Hepatic Riboflavin and Tumor Formation in Rats Fed Azo Dyes in Various Diets*

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It is well recognized that rats fed *p*-dimethylaminoazobenzene (DAB) develop tumors at rates that depend upon the composition of the basal diet. Dietary factors reported to increase the rate of tumor formation under certain circumstances include biotin (2, 4), pyridoxine (16), cystine (18), and the rice bran concentrate "Vitab" (12, 13, 16). The dietary factors most effective in retarding tumor formation include riboflavin (8, 12, 16) and hydrogenated coconut oil (13, 14). The evidence for these effects has been summarized in a recent review (17). Later experiments, however, suggest that riboflavin may be more important in the development of hepatic tumors than most of the other dietary factors that have been studied. When *m'*-methyl-*p*-dimethylaminoazobenzene (*m'*-Me-DAB) was fed as the carcinogen, the rate at which tumors developed was not increased by the feeding of the rice bran concentrate, nor did hydrogenated coconut oil exert any considerable retarding effect (6). Riboflavin, however, retarded the development of tumors due to the *m*-methyl dye, though not as much as when DAB was the carcinogen. Further evidence for a peculiar role of riboflavin in the formation of hepatic tumors is the observation that certain of the azo dyes markedly lower the hepatic concentration of this vitamin (7, 9) while chemically similar non-carcinogenic dyes do not (7). Indeed, there appears to be a rough correlation between the carcinogenic potencies of the various azo dyes and their effects upon the concentration of riboflavin in the liver in relatively short-term experiments (7). The question, therefore, arose whether diets known to alter the rate of hepatic tumor formation might not exert their effects primarily by modifying the ability of the liver to store riboflavin.

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METHODS

The experiments were of two general types. The first involved series in which hepatic tumors were produced in rats by feeding them diets similar to those used in previous experiments but designed to indicate the relative effectiveness of riboflavin, hydrogenated coconut oil, and the rice bran concentrate "Vitab" in altering the rate of formation of tumors due to *p*-dimethylaminoazobenzene. The diets contained 5 per cent of either corn oil or hydrogenated coconut oil (HCNO), in which enough *p*-dimethylaminoazobenzene was dissolved to furnish a concentration of 0.06 per cent of the dye in the ration. The B vitamins were added either as the synthetic mixture (16, 17) used routinely in previous experiments, or as the rice bran concentrate, "Vitab." Comparisons between these two sources of the B vitamins were made at two concentrations of riboflavin in the diet, 0.3 and 2.0 mgm./kgm. When the diet contained corn oil, the dye was fed for 4½ months, at which time the livers of a few animals in each group were examined by laparotomy. Thereafter, the corresponding basal diets free from the dye were fed for two more months, when the animals were killed and the livers removed for examination. The animals fed hydrogenated coconut oil received the dye for 5 months. The composition of the various diets is indicated in Table I.

The second type of experiment was primarily analytical. Rats of various ages were fed either *p*-dimethylaminoazobenzene or *m'*-methyl-*p*-dimethylaminoazobenzene in diets selected for their known ability to modify the rate of tumor formation. The diets (Table I) included our standard synthetic ration (16, 17) containing corn oil (diet 1), a similar diet in which the corn oil was replaced by hydrogenated coconut oil (13, 14) (diet 3), a diet containing the rice bran concentrate "Vitab" (diet 2), and another containing additional amounts of riboflavin (16) (diet 1). In other diets the level of casein was raised from 12 to 24 per cent, or higher amounts of riboflavin or of the rice bran

concentrate were incorporated (Tables III and IV). Such additions were made at the expense of the glucose. After 3 to 6 weeks the animals were killed by decapitation and the livers removed, weighed rapidly, and homogenized in a Waring blendor with 10 volumes of 0.1 *N* H_2SO_4. The mixture was autoclaved for 15 minutes at 15 pounds pressure, and the riboflavin determined fluorometrically according to the method of Conner and Straub (3), as modified by Andrews (1).

EXPERIMENTAL

Relative effectiveness of riboflavin, hydrogenated coconut oil, and the rice bran concentrate "Vitab" on tumor formation.—In line with previous observations in this laboratory, tumors due to *p*-dimethylaminoazobenzene developed more rapidly on a diet containing the rice bran concentrate, "Vitab," than when the standard synthetic diet was fed. These two diets are known to differ in their content of riboflavin. In a subsequent series 0.06 per cent *p*-dimethylaminoazobenzene in corn oil was fed in either the "Vitab" diet, or the synthetic diet, modified to contain equivalent amounts of riboflavin. Comparisons were made at 2 levels of the vitamin: 2.0 mgm. per kgm. and 0.3 mgm. per kgm. Tumors developed most rapidly at the lower level of riboflavin regardless of the source of the other B-vitamins. When the diet contained 2 mgm. of riboflavin per kgm., the incidence of tumors at 6½ months was 30 per cent on both the synthetic and the "Vitab" diets (Table II, groups 5 and 6). When the diets contained 0.3 mgm. of riboflavin per kgm. the incidence of tumors on the two diets was approximately 87 per cent at this time (Table II, groups 7 and 8). In other words, the incidence of tumors was increased by a reduction in the concentration of riboflavin in the ration whether synthetic B vitamins were fed or whether the diet contained the rice bran concentrate.

TABLE I: COMPOSITION OF DIETS

	gm./kgm. Corn oil synthetic	Corn oil vitab*	HCNO† synthetic	HCNO† vitab*
	1	2	3	4
Cerelose‡	790	770	790	770
Casein (purified)	120	120	120	120
Salts	40	40	40	40
Corn oil§	50	50		
Hydrogenated coconut oil			50	50
Rice bran concentrate		20		20
Thiamine	.003		.003	
Pyridoxine	.0025		.0025	
Choline	.030		.030	
Calcium pantothenate	.007		.007	
Riboflavin	.0003–.02	0–.002	.0003–.002	0–.002

*Vitab = Rice bran concentrate, National Oil Products Company, Harrison, New Jersey.
†HCNO = Hydrogenated coconut oil, Lever Brothers, Cambridge, Massachusetts.
‡Cerelose = Pure glucose monohydrate.
§ Corn oil = Mazola, Corn Products Refining Company.

TABLE II: RELATIVE IMPORTANCE OF RIBOFLAVIN AND OTHER NUTRIENTS IN MODIFYING THE DEVELOPMENT OF TUMORS DUE TO *p*-DIMETHYLAMINOAZOBENZENE

Group	Diet fed: Vitamin source		Ribo-flavin conc. mgm./kgm.	Time dye was fed months	Av. initial wt. gm.	Av. wt. at end of dye feeding gm.	Av. food consumption gm./rat/day	Survival at end of dye feeding	Tumor incidence: at end of dye feeding	Tumor incidence: 2 months later	Cirrhosis 2 months after end of dye feeding
1	Corn oil	Vitab	0.7	4	225	196	10.0	12/15	0/12	7/12	5
2	Corn oil	Synthetic	2.0	4	224	221	10.8	14/15	1/14	5/13	0
3*	Corn oil	Vitab	0.7	3	100	130	9.3	10/10	1/10	4/8	3
4*	Corn oil	Synthetic	2.0	3	97	164	9.8	10/10	1/10	5/10	5
5	Corn oil	Synthetic	2.0	4.5	160	194	10.0	10/10		3/10	5
6	Corn oil	Vitab	2.0	4.5	158	250	9.7	10/10		3/10	3
7	Corn oil	Synthetic	0.3	4.5	159	157	7.5	7/10	4/10	6/7	6
8	Corn oil	Vitab	0.3	4.5	161	162	8.7	9/9		7/8	7
9	HCNO	Synthetic	2.0	5	172	202	11.6	15/15		0/15	0
10	HCNO	Vitab	2.0	5	171	212	11.4	14/15		0/14	0
11	HCNO	Synthetic	0.3	5	165	142	8.7	14/15		7/12	mild
12	HCNO	Vitab	0.3	5	174	159	8.5	13/15		7/13	mild

*All groups except 3 and 4 were fed 0.06 per cent *p*-dimethylaminoazobenzene; groups 3 and 4 received 0.032 per cent *m'*-methyl-*p*-dimethylaminoazobenzene.
Corn oil—Mazola, Corn Products Refining Company.
Vitab—Rice bran concentrate, National Oil Products Company, Harrison, New Jersey.
HCNO—Hydrogenated coconut oil, Lever Brothers, Cambridge, Massachusetts.

This conclusion was verified in another series in which the fat of the diet was hydrogenated coconut oil (Table II, groups 9 to 12). Hepatic tumors due to *p*-dimethylaminoazobenzene are known to develop more slowly in the presence of hydrogenated coconut oil than of corn oil (13, 14). Hence in the present series the dye was fed for 5 months, and an additional 2 months permitted on the basal diets free from dye before the final examination for tumors was made. With hydrogenated coconut oil no tumors developed on either the synthetic diet or the "Vitab" diet when 2.0 mgm. of riboflavin were present per kgm. of diet (Table II, groups 9 and 10) although in the presence of corn oil an incidence of 30 per cent had been noted in the previous series in which the carcinogen was fed for a shorter period of time (Table II, groups 5 and 6). Thus, the protective effect of hydrogenated coconut oil against tumors due to *p*-dimethylaminoazobenzene (13, 14) was again confirmed. At the lower level of riboflavin intake, however, tumors developed in the presence of hydrogenated coconut oil on both the "Vitab" and synthetic diets. The incidences were nearly identical, 58 and 54 per cent respectively at 7 months (Table II, groups 11 to 12). This similarity in response would appear to indicate that the rice bran concentrate does not possess any peculiar factors that stimulate hepatic tumor formation other than the relatively low riboflavin content of the preparation.

Incidentally, at the lower level of riboflavin intake, hydrogenated coconut oil also retarded tumor development: 56 per cent of the animals on the coconut oil diet developed tumors after ingesting the dye for 5 months (Table II, groups 11 and 12) as compared to an incidence of 87 per cent when the dye was fed for 4½ months in comparable diets containing corn oil (Table II, groups 7 and 8).

Effect of various diets on the storage of riboflavin in the liver.—In general those diets on which rats are known to resist tumor formation tended to maintain relatively normal concentrations of riboflavin in the liver; those on which tumor formation is rapid tended to depress the concentration of hepatic riboflavin. The results were not always consistent, variations within groups were sometimes wide, and in individual rats in which the liver was enlarged abnormally under the influence of the azo dye, the concentration of hepatic riboflavin was sometimes depressed even though the total amount of the organ may have been quite high. A summary of the results is as follows: The livers of rats fed the rice bran concentrate, "Vitab," and 0.06 or 0.09 per cent of *p*-dimethylaminoazobenzene averaged 14.0 μgm. of riboflavin per gm. (11.7-16.8) and 74 μgm. per total liver (37-126) (Table III, groups 17, 26, 31, 37) while livers from corresponding rats fed the synthetic diet averaged 16.2 μgm. of riboflavin per gm. (13.7-18.3) and 97 μgm. per total liver (37-126) (Table III, groups 15, 24, 29, 35). In corresponding pairs of groups either the concentration or the total amount of riboflavin was higher on the synthetic diet than on that containing the rice bran concentrate (Table

TABLE III: EFFECT OF CERTAIN DIETS ON THE RIBOFLAVIN CONTENT OF LIVERS OF RATS FED *p*-DIMETHYLAMINOAZOBENZENE (DAB)

Group	Diet and dye fed	Casein level gm./kgm.	Feeding period wks.	μgm. B_2 per gm. /diet	No. of animals	Initial wt. gm.	Growth increment gm./wk.	Average daily food intake gm.	Average liver wt' gm.	Liver riboflavin μgm./gm.	Range	Total μgm.
13	Corn oil Syn. 0.06% DAB	120	3	2.0	5	108	0	8.0	5.3	14.4	13.5–15.5	75
14	HCNO Syn. 0.06% DAB	120	3	2.0	5	109	+5	9.0	5.9	17.1	15.7–19.6	101
15	Corn oil Syn. 0.09% DAB	120	3	2.0	3	200	−7	9.2	7.6	15.8	14.3–16.8	121
16	HCNO Syn. 0.09% DAB	120	3	2.0	3	185	0	10.6	7.3	18.3	16.7–20.0	134
17	Corn oil Vitab 0.09% DAB	120	3	0.7	3	200	−3	10.0	6.2	15.3	15.0–15.8	95
18	Corn oil Vitab (4%) 0.09% DAB	120	3	0.85	3	200	−5	9.4	7.4	14.4	13.2–15.8	107
19	Corn oil Syn. 0.09% DAB	120	3	10.0	3	200	−5	11.0	7.3	19.2	17.8–20.8	139
20	Corn oil Syn. 0.06% DAB	120	3	2.0	3	143	−1	11.0	7.0	16.2	13.8–17.5	113
21	HCNO Syn. 0.06% DAB	120	3	2.0	3	147	−2	9.5	6.5	18.2	17.3–18.9	118
22	Corn oil Vitab 0.06% DAB	120	3	2.0	3	148	−4	9.0	6.2	17.4	17.3–17.5	108
23	Corn oil Syn. 0.06% DAB	240	3	2.0	3	143	−11	10.0	6.1	20.2	19.0–21.0	123
24	Corn oil Syn. 0.09% DAB	120	6	2.0	3	215	−6	9.5	6.5	15.0	13.7–17.0	98
25	HCNO Syn. 0.09% DAB	120	6	2.0	3	228	−4	9.9	9.2	13.6	13.0–14.2	125
26	Corn oil Vitab 0.09% DAB	120	6	0.7	3	212	−10	9.0	5.3	13.3	12.5–14.6	70
27	Corn oil Syn. 0.09% DAB	120	6	10.0	3	225	−3	11.4	8.0	20.1	17.0–22.7	161
28	Corn oil Syn. No dye	120	6	2.0	6	190	+11	13.0	9.0	17.2	15.0–19.0	155
29	Corn oil Syn. 0.09% DAB	120	6	2.0	3	186	−3	8.8	5.5	17.0	16.6–17.9	95
30	HCNO Syn. 0.09% DAB	120	6	2.0	3	205	−1.5	11.6	7.5	17.5	16.8–18.2	130
31	Corn oil Vitab 0.09% DAB	120	6	0.7	3	188	−3	10.3	6.3	15.2	13.5–16.8	96
32	Corn oil Vitab (4%) 0.09% DAB	120	6	0.85	3	170	+2	9.4	6.3	15.5	14.6–17.0	97
33	Corn oil Syn. 0.09% DAB	120	6	10.0	3	195	−5	9.0	5.8	26.0	22.0–30.0	150
34	Corn oil Syn. No dye	120	6	2.0	5	125	+12.5	14.1	6.5	19.0	19.0–21.0	124
35	Corn oil Syn. 0.09% DAB	240	6	2.0	3	57	+5	6.3	4.4	16.7	15.2–18.3	75
36	HCNO Syn. 0.09% DAB	240	6	2.0	3	56	+7	6.9	5.0	19.0	15.0–25.0	94
37	Corn oil Vitab 0.09% DAB	240	6	0.2	3	56	+2	5.4	3.1	12.3	11.7–12.9	37
38	Corn oil Syn. 0.09% DAB	240	6	20.0	3	55	+9	8.4	6.0	23.5	23.4–23.5	141

Corn oil—Mazola, Corn Products Refining Company; HCNO—Hydrogenated coconut oil; Vitab—Rice bran concentrate.

III, group 15 vs. 17, 24 vs. 26, 29 vs. 31). The differences are regarded as suggestive, rather than as completely significant. When the riboflavin content of the diet was increased, the storage of the vitamin in the liver increased significantly (Table III, groups 19, 27, 33 and 38 versus groups 15, 24, 33 and 35), even though fairly high amounts of *p*-dimethylaminoazobenzene were present in the diet. This is the counterpart to the well-known effect of riboflavin in retarding the development of tumors due to this dye.

In the presence of *m'*-methyl-*p*-dimethylaminoazobenzene the storage of riboflavin in the liver was low whether the corn oil synthetic diet or the diet containing the rice bran concentrate was fed (Table IV, groups 39 versus 41, 51 versus 53). Variations within groups were usually wider than variations between groups although the average riboflavin storage was slightly lower on the "Vitab" diets. Previous experiments have indicated that the incidence of tumors is essentially the same on these two types of diets when the carcinogen is *m'*-methyl-*p*-dimethylaminoazobenzene (6). The addition of comparatively high levels of riboflavin to the synthetic diets containing the *m'*-methyl dye resulted in increased hepatic riboflavin storage, *e.g.*, 12.8 μgm./gm. versus 14.9 μgm./gm., and 60 μgm. versus 76 μgm. per total liver (Table IV, groups 39 and 43). Thus the improvement in riboflavin retention was not so marked with *m'*-methyl-*p*-dimethylaminoazobenzene as with *p*-dimethylaminoazobenzene (Table III), and this difference between dyes parallels the degrees to which their carcinogenic activities can be counteracted with dietary riboflavin (6, 16).

When hydrogenated coconut oil was substituted for corn oil in diets containing *p*-dimethylaminoazobenzene, an increase in hepatic riboflavin usually resulted. This was true with both weanling and adult rats, whether the dye was fed as 0.06 per cent or 0.09 per cent of the ration, or whether the feeding time was 3 or 6 weeks (Table III, groups 15 and 20 versus 16 and 21). Thus, for groups 15 and 20 the concentration of riboflavin per gram of liver averaged 16.0 μgm./gm. (15.8-16.2) on corn oil as contrasted to 18.3 μgm./gm. for groups 16 and 21 on hydrogenated coconut oil. Maximum differences due to these fats were observed in two other series in which 0.09 per cent of the azo dyes was fed. The total amounts of riboflavin per liver averaged 96 μgm. on corn oil as contrasted to 127 μgm. on hydrogenated coconut oil (Table III, groups 24 and 29 versus 25 and 30).

TABLE IV: EFFECT OF CERTAIN DIETS ON THE RIBOFLAVIN CONTENT OF LIVERS OF RATS FED *m'*-METHYL-*p*-DIMETHYLAMINOAZOBENZENE (*m'*-ME-DAB)

Group	Diet and dye fed	Casein level gm./kgm.	Feeding period wks.	μgm. B_2 added per gm. diet	No. of animals*	Initial wt. gm.	Growth increment gm./wk.	Average daily food intake gm.	Average liver wt. gm.	Liver riboflavin μgm./gm.	Range	Total μgm.
39	Corn oil Syn. 0.096% *m'*-Me-DAB	120	3	2.0	2/4	140	−9	6.5	4.7	12.8	11.8–13.9	60
40	HCNO Syn. 0.096% *m'*-Me-DAB	120	3	2.0	4	140	−7.7	6.6	5.0	11.7	10.0–14.0	59
41	Corn oil Vitab 0.096% *m'*-Me-DAB	120	3	0.7	4	142	−7.7	6.3	4.5	12.3	10.7–14.0	55
42	Corn oil Vitab—5% 0.096% *m'*-Me-DAB	120	3	0.5	4	123	−7.2	5.6	3.7	11.4	9.8–14.0	42
43	Corn oil Syn. 0.096% *m'*-Me-DAB	120	3	10.0	5	142	−7.6	6.9	5.1	14.9	12.0–17.9	76
44	Corn oil Syn. control No dye	120	3	2.0	5	123	+12.5	14.1	6.4	19.3	17.6–21.0	124
45	Corn oil Syn. Restricted No dye	120	3	2.0	5	142	−10.0	4.0	3.4	29.0	21.0–34.0	99
46	Corn oil Vitab No dye	120	3	0.7	5	132	+10.0	13.9	5.8	18.8	17.2–21.0	110
47	Corn oil Syn. 0.096% *m'*-Me-DAB	120	6	2.0	3/10	250	−9	7.2	6.9	13.6	12.8–14.7	94
48	HCNO Syn. 0.096% *m'*-Me-DAB	120	6	2.0	5/10	275	−14	7.2	6.7	13.5	12.0–15.5	91
49	Corn oil Vitab 0.096% *m'*-Me-DAB	120	6	0.7	2/10	276	−15	7.0	7.0	11.6	10.3–12.8	82
50	Corn oil Syn. 0.096% *m'*-Me-DAB	120	6	10.0	5/10	268	−9	8.2	8.3	14.8	13.5–16.4	123
51	Corn oil Syn. 0.064% *m'*-Me-DAB	240	6	2.0	3/10	53	+5	6.0	5.7	10.7	10.5–11.0	61
52	HCNO Syn. 0.064% *m'*-Me-DAB	240	6	2.0	3/10	53	+6	5.8	5.6	11.8	11.0–13.5	66
53	Corn oil Vitab 0.064% *m'*-Me-DAB	240	6	0.7	3/10	55	+3	5.5	4.3	11.7	9.8–15.0	50
54	Corn oil Syn. 0.064% *m'*-Me-DAB	240	6	10.0	3/5	57	+7	6.7	9.3	13.2	12.0–15.0	123
55	Corn oil Vitab 0.064% *m'*-Me-DAB	240	6	2.2	3/5	47	+7	5.5	6.0	11.3	9.5–13.0	68
56	Corn oil Syn. control No dye	240	6	2.0	3	61	+29	14.0	7.7	18.0	15.3–21.5	139
57	Corn oil Syn. control No dye	240	6	10.0	5	50	+20	13.8	6.5	24.8	21.0–27.0	160

Corn oil—Mazola, Corn Products Refining Company; HCNO—Hydrogenated coconut oil; Vitab—Rice bran concentrate.
* Fractions indicate number surviving the experimental period. Analytical results apply only to those that survived the full period.

In contrast to this increase in liver riboflavin observed in the presence of *p*-dimethylaminoazobenzene, hydrogenated coconut oil failed completely to maintain riboflavin storage when the carcinogen was *m'*-methyl-*p*-dimethylaminoazobenzene (Table IV, groups 39 versus 40, 47 versus 48). This observation is the counterpart to previous data (6) indicating that hydrogenated coconut oil does not diminish the rate of tumor formation when the *m'*-methyl dye is the carcinogen.

DISCUSSION

It has been suggested that several of the dietary combinations known to modify tumor formation may do so through a common mechanism, and present results indicate that this is at least partly true, since the "Vitab" diet, which accelerates tumor for-

mation due to *p*-dimethylaminoazobenzene, tended to decrease the concentration of hepatic riboflavin while diets containing hydrogenated coconut oil, which retard tumor formation, permit a greater retention of the vitamin than the control synthetic diet containing corn oil. Alterations in the dietary intake of riboflavin, as expected, alter the amount in the liver and change the rate of tumor formation correspondingly. Thus the original experiments of Kensler and associates (9) indicating a loss of hepatic riboflavin in the presence of azo dyes, are confirmed and extended, and it now appears that there is an inverse relationship between the rate of tumor formation on any particular dietary combination and the concentration of hepatic riboflavin retained on that diet. Experiments with the more active carcinogen, *m'*-methyl-*p*-dimethylaminoazobenzene also support this conclusion. Hepatic riboflavin was particularly low when this potent carcinogen was fed ([7], and Table IV versus Table III) and the storage of the vitamin was not modified by the rice bran diet, nor by hydrogenated coconut oil. This parallels previous results indicating that tumor formation due to the *m'*-methyl dye is relatively insensitive to these dietary factors (6). However, additional riboflavin in the diet decreased the rate of tumor formation due to *m'*-methyl-*p*-dimethylaminoazobenzene (6), while present data (Table IV) indicate that riboflavin retention is also increased somewhat when more of the vitamin is fed.

The inverse relationship between hepatic riboflavin and tumor formation raises the question whether the loss of hepatic riboflavin, at least locally, may be a necessary prerequisite for tumor formation. One might postulate a competitive inhibition between the azo dyes and riboflavin (or between their derivatives) for a critical spot on an enzyme molecule with the excess dye crowding out the riboflavin and exerting adverse effects. Conversely, excess riboflavin might crowd out the carcinogen and prevent it from acting. Substances such as hydrogenated coconut oil would then function by altering the amount of riboflavin available.

Another possibility is that the carcinogenic dyes damage a fundamental liver constituent, *e.g.*, protein, directly, and that the damaged protein is unable to retain riboflavin in normal concentration. In a sense, then, hepatic riboflavin would serve merely as an indicator of changes in other substances in the liver. Dietary factors such as hydrogenated coconut oil, low fat (10), or high levels of corn oil (10), might then exert their effects upon the primary carcinogenic reaction without themselves reacting with riboflavin. It would then also be possible, as observed by Kensler and his associates (8), for extremely high supplements of riboflavin (5 mgm./rat/day) to fail to prevent tumor formation provided the basal diet were otherwise inadequate. However, some other explanation would be needed for the fact that riboflavin alone retards tumor formation when added to a diet moderately low in protein ([16] and Table II). A connection between hepatic riboflavin and resistance to tumor formation has also been established in extensive experiments by Miller, Miller, Kline, and Rusch (11, 15).

SUMMARY

1. Hepatic tumors due to *p*-dimethylaminoazobenzene (DAB) developed more rapidly on a diet containing the rice bran concentrate, "Vitab," than on a standard synthetic diet. The two diets contained 0.7 and 2.0 mgm. of riboflavin per kgm., respectively. When the riboflavin content of the two diets was equalized, tumor formation was also equal. Reduction in riboflavin intake on both diets accelerated tumor formation.

2. When DAB was fed to rats in the "Vitab" diet low in riboflavin, the concentration of this vitamin in the liver tended to decrease more rapidly than when the control synthetic diet was fed. The addition of riboflavin to either diet increased hepatic retention of the vitamin and decreased tumor incidence. Thus, retention of riboflavin in the liver paralleled resistance to tumor formation with this carcinogen.

3. When *m'*-methyl-*p*-dimethylaminoazobenzene (*m'*-Me-DAB) was fed in the "Vitab" and synthetic diets respectively, the amounts of hepatic riboflavin retained were equally low. These diets do not affect the rate at which tumors develop when *m'*-Me-DAB is fed.

4. Livers of rats fed DAB in hydrogenated coconut oil contained more riboflavin than when corn oil was fed. The coconut oil retards tumor formation by this dye. However, when *m'*-Me-DAB was fed in the two oils, the amounts of hepatic riboflavin were equally low; tumor formation due to *m'*-Me-DAB is essentially the same on both oils.

5. The addition of 10 mgm. of riboflavin per kgm. of diet resulted in a marked increase in hepatic riboflavin when the carcinogen was DAB, but in only a moderate increase in the presence of *m'*-Me-DAB. Riboflavin is more effective in retarding tumor formation due to the former dye than to the latter.

6. These results, and the observation that the

degree of carcinogenicity of various azo dyes parallels their effectiveness in lowering hepatic riboflavin, suggest the general conclusion that there is an inverse relationship between the rate of tumor development and the level of hepatic riboflavin maintained on any particular dietary regimen.

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