The Effect of Pyridoxin and Other B Vitamins on the Production of Liver Cancer with *p*-Dimethylaminoazobenzene*†

D. L. Miner, M.A., J. A. Miller, M.S., C. A. Baumann, Ph.D., and H. P. Rusch, M.D.

(From the McArdle Memorial Laboratory, Medical School, and the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wis.)

(Received for publication December 18, 1942)

INTRODUCTION

It appears well established that the incidence of tumors resulting from the feeding of *p*-dimethylaminoazobenzene depends upon the character of the diet fed during the precancerous period. Factors reported to retard tumor development include liver (19), yeast (18), grain (16), the combination of casein and riboflavin (11), the combination of protein and B vitamins (16), and the combination of cystine and choline (6). Biotin (5) and cystine (25) are reported to be cocarcinogenic for this type of tumor. As might be expected, the cocarcinogenic and the anticarcinogenic effects observed depend upon the character of the basal rations fed. Casein and riboflavin are particularly anticarcinogenic when the basal diet consists of rice and carrot, or when the source of the vitamin B complex is a rice bran extract such as vitab (16), which contains all the B vitamins in abundance except riboflavin. Thus the effectiveness of the riboflavin could be essentially fortuitous, depending upon the specific deficiency in the crude materials employed in the basal ration. The effect of the other B vitamins in the genesis of hepatic tumors is still unknown. Some effect might be inferred, however, from the observation that the split products of the carcinogenic azo dyes inhibit certain enzymes that depend upon nicotinamide coenzymes or upon thiamin pyrophosphate for their activity (10,12).

The present study represents an attempt to determine the effect of each of the B vitamins in the production of hepatic tumors due to *p*-dimethylaminoazobenzene. This, of necessity, required the feeding of highly purified synthetic diets. As in our previous studies (16), young, mature albino rats 150 to 200 gm. in weight were fed 0.06 per cent of *p*-dimethylaminoazobenzene for a period of 120 days. The animals were kept in groups of 8 to 15 in screen-bottomed cages, and food and water were given ad libitum. The rations were mixed in amounts sufficient for 2 to 4 weeks and stored in the refrigerator. After 120 days the livers were examined by laparotomy and the animals continued for an additional 2 months on the same diet as before, but minus the azo dye.

Relative difficulty in the production of liver tumors in rats on synthetic diets.—Several workers have noted a decreased tendency toward tumor formation in animals maintained on synthetic diets as contrasted with nutritionally poorer diets consisting of crude materials. György, Poling, and Goldblatt (6) reported "cirrhosis, atypical nodular proliferation of bile ducts, adenocarcinoma, malignant hepatoma" in 80 to 100 per cent of their animals on a rice-carrot diet containing *p*-dimethylaminoazobenzene and crystalline thiamin, riboflavin, pyridoxin, and pantothenic acid, although no tumors were observed in rats fed a synthetic diet containing the azo dye. Du Vigneaud and his coworkers (5) observed that the addition of biotin definitely increased tumor production, but that in the absence of added biotin no tumors developed in 5 rats receiving a synthetic ration containing 0.1 per cent of *p*-dimethylaminoazobenzene. Antopol and Unna (1) fed 0.02 per cent of the azo dye in synthetic diets for periods up to 338 days and observed tumors in only 4 of 52 animals.

We have encountered a similar resistance toward tumor formation in animals fed synthetic diets of the following composition:

DIET A

Casein (vitamin-free)	180
Glucose	730
Corn oil	50
Salts mixture	40
p-Dimethylaminoazobenzene	0.6

* This investigation was aided by grants from the Jonathan Bowman Fund for Cancer Research and the Wisconsin Alumni Research Foundation. A gift of crystalline B vitamins from Dr. D. F. Robertson, of Merck & Company, is gratefully acknowledged.

† Published with the permission of the Director of the Wisconsin Agricultural Experimental Station.

Each rat received one drop of halibut liver oil per week. Crystalline vitamin B supplements added per kilogram of ration were:

Thiamin hydrochloride	1.0 mgm.
Riboflavin	1.5 "
Pyridoxin hydrochloride	1.0 "
Calcium pantothenate	5.0 "
Nicotinic acid	1.0 "

These levels of the B vitamins are just sufficient for the growth of young rats (2, 3, 4, 21, 24). The vitamin mixture was demonstrated to be adequate for maintenance by feeding a somewhat similar ration free of the azo dye to adult rats for a period of 6 months. The maintenance ration, diet B, contained no nicotinic acid and the level of casein was reduced to 12 per cent. Other rats were maintained on similar rations from which various B vitamins were omitted (Table I). The control group of rats not only remained in good health for 6 months, but actually gained in weight. In the absence of thiamin, riboflavin, or pantothenate, however, the animals died after 2, 3, and 4 months respectively. When 2 μgm. of pyridoxin or less were fed daily, the animals survived for over 6 months, although they lost some weight. However, when the level of pantothenate was reduced from 5 to 1.5 mgm. per kg. of ration, the animals survived without any weight loss.

Nevertheless, the addition of 0.06 per cent of *p*-dimethylaminoazobenzene to the diet for 4 months failed to produce tumors. Numerous variations in the vitamin levels were introduced with each vitamin fed at both a very high and a very low level. The survival of the animals was relatively poor in all groups and on no diet did more than one tumor appear (Table II). This relatively low tumor incidence stands in sharp contrast to our previous results with cruder diets, when a tumor incidence of over 80 per cent was observed consistently (16).

The reason for the relatively low incidence of tumors on the synthetic diet is not yet clear. Part of the difference may have been due to the effect of the biotin present in the rice bran concentrate previously employed. It is also possible that the azo dye may have increased the requirement of the animals for any or for several of the B vitamins, and that the animals correspondingly were in too poor a nutritive

TABLE I: THE MAINTENANCE OF ADULT RATS ON VARIOUS SYNTHETIC DIETS IN THE ABSENCE OF *p*-DIMETHYLAMINOAZOBENZENE

Group	Variation in B vitamins from control level, mgm. per kg.	Average starting weight, gm.	Average weight at 6 months, gm.	Condition at 6 months
Control diet B		267	302	Healthy
Pantothenate	Reduced from 5.0 to 1.5	276	288	"
Riboflavin	Reduced from 1.5 to 0.5	258	222	"
Pyridoxin	Reduced from 1.0 to 0.2	262	217	"
No pyridoxin		242	220	"
No thiamin		247	135 *	Died after 2 months
No riboflavin		253	146 *	Died after 3 months
No pantothenate		254	133 *	Died after 4 months

* Weight at death.

TABLE II: INCIDENCE OF HEPATIC TUMORS IN RATS FED MODERATE AMOUNTS OF SYNTHETIC B VITAMINS

Group	Variation in B vitamins from control level, mgm. per kg. ration	Average food consumption, gm. per rat per day	Average starting weight, gm.	Average weight at 4 months, gm.	Survival * at 4 months	Tumors *	
						4 months	6 months
Control diet A	—	7.0	229	179	8/24	0/8	0/8
Low riboflavin	0.15	6.0	240	160	8/15	1/8	1/8
High riboflavin	15	6.5	210	155	11/14	0/11	0/11
Low thiamin	0.3	6.7	227	160	4/15	1/4	1/4
High thiamin	10	7.7	218	170	8/15	1/8	1/8
Low pyridoxin	0.1	5.0	215	159	4/15	0/4	0/4
High pyridoxin	10	6.7	222	169	8/15	1/8	1/8
Low pantothenate	0.5	6.1	227	141	6/15	0/8	0/8
High pantothenate	50	5.6	167	127	8/15	0/8	0/8
Low nicotinic acid	0.1	5.6	290	190	5/15	0/5	0/5
High nicotinic acid	10	6.2	245	165	8/15	0/8	0/8
12 per cent casein (as in diet A)	—	7.6	230	180	6/15	0/6	0/6

* Survival = Number of living over number at start.
Tumors = Number with tumors over number surviving at 4 months.

state for the effective production of hepatic tumors (25). Thus although borderline levels of riboflavin tend to favor tumor formation (11, 16), similar levels of all the B vitamins at once tend to suppress it.

Tumor production on synthetic diets.—Du Vigneaud and his coworkers (5) succeeded in producing tumors in 3 of 5 rats on a synthetic diet containing biotin in addition to their mixture of the regular crystalline B vitamins. We have been able to produce a high percentage of liver tumors in the absence of added biotin when the levels of the other B vitamins were increased significantly above those amounts needed for maintenance. The control ration, diet C, was patterned after our previously successful crude diet (16) and consisted of:

Casein (vitamin-free)	120
Glucose	790
Corn oil	50
Salts mixture	40
p-Dimethylaminoazobenzene	0.6

The crystalline B vitamins were incorporated in the ration at levels approximately equivalent to those in a diet containing 2 per cent of rice bran concentrate. Supplements added per kilogram of ration were:

Thiamin hydrochloride	3.0 mgm.
Riboflavin	2.0 "
Pyridoxin hydrochloride	2.5 "
Calcium pantothenate	7.0 "
Nicotinic acid	30.0 "
Choline chloride	30.0 "

One drop of halibut liver oil per week was given by dropper to each rat. In the absence of the azo dye this low protein diet was demonstrated to be adequate for the growth of young rats. A gain in weight from an average of 61 gm. to 213 gm. during 100 days of feeding was observed, an average daily increase of 1.5 gm.

In the carcinogenic diets other than the control the level of each of the B vitamins was lowered in turn, and nicotinic acid and choline were completely omitted from diets 6 and 7 respectively (Table III). One group of animals received 2 per cent of rice bran concentrate (diet 8, Table III) and another received 2 per cent of the concentrate in addition to a mixture of crystalline vitamins somewhat similar to that of the control ration (diet 9, Table III). The total amount of B vitamins in the latter diet was approximately equal to that in a ration containing 4 per cent of rice bran concentrate. The effect of rice protein was measured in diet 10, which contained 80 per cent of ground polished rice and 5 per cent of casein (total crude protein approximately 12 per cent). The rest of the diet consisted of glucose 6, salts 4, and corn oil 5 per cent respectively, and the B vitamins added were those of the control ration.

The animals on most of the diets survived well and some actually gained weight in the presence of the azo dye (Table IV). In the control group the tumor incidence was 33 per cent at 4 months and 66 per cent

Table III: The Vitamin B Content of the Diets Fed to Groups Listed in Tables IV and V

Group	Vitamins, mgm. per kg. of diet: Riboflavin	Thiamin hydrochloride	Pyridoxin hydrochloride	Calcium pantothenate	Nicotinic acid	Choline chloride
1. Control diet C	2.0	3.0	2.5	7.0	30.0	30.0
2. Low riboflavin	0.5	3.0	2.5	7.0	30.0	30.0
3. Low thiamin	2.0	0.6	2.5	7.0	30.0	30.0
4. Low pyridoxin	2.0	3.0	0.2	7.0	30.0	30.0
5. Low pantothenate	2.0	3.0	2.5	1.5	30.0	30.0
6. No nicotinic acid	2.0	3.0	2.5	7.0	0	30.0
7. No choline chloride	2.0	3.0	2.5	7.0	30.0	0
8. 2 per cent rice bran concentrate	0	0	0	0	0	0
9. 2 per cent rice bran concentrate plus vitamins	0.5	3.0	2.5	7.0	30.0	30.0
10. 80 per cent rice	2.0	3.0	2.5	7.0	30.0	30.0
11. 2 per cent bran concentrate plus high riboflavin	10.0	0	0	0	0	0
12. Control diet C	2.0	3.0	2.5	7.0	30.0	30.0
13. High thiamin	2.0	10.0	2.5	7.0	30.0	30.0
14. No pyridoxin	2.0	3.0	0	7.0	30.0	30.0
15. No pyridoxin	2.0	3.0	0	7.0	30.0	30.0
High pyridoxin *	2.0	3.0	10.0 *	7.0	30.0	30.0
16. 18 per cent casein	2.0	3.0	2.5	7.0	30.0	30.0
17. 18 per cent casein High pyridoxin	2.0	3.0	10.0	7.0	30.0	30.0

* Pyridoxin was fed only after the 3rd month.

at 6 months. No pronounced effect on tumor incidence was noted when the content of thiamin was either greatly decreased or increased (diets 3 and 13, Table IV), nor did a reduction in the amounts of riboflavin, calcium pantothenate, or choline chloride (diets 2, 5, 7, Table IV) from the control level notably affect tumor production. The omission of nicotinic acid from the control ration appeared to increase the incidence of tumors somewhat (diet 6, Table IV). In the presence of 2 per cent of the rice bran concentrate the incidence of tumors was 100 per cent (diet 8, Table IV) as in our previous experiments (16), and the addition of 10 mgm. of riboflavin per kg. of ration completely prevented the appearance of tumors by 6 months (diet 11, Table IV).

Kensler and his coworkers (11) failed to observe much protection against tumor formation when riboflavin alone was added to a rice-carrot diet containing the azo dye. Presumably the limiting factor in this particular combination was protein. With crude diets we have observed a considerable lowering in tumor incidence when the level of casein in the diet was raised above 12 per cent (16). Where riboflavin was the limiting factor in our present experiments (diets 8 and 11, Table IV, and diets 1 and 3, Table V), an increase in this vitamin alone completely prevented tumor formation by 6 months. Furthermore, tumor incidence on synthetic diets containing 18 per cent of casein (diets 16 and 17, Table IV) was essentially the same as when 12 per cent of casein was fed (diets 1 and 12, Table IV).

As indicated in our previous work (16), protection at 6 months does not necessarily imply abolition of all subsequent tumor formation. We have occasionally noted some tumors developing as long as 7 months after the azo dye had been withdrawn from the diet.

TABLE IV: INCIDENCE OF HEPATIC TUMORS IN RATS FED HIGH AMOUNTS OF SYNTHETIC B VITAMINS

Group	Average starting weight, gm.	Weight at 4 months, gm.	Average food consumption, gm. per rat per day	Survival * at 4 months	Tumors *		Cirrhosis
					4 months	6 months	
1. Control diet C	190	167	6.5	12/14	4/12	8/12	Severe
2. Low riboflavin	194	144	6.2	11/14	2/11	6/11	Severe
3. Low thiamin	155	130	5.6	14/15	4/14	9/14	Moderate
4. Low pyridoxin	191	195	8.2	15/15	0/15	1/15	None or mild
5. Low pantothenate	197	223	10.0	14/14	4/14	7/14	Mild
6. No nicotinic acid	192	183	8.0	14/15	7/14	13/14	Severe
7. No choline	192	170	7.2	10/15	5/10	8/10	Severe
8. 2 per cent rice bran concentrate	192	153	7.7	8/12	3/8	8/8	Severe
9. 2 per cent rice bran concentrate plus vitamins	168	190	9.8	13/14	11/13	13/13	Mild
10. 80 per cent rice	219	283	11.6	10/14	5/10	6/10	Mild
11. 2 per cent bran concentrate plus high riboflavin	209	207	8.7	13/15	0/13	0/13	None or mild
12. Control diet C	161	166	7.5	15/15	2/15	8/15	Moderate–severe
13. High thiamin	166	170	8.0	15/15	4/15	10/15	Moderate
14. No pyridoxin	167	121	6.2	14/15	0/14	1/14	None
15. No pyridoxin	171	135 †	6.7 †				
High pyridoxin		169	7.8	14/14	1/14	7/14	Mild–moderate
16. 18 per cent casein	178	187	8.5	15/15	2/15	8/15	Moderate–severe
17. 18 per cent casein High pyridoxin	178	196	8.0	12/15	2/12	7/12	Moderate–severe

Groups 1 to 11 constitute one series of experiments and are directly comparable; groups 12 to 17 comprise a second series of experiments.

* Survival = Number living over number at start.
Tumors = Number with tumors over number surviving at 4 months.

† After 3 months' feeding of the azo dye.

The effect of pyridoxin.—When the amount of pyridoxin in the control ration was lowered, tumor formation was greatly reduced. The survival of the animals was 100 per cent in the presence of the azo dye when the level of pyridoxin was reduced to 0.2 mgm. per kg. of ration (diet 4, Tables III and IV). The average daily food consumption was high, and hepatic cirrhosis was either absent or very mild. Only one of the 15 animals on the low pyridoxin ration developed a tumor. This apparent cocarcinogenic effect of pyridoxin was confirmed in two other experiments. The same control ration and the same level of the B vitamins were fed as before, and 8 of 15 animals in the control group developed tumors (diet

12, Table IV). When pyridoxin was omitted entirely from the ration, the animals lost weight in the presence of *p*-dimethylaminoazobenzene, but cirrhosis was largely prevented, and only one of 14 animals developed a tumor by the end of 6 months (diet 14, Table IV). However, when pyridoxin was omitted from the ration for only 3 months but was added at a level of 10 mgm. per kg. of ration during the 4th month of feeding the dye and for 2 months thereafter, 7 of 14 animals developed tumors by 6 months, and the degree of cirrhosis was also greater than when pyridoxin was omitted throughout the experiment (diet 15, Table IV).

In a third series of experiments adult rats with large stores of vitamins (see below) were employed. The control ration and the levels of vitamins fed with the azo dye were the same as in diet C of the previous series, and the carcinogen was administered for 6 months instead of the usual 4. Tumors failed to develop in the control group, but 3 of 10 animals that received 10 mgm. of pyridoxin per kg. of ration developed tumors by the end of the 6 month period (Table V).

TABLE V: PRODUCTION OF LIVER TUMORS WITH SYNTHETIC DIETS FED TO RATS THAT HAD BEEN RAISED TO MATURITY ON FORTIFIED MILK

Group	Average starting weight, gm.	Weight at 4 months, gm.	Average food consumption, gm. per rat per day	Survival * at 6 months	Tumors * † at 6 months	Cirrhosis at 6 months
1. Control, diet C	227	247	11.0	10/15	0/10	None
2. Control, no nicotinic acid, no choline	229	240	10.5	7/15	0/7	Very mild
3. Low riboflavin	249	233	9.5	11/15	8/11 ‡	Mild
4. High riboflavin	216	230	12.0	8/15	0/8	None
5. Low pyridoxin	227	214	9.7	10/15	0/10	None
6. High pyridoxin	242	222	10.0	10/15	3/10	Very mild
7. Low pantothenate	231	223	10.3	7/15	0/7	Mild

* Survival = Number living over number at start.
Tumors = Number with tumors over number surviving at 4 months.
† Tumor incidence in this series was unchanged after an additional 2 months (total = 8 months) in the absence of the dye.
‡ The only tumor appearing in the entire series at 4 months was in this group.

Previous nutritional history.—The animals employed in most of our studies had been raised on crude diets relatively low in vitamins, and they were obtained directly from the Sprague-Dawley colony. Such rats readily developed tumors when they were subsequently fed the synthetic diet (diet C) containing *p*-dimethylaminoazobenzene. However, this diet failed to produce tumors when fed for 6 months to rats that had been raised to maturity on reconstituted milk fortified with crystalline B vitamins. The various modifications of the carcinogenic synthetic diet are indicated in Table V. Tumors appeared in only 2 of the 7 groups, those fed high amounts of pyridoxin and those fed low amounts of riboflavin (diets 3 and 6, Table V).

DISCUSSION

The means by which dietary constituents alter the carcinogenic potency of the azo dyes is as yet unknown, but at least three possibilities suggest themselves. For one thing, the dietary factor may either hasten or retard the destruction of the carcinogen. György and his associates (7) observed that diets made highly rancid with crude linoleic acid appeared to be anticarcinogenic, but that the dye was actually destroyed in the ration *in vitro*. However, no such destruction was observed in any of the diets employed in our laboratories. All uniformly contained 5 per cent of corn oil, which is rich in antioxidants (15). The rations were mixed at frequent intervals and stored in the cold. Dietary constituents might alter the stability of the dye in the digestive tract, and they might also affect its rate of absorption. However, liver is reported to prevent the formation of tumors due to the intraperitoneal administration of the carcinogen (17).

The destruction of the carcinogen within the body is a more likely possibility. Should this destruction prove to be oxidative, the greatest protection against the dye should result from dietary factors directly involved in physiological oxidations. Riboflavin and nicotinic acid seem to fall within this category, although thiamin, which is indispensable in the oxidative catabolism of carbohydrate, did not alter the carcinogenicity of *p*-dimethylaminoazobenzene.

Another destructive pathway is suggested by the observation that the methyl groups of *p*-dimethylaminoazobenzene may function to prevent hemorrhagic kidneys in choline-deficient rats (9); a dietary factor capable of serving as a methyl acceptor might retard tumor formation by demethylating the carcinogen. Nicotinic acid, which is largely excreted as the methylated derivative trigonelline (8), retards tumor

formation somewhat (11), and in our present experiments the absence of nicotinic acid from the diet appeared to increase tumor incidence.

Another consideration involves the high degree of specificity that the azo dyes exhibit by forming only liver tumors, a specificity all the more remarkable since the liver is so highly resistant to the action of the carcinogenic hydrocarbons (22). The liver is the site of many normal metabolic reactions of importance to the organism as a whole, and hence possesses specialized enzymatic mechanisms. The liver is also the site of many detoxification reactions. Hence it is possible that in the process of being itself detoxified, the dye alters an essential liver constituent and that the protective dietary factor tends to maintain that constituent at an adequate concentration, even in the face of the detoxification reaction. Riboflavin may have some such function, since excretory losses of this vitamin are increased when the azo dye is fed (11). Perhaps the carcinogen reacts with one of the proteins to which riboflavin is normally bound in the liver.

A third possibility is that the dietary factor enters directly into the carcinogenic process itself. Presumably the essential carcinogenic reaction is a conversion of ordinary tissue protein into an altered but reproducible type (14). The split products of the carcinogenic azo dyes inhibit the activity *in vitro* of several enzyme systems (10, 12, 20) including the very simple one urease-urea. It has been suggested that this inhibition is due to a combination of the oxidized split product with the sulfhydryl group of the enzymatically active protein (20). The role of the B vitamins in such a reaction is at present obscure and would appear to be at best of only secondary importance. In fact, no enzymatic activity whatever has as yet been demonstrated for pyridoxin. Incidentally, results on the cocarcinogenic effect of pyridoxin on liver tumors due to *p*-dimethylaminoazobenzene should not be extended to other tumor types until the appropriate experiments have been performed.

It is curious that most of the diets employed for the consistent production of tumors with *p*-dimethylaminoazobenzene are nutritionally abnormal. Thus both White and Edwards (25, 26) and György, Poling, and Goldblatt (6) employed diets very low in protein and high in fat. The Japanese were most successful with multiple-deficient diets, and their rice-carrot mixture (13, 18, 19) or imitations thereof (11, 16, 23) have frequently been employed in this country. Thus the over-all effect of diet on hepatic tumor production might seem to be that factors which tend to correct the initial deficiency of the diet also tend to reduce the carcinogenicity of the azo dyes. In our present experiments, however, tumors were most readily produced when the levels of all the B vitamins were high, and the addition of factors like thiamin hydrochloride, calcium pantothenate, or choline chloride to diets low in these factors offered no anticarcinogenic effect whatever.

In general, diets that failed to protect an animal against severe nodular cirrhosis also failed to protect against hepatic tumor formation. However, on several diets (5, 9, 10, 15, Table IV) cirrhosis was relatively mild even though the incidence of tumors was high.

SUMMARY

1. Thirty-six groups of 15 rats each were fed highly purified diets containing *p*-dimethylaminoazobenzene and crystalline synthetic B vitamins. The dye was fed for 4 months and the livers were inspected by laparotomy at 4 and 6 months. The incidence of tumors was low when moderate amounts of the vitamins were fed. These amounts, however, were adequate for the maintenance of adult rats for 6 months. When the levels of all the B vitamins were raised well above the amounts necessary for maintenance, the tumor incidence in the presence of the azo dye reached 66 per cent at 6 months.

2. Under the conditions of the experiment the incidence of tumors due to *p*-dimethylaminoazobenzene was greatly lowered when the level of pyridoxin in the diet was reduced, or when the vitamin was omitted entirely. Large amounts of pyridoxin fed to resistant rats tended to increase the incidence of tumors.

3. The addition of large amounts of riboflavin completely prevented the appearance of tumors in rats receiving only 12 per cent of casein. At carcinogenic levels of the synthetic B vitamins, the incidence of tumors was essentially the same with 12 per cent as with 18 per cent of casein in the diet.

4. The production of tumors appeared to be more difficult in animals raised to maturity on a diet consisting of fortified milk than in animals raised on a diet relatively low in the B vitamins.

REFERENCES

1. Antopol, W., and Unna, K. The Effect of Riboflavin on the Liver Changes Produced in Rats by *p*-Dimethylaminoazobenzene. Cancer Research, **2**:694-696. 1942.
2. Dann, M., and Cowgill, G. R. The Vitamin B Requirement of Female Albino Rats for Maintenance and Growth. Am. J. Physiol., **109**:27-28. 1934.
3. Dann, W. J., and Kohn, H. I. The Factor V (Coenzyme I and II) Content of Rat Tissues: Evidence for Synthesis of Nicotinic Acid by the Rat. J. Biol. Chem., **136**:435-442. 1940.
4. Day, P. L., and Darby, W. J. The Riboflavin Requirement for Growth of the Rat. J. Biol. Chem., **123**:xxviii-xxix. 1938.
5. du Vigneaud, V., Spangler, J. M., Burk, D., Kensler, C. J., Sugiura, K., and Rhoads, C. P. The Procarcinogenic Effect of Biotin in Butter Yellow Tumor Formation. Science, **95**:174-176. 1942.

6. GYÖRGY, P., POLING, E. C., and GOLDBLATT, H. Necrosis, Cirrhosis and Cancer of Liver in Rats Fed a Diet Containing Dimethylaminoazobenzene. Proc. Soc. Exper. Biol. & Med., **47**:41-44. 1941.
7. GYÖRGY, P., TOMARELLI, R., OSTERGARD, R. P., and BROWN, J. B. Unsaturated Fatty Acids in the Dietary Destruction of N,N-Dimethylaminoazobenzene (Butter Yellow) and in the Production of Anemia in Rats. J. Exper. Med., **76**:413-420. 1942.
8. HUFF, J. W., and PERLZWEIG, W. A. Studies in Nicotinic Acid Metabolism. III. Metabolism and Synthesis of Nicotinic Acid in the Rat. J. Biol. Chem., **142**:401-416. 1942.
9. JACOBI, H. P., and BAUMANN, C. A. Choline in Tumor-Bearing Animals and a Choline-Like Effect of Butter Yellow. Cancer Research, **2**:175-180. 1942.
10. KENSLER, C. J., DEXTER, S. O., and RHOADS, C. P. The Inhibition of a Diphosphopyridine Nucleotide System by Split Products of Dimethylaminoazobenzene. Cancer Research, **2**:1-10. 1942.
11. KENSLER, C. J., SUGIURA, K., YOUNG, N. F., HALTER, C. R., and RHOADS, C. P. Partial Protection of Rats by Riboflavin with Casein against Liver Cancer Caused by Dimethylaminoazobenzene. Science, **93**:308-310. 1941.
12. KENSLER, C. J., YOUNG, N. F., and RHOADS, C. P. The Inhibition of Yeast Carboxylase by Split-Products of N,N-Dimethylaminoazobenzene. J. Biol. Chem., **143**: 465-472. 1942.
13. KINOSITA, R. Special Report. Studies on the Carcinogenic Chemical Substances. Tr. Soc. path. jap., **27**:665-727. 1937.
14. LAVIK, P. S., MOORE, P. R., RUSCH, H. P., and BAUMANN, C. A. Some Additive Effects of Carcinogenic Hydrocarbons. Cancer Research, **2**:189-192. 1942.
15. MATTILL, H. A., and CRAWFORD, B. Autoxidation of Corn Oil as Related to Its Unsaponifiable Constituents. Indust. & Engin. Chem., **22**:341-344. 1930.
16. MILLER, J. A., MINER, D. L., RUSCH, H. P., and BAUMANN, C. A. Diet and Hepatic Tumor Formation. Cancer Research, **1**:699-708. 1941.
17. MORI, K., and NAKAHARA, W. Effect of Liver Feeding on the Production of Malignant Tumors by Injections of Carcinogenic Substances. Gann, **34**:48-58. 1940.
18. NAKAHARA, W., FUJIWARA, T., and MORI, K. Inhibiting Effect of Yeast Feeding on the Experimental Production of Liver Cancer. Gann, **33**:57-65. 1939.
19. NAKAHARA, W., MORI, K., and FUJIWARA, T. Inhibition of Experimental Production of Liver Cancer by Liver Feeding. A Study in Nutrition. Gann, **33**:406-428. 1939.
20. POTTER, V. R. The Inhibition of Sulfhydryl-Containing Enzymes by Split Products of *p*-Dimethylaminoazobenzene. Cancer Research, **2**:688-693. 1942.
21. REEDMAN, E. J., SAMPSON, W. L., and UNNA, K. Identity of Natural and Synthetic Crystalline Vitamin B_6. Proc. Soc. Exper. Biol. & Med., **43**:112-115. 1940.
22. SHEAR, M. J., STEWART, H. L., and SELIGMAN, A. M. Studies in Carcinogenesis. XIII. Tumors of the Spleen and Liver in Mice Following the Introduction of Hydrocarbons into These Organs. J. Nat. Cancer Inst., **1**: 291-302. 1940.
23. SUGIURA, K., and RHOADS, C. P. Experimental Liver Cancer in Rats and Its Inhibition by Rice-Bran Extract, Yeast, and Yeast Extract. Cancer Research, **1**:3-16. 1941.
24. UNNA, K., and RICHARDS, G. V. Relationship between Pantothenic Acid Requirement and Age in the Rat. J. Nutrition, **23**:545-553. 1942.
25. WHITE, J., and EDWARDS, J. E. Effect of Dietary Cystine on the Development of Hepatic Tumors in Rats Fed *p*-Dimethylaminoazobenzene (Butter Yellow). J. Nat. Cancer Inst., **2**:535-538. 1942.
26. WHITE, J., and EDWARDS, J. E. Effect of Supplementary Methionine or Choline plus Cystine on the Incidence of *p*-Dimethylaminoazobenzene-Induced Hepatic Tumors in the Rat. J. Nat. Cancer Inst., **3**:43-59. 1942.