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The Carcinogenicity of *p*-Monomethylaminoazobenzene in Various Diets and the Activity of this Dye Relative to *p*-Dimethylaminoazobenzene*

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In studies on the carcinogenic activity of compounds related to *p*-dimethylaminoazobenzene it has been observed that *p*-monomethylaminoazobenzene is about as active as the dimethyl compound (10, 17). More recently Giese, Miller, and Baumann (4) showed that when these compounds were fed *ad libitum* at either of two levels for $2\frac{1}{2}$ to $3\frac{1}{2}$ months, the monomethyl compound always caused a higher incidence of hepatomas than the dimethyl compound. However, rats fed *p*-monomethylaminoazobenzene voluntarily consumed more diet and, therefore, more of the carcinogen. Other studies (13) demonstrated that *p*-dimethylaminoazobenzene was reversibly demethylated *in vivo* to *p*-monomethylaminoazobenzene, and that the latter compound appeared to be irreversibly demethylated to *p*-aminoazobenzene. Since both methylated compounds are present in the animal body when either dye is fed, it is difficult to determine which is the closer to the true carcinogen. The present studies were designed with this question in mind. The effect of various diets on the carcinogenicity of *p*-monomethylaminoazobenzene was determined, and a more rigid comparison was made of the activity of the monomethyl and dimethyl dyes.

The diets selected were of two types. In the first group were diets high in riboflavin or in which hydrogenated coconut oil was substituted for corn oil; these rations are known to inhibit the induction of tumors when *p*-dimethylaminoazobenzene is the carcinogen (7, 11, 12, 14). The other diets represented an attempt to affect the equilibrium *in vivo* between *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene by altering the concentration of methyl groups in the tissues of the rat. For this purpose guanidoacetic acid or nicotinamide was fed to deplete the stores of labile methyl; guanidoacetic acid is methylated in the liver to form creatine (1), while nicotinamide is excreted by the rat as N^1-methylnicotinamide (15). Choline was added to the diets as a source of labile methyl groups (16).

METHODS

Groups of young, mature, male rats of the Sprague-Dawley strain, 170 to 200 gm. in weight, were fed 0.056 per cent of *p*-monomethylaminoazobenzene or 0.060 per cent of *p*-dimethylaminoazobenzene (equimolar levels) for 13 to 14 weeks. A single group was fed 0.106 per cent of *p*-aminoazobenzene for 11 months. The animals were kept in groups of 5 to 8 in screen-bottom cages, and food and water were given *ad libitum* (see below for the conditions used in the paired-feeding experiment, Table IV). The dye was incorporated in the rations by dissolving it with heat in the fat of the diet. The rations were mixed in amounts sufficient for 3 to 4 weeks, and stored at 0° C. At the end of the dye-feeding period the livers were examined by laparotomy, and the rats were continued on the same diets without the dye for an additional 2 months. At this time the animals were killed for a final tumor count.

The constituents of the various diets are listed in Table I. The control diet was the synthetic ration used

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previously (14), except that no choline was added; Miner and his co-workers (14) demonstrated that this vitamin was not necessary for efficient tumor production when *p*-dimethylaminoazobenzene was fed. Each rat also received 1 drop of halibut liver oil monthly. Rations 3 and 10 contained 5 per cent of hydrogenated coconut oil in place of a like amount of corn oil, while diets 4 and 11 contained 10 and 20 mgm. respectively of riboflavin per kgm. rather than the 2 mgm. per kgm. incorporated in the other diets. Three-tenths or 0.5 per cent of choline chloride was added to diets 5, 8, and 12, while 0.35 per cent of nicotinamide or 0.30 per cent of guanidoacetic acid was included in diets cages and fed diets containing *p*-monomethylaminoazobenzene or *p*-dimethylaminoazobenzene (Table I, Diets 1 and 2) for 13 weeks. The food consumption of the rats of each pair was equalized throughout the experiment by restricting both to the voluntary consumption of the animal eating the least. The livers were examined by laparotomy at 13 weeks, and the animals continued on the control ration without the azo dyes. A second operation was performed on the members of each pair when a tumor was palpated in either of the rats. The surviving animals were killed for examination 9 weeks after the end of the dye-feeding period.

TABLE I: COMPOSITION OF DIETS *

Diet No.	Gm. per kgm.									
	1 and 9	2	3 and 10	4	5	6	7	8	11	12
Cerelose †	790	790	790	790	787	786	787	786	790	785
Casein (purified ‡)	120	120	120	120	120	120	120	120	120	120
Salts mixture	40	40	40	40	40	40	40	40	40	40
Corn oil	50	50		50	50	50	50	50	50	50
Hydrogenated coconut oil			50							
Choline chloride					3			3		5
Nicotinamide						3.5 §				
Guanidoacetic acid							3 §			
Riboflavin	0.002	0.002	0.002	0.010	0.002	0.002	0.002	0.002	0.020	0.002
p-Dimethylaminoazobenzene		0.60								
p-Monomethylaminoazobenzene	0.56		0.56	0.56	0.56	0.56	0.56		0.56	0.56
p-Aminoazobenzene								1.06		

* Each kgm. of diet contained also 3.0 mgm. thiamine chloride, 2.5 mgm. pyridoxine hydrochloride, and 7.0 mgm. calcium pantothenate.
† Pure glucose monohydrate obtained from Corn Products Refining Company.
‡ For preparation see reference 14.
§ The levels of methyl acceptors were lowered at the end of the dye-feeding period to 2.8 gm. nicotinamide and 2.9 gm. guanidoacetic acid per kgm. of diet.

6 and 7 respectively. If one assumes that the methyl group of *p*-monomethylaminoazobenzene is physiologically available, and that the only other methyl groups in the diet were furnished by 3.25 per cent of methionine in the casein (3), the nicotinamide was fed at a level equivalent to the methyl groups contained in the diet. On the same basis the guanidoacetic acid fed was equivalent to 87 per cent of the dietary methyl groups. These levels were chosen arbitrarily. If the methyl group from the azo dye is not completely available to the animal, then the proportion of acceptor to dietary methyl groups in these diets was larger than calculated. The rats on these levels of methyl acceptor lost weight, and accordingly after the carcinogen was removed from the diets at 14 weeks both compounds were fed at levels equivalent to 90 per cent of the methyl groups furnished by the methionine in the casein; *i.e.*, 0.28 per cent of guanidoacetic acid and 0.29 per cent of nicotinamide.

In another series 2 groups of Sprague-Dawley rats, paired by sex and weight, were kept in individual

In addition to the experimental groups described above other rats, which had received diets 1, 6, 7, or 12 for 2 to 16 weeks, were used for analytical purposes. These animals were kept in individual cages and fed 8 gm. of diet per day. At intervals rats from each group were killed and the content of azo dye in the blood and liver was determined. The method of analysis was essentially that described previously (9), except that 4 cc. each of 11 *N* potassium hydroxide, water, and 95 per cent ethanol were used for saponification in place of the proportions suggested in the original paper. Each liver was perfused *in situ* with 100 cc. of 2 per cent sodium citrate in order to remove as much blood as possible, and thus make the results more representative of the liver tissue.

Two of the compounds used were synthesized in this laboratory. The *p*-monomethylaminoazobenzene, prepared as described by Miller and Baumann (9), had a m. p. of 87-88° C.; the guanidoacetic acid, synthesized and reprecipitated according to Brand and Brand (2), had a decomposition point of 281-284° C.

RESULTS

In 10 of the 12 groups (Table II) the rats remained in good health, ate more than 10 gm. of diet per day, and gained 40 or more gm. during the dye feeding period. The rats receiving the methyl acceptors (Groups 6 and 7) consumed 9 gm. of ration per day, but began to lose weight after 3 months. Ninety-three to 100 per cent of the rats on each diet survived until the laparotomies were performed, and no more than 2 animals from any group died without tumors during the subsequent 2 month period on the dye-free rations.

The effect of hydrogenated coconut oil and riboflavin.—When 0.056 per cent of *p*-monomethylaminoazobenzene was fed for 14 weeks in the usual corn oil diet, the incidence of tumors at 22 weeks was 87 per cent (Table II, Group 1). Of the rats on hydrogenated coconut oil (Group 3) 31 per cent had hepatomas at 22 weeks, while the incidence of neoplasms in Group 4, which received 10 mgm. of riboflavin per kgm., was 58 per cent. When 0.056 per cent of *p*-monomethylaminoazobenzene was fed for only 13 weeks, 60 per cent of the rats on the corn oil diet (Group 9) developed neoplasms by 22 weeks, while 27 per cent of those receiving hydrogenated coconut oil (Group 10) had hepatomas at this time. However, only 7 per cent of the animals fed 20 mgm. of riboflavin per kgm. of ration (Group 11) had liver tumors by the end of the experimental period. Thus the substitution of hydrogenated coconut oil for corn oil definitely inhibited tumor induction due to *p*-monomethylaminoazobenzene. Increasing the level of riboflavin from 2 to 10 mgm. per kgm. reduced the number of hepatomas slightly, while a further increase to 20 mgm. per kgm. considerably inhibited tumor formation. Nevertheless, these dietary effects were less pronounced than those previously observed when *p*-dimethylaminoazobenzene was used as the carcinogen (7, 11, 12, 14). In general gross cirrhosis paralleled tumor incidence; it was most severe in the rats on the corn oil diet and less pronounced in those receiving either hydrogenated coconut oil or high levels of riboflavin.

TABLE II: CARCINOGENICITY OF *p*-MONOMETHYLAMINOAZOBENZENE IN VARIOUS DIETS

Group no. *	Dye fed	Diet	Dye feeding period, wks.	Average initial weight, gm.	Gain in weight 13-14 wks., gm.	Ave. food consumption, gm./rat/day	Survival † 13-14 wks.	Hepatomas ‡ 13-14 wks.	Hepatomas ‡ 22 wks.	Neg. survivors at 22 wks.	Gross cirrhosis at 13-14 wks. none	very mild	mild-mod.	severe
1	MAB §	Control	14	185	58	11.5	15/15	6/15	13/15	1	1		9	5
2	DAB §	Control	"	183	42	10.4	15/15	1/15	9/15	4	2		12	1
3	MAB	HCNO ‖	"	188	59	13.3	13/13	1/13	4/13	9	8		5	0
4	"	10 mgm. riboflavin/kgm.	"	187	66	12.1	12/12	2/12	7/12	3	3		8	1
5	"	0.3% choline	"	182	63	10.9	14/15	8/14	12/14	1	5		9	1
6	"	0.35% nicotinamide	"	186	— 11	9.6	15/15	5/15	12/15	1	0		0	15
7	"	0.3% guanidoacetic acid	"	189	4	9.0	14/15	2/14	9/14	3	0		5	10
8	AB §	0.3% choline	"	182	67	13.0	12/12	0/12	0/12 **	7	12		0	0
9	MAB	Control	13	186	51	11.4	15/15	1/15	9/15	5	2		8	5
10	"	HCNO	"	185	65	12.3	15/15	1/15	4/15	9	5		10	0
11	"	20 mgm. riboflavin/kgm.	"	179	83	13.4	14/15	0/14	1/14	13	8		6	0
12	"	0.5% choline	"	186	53	11.7	15/15	3/15	11/15	3	6		9	0

* Groups 1 to 8 constitute one series of experiments and are directly comparable; Groups 9 to 12 comprise a second series.
† Survival = number living over number at start.
‡ Hepatomas = number with hepatomas over number alive at time of operation.
§ DAB = *p*-dimethylaminoazobenzene.
MAB = *p*-monomethylaminoazobenzene.
AB = *p*-aminoazobenzene.
‖ HCNO = hydrogenated coconut oil.
** This group was fed the dye continuously for 11 months; no tumors were noted even at this time.

The effect of methyl donors or acceptors.—In the presence of the azo dye the most obvious effects of choline, as a methyl donor, and of guanidoacetic acid or nicotinamide as methyl acceptors, were upon the degree of liver damage. Choline tended to minimize the gross cirrhotic changes normally appearing when *p*-monomethylaminoazobenzene is fed to rats (Table II, Group 5 versus Group 1; Group 12 versus Group 9). This is in agreement with the observations of White and Edwards (18), who fed *p*-dimethylaminoazobenzene with or without 0.2 per cent of choline in a diet containing 6 per cent of casein and 0.5 per cent of cystine.

The methyl acceptors, on the other hand, tended to aggravate the cirrhotic condition. The livers of rats fed *p*-monomethylaminoazobenzene and either guanidoacetic acid or nicotinamide (Table II, Groups 6 and 7) were very much more cirrhotic after 14 weeks

than those of the rats receiving the azo dye alone (Group 1). Furthermore, when the corresponding rations without the azo dye were fed during the subsequent 8 weeks the cirrhosis receded, and the livers of Group 1 tended to resume a smooth, normal appearance. However, in the presence of nicotinamide or guanidoacetic acid the cirrhosis did not regress appreciably, even after 8 weeks on the dye-free diets. The amounts of nicotinamide and guanidoacetic acid fed were about the maximum that can be tolerated by adult rats given the methyl acceptor and carcinogen simultaneously. The animals in both groups gained about 20 gm. during the first 4 weeks and remained in fair health for the next 8 to 10 weeks. Thereafter some of the rats, especially those fed nicotinamide, began to lose weight. Removing the carcinogen at 14 weeks and reducing the amount of methyl acceptors slightly (see Methods) permitted survival for the following 2 months.

But in spite of their effects on the degree of liver damage, neither the methyl donor nor the methyl acceptors altered greatly the number of tumors caused by the ingestion of *p*-monomethylaminoazobenzene. Thus while 87 per cent of the rats receiving no supplement (Table II, Group 1) had hepatomas at 22 weeks, the incidence was 86 per cent when 0.3 per cent of choline chloride (Group 5) was added to the diet, 80 per cent when 0.35 per cent of nicotinamide (Group 6) was included, and 64 per cent with 0.3 per cent of guanidoacetic acid (Group 7) in the diet. When the level of choline chloride was raised to 0.5 per cent and the carcinogen was fed for only 13 weeks, 73 per cent of the rats (Group 12) had neoplasms at 22 weeks as compared to an incidence of 60 per cent in the unsupplemented group (Group 9).

The methyl acceptors and methyl donor also failed to alter appreciably the amounts of azo dye in the blood and livers of rats fed *p*-monomethylaminoazobenzene (Table III). The blood of rats that had received these diets for 2 weeks contained an average of 15 to 18 μgm. of *p*-aminoazobenzene per cc.; no more than traces of the methylated dyes were detected. The livers of these rats contained 2 to 3 μgm. of *p*-dimethylaminoazobenzene, 2 to 5 μgm. of *p*-monomethylaminoazobenzene, and 4 to 8 μgm. of *p*-aminoazobenzene; there was no significant difference between the values for the rats fed the control diet or high levels of choline, guanidoacetic acid, or nicotinamide for only 2 weeks. When the same diets were fed for 10 to 16 weeks, the *p*-aminoazobenzene content of the blood again averaged 15 to 18 μgm. per cc., and the level of the dimethyl compound in the liver was the same as at 2 weeks: 2 to 3 μgm. per liver. The amount of *p*-aminoazobenzene in these livers was slightly higher, 8 to 9 μgm. per liver, than at 2 weeks, possibly because these livers were cirrhotic and there was less assurance that the perfusion had been complete; any blood not removed would raise the apparent content of *p*-aminoazobenzene in the organ. The amount of the monomethyl compound in the livers increased to an average of 6 μgm. for the rats fed either guanidoacetic acid or nicotinamide, whereas the livers of the rats on the control diet for the same period contained only 2.5 μgm. Again the cirrhosis, with a consequent decrease in normal liver tissue, may have been responsible for the difference.

p-Aminoazobenzene and choline.—Feeding 0.3 per cent of choline chloride with 0.106 per cent of *p*-aminoazobenzene did not appear to promote the formation of liver tumors by this dye. Of the 12 rats fed the compound continuously 5 survived for 11 months, but neither tumors nor gross cirrhosis were found. Analyses of tissues from 2 of the rats fed the diet for 11 months showed the presence of appreciable amounts of *p*-aminoazobenzene, but neither of the methylated dyes could be detected. On the average there were 18 μgm. per cc. of blood, 22 μgm. per liver, and 9 μgm. per gm. of perirenal fat.

While other investigators (6, 10) have also failed to induce neoplasms with *p*-aminoazobenzene, Kirby (8) produced liver tumors (2 macroscopic, 1 microscopic) in 3 of 16 rats by feeding 0.2 to 0.3 per cent of this compound (3.8 to 5.5 times the molar level used for the dimethyl compound) for 17 months. His conclusion that ". . . methylation of the primary amine, *p*-aminoazobenzene, enhances but does not initiate carcinogenic activity . . ." may be valid, but other considerations should be mentioned. If small amounts of *p*-monomethylaminoazobenzene or *p*-dimethylaminoazobenzene either contaminated the dye fed, or were formed *in vivo,* the quantities in the tissues might have been sufficient to cause tumors in such a long experiment. Maruya (6) reported 2 hepatomas after feeding 0.006 per cent of *p*-dimethylaminoazobenzene for 371 days, but none from 0.002 per cent in 400 days. If 1 to 2 per cent of the *p*-aminoazobenzene fed at the 0.2 per cent level were methylated *in vivo,* this would be equivalent to feeding 0.002 to 0.005 per cent of the methylated dyes in the diet. It would be very difficult to detect analytically such small amounts of one dye in the presence of 50 to 100 times as much of another derivative. For example, in the present experiment the livers of the rats fed 0.106 per cent of *p*-aminoazobenzene (Group 8) contained 22 μgm. of the dye; the chromatographic detection of bands containing 0.2 to 0.4 μgm. (1 and 2 per cent of the total) of either of the two methylated dyes in the presence of this amount of *p*-aminoazobenzene is very uncertain with the present methods.

Relative carcinogenicity of p-dimethylaminoazoben-

zene and p-monomethylaminoazobenzene.—In agreement with previous results from this laboratory (4) the rats fed *p*-monomethylaminoazobenzene *ad libitum* paired-feeding conditions, however, these compounds had equal activity (Table IV). Of the 15 pairs started both members of 13 pairs survived until at least 1 had

TABLE III: LEVELS OF *p*-MONOMETHYLAMINOAZOBENZENE AND ITS METABOLITES IN TISSUES OF RATS FED THIS DYE WITH CHOLINE OR METHYL ACCEPTORS

Diet	Period dye was fed	No. of rats/ group	Blood AB *	Liver (perfused) DAB *	Liver (perfused) MAB *	Liver (perfused) AB
	wks.		μgm./cc.†	μgm. per liver †		
Control	2	3	18.3 (14.2-22.7)	3.0 (2.1-4.0)	2.7 (1.9-3.5)	6.2 (4.3-8.1)
0.3% Guanidoacetic acid	"	"	15.6 (15.4-16.0)	2.7 (2.2-3.1)	3.8 (2.4-4.6)	4.8 (3.9-5.5)
0.35% Nicotinamide	"	"	16.5 (15.2-17.8)	2.0 (1.8-2.4)	3.5 (2.6-3.9)	4.8 (4.3-5.2)
0.5% Choline	"	"	14.2 (11.2-17.5)	2.0 (1.9-2.1)	4.5 (4.3-4.8)	7.2 (6.3-8.9)
Control	10-16	5	15.2 (8.1-20.8)	2.7 (1.4-3.7)	2.5 (1.4-3.0)	8.3 (6.1-9.3)
0.3% Guanidoacetic acid	"	"	17.1 (13.8-20.6)	1.9 (1.2-3.3)	5.7 (3.0-10.4)	9.2 (6.1-12.3)
0.35% Nicotinamide	"	"	18.2 (12.8-22.0)	1.9 (1.2-3.0)	6.8 (4.6-8.6)	8.8 (7.5-9.7)

* DAB = *p*-dimethylaminoazobenzene.
MAB = *p*-monomethylaminoazobenzene.
AB = *p*-aminoazobenzene.
† The single value is the average for the group; figures in parentheses indicate the ranges found.

TABLE IV: THE RELATIVE CARCINOGENICITIES OF *p*-DIMETHYLAMINOAZOBENZENE AND *p*-MONOMETHYLAMINOAZOBENZENE AS DETERMINED BY PAIRED-FEEDING *

Pair no.	Sex	Average initial weight, gm.	Average weight increment, gm.	Dye consumed in 13 weeks, millimols/rat	*p*-Monomethylaminoazobenzene Hepatoma, 13 wks.	*p*-Monomethylaminoazobenzene Hepatoma, 22 wks.	*p*-Monomethylaminoazobenzene Gross cirrhosis, 13 wks.	*p*-Dimethylaminoazobenzene Hepatoma, 13 wks.	*p*-Dimethylaminoazobenzene Hepatoma, 22 wks.	*p*-Dimethylaminoazobenzene Gross cirrhosis, 13 wks.
1	M	205	22	2.12	0	0	normal	0	0	normal
2	F	162	14	1.89	0	+	mild	0	+	moderate
3	F	172	— 19	1.76	0	+	moderate	0	+	severe
4	M	181	0	1.72	0	+	severe	0	+	normal
5	M	167	8	2.05	0	+	moderate	0	+	severe
6	M	178	30	2.07	0	+	normal	0	+	mild
7	M	216	— 9	1.95	+	+	moderate	0	0	normal
8	F	184	— 14	1.86	+	+	mild	0	0	mild
9	F	164	— 19	1.92	0	+	severe	0	0	normal
10	M	168	17	1.98	0	+ †	moderate	0	? †	mild
11	M	201	— 24	1.99	0	? ‡	moderate	+	+	mild
12	F	160	7	1.89	0	0	mild	+	+	moderate
13	M	202	— 30	1.80	0	0	normal	0	+	moderate
Average	..	182	— 1	1.93	2/13	9/13	....	2/13	8/13	....

* Two pairs in which 1 rat died before either animal developed a tumor are not included in this table.
† In this pair the rat receiving *p*-monomethylaminoazobenzene had a liver tumor when the animal on *p*-dimethylaminoazobenzene died after 18 weeks on the experiment.
‡ Died after 14 weeks.

developed more tumors than those on the dimethyl compound. The incidences were 40 and 87 per cent for the monomethyl dye at 14 and 22 weeks, and 7 and 60 per cent for the animals receiving *p*-dimethylaminoazobenzene (Table II, Groups 1 and 2). Under a hepatoma. Two rats on each compound had liver tumors at 13 weeks, and at 22 weeks the total number was 8 for the rats fed *p*-dimethylaminoazobenzene and 9 for the group receiving the monomethyl compound. There was no significant difference in the rate of

formation or growth of the neoplasms on the two diets; likewise the cirrhosis at the end of the dye-feeding period was generally mild to moderate in both cases. Further, there was no correlation between the amount of dye consumed by a given rat and the rate of tumor formation. While these data demonstrated that the two compounds had equal carcinogenic potency as determined by the average of 13 pairs of rats, they also indicated the heterogeneity of such rats with respect to susceptibility to the induction of hepatomas by the azo dyes. Both members of 1 pair were tumor-free at 22 weeks and both members of 5 pairs had tumors at this time. In 7 of the 13 pairs, however, only 1 rat of each pair had developed a neoplasm by the end of the experimental period: 4 on the monomethyl compound and 3 on *p*-dimethylaminoazobenzene.

While the rats remained in generally good health, the average weight change was −1 gm. (Table IV) during the dye feeding period, in contrast to gains of 42 and 58 gm. for the rats fed *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene *ad libitum* (Table II). This difference was probably due to the fact that both rats of each pair were continually restricted to the lower voluntary food consumption of the two, so that both animals were penalized if either went off feed for a few days. The average food intake was 8 gm. per rat per day.

DISCUSSION

The results of these experiments cannot be interpreted as favoring either *p*-dimethylaminoazobenzene or *p*-monomethylaminoazobenzene as being closer to the true carcinogen. The equal activity of these compounds, when compared under controlled feeding conditions, suggests either that both compounds are carcinogenic or that they are both converted at the same rate *in vivo* to the active compound. Or, if only one of these two dyes is the actual carcinogen, then the rate of conversion of the noncarcinogenic one to the active compound must be very efficient indeed. On the other hand the greater effect of such dietary constituents as hydrogenated coconut oil or riboflavin on carcinogenesis due to the dimethyl dye, as compared to *p*-monomethylaminoazobenzene, might be interpreted as indicating that the latter compound was nearer to the true carcinogen. However, it is more likely that this is another expression of the difference between *ad libitum* and controlled feeding with rats receiving the monomethyl compound. Under identical conditions it is doubtful if any difference in the inhibition by riboflavin and hydrogenated coconut oil would be observed when the two dyes were compared. Incidentally, it is still not clear why rats fed *p*-monomethylaminoazobenzene voluntarily consume more food than those receiving equimolar amounts of the dimethyl dye in the diet.

Since *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene are formed from each other *in vivo,* it is quite possible that a transmethylation reaction may be involved. However, the conversion of *p*-monomethylaminoazobenzene to *p*-aminoazobenzene is not reversible to any detectable extent, and part of or all the monomethyl compound may be degraded by a mechanism that does not make the methyl group available to other molecules such as guanidoacetic acid or nicotinamide. Liver preparations, for instance, have been observed to demethylate sarcosine by oxidation of the methyl group to formaldehyde (5). If the methylated aminoazo dyes undergo a similar degradation, feeding large quantities of methyl acceptors with *p*-monomethylaminoazobenzene would probably not alter the rate of formation of the completely demethylated compound. The inability to demonstrate a difference in the quantity of the dimethyl compound in the liver may be due also to the difficulty of altering the methyl balance of animal tissues sufficiently to affect a given reaction to a noticeable extent.

SUMMARY

1. Hepatomas developed by 22 weeks in 60 to 87 per cent of rats fed a synthetic diet containing corn oil and *p*-monomethylaminoazobenzene for 13 to 14 weeks, whereas only 30 per cent of the rats fed hydrogenated coconut oil developed tumors by this time. Raising the riboflavin content of the ration from 2 to 10 mgm. per kgm. reduced the tumor incidence slightly; when 20 mgm. per kgm. were fed, only 1 of 14 rats had a hepatoma at 22 weeks.

2. Rats receiving diets that contained 0.3 per cent of guanidoacetic acid, 0.35 per cent of nicotinamide, or 0.3 or 0.5 per cent of choline with *p*-monomethylaminoazobenzene developed approximately the same number of neoplasms as the animals on the control diet, although the methyl acceptors caused a more severe gross cirrhosis than the control diet, whereas the addition of choline minimized the cirrhosis. Analyses of the livers and blood from rats on each of these diets indicated that the methyl acceptors and donor did not alter greatly the levels of *p*-dimethylaminoazobenzene, *p*-monomethylaminoazobenzene, and *p*-aminoazobenzene in the tissues.

3. No liver tumors or gross cirrhosis were found in rats fed 0.106 per cent of *p*-aminoazobenzene with 0.3 per cent of choline for 11 months.

4. When *p*-dimethylaminoazobenzene and *p*-monomethylaminoazobenzene were fed *ad libitum,* the rats receiving the monomethyl compound developed more tumors than those on the dimethyl dye. However,

when the two compounds were compared by the paired-feeding technic the carcinogenic activities were found equal.

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