EFFECTS OF HEXACHLORONAPHTHALENE ON VITAMIN A METABOLISM IN THE RAT

by

George Edwin Bunce

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APPROVED:

Director of Graduate Studies

Dean of Applied Science and Business Administration

APPROVED:

Head of Department

Major Professor

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CHAPTER I

THE PROBLEM

Literature Review

Polychlorinated naphthalenes are fire, heat, and water resistant wax-like compounds. For the past 25 years they have been employed as lubricant and crankcase oil additives and as insulation for wires, coils, and small transformers (9). They were found to be highly effective as insecticides against thrips, scales, aphids, and red spiders but were too damaging to plants for practical field use (4).

It was noticed that workers handling the Halowaxs, as the polychlorinated naphthalenes are known commercially, often became afflicted with a skin condition referred to as cable rash, cable itch, or chloracne. Acute yellow atrophy of the liver and jaundice were also detected in advanced cases (9). Such observations stimulated research on the toxicity of these compounds in small animals. Bennett and associates (3), noted that inhalation or ingestion of penta- and hexa- isomers of the polychlorinated naphthalenes at levels between 50 to 100 mg daily intake caused

extensive damage to the liver, especially in the area of the bile capillaries, in rats. Cleary et al (6), observed an increase in urinary inorganic chloride when the same compounds were ingested by rats or dogs. They postulated that the intracellular liberation of halogen was the primary reason for the toxicity of the Halowaxs.

Following this work, however, there were few references to research on the deleterious properties of the chlorinated naphthalenes until 1952 when they were identified by Bell as the causative agent in a lubricant which produced the X-disease syndrome in cattle (2).

X-disease or hyperkeratosis of cattle was first recognized by Olafson in New York in 1941 and has since been reported in all states east of the Rocky Mountains (18). The symptoms consist of excessive lacrimation, diarrhea, polyuria, marked salivation, nasal discharge, red swollen areas on the mouth and muzzle, and loss of weight. Thickening and wrinkling of the skin may occur on the neck and withers and sometimes over the entire body. Internal lesions include a cystic dilatation of the renal tubules; squamous

metaplasia of the epididymis, seminal vesicles, and salivary gland ducts; mucoid papillary proliferations in the larger bile ducts and gall bladder; fibrosis in the pancreas and the kidney; and proliferation of the small bile ducts in the liver (9).

Olafson et al (19), reported that plasma vitamin A declined rapidly following ingestion of the toxic substance and remained depressed for a month subsequent to the cessation of the intake of the causative agent. This depression of plasma vitamin A and the similarity of some of the X-disease lesions to typical avitaminosis A symptoms and lesions (proliferative epithelial disturbances, lacrimation, abortion) suggested that the chlorinated naphthalenes were involved in an interference with vitamin A metabolism (9). A number of hypotheses have been advanced to explain the nature of this disturbance.

Olafson and co-workers at Cornell (19), noted that plasma carotene rose following the feeding of large doses of carotene but that plasma vitamin A remained depressed. Feeding of preformed vitamin A resulted in increases in both plasma and liver levels of the vitamin until feeding of the supplement was

discontinued. From these data they suggested that the hyperkeratosis factor exerted an anti-vitamin A effect, in part at least, by interfering with the conversion of carotene to vitamin A.

Both Webster et al (22), and Hoekstra et al (12), found vitamin A therapy to be of value in delaying the appearance of hyperkeratosis in calves and alleviating the condition in older animals. Vitamin A supplementation appeared to decrease diarrhea, relieve coughing, and improve appetite and general condition, but there was no noticeable effect on the lacrimation, mouth lesions, or skin thickening. Hoekstra's group, in addition, determined plasma carotene; vitamins A, B_6, B_{12}, and C; niacin; sodium; and potassium and found that only vitamin A was significantly altered. They were not able to show a depression of vitamin A in homogenized fresh cow liver or in corn oil after as much as 24 hours incubation at 37°C with polychlorinated naphthalene. They proposed the following theories to explain the depressed vitamin A: inhibited absorption, greatly increased excretion, chemical destruction or inactivation of the vitamin A molecule, an anti-vitamin like effect, or a greatly increased requirement for the vitamin.

The observation by Hove (13), that there was a concurrent drop in vitamin E in the plasma of hyperkeratotic calves as the vitamin A concentration decreased led him to suggest an in vivo pro-oxidant property of the chlorinated naphthalenes which acted on unsaturated fat systems.

Engel et al (9), investigated the effect of protein on the toxicity of hexachloronaphthalene in the rat. Their results showed that ingestion of a total of only three to four mg of the toxic factor in a diet containing nine per cent casein produced a real liver hypertrophy and a substantial increase in liver fat content. Elevation of the casein from 18 or 27 per cent at the expense of sucrose, however, afforded a significant protection against liver fat accumulation. Substitution of 20 per cent Crisco for an equal amount of sucrose in the diet or omission of the vitamin A and D supplement had no effect on the liver changes induced by hexachloronaphthalene.

Joyce in 1955 (14), was interested in the relative ability of rat and calf liver tissue to metabolize the toxic substance. He found that the liver tissue of the calf could metabolize three and one

half times as much hexachloronaphthalene as could rat liver tissue. He also analyzed the feces and urine of mature rats injected subcutaneously with 15 mg of hexachloronaphthalene and detected hydrolyzable, conjugated products of the toxic compound in the former but not the latter. The fact that conjugated products of chlorinated naphthalene metabolism appeared in the feces while the products of naphthalene itself appear in the urine (11) suggested a difference in the modes of metabolism of naphthalene and its chlorinated derivatives.

Importance of the Problem

Sufficient evidence is available to show that in cattle the intake of polychlorinated naphthalene is followed by a decrease in plasma and liver vitamin A and by appearance of symptoms and lesions resembling those of avitaminosis A. This investigation is needed to define the relation between hexachloronaphthalene and vitamin A in the rat, a more economical laboratory animal.

Although the participation of vitamin A in the visual cycle has been elucidated, little is known

about the specific physiological roles which make it essential for life in animals. Thus, compounds which prove to be antagonistic to vitamin A or its normal metabolism are of possible value for research of a fundamental nature into the mechanics of vitamin A metabolism.

Approach to the Problem

A study was made of the effect of hexachloronaphthalene on preformed residual vitamin A in the rat. The effect of the toxic agent on the efficiency of assimilation of dietary carotene and vitamin A was also investigated. The state of vitamin A nutrition in the animals was determined by visual observation for typical deficiency symptoms and by analysis of various tissues following specific dietary treatments.

CHAPTER II

THE INVESTIGATION

Experimental Procedure

Weanling rats of the Holtzman strain weighing between 30 and 55 grams were used for these studies. From conception to weaning of the experimental animals, their dams were fed a stock ration in which the sources of vitamin A had been omitted. When the young animals were placed on experiment, they were segregated into equal groups on the basis of weight, sex, and litter.

The purified vitamin A deficient diet used in all experiments was derived from the diet recommended in the U. S. Pharmocopeia XIV (21) for the preparation of rats for the biological assay of vitamin A activity. The composition of the diet appears in Table 1. When desired, beta-carotene[1], hexachloronaphthalene[2], and vitamin E[3] were added to the diet in petroleum ether

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1 Beta, 90 per cent; alpha, 10 per cent. Nutritional Biochemicals Corporation.

2 Hexa, 95 per cent; penta and hepta, 5 per cent. Donated by Union Carbide and Carbon Corporation.

3 Alpha tocopherol, 50 per cent; alpha tocopherol acetate, 50 per cent. Donated by Merck and Company.

TABLE 1

Composition of Vitamin A Deficient Diet

Ingredient	Per Cent
Purified casein	18
Cottonseed oil[1]	5
Sucrose	68
Salts 5[2]	4
Vitamin mix[3]	5

1 Wesson oil

2 Supplies in mg per kg of diet: $Ca_3(PO_4)_2$, 2050; K_2HPO_4, 1030; $MgSO_4 \cdot 7H_2O$, 600; NaCl, 500; Fe citrate, 130; $MnSO_4 \cdot H_2O$, 20; $CuSO_4 \cdot 5H_2O$, 5; $ZnCO_3$, 10; KI, 5.

3 Supplies in mg per kg of diet: thiamine·HCl, 5; riboflavin, 10; pyrdoxine·HCl, 5; calcium pantothenate, 20; niacin, 40; inositol, 200; choline chloride, 2000; and Vitamin D, 1.

(30 to 60°C B.P.) solution. The solvent was allowed to evaporate and the additives were then mixed into the diet with a blender. The diet was stored under refrigeration in closed glass jars.

The vitamin A status of the animals was determined by observation for gross symptons of xerophthalmia and by tissue analysis of liver, kidney, and small intestime by the method of Ames et al (1). The animals were sacrificed by a sharp blow on the head and immediately exsanguinated. The small intestine was removed by ligation at the pyloric sphincter and the ileocecal valve. It was then split open, washed clean of its contents with a solution of 0.9 gram NaCl per 100 ml distilled water, and placed in a clean glass jar to be frozen until it could be analyzed for vitamin A. The liver and kidneys were removed and frozen until they could be submitted to the same analysis.

Single oral doses of alpha carotene[1] or beta carotene were prepared in the following manner. First, the compound was dissolved in approximately 10 ml of

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[1] Alpha, 90 per cent; beta, 10 per cent. Nutritional Biochemicals Corporation.

warm petroleum ether. The petroleum ether solution was then added to 20 ml of Wesson oil. The ether was evaporated from the oil on a hot plate leaving the carotene in solution in the Wesson oil. One gram of vitamin E and one ml of Tween 80[1] were added directly to the oil prior to the heating. This solution is similar to the one described by Mattson et al (16). The vitamin A palmitate[2] solution was prepared by adding the compound directly to 20 ml of Wesson oil containing the Tween 80 and vitamin E. The forced feeding was accomplished by injecting one ml of the solution through a small rubber tube into the esophagus.

Effect of Hexachloronaphthalene on Preformed Vitamin A

Previous work (19), had indicated that in cattle the ingestion of polychlorinated naphthalene caused a more rapid disappearance of vitamin A from the liver and blood than resulted from uncomplicated avitaminosis A. The first trial in this experiment

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1 Polyoxyethylene sorbitan monooleate. Atlas Powder Company.

2 Endo Products, Inc.

was designed to determine if a vitamin A deficiency would be hastened in rats when hexachloronaphthalene was included in a purified vitamin A deficient diet. Twenty-four rats were divided into three groups. Group one was fed the USP purified vitamin A deficient diet plus 15 ppm (parts per million) of hexachloronaphthalene. The second group received the USP diet alone, while the third group received the USP diet and a supplement of five mcg of beta carotene per gram of feed. The feed provided for each animal in the second and third groups was limited to the amount consumed by the corresponding animal in group one. Feed intake averaged approximately 10 to 12 grams per day per rat at the beginning of the experiment but by the 25th day had dropped to half of this and remained there until the end of the study. The treatment was continued until severe xerophthalmia developed in the animals in group one. At this time the group one rat and its diet mates in groups two and three were sacrificed and their livers were analyzed for vitamin A. All animals were sacrificed within 35 to 45 days after the beginning of the experiment. The results of the liver analyses appear in Table 2. All of the animals gained two to three grams per day

TABLE 2

Influence of Dietary Hexachloronaphthalene on Total Liver Vitamin A, Liver Weight, and Liver Weight Per Cent Body Weight

Animal	Ration								
	USP + 15 ppm 6C1ØØ[1]			USP Alone			USP + 5 ppm Beta Carotene		
	mcg	gm	%	mcg	gm	%	mcg	gm	%
1	6.7	3.4	5.1	3.7	2.8	4.3	11.6	2.6	3.2
2	9.8	3.7	5.1	5.5	2.8	3.5	8.6	2.4	4.0
3	33.6	3.7	5.3	15.0	3.1	5.0	15.2	3.4	3.8
4	24.7	3.3	4.3	9.0	2.4	3.8	24.4	3.5	3.9
5	7.0	2.7	4.4	2.4	2.9	3.8	2.5	2.6	4.1
6	7.6	3.9	4.7	6.7	3.4	3.8	3.0	2.3	4.2
7	6.5	3.0	4.6	3.2	3.9	4.1	3.7	2.5	3.7
8	12.0	3.4	4.7	4.0	2.2	3.6	5.8	3.9	4.4
Av.	13.6	3.4	4.8	6.2	2.9	4.0	9.4	2.9	3.9

[1] Hexachloronaphthalene

until the feed intake dropped, at which time they began to lose weight at an equal rate.

With the exception of rat number five in group two, no animals in groups two and three exhibited any sign of xerophthalmia at any time during the experiment. It is interesting to note that although the appearance of xerophthalmia was accelerated by the addition of the toxic agent to the diet, the average vitamin A per liver in group one was higher than in either of the other groups.

The suggestion by Moore (17), that vitamin E might protect against depletion of stored vitamin A and the theory of Hove (13), that polychlorinated naphthalenes may act as in vivo pro-xoidants, were considered in the design of the second trial of experiment one. This trial was planned to investigate the possible advantage of a vitamin E supplement in delaying xerophthalmia in rats on a vitamin A deficient diet both in the presence and absence of hexachloronaphthalene. Twenty-four rats were divided into four equal groups. Group one was fed the USP diet alone while group two received the USP diet with a supplement of 0.5 mg vitamin E per gram of feed.

Group three was given the USP ration plus 15 ppm of hexachloronaphthalene. The remaining rats were given USP diet to which both vitamin E and the toxic agent had been added. Feed intake was again controlled by the animals receiving the chlorinated naphthalene, and the amount consumed was approximately the same as in the first trial. All animals were maintained on this treatment for 35 days. At this time all were sacrificed and their livers removed for vitamin A analysis. The results are given in Table 3.

Again it was evident that xerophthalmia developed more rapidly in the animals which received the hexachloronaphthalene than in those on the deficient diet alone. The addition of vitamin E to the toxic and non-toxic diets had little effect on either the liver store of vitamin A or the severity of xerophthalmia. As in the first trial, the amounts of vitamin A in the livers of the animals on the toxic ration were slightly greater than the amounts detected in the livers of rats which consumed the non-toxic diet.

TABLE 3

Influence of Dietary Hexachloronaphthalene and Vitamin E on Total Liver Vitamin A, Liver Weight, Liver Weight Per Cent Body Weight, and Severity of Xerophthalmia

Ration	Animal						
	1	2	3	4	5	6	Av.
USP Alone							
mcg	10.7	8.7	2.6	5.8	1.3	0.7	5.0
gm	5.1	4.0	3.6	4.0	3.5	3.8	4.0
%	4.5	3.6	4.1	4.0	3.7	4.6	4.1
xero	+	+	++	++	++	+	
USP + 500 ppm Vitamin E							
mcg	17.2	6.4	4.3	4.0	3.2	lost	5.9
gm	4.3	3.8	3.3	3.9	4.3	3.6	3.9
%	5.0	6.0	5.0	5.8	4.4	4.0	5.0
xero	++	++	++	++	+	+	
USP + 15 ppm 6C1ØØ[1]							
mcg	9.6	17.5	8.1	9.5	1.8	0.4	7.8
gm	4.6	5.5	4.3	4.3	3.6	4.0	4.4
%	4.2	5.1	6.1	4.5	4.0	5.7	4.9
xero	+++	+++	+++	+++	+++	+++	
USP + 15 ppm 6C1ØØ + 500 ppm Vitamin E							
mcg	14.5	17.9	10.9	10.3	1.2	0.8	9.3
gm	4.2	4.1	4.6	4.3	4.0	4.2	4.2
%	3.8	4.0	6.4	5.1	4.8	6.2	5.1
xero	+++	+++	+	+++	++	+++	

\+ No xerophthalmia

++ Slight inflammation around eyes

+++ Severe inflammation and exudation

[1] Hexachloronaphthalene

Effect of Hexachloronaphthalene on Assimilation of Dietary Carotene

Twenty-four rats were divided into four equal groups. Group one was fed the USP diet without the addition of the natural anti-oxidant. Group two received the USP diet plus a supplement of 0.5 mg vitamin E per gram of feed. Groups three and four received the same rations, respectively, except that 30 ppm hexachloronaphthalene was included in each. Carotene was added to all the diets at a level of 4.8 mcg per gram of feed.

The intake of the group consuming the toxic diet without the added vitamin E limited the amount of feed supplied to the other three groups. All animals were sacrificed after 35 days and liver samples were analyzed for vitamin A. The results are given in Table 4.

Comparison of the average vitamin A content of the livers of the four groups by means of the Duncan Multiple Range Test (8) indicated that the storage of vitamin A by group two (the animals receiving the tocopherol supplemented, non-toxic diet) was significantly greater ($P = <0.01$) than the storage

TABLE 4

Influence of Dietary Hexachloronaphthalene and Vitamin E on Total Liver Vitamin A, Liver Weight, and Liver Weight Per Cent Body Weight in Rats Fed a Carotene Enriched Diet

Ration[1]	Average[2] Vitamin A	Average Liver Weight	Average Liver Weight Per Cent Body Weight
	mcg	gm	%
USP Alone	22.9	6.1	3.8
USP + 500 ppm Vitamin E	32.9[3]	5.7	3.7
USP + 30 ppm 6Cl$\phi\phi$[4]	17.5	6.6	4.3
USP + 30 ppm 6Cl$\phi\phi$ + 500 ppm Vitamin E	20.6	7.2	4.6

1 All rations included 4.8 ppm beta carotene

2 Average values from six animals per ration

3 $p = < 0.01$

4 Hexachloronaphthalene

resulting from all other treatments. None of the remaining groups exhibited any statistically significant margin over another.

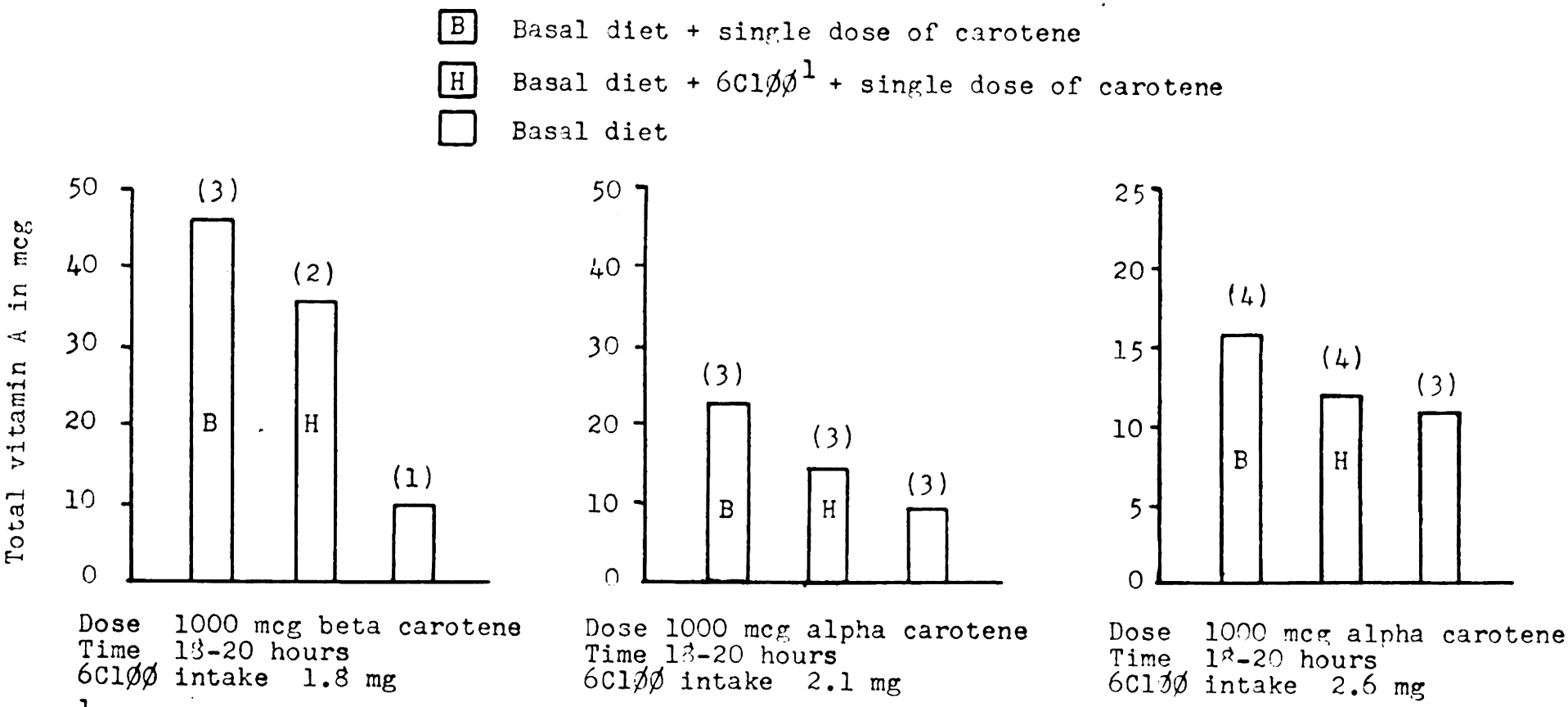
Effect of Hexachloronaphthalene on the Assimilation of a Single Oral Dose of Carotene

In order to investigate further the poorer assimilation of dietary carotene by the rats receiving hexachloronaphthalene as compared to normal rats a series of studies were conducted in which the increase in tissue vitamin A was determined at various times following forced feeding of carotene solutions.

The first three trials were similar in the following respects. Weanling rats were divided into two groups of two to four rats each. One group was fed the USP vitamin A deficient diet alone while the second received the ration plus 30 ppm hexachloronaphthalene. Feed intake was limited by the amount consumed by the latter group. This treatment was continued until 1.8 to 2.6 mg of the chlorinated naphthalene had been ingested. One or two animals representing each litter were removed and sacrificed at this time, and the liver and small intestine of

each were analyzed for vitamin A. It was assumed that the average vitamin A detected in these animals approximated the level of the vitamin in the remaining litter mates prior to their exposure to carotene. The remaining rats were force fed one ml of solutions of alpha or beta carotene at a concentration of 1,000 mcg per ml. These animals were sacrificed at times varying from eighteen to twenty hours after dosage, and the liver and small intestine of each were removed and analyzed for vitamin A. The difference between the average initial vitamin A and the amount recovered following the oral feeding of carotene was termed the increase in vitamin A. Figure 1 shows the average increase in vitamin A per group in the first three trials.

A fourth trial was undertaken to determine if the inclusion of hexachloronaphthalene in the carotene solution would be sufficient to exert a depressing effect on carotene assimilation when the test animals had not been previously exposed to the toxic agent. The treatment was the same as in the previous studies except that the dose of carotene was reduced to 500 mcg, the dose-sacrifice interval was 17 to 22 hours, the total amount of hexachloronaphthalene (3.6 mg)



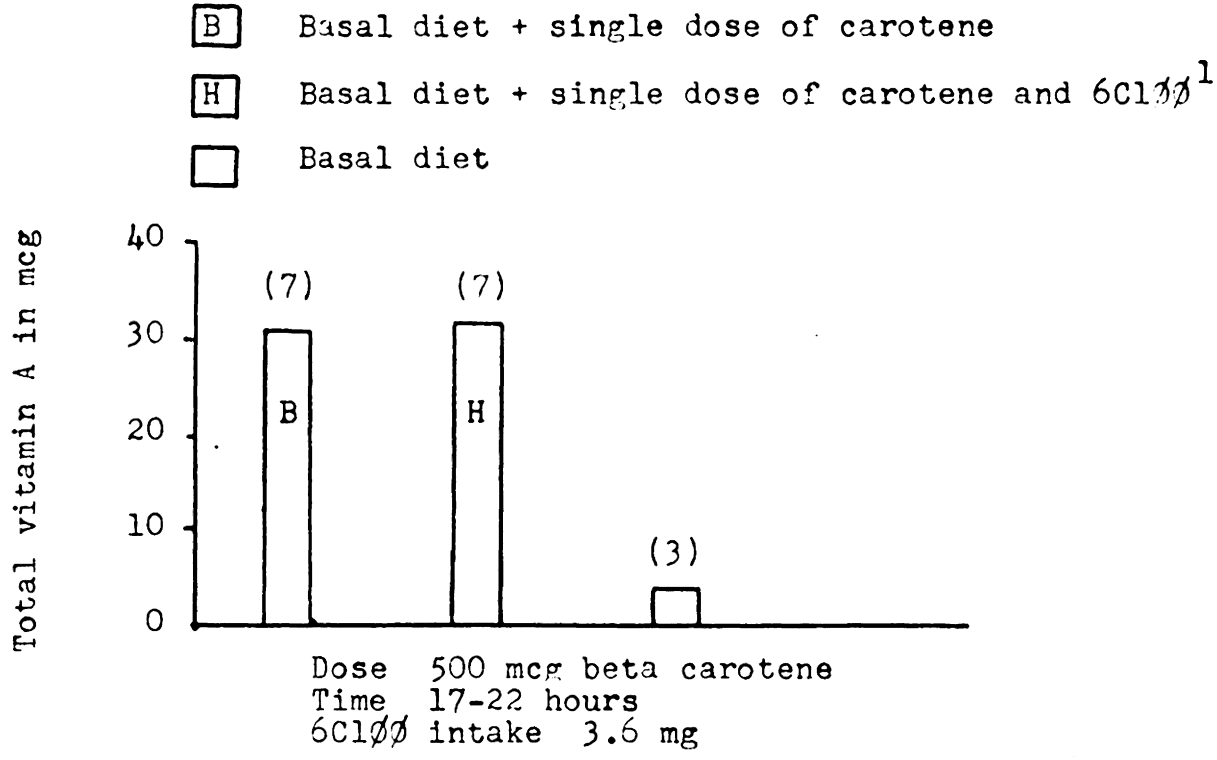
[1] Hexachloronaphthalene

Numbers in ( ) are number of rats per treatment

FIGURE 1. EFFECT OF HEXACHLORONAPHTHALENE ON INCREASE OF VITAMIN A IN RAT LIVER AND INTESTINE FOLLOWING INGESTION OF CAROTENE

was included in the carotene solution instead of being added in the diet, and the liver alone was analyzed for vitamin A. The average increase in vitamin A per group in this trial is shown in Figure 2.

Finally, the effect of omission or addition of a surface active agent to the carotene solution was determined. Thirty-two rats were employed in this trial. Six animals were used to obtain the average level of vitamin A prior to dosage as described for the first trials in this experiment. The remaining animals were divided into two equal groups. Again, one group received the USP ration alone while the other was fed the same ration plus 30 ppm hexachloronaphthalene until 3.6 mg of the additive had been ingested. Six rats from each group were force fed a carotene solution in which the Tween had been omitted. The remaining seven in each group were force fed an equal amount of the solution to which had been added 400 mcg per ml of sodium taurocholate. Thirteen to sixteen hours later the animals were sacrificed and the liver and kidneys of each were removed and analyzed for vitamin A. The average increase in vitamin A in each rat is shown in Figure 3.



[1] Hexachloronaphthalene

Numbers in ( ) are number of rats per treatment

FIGURE 2. EFFECT OF SIMULTANEOUS ADMINISTRATION OF HEXACHLORONAPHTHALENE AND CAROTENE ON INCREASE OF VITAMIN A IN RAT LIVER

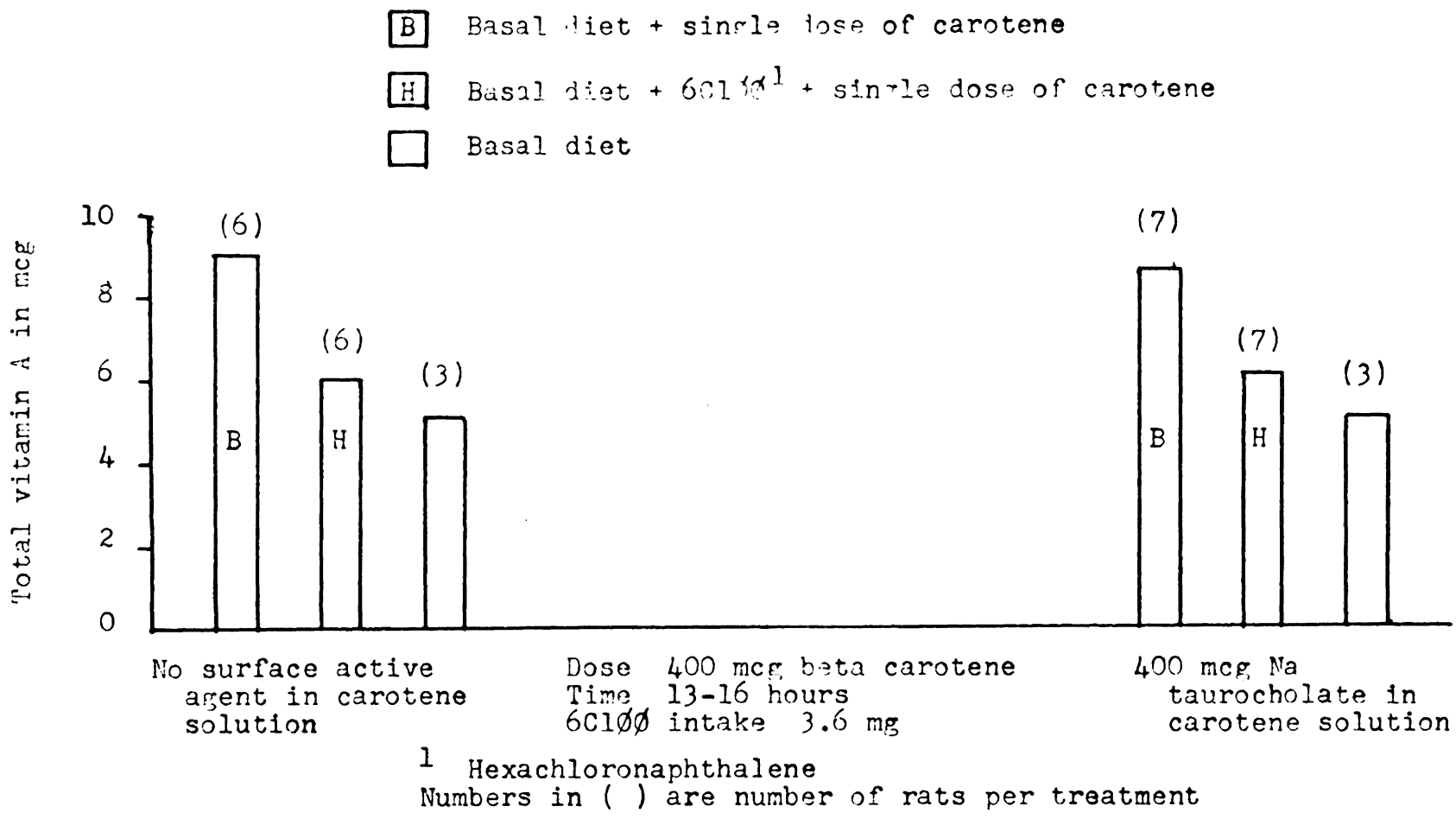


FIGURE 3. EFFECT OF HEXACHLORONAPHTHALENE AND SODIUM TAUROCHOLATE ON INCREASE OF VITAMIN A IN RAT LIVER AND KIDNEY FOLLOWING INGESTION OF CAROTENE

The data from this experiment indicate that ingestion of hexachloronaphthalene prior to a single oral does of carotene depressed the ability of the rat to assimilate the pro-vitamin. The addition of a bile salt to the carotene solution failed to ameliorate this depression to any great extent. No difference in carotene conversion was observed when the total dose of the toxic compound was included in the carotene solution rather than in the diet prior to the forced feeding.

Effect of Hexachloronaphthalene on the Assimilation of a Single Oral Dose of Vitamin A

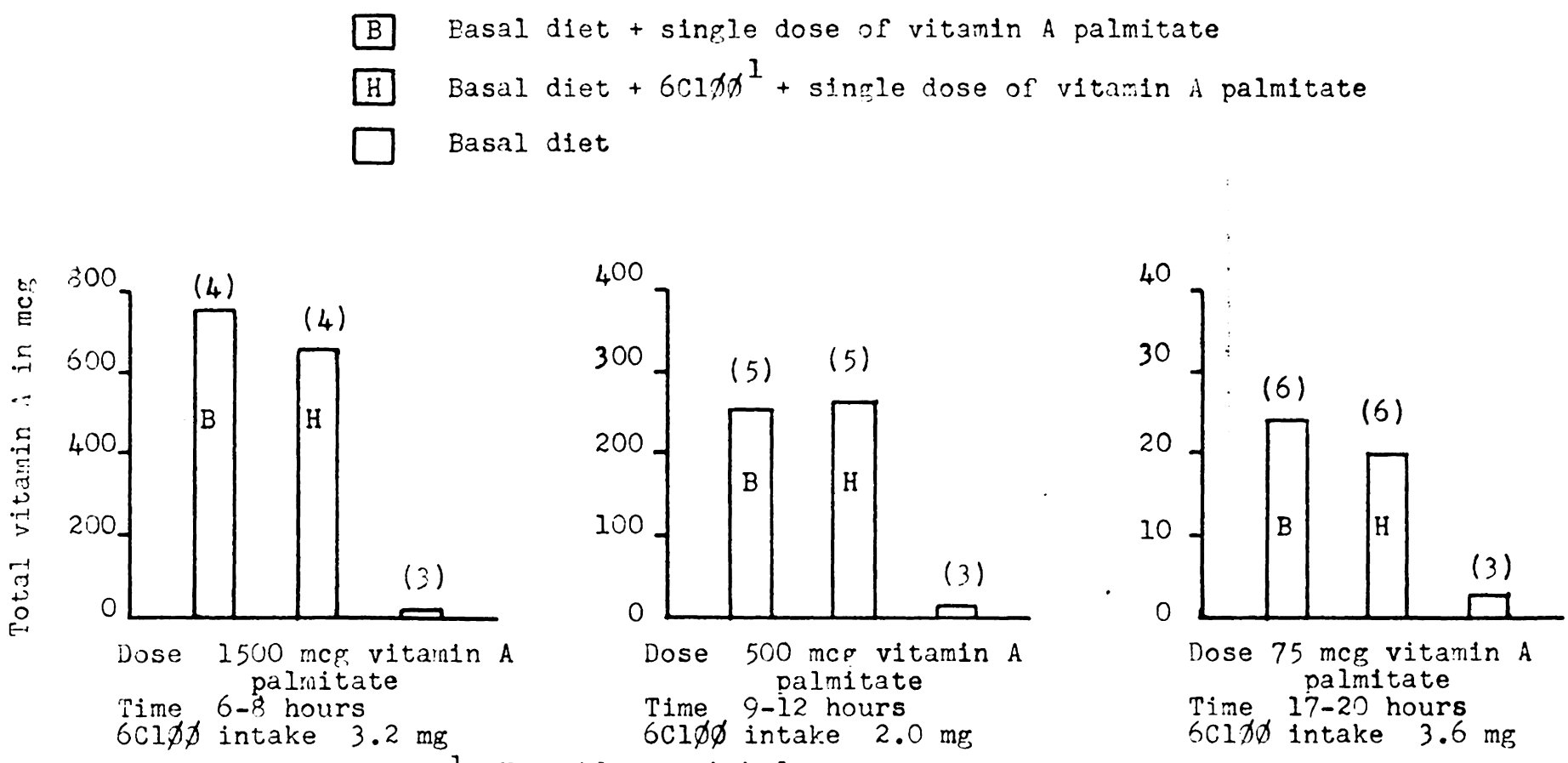
Weanling rats were again employed for this work. The animals were paired into two groups of five to seven each. One group was given the USP purified vitamin A deficient diet. The remaining rats were fed the same diet to which had been added 30 ppm hexachloronaphthalene. Feed intake was limited by the amount consumed by the latter group. This treatment was continued until 2.0 to 3.6 mg of the toxic compound had been ingested. At this time one animal representing each litter was removed to serve as the control. The

average vitamin A found in the tissues of the control animal was assumed to be the approximate amount of the vitamin present in the tissues of the treated rats prior to dosage.

After removal of the control animals, the remaining rats were given one ml of the vitamin A palmitate in Wesson oil solution by stomach tube. They were sacrificed at varying times subsequent to the forced feeding. The tissue samples for vitamin A analysis were removed and frozen immediately. The study was performed three times, each time utilizing different concentrations of the vitamin and different intervals between dosage and sacrifice. The intestine was not analyzed when the dosage-sacrifice interval was as much as 17 to 20 hours since previous work indicated that little vitamin A remained in that tissue at that time. The results of the analyses are graphically presented in Figure 4.

At a dosage of 1500 mcg there is little difference in the average amount of vitamin A recovered in the liver and intestine of the two groups.

When the dosage was reduced to 500 mcg per rat, the recovery of vitamin A in both the liver and



[1] Hexachloronaphthalene
Numbers in ( ) are number of rats per treatment

FIGURE 4. EFFECT OF HEXACHLORONAPHTHALENE ON INCREASE OF VITAMIN A IN RAT LIVER AND INTESTINE FOLLOWING INGESTION OF VITAMIN A PALMITATE

intestine of the experimental animals was very similar. It should be pointed out, however, that the rats in this particular study averaged 20 to 25 grams larger than the ones receiving the other levels of the vitamin and that they also ingested the smallest amount of the toxic compound prior to dosage. It has been often noticed in this laboratory that, in rats, an increase in resistance to hexachloronaphthalene accompanies maturity and increase in weight. The possibility could then be considered that the rats on the toxic diet failed to take in an amount of the compound necessary to exert a detectable influence on the vitamin A mechanisms involved.

In the third trial, the dosage of the vitamin was further reduced to 75 mcg per rat but the hexachloronaphthalene ingested per rat was increased to 3.6 mg per animal. Again, the average difference between the two groups was not greater than one should expect from animal to animal variation.

Addendum

The observation that hexachloronaphthalene accelerated a vitamin A deficiency in rats motivated

investigation of the possible changes in quantity and electrophoretic mobility of the plasma proteins. The albumin fraction of plasma protein has been suggested as the carrier of blood vitamin A. Since an experiment with hexachloronaphthalene in cattle was underway at that time and the blood samples were readily available from these animals, it was decided to initiate the study with the bovine rather than the rat.

Blood samples were taken from four bull calves: one Jersey, one Guernsey, and two Holsteins; prior to oral dosage with gelatin capsules containing hexachloronaphthalene dissolved in corn oil. The calves weighed approximately 150 pounds at the beginning of the experiment. Only three calves received the toxic substance; one Holstein was spared to serve as the control. The capsules were administered every other day for ten days until the total dose of the chlorinated naphthalene amounted to one mg per pound body weight. Blood samples were obtained from each calf at intervals of two to five days for the duration of the experiment. The treated Holstein died 29 days after receiving the initial dose. The Jersey calf

succumbed after 34 days while the Guernsey and the control were sacrificed on the 47th day.

Ten mcl aliquots of the blood samples were subjected to paper electrophoretic analysis on a Spinco model R, series B, electrophoresis apparatus. A 0.075 molar veronal buffer at a pH of 8.6 was employed. Current was held constant at 5.0 milliamps. The resultant strips were scanned with the Spinco Analytrol. Typical graphs from this apparatus are shown in Figure 5.

The graphs indicated that while the motility of the proteins was not altered, there was a distinct depression of the quantity of plasma protein, especially in the albumin fraction, by the time that half of the toxic dose had been administered.

In order to confirm this observation, the 10 per cent trichloroacetic acid precipitable protein in two ml aliquots of the previously examined blood samples was dried and weighed on tared watch glasses. The results are presented in Table 5.

From this evidence, it appears that a depression of the level of plasma protein in general and of the albumin fraction in particular is a manifestation of hexachloronaphthalene poisoning in cattle.

TABLE 5

Effect of Hexachloronaphthalene on Total TCA Precipitable Plasma Protein in the Calf

Days After Dosage of 6C1ØØ [1]	Animal			
	1898 Control	1892	1880	1870
	gm	gm	gm	gm
0	0.169	0.161	0.153	0.141
5	0.149	0.139	0.130	0.117
10	0.136	0.116	0.130	0.123
13	0.143	0.111	0.127	0.126
17	0.141	0.111	0.128	0.124
22	0.138	0.101	0.125	0.121
29	0.147	died	0.124	0.124
34	--	--	0.154 [2]	--
36	0.159	--	--	--
43	0.143	--	--	0.116
47	--	--	--	0.116

1 Hexachloronaphthalene

2 Died soon after sampling

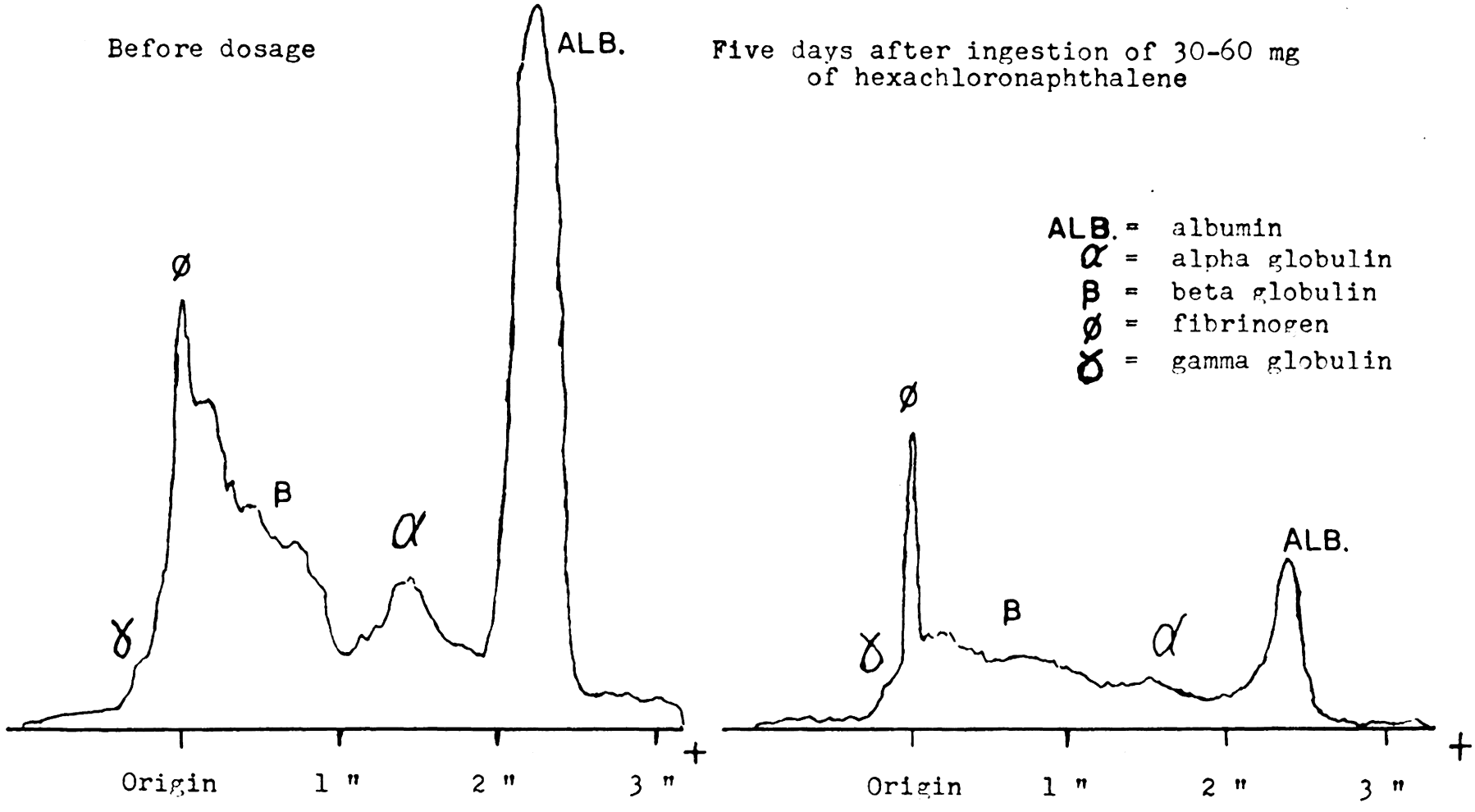


FIGURE 5. TYPICAL ELECTROPHORETIC PATTERNS OF 0.01 ML ALIQUOTS OF CALF BLOOD PLASMA PROTEINS BEFORE AND AFTER INGESTION OF HEXACHLORONAPHTHALENE

CHAPTER III

DISCUSSION OF RESULTS

A number of conclusions can be drawn from an evaluation of the data obtained in this investigation.

In the first experiment, it was apparent that the toxic properties of hexachloronaphthalene were reflected in a disruption of the metabolism of preformed vitamin A in the rat. The appearance of xerophthalmia was hastened in weanling rats with initially low stores of liver vitamin A when only 15 ppm of hexachloronaphthalene was added to the purified vitamin A deficient ration. Dietary supplements of vitamin E failed to alleviate this effect. Despite the more rapid appearance of xerophthalmia in the rats receiving the toxic substance, however, analysis of their livers showed that either approximately equal or slightly higher amounts of vitamin A were in that organ. This suggests that the hexachloronaphthalene does not hasten depletion of the vitamin A store in the liver directly but rather increases the requirement of the vitamin in the peripheral tissues.

In conjunction with this, per cent ether extract was determined for all the rat livers in the first

experiment. No correlation could be ascertained between the per cent ether extract and the liver vitamin A. There did appear to be, however, an inverse relationship between per cent lipid in liver and the more rapid appearance of xerophthalmia. In general, the rats with the most severe xerophthalmia had lower liver lipid. This is further evidence that hexachloronaphthalene exerts its depressant effect on preformed vitamin A through an extra-hepatic mechanism.

A significant increase in vitamin A storage from dietary carotene was observed in the second experiment when a complete, non-toxic ration was supplemented with vitamin E as would be expected. The stimulatory effect of vitamin E on carotene conversion is generally attributed to its ability to shield the carotene from oxidation in the gut (14). When hexachloronaphthalene was added to the ration, however, no stimulation of carotene conversion to vitamin A was evident from vitamin E supplementation. If hexachloronaphthalene were an in vivo pro-oxidant as Hove has suggested (13), the vitamin A stores should have been significantly greater when both the toxic compound and vitamin E

were present, than when only the toxic substance was included in the ration. The failure of vitamin E to induce an increase in liver vitamin A when it was added to the toxic diet implies that although more carotene was presumably made available for absorption and conversion by the intestine, the carotene conversion mechanism could not handle it. It would then appear that ingestion of hexachloronaphthalene lowers the ability of the rat to convert carotene to vitamin A.

The first three studies of the third experiment support the postulation that carotene conversion is less efficient in the rat following the intake of hexachloronaphthalene. A depression of carotene utilization was not demonstrable, however, when the total amount of the chlorinated compound and the dose of carotene were introduced into the stomach simultaneously. It can therefore be concluded that the decrease in carotene conversion following the feeding of hexachloronaphthalene is a consequence of either physiological changes which accompany the metabolism of the compound or the direct action of a resultant

metabolite rather than a specific interaction of carotene with hexachloronaphthalene.

The addition of sodium taurocholate to the carotene solution did not improve the yield of vitamin A in the rats fed the toxic ration. Neither, however, did it increase the yield in the normal rats. The latter observation, since it is in opposition to other work (20), makes it difficult to draw a conclusion from this particular trial.

A similarly designed experiment gave evidence that the ingestion of hexachloronaphthalene did not specifically impair the ability of the rat to absorb and store a single oral dose of vitamin A palmitate. Since the intestinal wall is generally accepted as the principal site of conversion of carotene to vitamin A, it can be concluded that any loss in efficiency of carotene utilization in the previous experiments must have resulted from a disturbance in either the absorption or the conversion of the pro-vitamin.

The observation that plasma protein in calves was depressed following intake of hexachloronaphthalene has stimulated further speculation on the physiological consequences of the metabolism of the

compound. It is logical to assume that either kidney damage, resulting in proteinuria; liver damage, resulting in a reduced rate of plasma protein synthesis; or a combination of the two could produce this effect. An experiment is underway at present with calves which is designed to aid in the elucidation of this problem. It is not unreasonable to relate the plasma vitamin A depression in cattle to the decrease of plasma protein considering the role of the latter in the circulatory transport of the carotenoids.

In summary, it appears that in the course of the metabolism of hexachlorinated naphthalene by the rat, physiological changes occur which among other things bring about an increased tissue requirement for vitamin A and a decreased capacity for carotene assimilation. The fact that a role for vitamin A in the detoxification of dibenzanthracene has been suggested (11), offers the possibility of a similar function in the case of hexachloronaphthalene. The lesions in the liver and bile duct implicate the biliary system as a possible factor in the decreased carotene assimilation despite the continued efficiency of vitamin A palmitate absorption and the failure of added bile salt to

alleviate the depression. The recent report (5) that vitamin A metabolism may be mediated in some respects by cortisone serves to suggest that a possible endocrine malfunction should not be overlooked.

As far as future work is concerned, it seems probable that in vitro tissue studies on the possible role of vitamin A in hexachloronaphthalene detoxification could prove to be fruitful. In vitro comparison of carotene absorption and conversion by intestines of treated and non-treated rats should also contribute valuable information. A more complete understanding of the physiological changes involved in hexachloronaphthalene metabolism will allow the present observations to be fitted into a logical pattern, and should enable us to define more clearly the normal metabolism of vitamin A.

CHAPTER IV

SUMMARY

Experiments were undertaken to study in vivo the effect of hexachloronaphthalene in vitamin A metabolism in the rat. The status of vitamin A in the animal was determined by tissue analysis for the vitamin and by visual observation for symptoms of vitamin A deficiency. Four studies were made. The effects of dietary hexachloronaphthalene on preformed residual vitamin A, on assimilation of dietary carotene, on assimilation of a single oral dose of carotene, and on the absorption and storage of a single oral dose of vitamin A palmitate were investigated. In addition, the effect of ingested hexachloronaphthalene on calf plasma protein was studied.

The following conclusions were derived from the results of the experiments.

1. The metabolism of dietary hexachloronaphthalene resulted in an increased requirement for vitamin A in peripheral tissue. This effect was apparently separate from the liver hypertrophy and fatty infiltration which are characteristic results of chlorinated naphthalene ingestion in rats. The mechanism

of this increased demand for vitamin A was not discerned. Vitamin E supplementation was of no apparent value in preventing this increase in the requirement for vitamin A.

2. The addition of vitamin E to carotene-rich diets normally produces an increased yield of vitamin A. This stimulus of carotene conversion was not apparent when hexachloronaphthalene was included in the ration.

3. The ingestion of dietary hexachloronaphthalene depressed the ability of the rat to convert carotene to vitamin A. This was not true when the total dose of the toxic compound was included in the carotene solution. The addition of a bile salt to the carotene solution was not of value in preventing the depression of carotene conversion. Neither, however, did it stimulate conversion in the normal animals as was expected.

4. The ingestion of dietary hexachloronaphthalene had no apparent effect on the ability of the rat to absorb and store a single oral dose of vitamin A palmitate.

5. Paper electrophoresis studies and analysis of changes in TCA precipitable plasma protein in calves indicated that a depression of the level of the plasma proteins, especially the albumins, was a manifestation of the ingestion of hexachloronaphthalene by these animals.

CHAPTER V

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