HEPATIC VITAMIN A IN THE RAT AS AFFECTED BY THE ADMINISTRATION OF DIBENZANTHRACENE*

By JULES C. ABELS,‡ M.D., ALICE T. GORHAM, SHIRLEY L. EBERLIN, ROBERT HALTER, PH.D., AND C. P. RHOADS, M.D.

(From the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York)

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Considerable experimental evidence exists to prove that the administration of the carcinogen dibenzanthracene to the rat results in a decrease of the content of vitamin A in the animal's liver (1, 2). This result is obtained when the animal receives either adequate or moderately excessive amounts of the vitamin (2), but the mechanism by which the effect is produced is entirely unknown. Recently, however, the possibility has been suggested (3) that a specific hepatic protein, to which vitamin A conceivably is bound, is in some manner impaired or absent from the liver of the animal which receives the carcinogen.

This suggestion of damage to a conjugated protein, of which vitamin A forms the prosthetic group, may be analogous to the concept of Kensler, Dexter, Young, and Rhoads (4, 5). They demonstrated that the presence of metabolites of the carcinogen butter yellow prevents *in vitro* both the diphosphopyridine nucleotide and cocarboxylase from functioning with their specific protein enzymes in the yeasts used. This prevention, in turn, can be avoided, within limits, by the introduction of excessive amounts of the specific coenzymes into the systems. It is conceivable, therefore, that a similar competitive relationship might exist in the rat liver between vitamin A and dibenzanthracene—a competition for a specific protein, perhaps of enzyme nature, and the success in that competition dependent upon the relative concentrations of the two competing substances.

On the other hand, since one of the recognized functions of the liver is to fabricate, store, and distribute vitamin A (6), the impaired hepatic storage of the vitamin due to dibenzanthracene feeding also might be explained by the production of a general hepatic insufficiency imposed on the organ by the carcinogen. The association of a defective distribution of vitamin A between the liver and plasma of patients with gastro-intestinal cancer (7) with the presence of a generalized hepatic damage in those patients (8) already has been demonstrated. Likewise, there is good evidence that some patients with hepatic cirrhosis or atrophy have lost, to a considerable extent, their ability to store the vitamin (9).

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‡ Finney-Howell Fellow.

In view of these facts, it became desirable to investigate the evidence in support of these two possible explanations for the defective storage of the vitamin in the dibenzanthracene treated rat: (1) that the carcinogen has a specific effect on some liver protein, or other anchor, to which the vitamin normally is attached, or (2) that the carcinogen causes a general hepatic insufficiency and among the functions lost is that of vitamin A storage. The results of this investigation form the subject of the present report.

Methods and Materials

The experiments were carried out on male albino rats of Sherman strain, which had an initial weight of from 90 to 110 gm.

The standard diet consisted of 8 to 9 gm. of Purina dog chow (Ralston Purina Company) per rat, per day.[1] To this daily ration were added 1 gm. of carrot and 1.5 gm. of lettuce. In certain experiments 1.5 gm. of Fleischmann's dried brewer's yeast, No. 20–40,[2] were added to the daily diet of each rat.

Vitamin A was given in the form of a vitamin A concentrate[3] diluted in corn oil so that 1 ml. contained 400 U.S.P. units of the vitamin. The required amount of this supplement was administered daily through a syringe inserted into the mouths of those animals which received the vitamin.

A 0.1 per cent emulsion of dibenzanthracene (10) was injected in doses of 3 mg. or 5 mg. at weekly intervals into the peritoneal cavity of those rats scheduled to receive the carcinogen. The total volume of each dose varied from 3 to 5 ml.

No animal was given either vitamin A supplement or dibenzanthracene until he had received the standard diet for 2 weeks in order to make sure that all the rats were in good condition and to allow them to become adjusted to their environment. Urine collections were obtained from each group of animals for 5 days before they were sacrificed. During this collection period, the animals to be killed were kept in metabolism cages.

All animals were weighed every week. In general, the nutrition of the control animals was found to be well maintained and their weight increased uniformly at about 15 gm. per rat, per week for 12 weeks.

Animals were sacrificed by decapitation to procure adequate amounts of blood. As soon as possible, the liver of each rat was removed and weighed. A portion of liver of about 600 mg. then was placed in a sealed, tared weighing bottle. This specimen was weighed accurately and homogenized into a brei for chemical determinations.

In the present study chemical determinations were made of:

1. Vitamin A in the plasma and liver. The methods used have been described previously by the authors (7, 11).

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[1] This ration contained from 150 to 170 U.S.P. units of vitamin A.

[2] The yeast was supplied through the courtesy of the Standard Brands, Inc. 1 gm. of yeast contained 520 mg. of protein, 160 μg. of thiamin, 70 μg. of riboflavin, 60 μg. of pyridoxin, 600 μg. of nicotinic acid, and about 650 μg. of choline. It is also known to contain appreciable amounts of sulfur amino acids.

[3] This concentrate was supplied through the courtesy of the Endo Products, Inc.

2. Total protein, albumin, and globulin of the serum and liver.[4] The technique of Robinson *et al.* (12) was used to measure the protein and its fractions in the serum and liver brei.

3. The total lipid carbon of the liver. This was determined by the technique of Van Slyke *et al.* (13).

4. The cholesterol and cholesterol esters of the serum. These were measured by the method of Schoenheimer and Sperry (14).

5. Phospholipids of the liver. The method of Sinclair (15) was used.

6. Riboflavin of the liver. The technique of Hodson and Norris (16), adapted by Kensler *et al.* (4) for the determination of the vitamin in liver brei was employed.

7. The urinary glucuronates. These were measured by the procedure of Maughan *et al.* (17).

8. The urinary phenol and phenol esters. The method of Folin and Denis was used (18). The final color developed was read by the Pfalz and Bauer Photoelectric colorimeter.

The material now to be presented includes the results of experiments to determine whether the administration of dibenzanthracene to the rat resulted in (*a*) a general hepatic insufficiency, or (*b*) an isolated effect on a specific liver protein (or other anchor) to which vitamin A normally is attached.

Experiments to Ascertain Whether or Not the Administration of Dibenzanthracene Produces a General Hepatic Insufficiency

For these experiments a colony of 58 Sherman strain, male, albino rats was used. These animals had an initial weight of from 90 to 110 gm. After they had taken the standard diet for 2 weeks, 10 unselected rats were sacrificed in order to ascertain the status of the hepatic functions of the animals in the colony.

Results of Preliminary Studies.—At the time the 10 animals were sacrificed their weights ranged from 126 to 140 gm. and averaged 134.5 gm. The livers of these animals weighed from 4.0 to 6.1 gm., and the average weight was 4.9 gm. Thus, the ratio of liver weight to body weight ranged from 0.035 to 0.039, and the average ratio was 0.037 (Table I).

The criteria used in this study as measures of normal hepatic function were the ability of the liver to (1) maintain a normal total lipid and phospholipid content, (2) to store and fabricate albumin, (3) to esterify cholesterol, (4) to synthesize and conjugate glucuronic acid, and (5) to conjugate phenol. The justification for the use of these criteria is presented in a later section of this communication.

Accordingly, determinations were made in these and all other animals of this colony which subsequently were sacrificed, of the hepatic concentrations of total

[4] By the term liver "albumin" is meant that fraction of the liver protein soluble in 22 per cent Na_2SO_4 at 37°C.; by the term liver "globulin" is meant the total liver protein less the liver "albumin."

lipid, phospholipid, total protein, "albumin," and "globulin." Likewise, the concentrations of protein, albumin, globulin, cholesterol, and cholesterol esters were measured in the serum, and the daily excretion of phenol, phenol esters, and glucuronates in the urine. As a part of the study it was necessary to determine the levels of vitamin A in the liver and plasma, and likewise desirable to measure the riboflavin content of the livers.

At the end of the 2 week preliminary period, before any of the rats had received vitamin A supplements or carcinogen, it was found that the animals had

TABLE I

The Results of Preliminary Studies Made on Two Groups of Five Unselected Rats of the Colony Used in the Present Study

	Group I	Group II
No. of animals	5	5
Body weight, *gm.*	126–140	126–136
Liver weight, *gm.*	4.0–6.1	4.1–5.8
Average LW/BW	0.039	0.035
Hepatic vitamin A, U.S.P. *units per gm.*	20.6–34.7	22.6–36.7
Hepatic total lipid carbon, *gm. per cent*	4.45–7.5	4.5–7.0
Average hepatic phospholipid, *gm. per cent*	2.54	2.60
Average hepatic total protein, *gm. per cent*	15.75	15.55
Average hepatic albumin, *gm. per cent*	2.1	2.2
Average hepatic globulin, *gm. per cent*	13.65	13.35
Average hepatic riboflavin, *μg. per cent*	16.6	24.2
Average plasma vitamin A, U.S.P. *units per 100 ml.*	0	0
Average total serum cholesterols, *mg. per cent*	115	100
Average cholesterol esters/total cholesterol, *per cent*	71	69
Average urinary output of glucuronates, *mg. per rat, per day*	2.4	2.7
Average urinary output of total phenol, *mg. per rat, per day*	3.0	3.2
Average urinary output of phenol esters, *mg. per rat, per day*	0.9	1.0

hepatic concentrations of vitamin A which ranged from 20.6 to 36.7, and averaged 28.5 U.S.P. units per gm. of wet liver. No vitamin A was found in the plasma of these rats at this time (Table I).

The total lipid in the livers of this group varied from 4.45 to 7.5 gm., and averaged 6.2 gm. per cent. The phospholipid content of the pooled liver breis of 2 groups of 5 animals each was measured and found to range from 2.54 to 2.60 gm. per cent, the average was 2.58 gm. per cent. Also, the total protein, "albumin," and "globulin" concentrations of the livers were measured in the pooled breis of 2 groups of 5 animals each. The contents of total protein varied from 15.55 to 15.75 gm. per cent, of "albumin" from 2.1 to 2.2 gm. per cent, and of "globulin" from 13.35 to 13.65 gm. per cent. The concentrations of riboflavin in these 2 pooled breis varied from 16.6 to 24.2 μg. per gm. of wet liver.

The levels of total serum cholesterols ranged from 100 to 115 mg. per cent, of which the esters formed from 69 to 71 per cent (Table I). At this time the concentrations of total protein, albumin, and globulin in the serum were not determined.

The urinary excretions of glucuronates varied from 2.40 to 2.70 mg. per rat, per day, over a 5 day test period. The daily urinary outputs of total phenol ranged from 3.0 to 3.2 mg. per rat, and that of phenol esters from 0.9 to 1.0 mg. (Table I).

After these basal data had been obtained, the remainder of the colony was divided into 4 equal groups of 12 animals each (A, B, C, D). All were maintained on the standard diet. The animals of group A received the diet alone and acted as the controls for the experiment, group B received, in addition to the diet, 200 U.S.P. units of vitamin A per rat, per day, each animal of group C was given every week 3 mg. of dibenzanthracene intraperitoneally, and the rats in group D received not only the 3 mg. dose of the carcinogen each week, but also the 200 U.S.P. units of the vitamin each day.

Four unselected animals of each of the 4 groups were sacrificed at the end of 4, 8, and 12 weeks after the preliminary results had been obtained. At these times studies were made to ascertain the effect of the carcinogen on the growth of the animals, on their hepatic functions, and on their ability to store vitamin A. Likewise, measurements were made of the riboflavin contents of the livers.

Results after the Administration of Dibenzanthracene, Vitamin A, or Both.—(*a*) *Rate of Growth:* The animals which received the carcinogen showed a definite retardation of growth. Whereas the rats of groups A and B during the first 4 weeks showed an average weight gain per week of about 20 gm., and of from 9 to 14 gm. during the next 4 weeks, those of groups C and D gained only 11 gm. during the first, and from 5.5 to 9.75 gm. during the second 4 week period. This growth retardation was more prominent in the rats which received the carcinogen alone, for during the third 4 week period the animals of this group actually lost weight, whereas those of the other 3 groups remained at relatively stable levels (Table II).

(*b*) *Liver Weight and Ratio of Liver Weight to Body Weight (LW/BW):* The average liver weights of groups C and D at all times were about 2 gm. heavier than those of groups A and B, and after the 4th week, the livers of the rats of group D were even larger than those of group C. When the average liver weight of each group was divided by the average body weight of that group, a ratio was obtained which emphasized particularly the fact that the administration of the carcinogen had produced a hepatomegaly. Thus, at the end of the 4, 8, and 12 weeks, the ratios of LW/BW for groups A were 0.035, 0.032, 0.022 respectively; and for group B, 0.036, 0.025, and 0.028. In sharp distinction were the values for group C of 0.052, 0.040, and 0.046, and also those for group D, of 0.050, 0.041, and 0.045. It is to be noted that no significant difference existed between the comparable values for groups A and B, or between those for groups C and D (Table III).

(*c*) *The Concentrations of Vitamin A in the Liver:* The effect of the administration of dibenzanthracene on the hepatic store of vitamin A is essentially that obtained by Goerner (1) and by Baumann (2, 3). Even at the end of 4 weeks, when each of the rats of groups C and D had received only 12 mg. of the carcinogen, significant decreases in their hepatic concentrations of vitamin A were noted. The average hepatic content of the vitamin of group A was 266 U.S.P. units; of group B, (which had received 200 U.S.P. units of vitamin A per rat per day) 404 U.S.P. units; of group C, 104 U.S.P. units; and of group D (which had received both carcinogen and 200 U.S.P. units of vitamin A per rat, per day), 190 U.S.P. units per gm. of wet liver (Table IV).

TABLE II

The Average Body Weight of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of		
	4 wks.	8 wks.	12 wks.
	gm.	*gm.*	*gm.*
A	210	265	270
B	213	250	245
C	181	213	170
D	179	218	210

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

TABLE III

The Average Liver Weight and Ratios of Liver Weights to Body Weights of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of					
	4 wks.		8 wks.		12 wks.	
	Liver weight	LW/BW	Liver weight	LW/BW	Liver weight	LW/BW
	gm.		*gm.*		*gm.*	
A	7.4	0.035	8.6	0.032	6.05	0.022
B	7.6	0.036	6.3	0.025	6.8	0.028
C	9.6	0.052	9.75	0.040	7.9	0.046
D	9.3	0.050	11.2	0.041	9.5	0.045

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

A consistent increase in the average hepatic concentration of vitamin A in all instances was associated with the increased age and weight of the animals. Nevertheless, the hindrance to vitamin A storage which the dibenzanthracene imposed upon the livers was a constant finding. At the end of 8 weeks, the average hepatic concentrations of the animals sacrificed from groups A, B, C, and D respectively were 1192, 1788, 479, and 306 U.S.P. units per gm., and those sacrificed at the end of 12 weeks, 2560, 2475, 645, and 554 U.S.P. units per gm.

At the end of the 4 and 8 week periods, the daily administration of 200 U.S.P. units of vitamin A to the rats of group B had increased the hepatic concentra-

TABLE IV

The Average Concentration of Vitamin A in the Livers of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of		
	4 wks.	8 wks.	12 wks.
	U.S.P. *units per gm.*	U.S.P. *units per gm.*	U.S.P. *units per gm.*
A	266	1192	2560
B	404	1788	2475
C	104	479	645
D	190	306	554

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day and 3 mg. of dibenzanthracene per rat, per week.

TABLE V

The Average Concentration of Vitamin A in the Plasma of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of		
	4 wks.	8 wks.	12 wks.
	U.S.P. *units per 100 ml.*	U.S.P. *units per 100 ml.*	U.S.P. *units per 100 ml.*
A	125	95	200
B	155	160	175
C	202	77.5	150
D	219	143.5	310

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

tions of the vitamin over those of group A. In contrast to this finding, it was noted that the administration of an equal amount of vitamin A to the rats of group D increased the hepatic levels of the vitamin over those of group C only until the end of the 4th week, and that thereafter, with the continued injections of dibenzanthracene, no increased stores of the vitamin could be effected in the rats of group D.

In summary, therefore, it would appear that the ingestion of vitamin A supplements temporarily increased the hepatic store of the vitamin, and that the parenteral administration of the carcinogen hindered the establishment of this store both in animals taking a normal diet and in those fed 200 U.S.P. units of vitamin A.

(*d*) *The Concentrations of Vitamin A in the Plasma:* At the end of the 4th week the average plasma levels of vitamin A in groups A, B, C, and D in that order were 125, 155, 202, and 219 U.S.P. units per cent. The ingestion of vitamin A supplements was reflected in its increased plasma levels of groups B and D, over those of groups A and C respectively (Table V).

The average concentration of the vitamin in the plasma of the group D rats remained higher than that of the group C animals throughout the experiment, probably due to the continued administration of the vitamin supplements. This relationship, however, did not persist between the animals of groups A and B whose liver concentrations of vitamin A had increased considerably as the experiment continued. A lack of correlation between the plasma levels of vitamin A in rats with normal and abnormally high hepatic concentrations of the vitamin has been found by others (19).

It is of interest to note that after the 4th week the levels of vitamin A in the plasma of groups C and D were no longer correspondingly higher than those of groups A and B.

(*e*) *Total Lipid Carbon and Phospholipid Content of the Liver:* The concentrations of total lipid carbon in the livers of the animals which received the carcinogen were not abnormally increased at the end of the 4th week. The average hepatic contents of total lipid carbon of groups A and B were 5.5 and 9.8 gm. per cent respectively, whereas those of groups C and D were 6.4 and 6.8 gm. per cent respectively. The average concentrations obtained for the livers of groups A, B, C, and D in that order at the end of 8 weeks were 8.8, 9.3, 10.6, and 7.4 gm. per cent; and at the end of 12 weeks 6.5, 5.5, 7.5, and 6.6 gm. per cent (Table VI).

This failure to find any significantly altered total lipid carbon content in the livers of the rats which received dibenzanthracene is compatible with the relatively constant concentration of phospholipid in those organs. For groups A, B, C, and D, respectively, the hepatic concentrations of phospholipid were at the end of 4 weeks 2.7, 2.7, 2.85, and 2.25 gm. per cent; at the end of 8 weeks 3.0, 2.8, 3.1, and 2.95 gm. per cent; and at the end of 12 weeks 2.65, 2.8, 2.9, and 2.75 gm. per cent (Table VI).

(*f*) *The Concentrations of Total Protein, "Albumin," and "Globulin" in the Liver:* No consistently significant changes in the concentrations of hepatic protein or the two fractions were noted in the groups of animals studied. At the end of the 4th week, the concentrations of liver total proteins were for groups A, B, C, and D 15.4, 14.6, 13.95, and 14.65 gm. per cent respectively. The low value of 13.9 gm. per cent of group C was due to a decrease in the "globulin" fraction. The average concentrations of "globulin" at this time were for groups A, B, C, and D respectively 13.7, 12.9, 12.1, and 12.9 gm. per cent, and those of "albumin" 1.7, 1.7, 1.85, and 1.75 gm. per cent (Table VII).

Four and 8 weeks later the average contents of total protein, "albumin," and

"globulin" in the livers of the group C rats were found to be within the limits of normal. The average values found for total liver protein at the end of 8

TABLE VI

The Average Concentrations of Total Lipid Carbon and of Phospholipid in the Livers of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of					
	4 wks.		8 wks.		12 wks.	
	Total lipid carbon	Phospholipid	Total lipid carbon	Phospholipid	Total lipid carbon	Phospholipid
	gm. per cent	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*
A	5.5	2.7	8.8	3.0	6.5	2.65
B	9.8	2.7	9.3	2.8	5.5	2.8
C	6.4	2.85	10.6	3.1	7.5	2.9
D	6.8	2.25	7.4	2.95	6.6	2.75

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

TABLE VII

The Average Concentrations of Total Protein, "Albumin," and "Globulin" in the Livers of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of								
	4 wks.			8 wks.			12 wks.		
	Total protein	"Albumin"	"Globulin"	Total protein	"Albumin"	"Globulin"	Total protein	"Albumin"	"Globulin"
	gm. per cent	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*
A	15.4	1.7	13.7	16.75	2.55	14.2	19.0	4.1	14.9
B	14.6	1.7	12.9	18.3	2.7	15.6	18.65	3.75	14.9
C	13.95	1.85	12.1	18.45	2.85	15.6	18.7	3.8	14.9
D	14.65	1.75	12.9	14.9	2.1	12.8	19.2	3.6	15.6

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

weeks for groups A, B, C, and D in that order were 16.75, 18.3, 18.45, and 14.9 gm. per cent, and at the end of 12 weeks 19.0, 18.65, 18.7, and 19.2 gm. per cent. The average concentrations of "albumin" at the end of 8 weeks were 2.55, 2.7, 2.85, and 2.1 gm. per cent and at the end of 12 weeks 4.1, 3.75, 3.8, and 3.6

gm. per cent for groups A, B, C, and D respectively. Finally, at the end of 8 weeks, the average hepatic contents of "globulin" were 14.2, 15.6, 15.6, and 12.8 gm. per cent, and at the end of 12 weeks 14.9, 14.9, 14.9, and 15.6 gm. per cent for groups A, B, C, and D respectively.

(*g*) *The Concentrations of Total Protein, Albumin, and Globulin in the Serum:* Likewise, no evidence of a significant hypoproteinemia or hypoalbuminemia was found in the animals which were injected with dibenzanthracene. During the 12 weeks of this experiment, the average total serum protein of group A varied from 6.2 to 7.2 gm. per cent; of group B from 6.0 to 7.1 gm. per cent; of group C from 6.1 to 6.9 gm. per cent; of group D from 6.0 to 7.3 gm. per cent.

TABLE VIII

The Average Concentrations of Total Protein, Albumin, and Globulin in the Serum of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of								
	4 wks.			8 wks.			12 wks.		
	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin	Total protein	Albumin	Globulin
	gm. per cent	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*
A	7.2	4.0	3.2	6.2	3.9	2.3	6.8	4.9	1.9
B	7.1	4.4	2.7	6.0	3.6	2.4	6.4	5.4	1.0
C	6.9	3.9	3.0	6.1	3.6	2.5	6.4	5.5	0.9
D	7.3	4.3	4.0	6.0	3.4	2.6	6.75	5.7	1.05

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

The average concentration of serum albumin of group A varied from 3.9 to 4.9 gm. per cent; of group B from 3.6 to 5.4 gm. per cent; of group C from 3.6 to 5.5 gm. per cent; and of group D from 3.4 to 5.7 gm. per cent (Table VIII).

Furthermore, the average ratios of albumin to globulin in the serum of groups C and D were not significantly lower than those of groups A and B. These values for groups A, B, C, and D in that order were at the end of 4 weeks 1.25, 1.63, 1.30, and 1.43; at the end of 8 weeks, 1.70, 1.50, 1.44, and 1.31; and finally at the end of 12 weeks, 2.46, 5.40, 6.34, and 5.43. The concentrations of globulin in the serum of the four groups varied considerably and accordingly these variations were reflected in the wide fluctuation of the A/G ratios.

(*h*) *The Ratios of the Concentration of Esterified to Total Cholesterol in the Serum:* At no time were the ratios of esterified to total cholesterol abnormally altered in the serum of those rats which received the carcinogen. After the

animals of groups C and D had each received the dibenzanthracene for 4 weeks, these ratios were 70 and 75 per cent respectively, whereas those for groups A and B were 75 and 70 per cent respectively. Again, at the end of 8 weeks, the ratios for groups A, B, C, and D in that order were 86, 84, 71, and 85 per cent; and at the end of 12 weeks 73, 62, 66, and 72 per cent (Table IX).

(*i*) *The Daily Urinary Excretion of Glucuronates, and of Total and Conjugated Phenols:* The urine was collected from each group of animals for 5 days during the 4th, 8th, and 12th weeks of the experiment. Determinations of the daily output of glucuronates, however, were made only during the 4th and 12th weeks, and at those times no significantly abnormal decrease in the amounts excreted by groups C and D were found. During the 4th week, the animals

TABLE IX

The Average Concentrations of Free and Esterified Cholesterol in the Serum of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of					
	4 wks.		8 wks.		12 wks.	
	Free	Esters	Free	Esters	Free	Esters
	mg. per cent	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent*	*mg. per cent.*
A	30	77.5	11	68	23	60
B	34	79	13	68	33	53
C	22	103	20	50	31	61
D	30	92	13	75	24	63

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

of groups A, B, and C excreted 4.5 mg. per rat per day, and those of group D 5.0 mg. per rat per day. During the 12th week, group A excreted 4.0 mg. per rat per day, group B 5.5 mg., group C 5.0 mg., and group D 4.25 mg. per rat, per day.

During the periods of collection the daily excretion of total and of conjugated phenol by the animals in groups C and D were within the limits of those excreted by the rats which did not receive the carcinogen. The urinary output of total phenols by groups A, B, C, and D respectively were 2.8, 4.0, 3.2, and 3.3 mg. per day during the 4th week; 4.1, 1.9, 4.7, and 3.55 mg. per day during the 8th weeks; and 3.8, 3.1, 4.2, and 3.8 mg. per day during the 12th week. Furthermore, the degree of phenol conjugation by the rats of groups C and D during these periods were at all times within the normal limits. The ratio of the outputs of total to conjugated phenol of groups A, B, C, and D in that

order were 23, 26, 30, and 24 per cent in the 4th week; 33, 22, 23, and 24 per cent in the 8th week; and 34, 34, 37, and 37 per cent in the 12th week (Table X).

(*j*) *The Concentration of Riboflavin in the Liver:* Goerner has demonstrated

TABLE X

The Average Excretion of Glucuronates, Total Phenol, and Phenol Esters in the Urine of the Rats in the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	During the course of the							
	4th wk.			8th wk.		12th wk.		
	Glucuronates	Total phenol	Phenol esters	Total phenol	Phenol esters	Glucuronates	Total phenol	Phenol esters
	mg./rat per day	*mg./rat per day*	*mg./rat per day*	*mg./rat per day*	*mg./rat per day*	*mg./rat per day*	*mg./rat per day*	*mg./rat per day*
A	4.5	2.8	0.6	4.1	1.7	4.0	3.8	1.3
B	4.5	4.0	1.05	1.9	0.4	5.5	3.1	1.05
C	4.5	3.2	1.0	4.7	1.1	5.0	4.2	1.6
D	5.0	3.3	0.8	3.55	0.85	4.25	3.8	1.4

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

TABLE XI

The Average Concentration of Riboflavin in the Livers of the Rats on the Basal Diet and of Those Which Received Supplements of Vitamin A and of Dibenzanthracene

Group	At the end of		
	4 wks.	8 wks.	12 wks.
	μg.	*μg.*	*μg.*
A	17	30	34
B	17	33	30
C	15	30.5	28
D	15	29	30

Group A received basal diet alone; group B received diet and 200 U.S.P. units of vitamin A per rat, per day; group C received diet and 3 mg. of dibenzanthracene per rat, per week; group D received diet and 200 U.S.P. units of vitamin A per rat, per day, and 3 mg. of dibenzanthracene per rat, per week.

that whereas the administration of dibenzanthracene to the rat depletes and prevents the establishment of the hepatic store of vitamin A, the carcinogen is without effect upon the concentrations of vitamin C in the liver (1). To investigate further the possibility that the primary effect of the dibenzanthracene is on the hepatic store of vitamin A, determinations were made of the concentrations of riboflavin in the livers of the animals studied.

At the end of the 4th week of the experiment, the hepatic concentrations of riboflavin were for groups A, B, C, and D respectively 17, 17, 15, and 15 μg. per gm. wet weight. At the end of the 8th week, the average concentrations were 30, 33, 30.5, and 29 μg. per gm., and at the end of the 12th week, 34, 30, 28, and 30 μg. per gm. wet weight for groups A, B, C, and D respectively (Table XI).

Thus, it would appear that the administration of the carcinogen depletes neither the hepatic store of vitamin C nor that of vitamin B_2.

The evidence at hand indicates, therefore, that the parenteral administration of dibenzanthracene to rats does produce an hepatomegaly but not a general hepatic insufficiency. This conclusion is based upon the observation that the rats which received the carcinogen did not have any significant fatty infiltration of their livers nor abnormally low hepatic content of phospholipids. The livers contain normal amounts of "albumin" and make normal amounts of serum albumin, esterify normal amounts of cholesterol and phenol, and synthesize and conjugate normal amounts of glucuronic acid. Furthermore, the administration of the carcinogen to the rats does not impair the ability of their livers to store all vitamins, since such livers retain normal concentrations of riboflavin and of vitamin C (1).

Experiments to Ascertain Whether or Not a Competition Exists Between Dibenzanthracene and Vitamin A for the Storage of the Vitamin in the Liver

From the data presented it would appear that the injection of dibenzanthracene into the rat in some manner impairs the ability of vitamin A to remain in the liver. A similar situation has been found to exist when the carcinogen, butter yellow, is fed to the rat (4). The administration of this carcinogen decreases the hepatic stores of diphosphopyridine nucleotide and of riboflavin. Furthermore, the introduction of certain metabolites of butter yellow into a suspension of yeast apozymase has been shown to reduce the activity of diphosphopyridine nucleotide, a derivative of nicotinic acid, probably by blocking the nucleotide from its attachment to its specific enzyme (20). This block, however, can be prevented *in vitro* by the introduction into the system of excess amounts of the nucleotide. This fact suggests the existence of a competition *in vitro* between the metabolite of butter yellow and the diphosphopyridine nucleotide for the specific enzyme protein, and perhaps a similar competition exists in the liver of the animal fed butter yellow. Hence, should it be possible to demonstrate that the ability of dibenzanthracene to reduce the liver content of vitamin A could be prevented by the simultaneous administration of excess amounts of vitamin A, then it would be reasonable to assume the existence of a similar competition between dibenzanthracene and vitamin A for the storage of the vitamin. Furthermore, it would appear

that the presence of dibenzanthracene, or its metabolites, specifically impairs the means by which vitamin A is anchored in the liver.

Accordingly, this hypothesis was subjected to a simple experiment. Four groups of Sherman strain rats, E, F, G, and H, whose initial weights varied from 85 to 105 gm. were placed on the same diet as that given the animals in the previous experiment. Each rat received at weekly intervals 3 mg. of dibenzanthracene intraperitoneally for 8 weeks. Group E consisted of 21 animals which received only the diet and the carcinogen. Group F, of 10 animals, received in addition an oral supplement of 200 U.S.P. units of vitamin A per rat, per day. Group G, of 11 animals, received an oral supplement of 400 U.S.P. units of vitamin A per rat, per day. Finally, the 11 animals of Group H received no supplements of vitamin A, but instead 1.5 gm. of Fleischmann's brewers' yeast each day. This last group was included in order to determine whether or not the administration of a crude supplement of the B vitamins and choline also might not counteract the effect of the carcinogen on the hepatic store of vitamin A.

At the end of the 8th week of this experiment, 5 unselected rats of each group were sacrificed and determinations made of their hepatic and blood levels of vitamin A. The average hepatic concentration of the vitamin of groups E, F, G, and H respectively were 750, 1125, 1560, and 394 U.S.P. units per gm. wet weight (Table XII). Thus, the values obtained for groups E, F, and G indicated at once that the simultaneous administration of increasing amounts of vitamin A exerted a graded countereffect on the ability of dibenzanthracene to impair the hepatic storage of this vitamin. Indeed, this countereffect was noted in the previous experiment at the end of the first 4 week period. At that time the average vitamin A in the livers of the rats which had received the carcinogen alone (group C) was 104 U.S.P. units per gm., whereas that of the animals which received both the carcinogen and 200 U.S.P. units of vitamin A daily was 190 U.S.P. units per gm. In the previous experiment, however, this relationship no longer existed at the end of the 8th or 12th weeks. Why the daily administration of 200 U.S.P. units of vitamin A in the first experiment counteracted the effect of dibenzanthracene for 4 weeks but not for 8 weeks, whereas it still did so in the second, cannot be explained at this time.

In this second experiment, at the end of 8 weeks the average levels of vitamin A in the plasma of groups E, F, G, and H respectively were 183, 300, 400, and 160 U.S.P. units per cent. For groups E, F, and G these increasing plasma levels probably reflected only the corresponding increase in the hepatic concentration of the vitamin (19) (Table XIII).

It is to be noted that the average concentration of the vitamin in both the liver and plasma of the animals which received yeast (group H) were even lower than those of group E. The animals of the former group had received no less vitamin A than had those of the latter.

Since it was found that the administration of excess amounts of vitamin A could counteract the ability of dibenzanthracene to impair hepatic storage of the vitamin, it was next desirable to determine whether or not the injection of larger amounts of the carcinogen, in turn, could counterbalance the "protective" effects of the vitamin A supplements. Should such a counterbalance be demonstrated, then the hypothesis that a competition exists between the carcinogen and vitamin A for the hepatic storage of the vitamin would have some further support.

TABLE XII

The Average Concentration of Vitamin A in the Livers of the Rats Which Received Dibenzanthracene and Supplements of Vitamin A or Yeast

Group	At the end of	
	8 wks.	12 wks.
	U.S.P. *units per gm.*	U.S.P. *units per gm.*
E	750	685
F	1125	664
G	1560	898
H	394	228

Group E received basal diet and 3 mg. of dibenzanthracene per rat, per week for 8 weeks and 5 mg. of dibenzanthracene per rat, per week thereafter. Group F received, in addition, 200 U.S.P. units of vitamin A per rat, per day; group G received 400 U.S.P. units per rat, per day; group H received 1.5 gm. of yeast per rat, per day.

TABLE XIII

The Average Concentration of Vitamin A in the Plasma of the Rats Which Received Dibenzanthracene and Supplements of Vitamin A or Yeast

Group	At the end of	
	8 wks.	12 wks.
	U.S.P. *units per 100 ml.*	U.S.P. *units per 100 ml.*
E	183	288
F	300	300
G	400	250
H	160	275

Group E received basal diet and 3 mg. of dibenzanthracene per rat, per week for 8 weeks and 5 mg. of dibenzanthracene per rat, per week thereafter. Group F received, in addition, 200 U.S.P. units of vitamin A per rat, per day; group G received 400 U.S.P. units per rat, per day; group H received 1.5 gm. of yeast per rat, per day.

In an attempt to provide this evidence the remaining animals of groups E, F, G, and H were given weekly injections of 5, instead of 3, mg. of dibenzanthracene. At the end of 12 weeks, after 4 of the larger injections had been administered to each rat, all of the animals were sacrificed and determinations made of vitamin A in their livers and plasma.

Previously it had been demonstrated that at the end of the 8th week the daily ingestion of 200 (group F) and 400 (group G) U.S.P. units of vitamin A by rats which received only 3 mg. of dibenzanthracene weekly had increased their average hepatic concentrations of the vitamin by 50 and by 108 per cent respectively over that found in the control group (E). However, after the weekly dose of carcinogen had been raised to 5 mg., the average hepatic concentration of the vitamin had fallen in groups E, F, G, and H respectively to 685, 664, 898,

and 228 U.S.P. units per gm. wet weight. Thus, the daily ingestion of 200 U.S.P. units of vitamin A by the rats which received 5 mg. of the carcinogen no longer was able to increase the hepatic store of the vitamin, and the daily ingestion of 400 U.S.P. units by these animals was able to effect only an increased hepatic concentration of vitamin A of 31 per cent instead of 108 per cent (Table XII).

Hence, the ability of the dibenzanthracene to interfere with the storage of vitamin A by the liver not only can be counteracted by the simultaneous administration of an excess of vitamin A, but this counteraction, in turn, can be overcome by the addition of larger amounts of the carcinogen. Such a relationship indicates the possible existence of a competition between vitamin A and dibenzanthracene, for some component of the liver, possibly protein, with which vitamin A normally is conjugated. The success in this competition apparently depends upon the relative concentrations of the two competing substances.

At the end of the 12th week, when each rat had received 8 injections of 3 mg. of dibenzanthracene, and 4 injections of 5 mg. of the carcinogen, the average plasma levels of the vitamin of groups E, F, G, and H were 288, 300, 250, and 275 U.S.P. units. There appears to be no obvious correlation between these levels and those of the livers of the corresponding groups (Table XIII).

It is of interest to note that the average hepatic concentration of vitamin A in the animals which received the supplements of yeast (group H) remained significantly lower than even that of the control group E. Thus, it would appear that the simultaneous administration of yeast to the dibenzanthracene treated rat certainly does not protect the animal from the effects of the carcinogen on the hepatic storage of vitamin A.

DISCUSSION

In the present study an attempt has been made to ascertain whether the ability of dibenzanthracene to impede the storage of vitamin A by the rat liver is due to a general hepatic insufficiency caused by the carcinogen or to an impairment of a specific function of the liver. Inasmuch as the liver performs multiple functions, it is reasonable to believe that no single test could measure adequately the efficiency of the whole organ. Therefore, by the quantitative measurement of various intermediary or end products of liver metabolism, and then by the consideration of the results as a whole, it was felt that the presence or absence of general hepatic damage could be recognized. In this study, measurements of liver function were selected arbitrarily and thus it is necessary to consider the evidence which would indicate that the tests employed in this study provided adequate indices.

1. The Hepatic Concentration of Total Lipid and Phospholipid.—It is now a well established fact that the liver is concerned intimately in the intermediary metabolism of lipoids (21) and that in many conditions associated with hepatic

damage there occurs a striking accumulation of fat in the liver cells. An increase in the concentration of neutral fat and a decrease of phospholipids has been found especially in the livers of patients with alcoholic hepatic cirrhosis (22), and in animals given various toxic agents (23).

2. Total Protein, Albumin, and Globulin.—The best evidence at hand thus far indicates that the liver is of primary importance in the formation, storage, and exchange of plasma protein. Fibrinogen, and probably the albumins, are produced only by the liver cells (24).

Abnormal levels of circulating plasma protein are reached only when the protein stores, chiefly in the liver, are decreased. The normal animal, depleted of its protein stores, readily can synthesize albumin, globulin, and fibrinogen from a proper mixture of amino acids, but those proteins are deposited first in the liver and other organs, and do not appear in the plasma until the body stores have been reestablished. In a damaged liver the synthesis of protein from amino acids is altered, and the circulating concentrations of plasma protein continue to decrease with further hepatic damage. The disappearance of the hepatic stores of protein with damage to the liver now has been shown both experimentally and in human individuals with hepatic cirrhosis (24).

3. Cholesterols and Cholesterol Esters.—The serum concentration of total cholesterol and the ratio of free to esterified cholesterol in the normal healthy animal is maintained within rather narrow limits. The esterification of this sterol is accomplished chiefly in the liver, and the ability of the damaged liver to perform this function is considerably decreased (25).

4. Glucuronic Acid.—It has been demonstrated that several aromatic compounds normally are conjugated with glucuronic acid and excreted as glucuronates in the urine. This conjugation has been considered to be a form of detoxification. The liver is the chief site both for the synthesis of glucuronic acid and its conjugation to other aromatic compounds. Livers of rats injured by phosphorus soon lose their capacity to synthesize glucuronic acid (26).

5. Phenol and Phenol Esters.—Detailed studies of the metabolism of phenol have demonstrated that the synthesis of phenol sulfuric and phenol glucuronic acids takes place only in the hepatic parenchyma. The exclusion of the liver from the normal circulation by an Eck fistula eliminates phenol conjugation. Likewise extensive liver injury due to various toxic agents always will lessen phenol conjugation. Extreme and fatal hepatic injury will reduce phenol conjugation to zero (27).

In conclusion, it would appear that adequate measures were taken to demonstrate the possible existence of hepatic insufficiency. The fact that none of the functions studied were altered in the animals indicates that dibenzanthracene is not a general hepatotoxin but, according to the data thus far available, apparently affects particularly that function of the liver which is concerned with the metabolism of vitamin A.

This particular effect of the carcinogen can be obviated to some measure by

the simultaneous administration of large supplements of vitamin A. This relationship between the carcinogen and the vitamin therefore suggested the existence of an anchor in the liver to which vitamin A normally is attached and which can be competed for by the dibenzanthracene or its metabolites. Such a hypothesis definitely could be proved if it were possible to show that (1) the dibenzanthracene-treated rat excretes or metabolizes increased amounts of vitamin A, (2) the hepatic concentration of dibenzanthracene and its metabolites is a function of the vitamin A simultaneously administered to the animals, and (3) that the administration of large supplements of vitamin A results in an increased excretion or utilization of dibenzanthracene. Unfortunately, trustworthy methods for these studies now are not available, though they are under investigation in this laboratory (28, 29).

In contrast to the effect of the carcinogen butter yellow, dibenzanthracene apparently does not impair the hepatic storage of riboflavin. Furthermore, whereas the simultaneous administration of yeast to rats given butter yellow prevents the depletion of riboflavin and diphosphopyridine nucleotide from their livers, the administration of yeast did not counteract the ability of dibenzanthracene to deplete the hepatic store of vitamin A. These observations thus suggested that different carcinogens selectively inhibit different reactions which occur in the liver cells, and further indicate the particular effect of dibenzanthracene on the hepatic metabolism of vitamin A.

In section B of this study it was noted that the simultaneous administration of yeast to the dibenzanthracene-treated rats apparently enhanced the ability of the carcinogen to deplete the hepatic stores of vitamin A. At present, no explanation for that observation is at hand, but it is conceivable that the effect was an additive one. Previous studies have shown that the administration of yeast to human individuals can increase the plasma levels of vitamin A (7), probably by stimulating a release of the vitamin from its hepatic stores. Likewise, there is evidence to show that normal rats fed choline, a substance contained in yeast, have abnormally low concentrations of vitamin A in their livers (30). Details of the relationship between choline, yeast, and vitamin A at present are under investigation in this laboratory.

CONCLUSIONS

1. The decreased concentrations of vitamin A in the livers of rats given dibenzanthracene probably are due to a particular effect of the carcinogen on the ability of the liver to store the vitamin and not to the production of general hepatic dysfunction.

2. The administration of dibenzanthracene to normal rats does not (*a*) increase significantly their hepatic content of total fat nor decrease that of phospholipid; (*b*) impair the ability of their livers to fabricate serum albumin; (*c*) impair the capacity of their livers to esterify cholesterol or phenol; (*d*) interfere with the hepatic synthesis and conjugation of glucuronic acid; or (*e*) interfere with the hepatic storage of riboflavin.

3. The simultaneous ingestion of yeast by the dibenzanthracene-treated rats further depletes their hepatic stores of vitamin A. This depletion conceivably is due to the fact that yeast alone also might deplete the liver of its vitamin A and thus a summation of two similar effects is attained.

4. The results suggest a competition between vitamin A and dibenzanthracene for some substance, possibly a protein, to which vitamin A may be bound in the liver.

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