hamsters, and is present in rat bile but not rat urine (Figs. 11 and 12). Because of its instability, the amount of the N-hydroxy-N-glucuronide is difficult to quantitate accurately, however we estimate that 4.5% of the DMAB dose is excreted in this form in rat bile, 1.2% is excreted in hamster bile and 4.2% is excreted in hamster urine in the first 24 hour period after dosing.

From the work of Radomski et al. (15, 16) and Kadlubar et al. (17, 18) it appears that the presence of the N-hydroxy-N-glucuronide of DMAB in hamster urine is directly related to the induction of urinary bladder tumors in this species. N-Hydroxy-N-glucuronides of arylamines such as 4-aminobiphenyl or 2-naphthylamine are regarded as transport forms and proximate carcinogens which release the ultimate carcinogen, the N-hydroxy aglycone, upon mild acid (pH 5-6) conditions such as may exist in the urinary bladder of some species (18), or upon hydrolysis with β-glucuronidase (15, 18). In five separate determinations, we found that the pH of normal, 24 hour hamster urine to vary from pH 6.2 to 6.6. Under these conditions the N-glucuronides of DMAB and N-hydroxy-DMAB may be extensively hydrolyzed (Fig. 17). The absence of bladder tumors in the rat in response to DMAB is likely due to the preferential excretion of the N-hydroxy-N-glucuronide in the bile rather than the urine in this species. Extensive investigations (19, 20) into the relative excretion of organic anions into the urine and bile of rats has determined that a

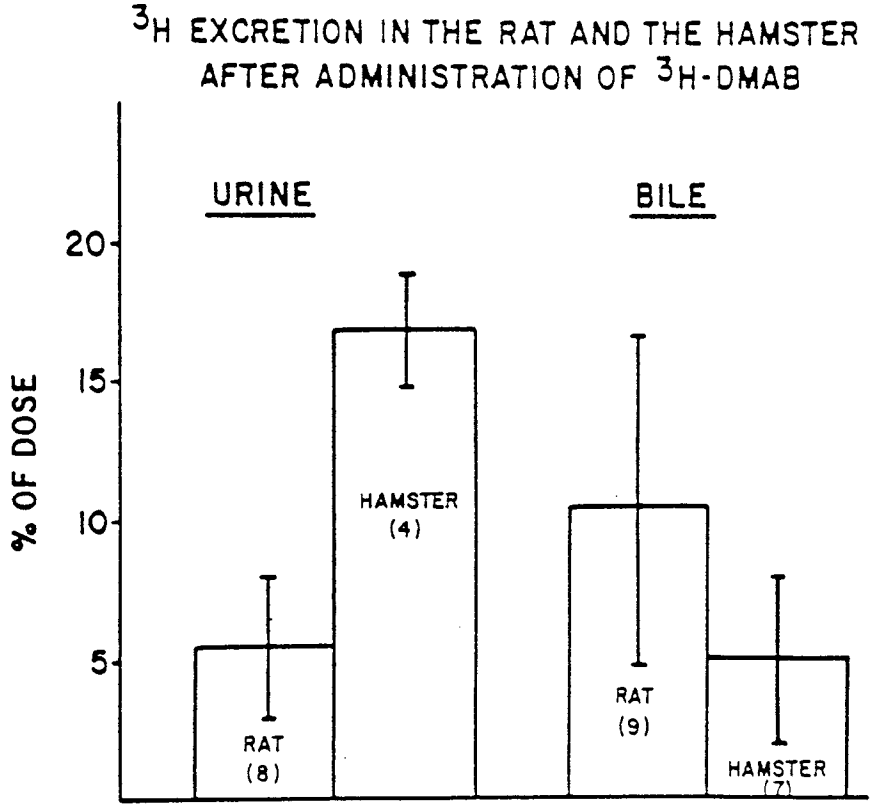


Fig. 10

Fig. 10 24 hour excretion of radioactivity in urine and bile following an s.c. injection of tritiated DMAB (75 mg/kg). Numbers in parentheses denote number of animals used.

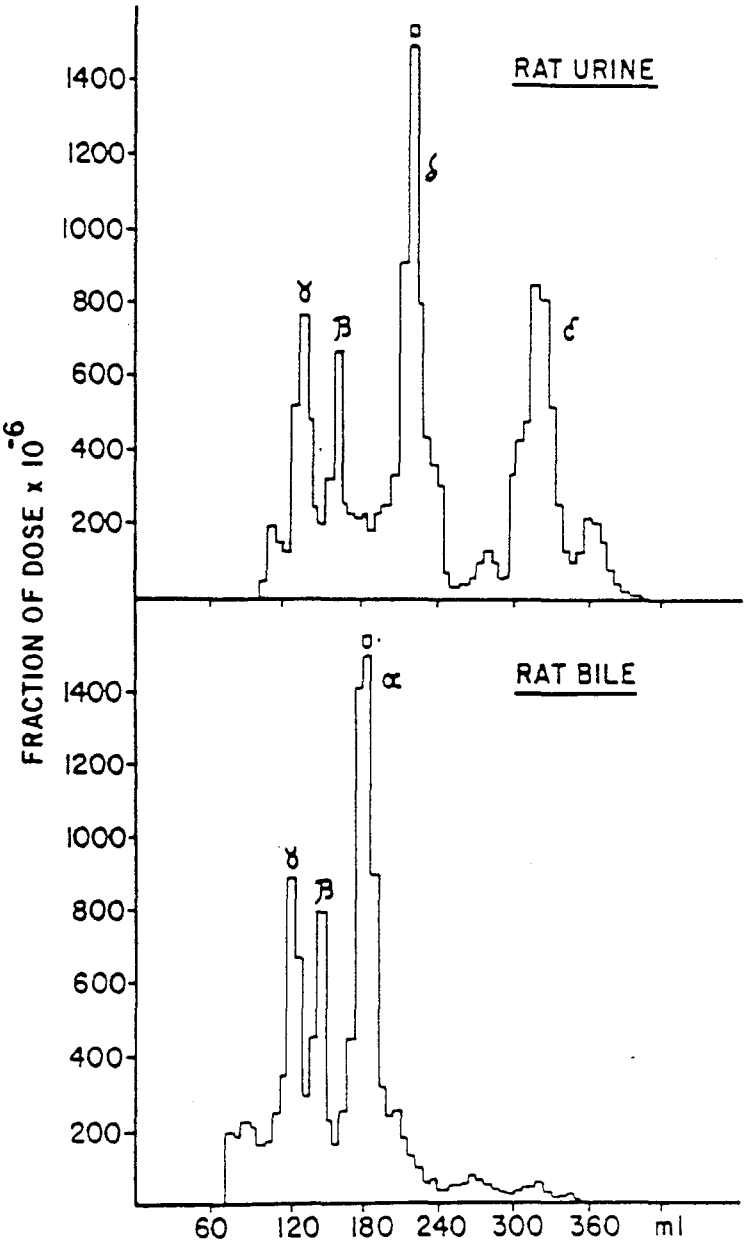


Fig. 11

Fig. 11 Sephadex LH-20 profiles of DMAB metabolites in rat urine and rat bile collected for 24 hours following tritiated DMAB administration.

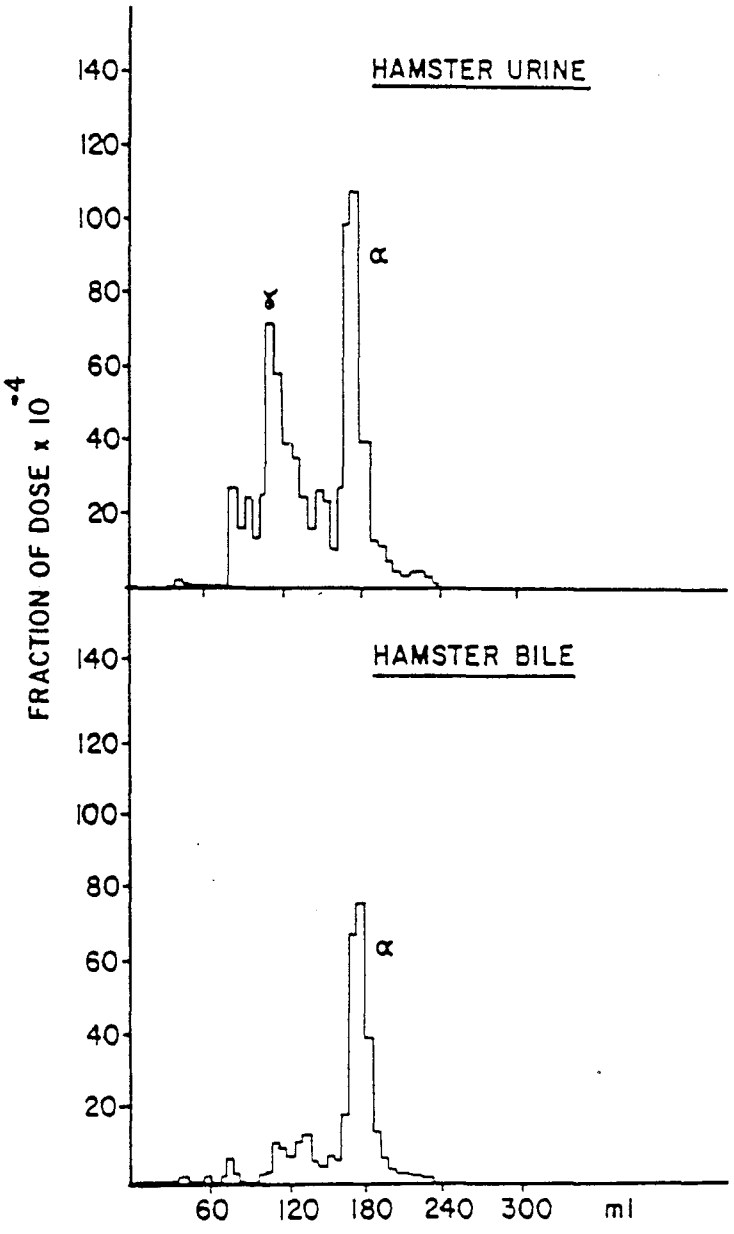


Fig. 12

Fig. 12 Sephadex LH-20 profiles of DMAB metabolites in hamster urine and hamster bile collected for 24 hours following tritiated DMAB administration.

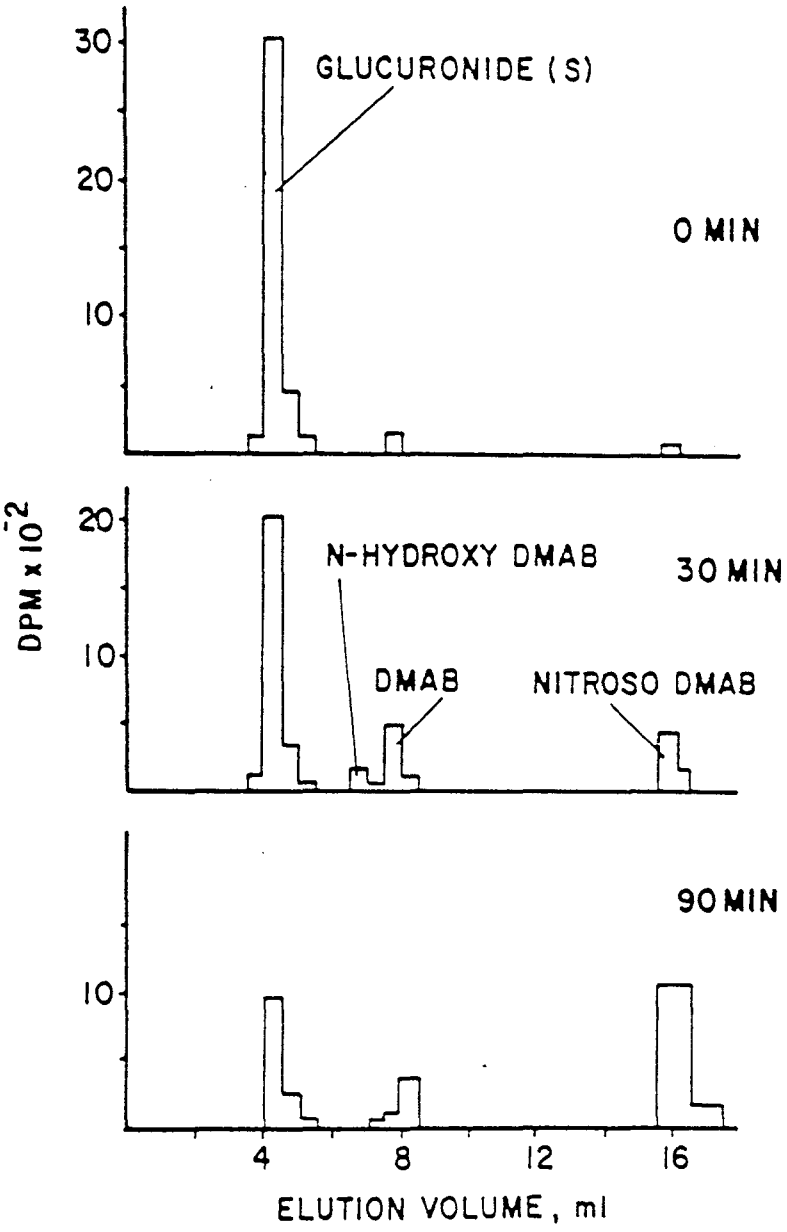


Fig. 13

Fig. 13 HPLC analyses of rat bile α peak incubated for varying times at pH 5 and 37°.

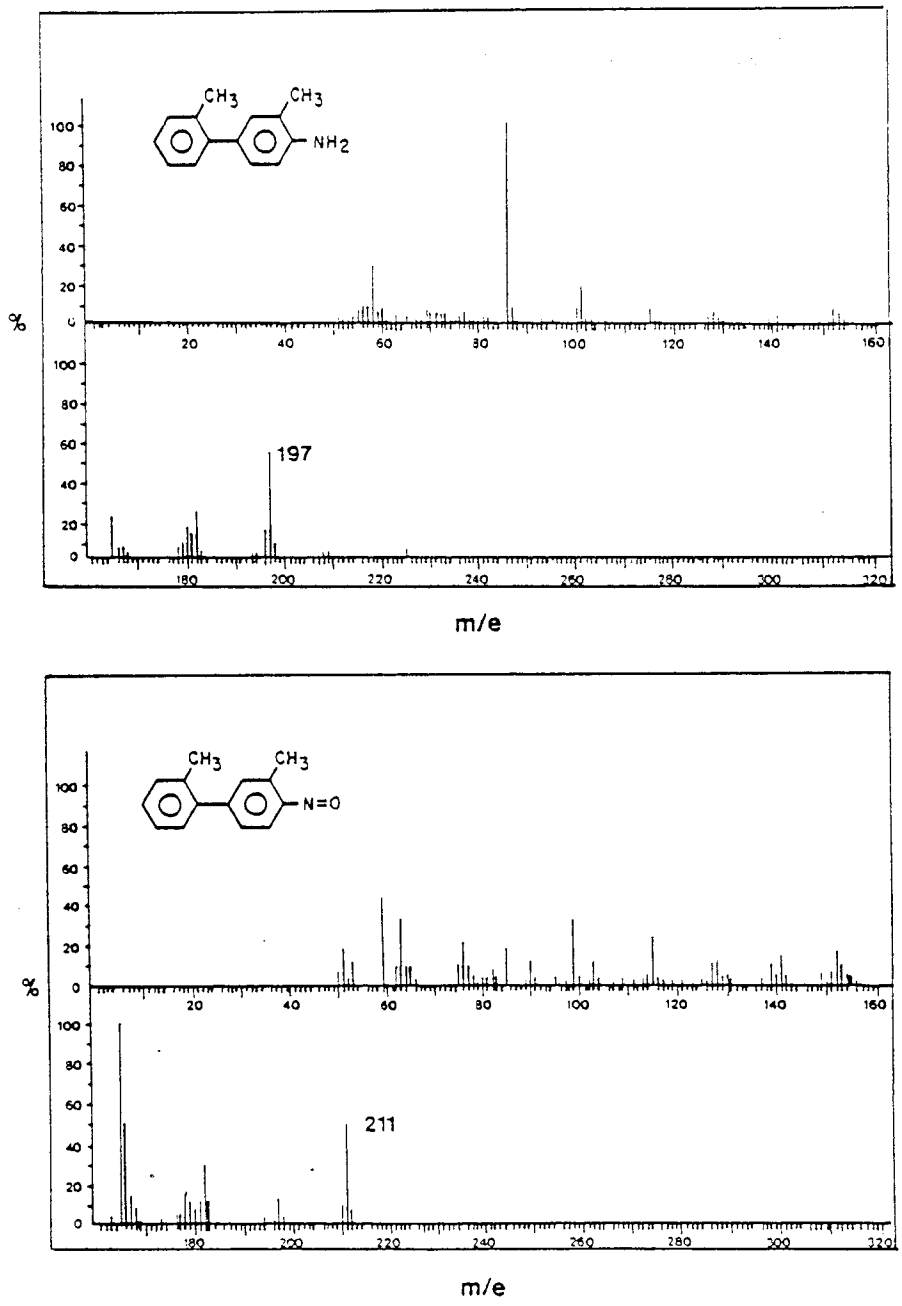


Fig. 14

Fig. 14 Mass spectra of aglycones recovered from mild acid (pH 5) hydrolysis of Sephadex LH-20 peak α.

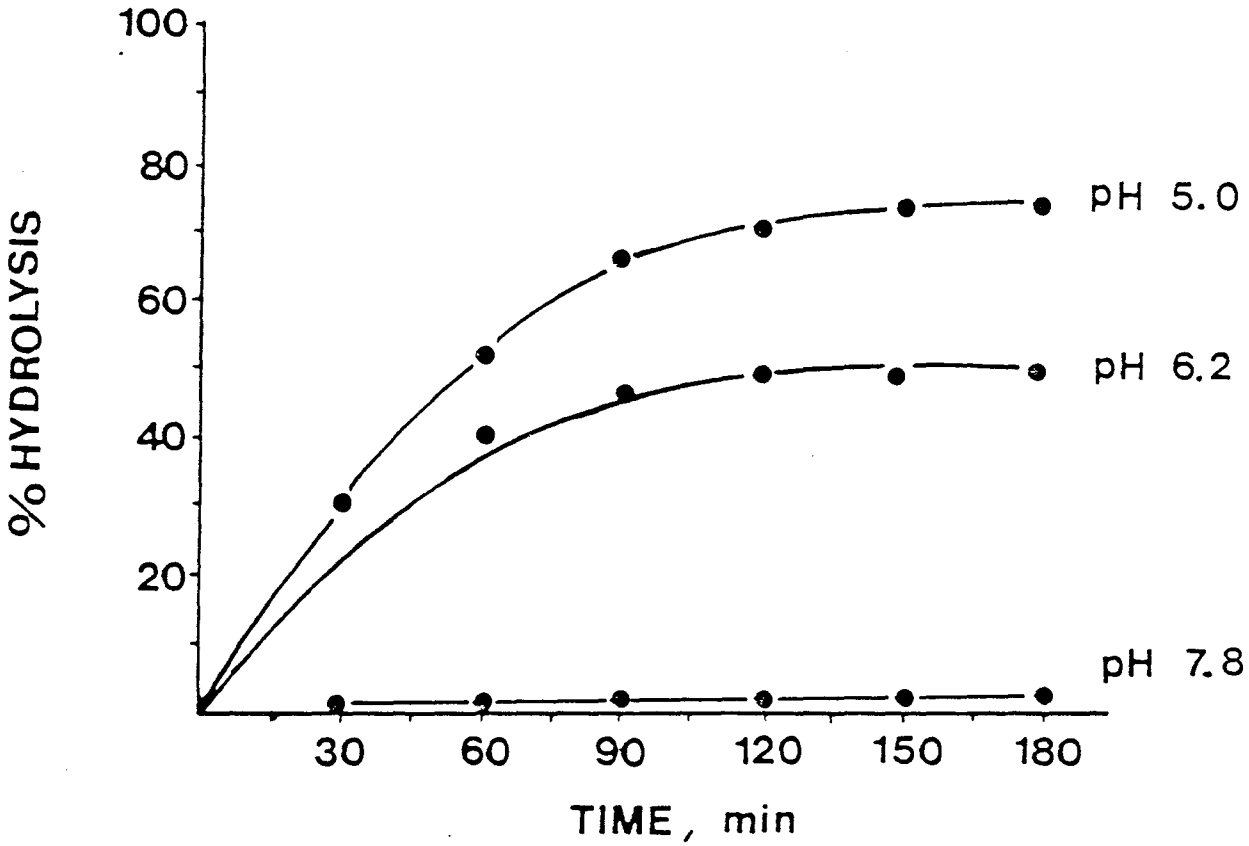


Fig. 15

Fig. 15 Time course of hydrolysis of Sephadex LH-20 peak α at pH 5, pH 6.2 and pH 7.8.

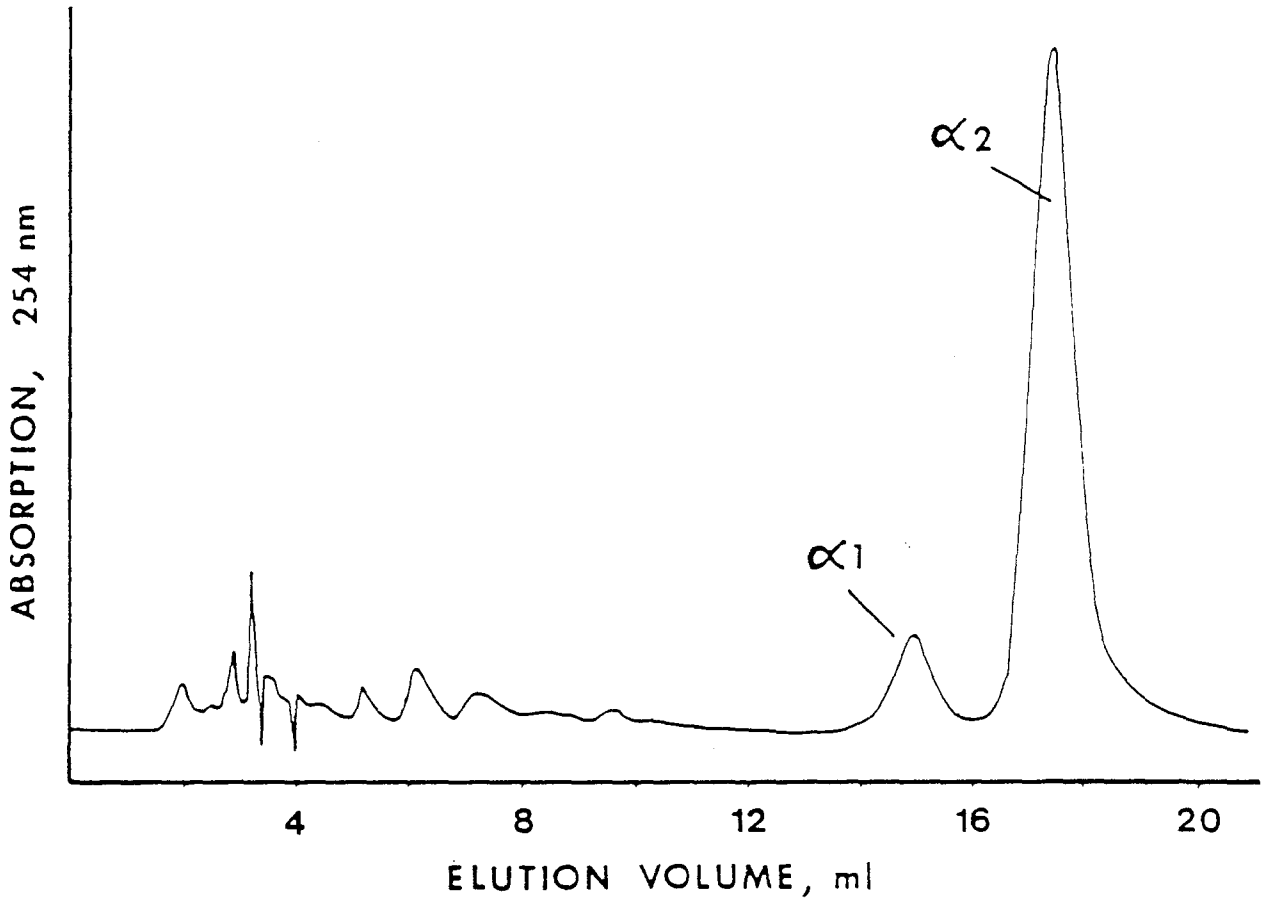


Fig. 16

Fig. 16 Resolution of Sephadex LH-20 peak α (rat bile) into glucuronides (α_1 and α_2) by HPLC. A μBondapak C_{18} column was eluted with 30% methanol.

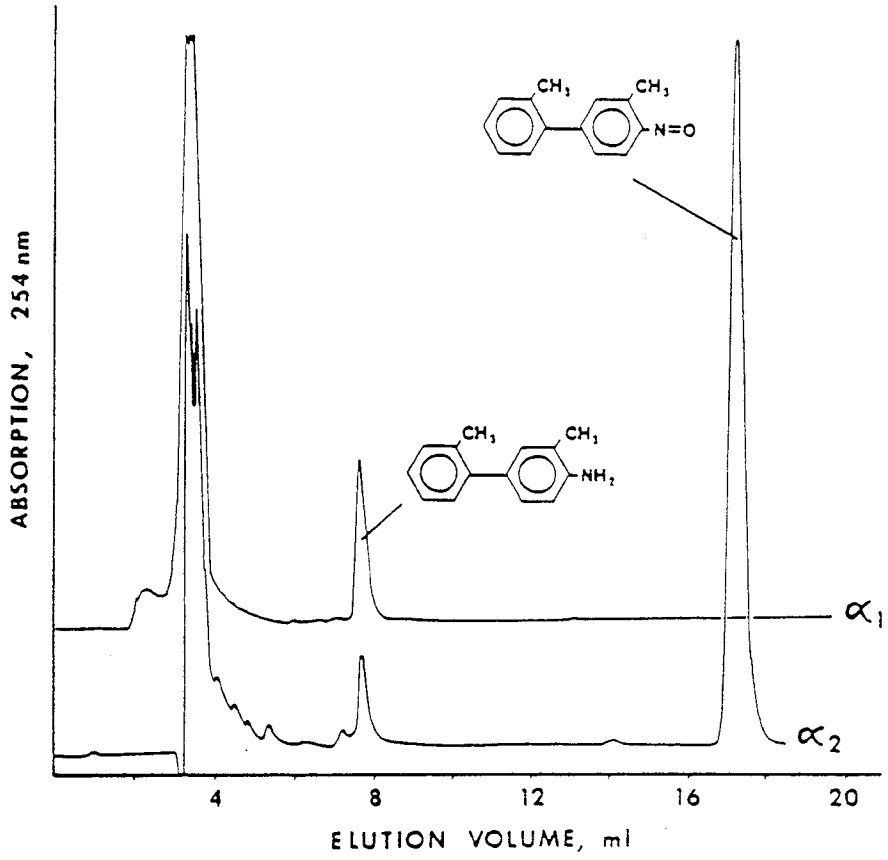


Fig. 17

Fig. 17 HPLC of pH 5 hydrolysis products of α_1 and α_2. A μBondapak C_{18} column was eluted with 75% methanol.

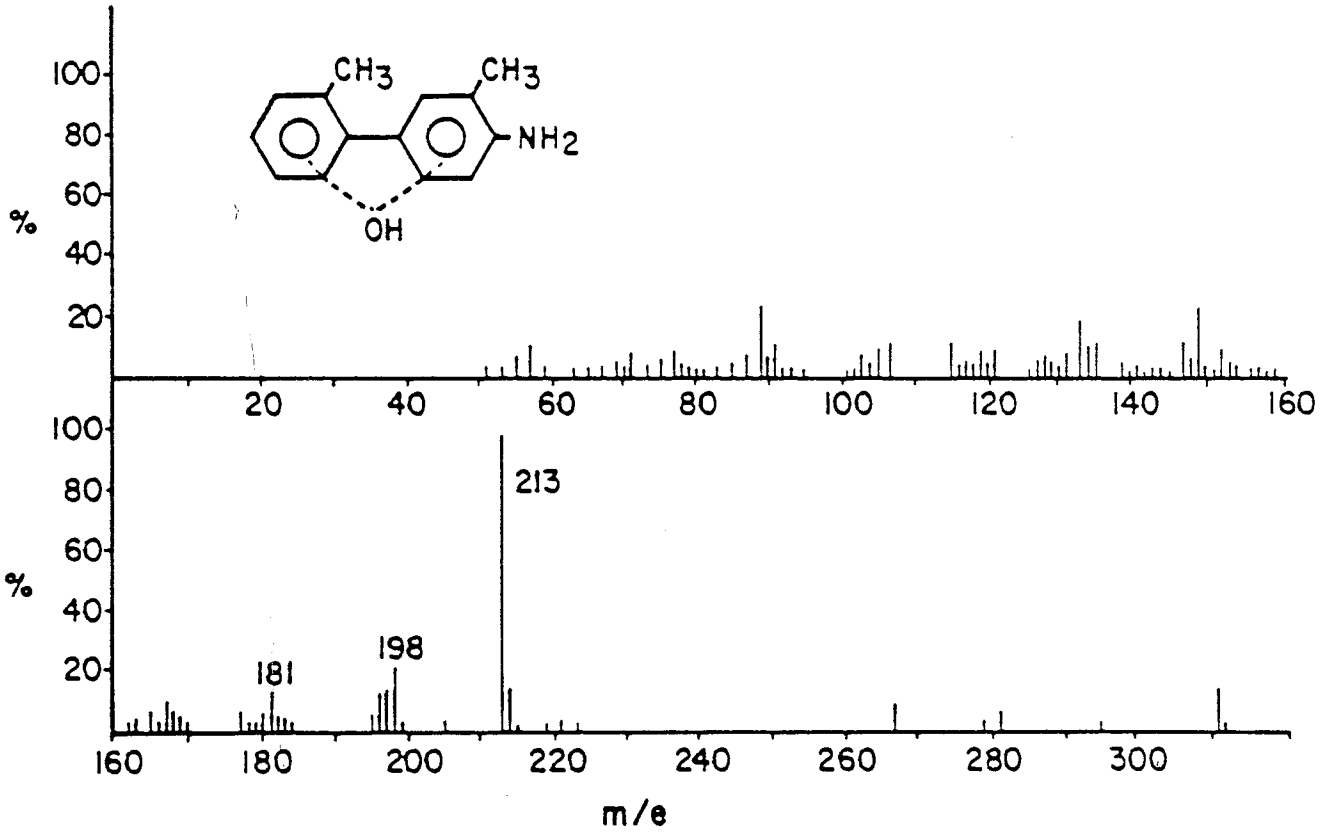


Fig. 18

Fig. 18 Mass spectrum of the aglycone of Sephadex LH-20 peak β. A ring hydroxylated metabolite is indicated; the position of the hydroxyl group cannot be determined by mass spectral analysis alone.

rather sharp molecular weight threshold for biliary excretion in this species exists; compounds below the molecular weight of approximately 350 being excreted almost entirely in the urine. Above 350 mol. wt., the extent of biliary excretion increases steeply as a function of the molecular weight. Thus in the rat, the extents of biliary excretion of the N-hydroxy-N-glucuronide of DMAB (mol. wt. 388) would be greater than the corresponding metabolite of 3-methyl-4-aminobiphenyl (mol. wt. 374) which in turn would be greater than that of N-hydroxy-N-glucuronide of 4-aminobiphenyl (mol. wt. 360). Interestingly, this correlates well with the carcinogenicity of the parent amines for the colon (Table I). The excretion of the N-hydroxy-N-glucuronide of DMAB in both hamster urine and bile could be due to a higher molecular weight threshold for biliary excretion in this species.

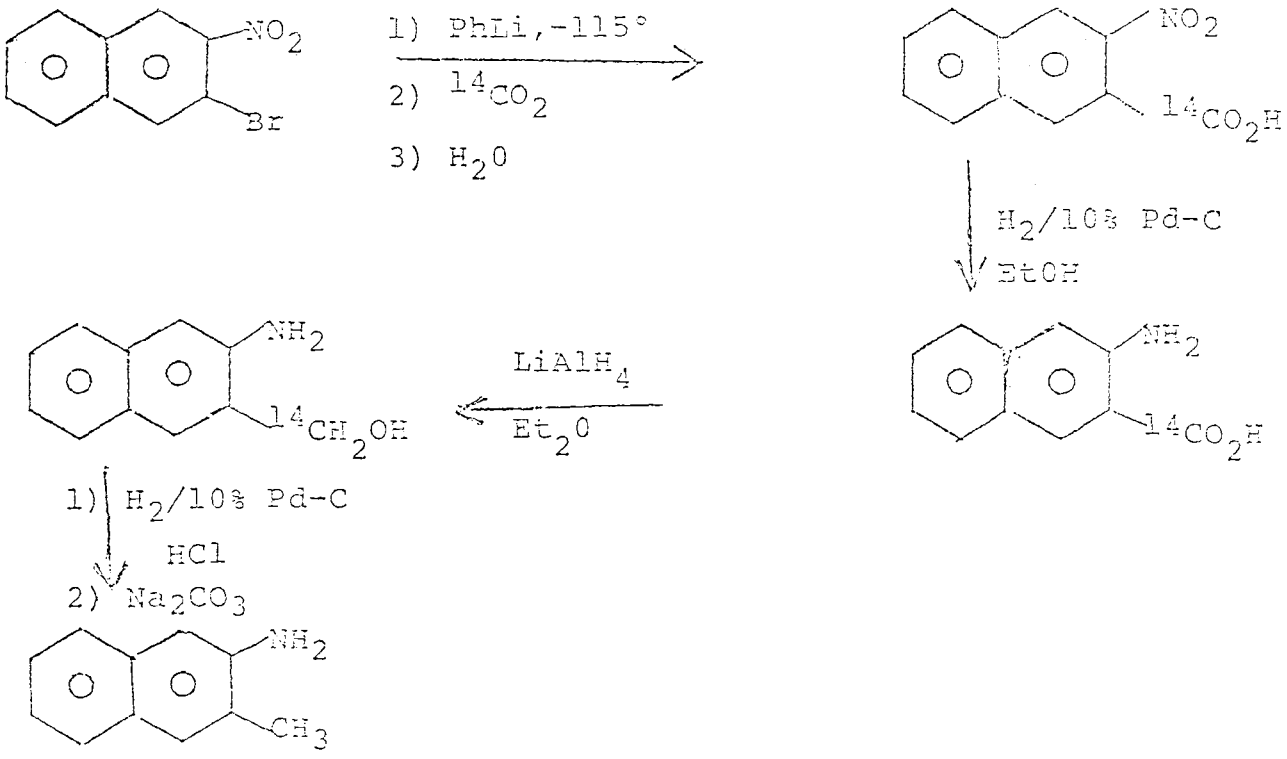
It is obvious that besides molecular weight other factors such as the reactivity of the ultimate carcinogen must also play a role in determining the organotropism and carcinogenicity of the 4-aminobiphenyl derivatives, since the 2-methyl and the 3-methoxy substituted compounds were observed to induce tumors only in the liver and bladder of rats, respectively (Table 5). As previously mentioned, we have demonstrated that DMAB is significantly more mutagenic in the Ames assay than is 2'-methyl-4-aminobiphenyl and that the same relationship holds for the corresponding N-oxidized derivatives.

With respect to the carcinogenicity of DMAB to the colon, bacterial enzymes may play a major role in the further activation of the N-hydroxy-N-glucuronide. Analogous metabolites of 2-naphthylamine and AB have been determined to be easily hydrolyzed to the aglycones by E. coli β-glucuronidase (17), moreover Reddy and Watanabe (5) have demonstrated that germfree status significantly reduces the incidence of DMAB induced intestinal tumors in F344 rats. Thus we conclude that the N-hydroxy-N-glucuronide of DMAB represents the proximate carcinogenic form of DMAB for the rat and hamster colon and for the hamster urinary bladder. In both organs, the glucuronide is cleaved, albeit by different mechanisms, to N-hydroxy-DMAB which represents the ultimate carcinogenic form.

Up to now relatively little information has been available as to the metabolism of DMAB. However, J.W. Gorrod, in unpublished work, has detected the N-glucuronide of DMAB in rat bile and has obtained evidence for the oxidation of both the 3 and 2' methyl groups as well as for hydroxylation at the 4' position (personal communication) thus providing supporting evidence for our results.

2. MNA metabolism in the F344 rat and the Syrian golden hamster.

Studies were also initiated into the in vivo metabolism of 3-methyl-2-naphthylamine (MNA). The ^{14}C-labeled compound was synthesized as summarized below:



The labeled carcinogen was given sc at a dose level of 60 mg/kg. In rats, 72 hours after injection, only 0.07-0.1% of the dose radioactivity appeared in the expired air as CO_2. Thus, extensive oxidative demethylation of MNA does not occur in the male F344 rat. In the same time interval only approximately 0.05% of the dose appears in the expired air as H_2SO_4-soluble volatile basic compound(s).

In intact rats (animals not submitted to bile duct cannulation), 42-58% of the dose (measured as radioactivity) was excreted in the urine in 72 hours (Fig. 19). High voltage paper electrophoresis (0.05 M Na borate, pH 9.2) indicated the presence of at least 5 metabolites.

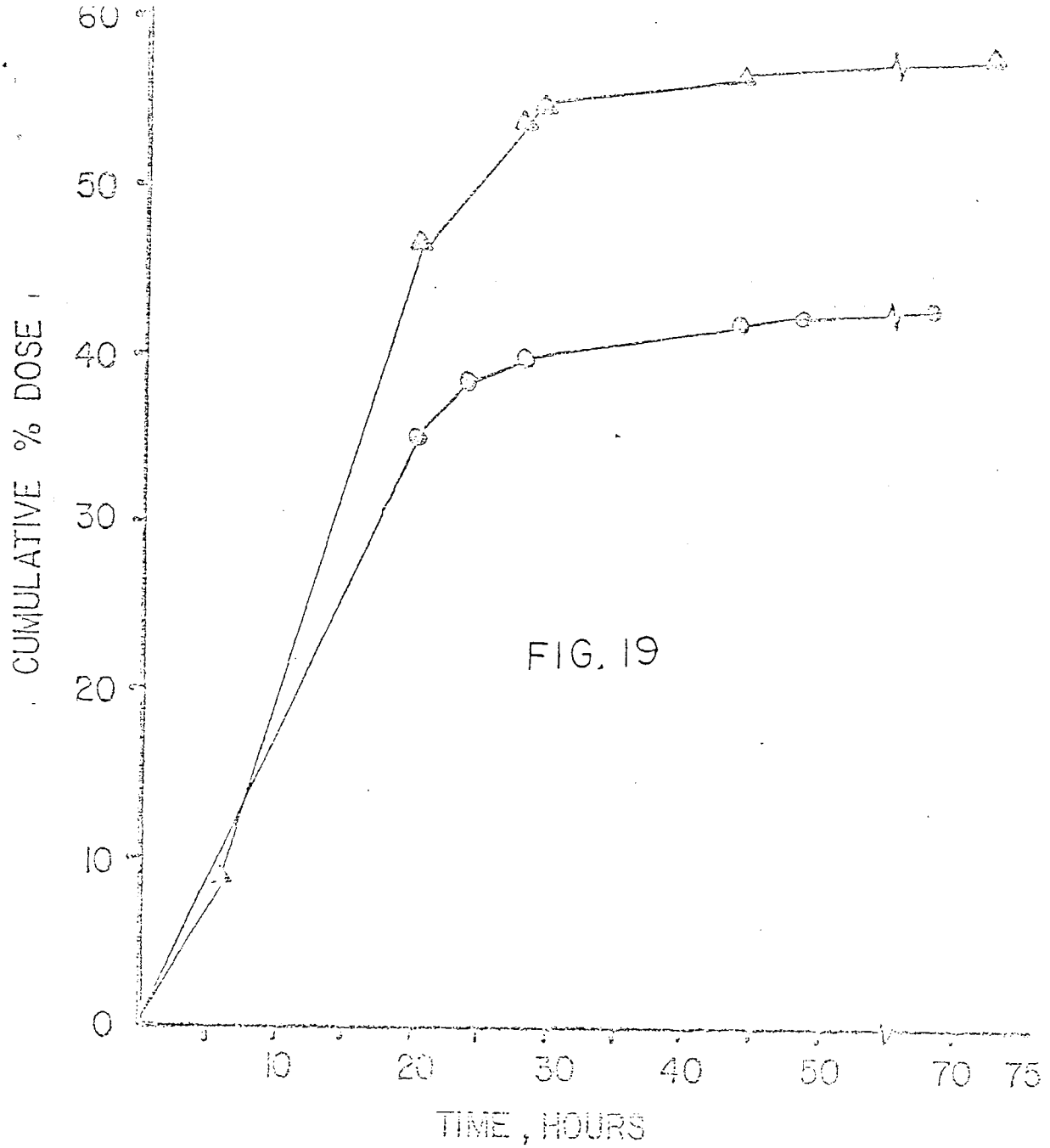


FIG. 19

Approximately 12% of the ^{14}C remained at the origin; approximately 88% moved toward the anode in 4 bands indicating the presence of conjugates, naphthoic acids and naphthols.

Extensive biliary excretion of MNA metabolites was observed in the rat. Approximately 25-28% of the total dose (as radioactivity) appeared in the bile over a 60 hour period (Fig 20). Analysis of pooled bile by high voltage paper electrophoresis showed 3 zones of radioactivity. One of these ("BN-1"), representing approximately 4% of the total biliary metabolites remained at the origin. The other two, BN-2 and BN-3, representing approximately 79% and 16% of the total biliary metabolites, respectively, migrated toward the anode at approximately 1/2 the mobility of picric acid standard.

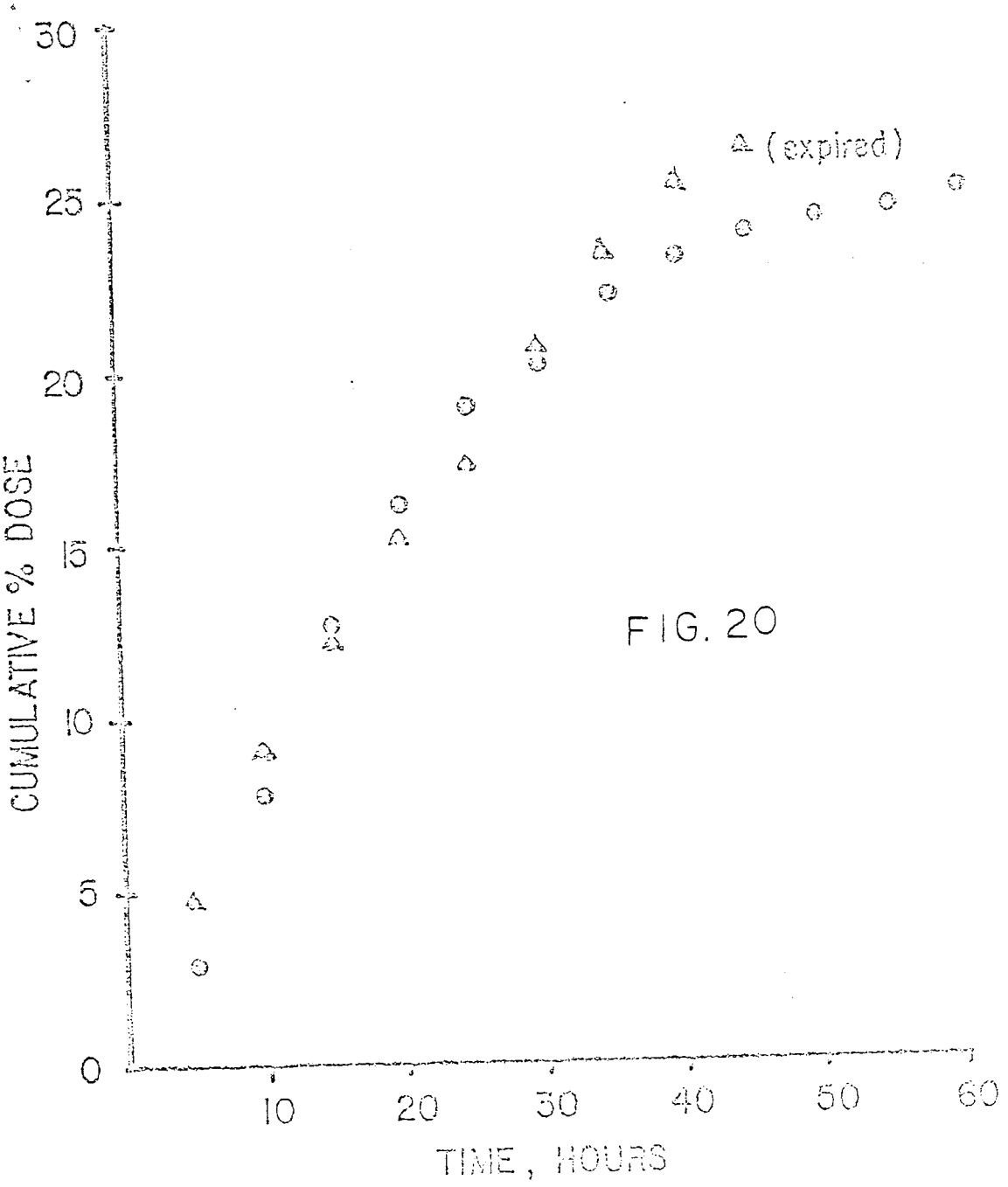


FIG. 20

In contrast to rat bile which shows a relatively simple metabolite pattern on Sephadex LH-20 chromatography (Fig 21), the urinary patterns of MNA treated rats (Fig 22) or hamsters (Fig 23) are much more complex. By treating the pooled, concentrated peak fractions with β-glucuronidase or aryl sulfatase, followed by ether extraction and TLC on silica gel plates ($CHCl_3$:benzene: ethyl acetate:methanol, 70:15:15:3), the sulfates and glucuronides of N-acetylated, C-hydroxylated were identified as indicated on the elution profiles. The mass spectrum of the aglycone is shown in Fig 24.

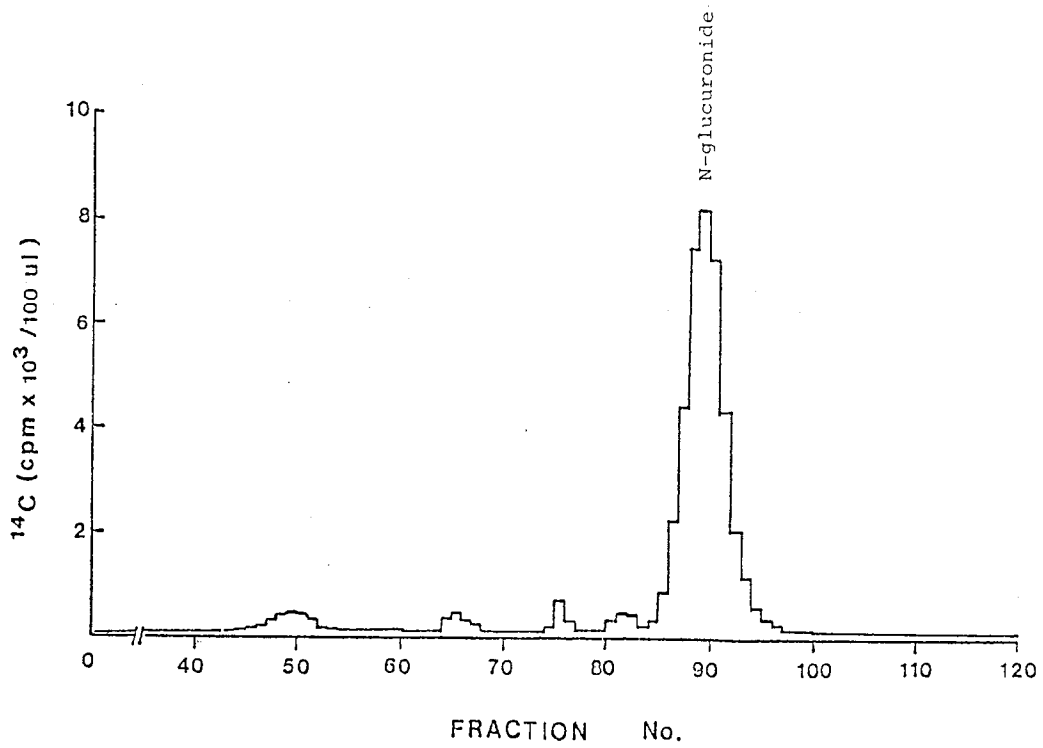


FIG.21. Sephadex LH-20 chromatography of pooled 0-24 hr bile of male F-344 rat which had received 60 mg/kg 3-Me-2-NA ($-^{14}CH_3$)

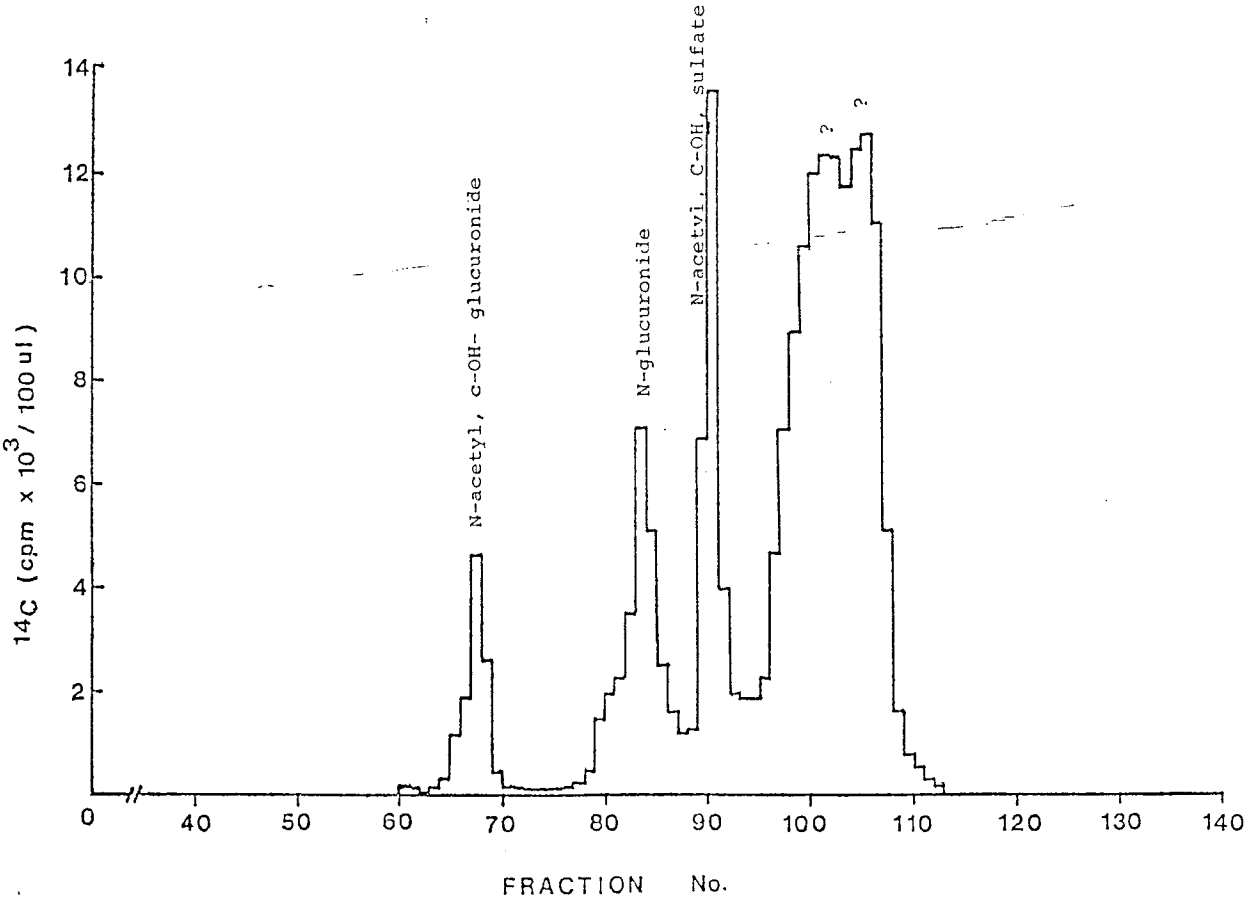


FIG.22. Sephadex LH-20 chromatography of pooled 0-24 hr urine of male F-344 rats treated with 60 mg/kg 3-Me-2-NA ($-^{14}CH_3$)

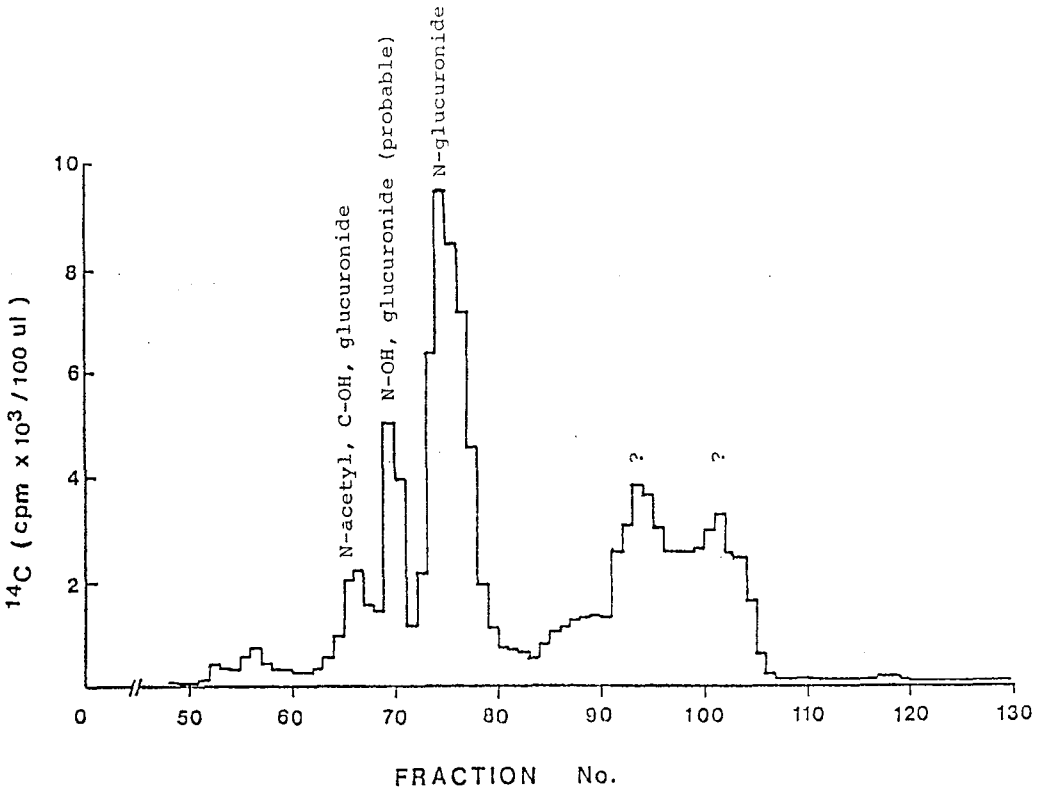


FIG.23. Separation of 3-Me-2-NA metabolites, hamster urine

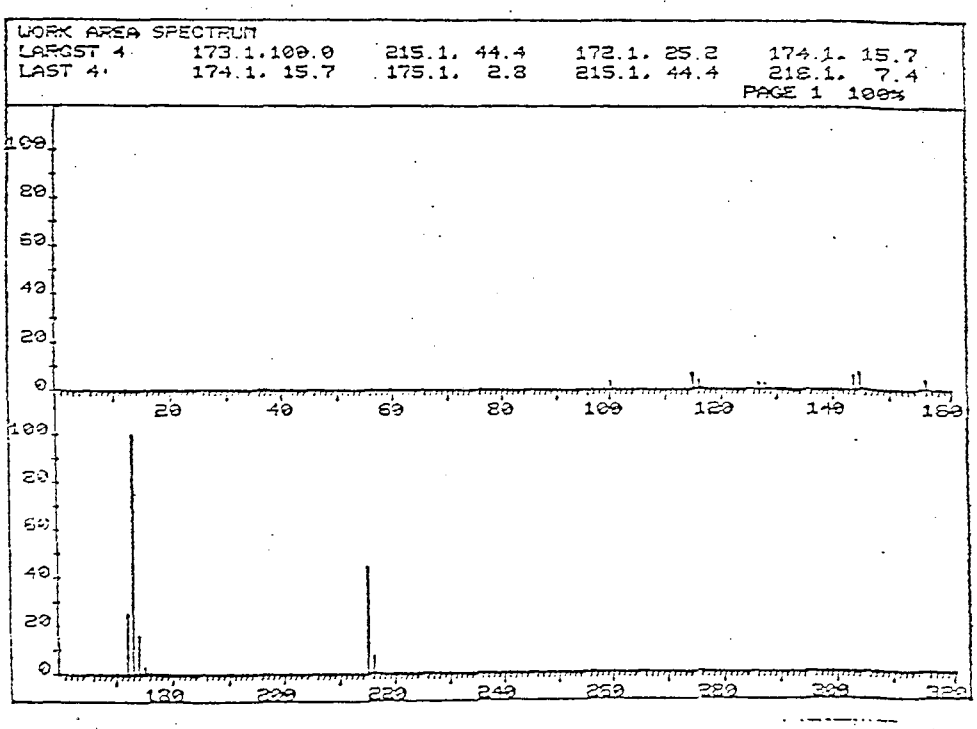


FIG.24. Mass spectrum of 3-Me-2-NA urinary metabolite after enzymatic hydrolysis and purification by TLC. A C-hydroxylated, N-acetylated species is indicated but position of the hydroxyl group isnot yet certain.

Indirect evidence for the activation of MNA by N-hydroxylation was obtained by isolating the azoxy derivative of MNA from hamster urine.

In these experiments, Syrian golden hamsters were pretreated with 3-methylcholanthrene, 20 mg/kg, i.p., once daily, 3 days and then treated with MNA ($^{14}CH_3$). Treatment of either whole urine (pre-extracted with ether), or the Sephadex LH-20 peak so indicated in Fig 23, with β-glucuronidase, extraction with ether and submission to TLC on silica gel (benzene:$CHCl_3$, 2:1), yielded a zone Rf-0.51) which, when eluted, concentrated and analyzed by mass spectrometry yielded the fragmentation pattern shown in Fig 25. The spectrum shows a prominent $[M-OH]^+$ (m/e 309) peak characteristic of o-substituted azoxy compounds as well as a large molecular peak at m/e 326. It appears, therefore, that the azoxy derivative is formed by oxidation of the N-OH derivative during or after enzymatic hydrolysis of the glucuronide. We conclude that the precursor peak in Fig 23 represents the N-hydroxy-N-glucuronide of MNA. It is of interest that this peak is present in hamster urine but not rat urine (Fig 22) or rat bile (Fig 21). In the latter, the N-glucuronide of MNA is the main metabolite. As in the case of DMAB, it is likely that the N-hydroxy-N-glucuronide of MNA is responsible for the induction of urinary bladder tumors in the hamster (Table 1). Unfortunately, the termination of this grant prevented acquisition of further data to examine this mechanism in more detail.

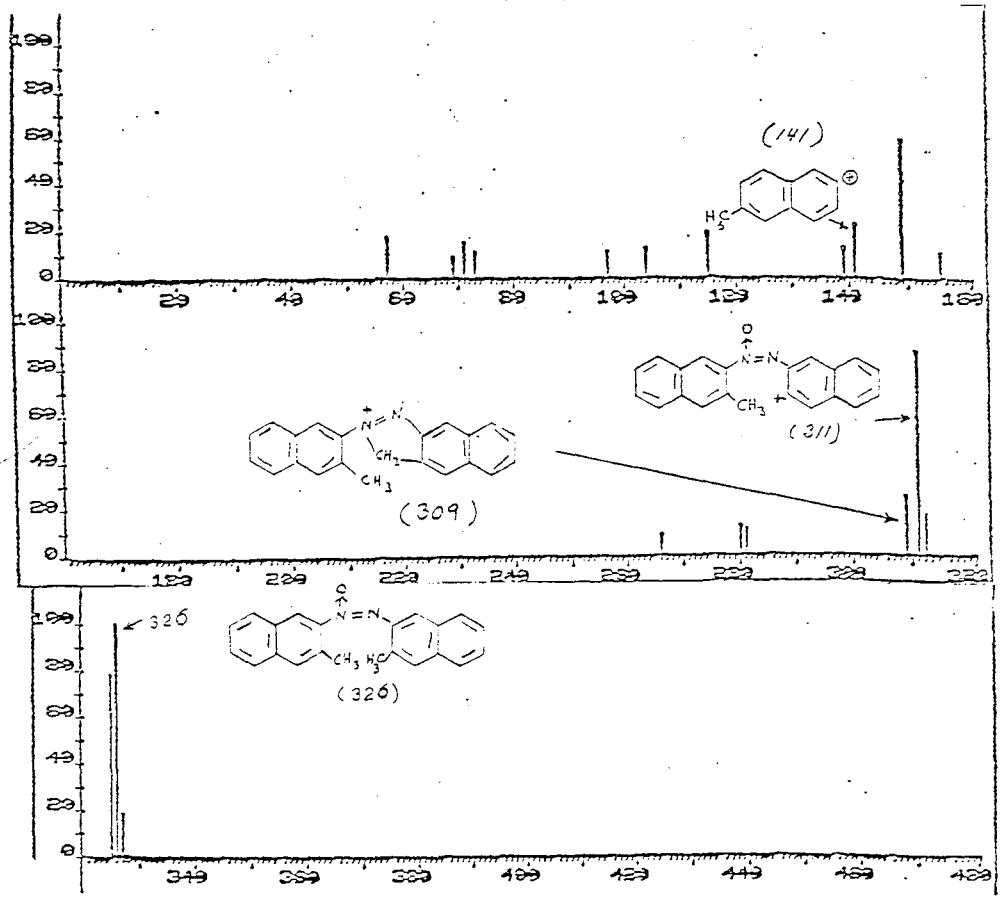


FIG.25. Mass spectrum of suspected bis (2-methyl-3-naphthyl) diazene N-oxide isolated from urine of 3-MC treated hamsters.

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PUBLICATIONS SUPPORTED BY GRANT OH-00611

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PB84-238310

REPORT DOCUMENTATION PAGE

1. REPORT NO.

2.

4. Title and Subtitle: Mechanism of Action: Carcinogenic o-Methylarylamines

5. Report Date: 82/00/00

6.

7. Author(s): Weisburger, J. H., E. S. Fiala, and S. S. Hecht

8. Performing Organization Rept. No.

9. Performing Organization Name and Address: NIOSH, U.S. Department of Health and Human Services, Cincinnati, Ohio

10. Project/Task/Work Unit No.

11. Contract(C) or Grant(G) No.

(C)

(G) OH-00611

12. Sponsoring Organization Name and Address: Same as box 9

13. Type of Report & Period Covered

14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

The carcinogenicity of 3,2'-dimethyl-4-aminobiphenyl (13394860) (DMAB) and 3-methyl-2-naphthylamine (10546244) (MNA) was investigated in F344-rats, Charles-River-CDF-rats, Syrian-golden-hamsters, and guinea-pigs. Mutagenicity of aniline (62533) derivatives, MNA, DMAB, and ortho-toluidine (95534) was determined in Salmonella-typhimurium strains TA-100, TA-1535, and TA-1538, with or without rat liver S9 fraction. Rats and hamsters were given subcutaneous injections of MNA or DMAB for various periods then killed and necropsied. Rats were compared to hamsters in terms of susceptibility to DMAB carcinogenesis. Tritiated DMAB was given to hamsters and F344-rats. Bile was collected in cannulated animals and urine and feces were collected from nonoperated animals. DMAB produced mainly urinary bladder tumors in hamsters; MNA was less active, but caused local sarcoma at the injection site. Treatment with disulfiram (97778) decreased DMAB carcinogenicity in the small intestine in hamsters, while rats developed bladder tumors. No effect was seen on colon cancers. Prostate carcinomas occurred in male rats given DMAB but none were seen in hamsters. In Salmonella, compounds having a methyl group ortho to the amine were more mutagenic. Greater amounts of DMAB were excreted in the bile of rats than in hamsters. Hamsters excreted DMAB in urine, whereas rats did not.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

NIOSH-Publication, NIOSH-Grant, Bladder-disorders, Carcinogens, Poisons, Industrial-chemicals, Cytotoxic-effects, Animal-studies, Safety-research, Grant-Number-OH-00611

c. COSATI Field/Group

18. Availability Statement:

19. Security Class (This Report)

21. No. of Pages: 39

20. Security Class (This Page)

22. Price