THE METABOLISM OF HIGHLY CHLORINATED NAPHTHALENES BY THE ALBINO RAT AND THE CALF

by

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Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute

in candidacy for the degree of

MASTER OF SCIENCE

in

Biochemistry

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March, 1955

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ACKNOWLEDGMENT

Sincere appreciation is expressed to Dr. R. W. Engel, V. P. I., for his guidance; to Dr. K. W. King, V. P. I., for his counsel and encouragement; to Dr. W. B. Bell, V. P. I., for his advice and for supplying the calf tissue; to the staff of the Biochemistry Department, Virginia Agricultural Experiment Station, for their help, particularly to W. N. Linkous, N. O. Price and J. Albert.

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CHAPTER I

THE PROBLEM

I. STATEMENT OF THE PROBLEM

It was the purpose of this research to study, *in vitro* and *in vivo*, the metabolism of highly chlorinated naphthalenes by the albino rat and the calf.

II. LITERATURE PERTINENT TO THE IMPORTANCE OF THE PROBLEM

In 1947, a disease of cattle characterized in part by the development of thick, toughened skin, first on the sides of the neck, back and rump, and then extending down the sides, was reported by Olafson (15). It was designated by him as Bovine Hyperkeratosis, and has been popularily called X-Disease. Lee (13), has estimated that from $2,000,000 to $4,000,000 worth of cattle died or had to be slaughtered, because of this disease, in the United States from 1948 to 1952.

The disease was produced successfully by four independent laboratories in 1951 (17), and through their findings, it was suggested that field outbreaks of the disease were caused by toxic agents in the feed. In 1952, Bell (2), reported that a lubricant was responsible for the disease in one outbreak. Further investigation (3), identified the toxic ingredient as a highly chlorinated naphthalene[1]. In one outbreak in Germany (19), a wood preservative was found to cause the disease. Later, the causative agent also was traced to a chlorinated naphthalene (16). That petroleum products containing these compounds could cause the disease also was shown by Sikes, Wise and Bridges (18). For a review on the nature of X-Disease in cattle see that by Engel and Bell (8).

Reports concerning the pathology of the disease in cattle can be found in the literature (15, 14) and will not be included here. Bennett, Drinker and Warren (5), have described the toxic effects of chlorinated naphthalenes administered to rats, the most prominent symptom being the production of a fatty liver.

The relative toxicities of chlorinated naphthalenes administered to cattle have been reported by Bell (4), who found that on a weight basis, the toxic effects increased with a corresponding increase in chlorine atoms, reaching a maximum toxicity with hexachloronaphthalene. Heptachloronaphthalene was as toxic as the hexa-compound, while octachloronaphthalene showed a decreased toxicity. This latter was believed due to its being absorbed to a lesser extent by the animal. Engel and Hash (10), have described the relative toxicities of these

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1. Chlorinated naphthalenes are used as chemical additives to lubricants, crankcase and cutting oils

compounds in rats, using % liver fat as an index of toxicity. Here again, an increase in chlorine content showed a corresponding increase in toxicity, with heptachloronaphthalene as the most toxic. Administration of vitamin B_{12} had no effect on the toxicity of hexachloronaphthalene.

The effect of protein on the toxicity of hexachloronaphthalene administered to rats has been described by Engel, Linkous and Bell (11). These workers found that the toxicity of hexachloronaphthalene as measured by % liver fat, was greater in rats maintained on low protein diets than in those on diets high in protein.

Since the symptoms of the disease in cattle resembled those seen in vitamin A deficiency, Olafson, McEntee and Hansel (16), determined the plasma and liver carotene and vitamin A concentrations of twenty-four hyperkeratotic calves and one cow. Their findings suggested that these agents in some way impair the conversion of carotene to vitamin A. On the other hand, it is interesting to note that cats, which do not have the enzymes for such a conversion, are none the less susceptible to the toxic effects of the chlorinated naphthalenes. This would seem to suggest that vitamin A itself is the target of the disease producing agent.

III. IMPORTANCE OF THE PROBLEM

Since highly chlorinated naphthalenes have been demonstrated to be at least one cause of hyperkeratosis, the investigation of their metabolism and mode of action is of particular interest since to date, there has been only one other report made of an anti-vitamin A active substance (1). The investigation of chlorinated naphthalene metabolism and method of action may possibly lead to more basic information about vitamin A itself, one of the oldest vitamins, but one about which much information is still lacking. Thus, research on a particular problem may suggest leads to research of a more fundamental type.

To date, only one report has appeared in the literature concerning the metabolism of chlorinated naphthalenes, that by Cleary, Maier and Hitchings (7). These workers postulated that the toxic properties of the compounds in question arise from the liberation of free chlorine during their metabolism, since a rise in urine inorganic chlorine levels was noted after feeding highly chlorinated naphthalenes to dogs. Another possible metabolic pathway was also suggested, that being through the formation of mercapturic acids by conjugation of the chlorinated naphthalenes with cysteine.

The apparent lack of information regarding the metabolism of chlorinated naphthalenes is best ascribed to the lack of a suitable, sensitive method for the determination of these compounds. Engel et al. (12), have described a colorimetric method for such a determination, but it is not readily adaptable to a metabolic study due to its lack of sensitivity.

Blinkenstaff and Callen (6), have recently reported the isolation of pentachloronaphthalene from contaminated cottonseed feed pellets, but their procedure is not suitable for microanalysis.

CHAPTER II

THE INVESTIGATION

I. THE APPROACH TO THE PROBLEM

The study can be divided into three major parts. The first phase of the investigation was designed to study *in vitro*, the metabolism of hexachloronaphthalene, and involved a determination of the amount of uptake of hexachloronaphthalene on incubation with rat and calf liver and kidney tissue. It was the hope that this study would give some insight into the relative amount of the compound the animal is capable of detoxifying. Liver and kidney were chosen as the substrates since they are the major organs of detoxification and because, in both the rat and calf, changes in these organs after chloronaphthalene poisoning are extensive. The study involved the use of tissue slices of these organs, since tissue slices are thought to represent organized surviving tissue, the metabolism of which qualitatively if not quantitatively, represents that of the original tissue.

As a second phase, balance studies to determine the relative amounts of hexa- and octachloronaphthalene absorbed by the rat after feeding, were undertaken in an effort to obtain experimental data relating the absorbability to toxicity.

This *in vivo* study was accomplished by quantitatively examining feces collected after feeding known doses of the compounds.

In the third phase, investigations as to the formation of hydrolyzable conjugates as a means of metabolism by the rat were made. Since naphthalene itself, and also bromobenzene, are metabolized in this manner, it is logical that such could also be the case for the chlorinated derivatives of naphthalene. By comparing the amounts of hexa- and octachloronaphthalene determined before and after acid hydrolysis of urine and fecal extracts, the desired information could be obtained.

II. METHODS

General Statement

The methods utilized for all of the studies followed the same general procedure; extraction of the chlorinated naphthalene from the substrate under investigation, followed by a quantitative determination of the amount present. Since the colorimetric method of Engel *et al*. was unsatisfactory for a metabolic study, another quantitative method had to be devised. This involved the use of the Beckman Universal Spectrophotometer, Model DU. Both hexa- and octachloronaphthalene[1] showed maxima

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1. Both the hexa- and octachloronaphthalene used in the studies contained about 5% of the compounds of adjacent chlorination levels, i.e. penta- and heptachloronaphthalene.

in their absorption spectra at 320 mu., and providing that interfering substances could be eliminated or standardized, as little as 2 ugs. could be determined readily. Standard curves relating optical density at 320 mu. to concentration for hexa- and octachloronaphthalene in petroleum ether are shown in Figures 1 and 2.

Both compounds were found to be quite soluble in petroleum ether, (B.P. = 60° C.), and moderately so in absolute methyl alcohol. These solvents, therefore, were used singly or in combination for the extraction.

Tissue Studies

In brief, the procedure for the tissue study experiments was first, to incubate a known amount of hexachloronaphthalene with liver or kidney slices suspended in Ringer's phosphate buffer, and then to extract the suspension and quantitatively determine the amount of hexachloronaphthalene remaining. From this value, the % loss or uptake of the compound could be calculated readily. After a number of experiments, a two-step extraction with absolute methyl alcohol and petroleum ether was found to give an extract in which interfering substances could be kept at a constant, minimum value. Thus, a standard blank tissue value for each liver and kidney examined could be achieved. A two-step

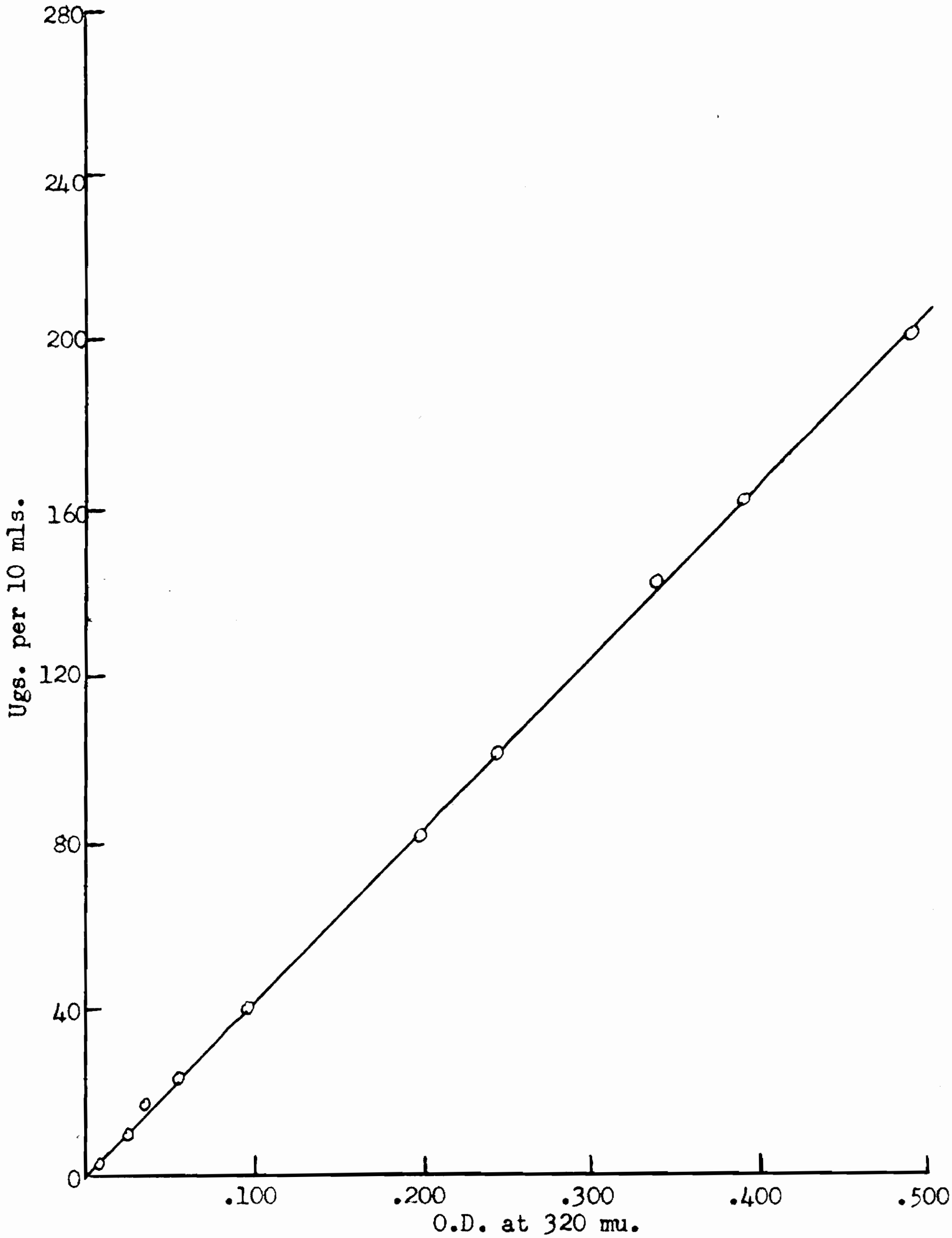

FIGURE 1.
STANDARD CURVE FOR HEXACHLORONAPHTHALENE IN PETROLEUM ETHER

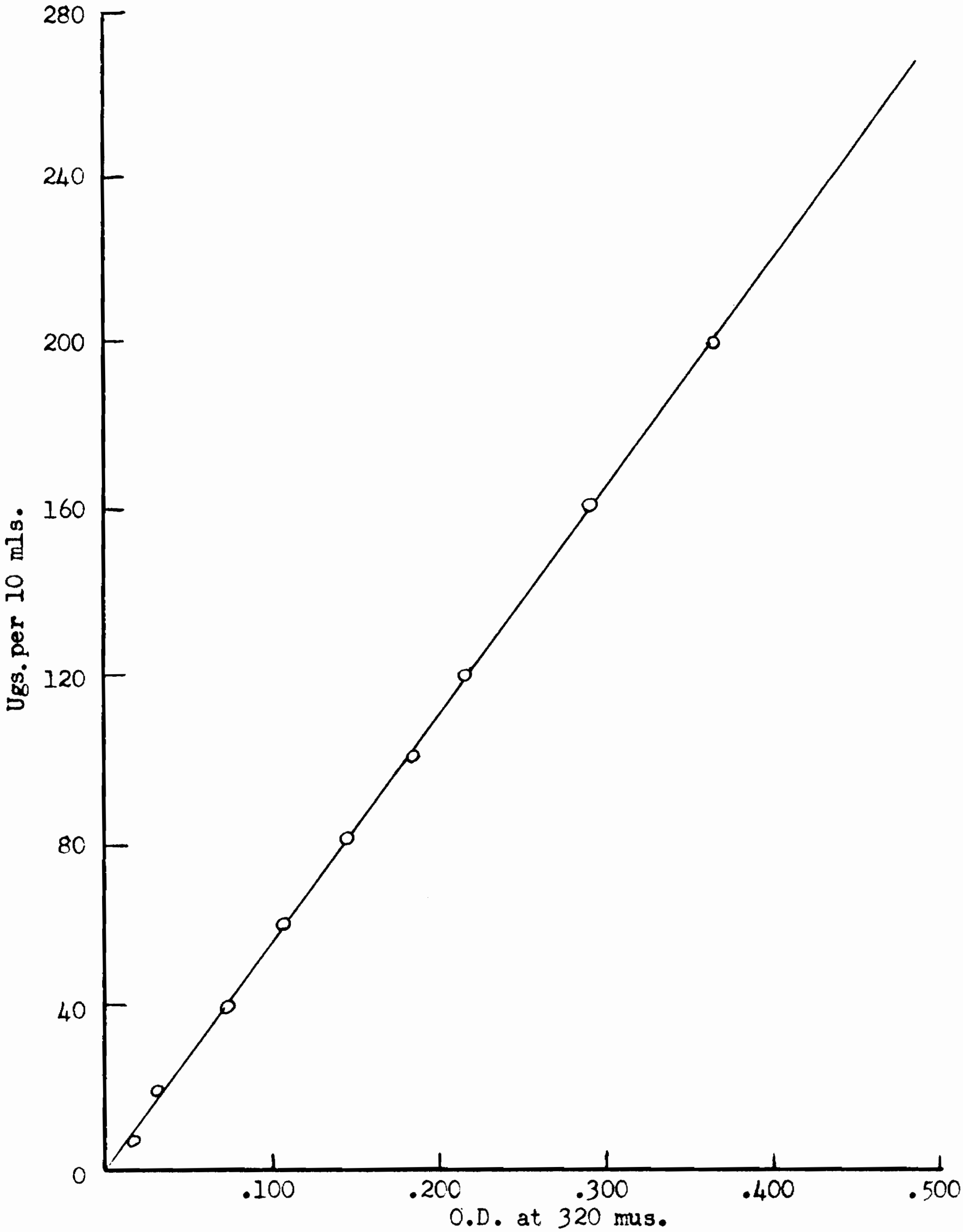

FIGURE 2.
STANDARD CURVE FOR OCTACHLORONAPHTHALENE IN PETROLEUM ETHER

extraction was necessary since a single extraction with petroleum ether invariably gave an emulsion which often gelled on shaking, while the alcohol extract was pigmented. The exact procedure for rat liver and kidney tissue follows:

An albino rat, seven or more weeks old, was killed by severing its jugular vein. After bleeding, the liver and kidneys were removed and placed in chilled Ringer's phosphate buffer. Tissue slices, ranging in weight from 180 to 210 mgs. for liver, and 100 mgs. for kidney, were made on a hand microtome, and each slice placed in a 20 ml. beaker containing 2 mls. of the phosphate buffer and 0.1 ml. of a solution of hexachloronaphthalene in ethyl alcohol, of concentration 400 ugs. per ml. The beakers were placed in a Dubnoff Metabolic Shaking Incubator, pre-set at 37.5° C., and incubated while shaking at 100 oscillations per minute for three hours. The contents of each beaker then were killed by adding 0.1 ml. of 10% HCl to each, and quantitatively transferred to ground glass stoppered tubes. Ten mls. of absolute alcohol were added to each, and the tubes were shaken mechanically for five minutes. Centrifugation in an International Clinical Centrifuge, Model C-50, at top speed, (8-place head), followed, after which an aliquot of exactly 5 mls. of each supernatant was drawn off and placed in another set of glass stoppered tubes. To each of these was added 1 ml. of distilled water and 10 mls. of petroleum ether, and the tubes again shaken for five minutes. After allowing for complete separation of the two layers which formed, an aliquot of the petroleum ether layer of each tube was drawn off, and its optical density determined at 320 mu. against a petroleum ether blank. In each series of slices, three tissue blanks were run, their values averaged and this final value taken as the tissue blank reading for that particular series. By subtracting this value from those obtained from slices incubated with the hexachloronaphthalene, and referring to the standard curve for this compound, the % uptake for each slice could be calculated.

The same procedure was used for liver and kidney tissue obtained from freshly slaughtered calves.

Experiments using slices inactivated with 10% HCl prior to incubation, showed that 100% of the hexachloronaphthalene added to these could be recovered repeatedly by the method described.

Balance Studies

The procedure utilized for the balance studies on the relative absorptions of hexa- and octachloronaphthalene was an adaptation of the method described above. Adult rats were fed known amounts of the compounds in question, their feces collected over twenty-four hour periods, dried and extracted with petroleum ether. The major interfering substances encountered after extraction, pigments due to chlorophylls, carotenes and fecal pigments, were easily removed by the use of absorption chromatography. The optical densities of the chromatographed extracts were determined, and the amounts of chlorinated naphthalenes present were calculated. Detailed procedure follows:

Two adult stock rats weighing approximately 400 grams each were placed in separate cages. A stock rat diet was fed to each, and fecal matter was collected on cheese cloth stretched beneath the wire bottoms of the cages. Table 1 shows the composition of the stock diet.

TABLE 1

COMPOSITION OF THE STOCK RAT DIET

Ingredient	% of Total
Wheat	55.4
Casein	11.8
Meat Scraps	9.8
Milk Powder	7.8
Molasses	4.9
Crisco	4.9
Quadrex	2.9
Alfalfa Meal	2.0
Salt	0.5

The feces were dried at 85° C. for four hours, pulverized and extracted for twenty-four hours in Bailey-Walker extractors with 35 mls. of petroleum ether. After cooling and filtering any precipitate which formed, the extracts were evaporated to dryness under reduced pressure in a hot water bath, and the residues taken up in 4 mls. of petroleum ether. These solutions were chromatographed on 1 cm. X 3 cms. columns of a one to one by weight mixture of activated alumina, (60-100 mesh), and Super-Cel[1]. The columns were washed with six 1 ml. portions of petroleum ether, and the combined eluate fractions made up to 10 mls. with petroleum ether. Their optical densities were then determined at 320 mu.

Fecal blanks were run for three successive days prior to feeding a diet containing the chlorinated naphthalenes. On the fourth day, one rat was given a diet consisting of 5 mgs. of hexachloronaphthalene in 50 gms. of stock feed and the other rat, a diet containing the same amount of octachloronaphthalene in 50 gms. of stock feed. The feces of each were analyzed by the method described until no more of the compounds appeared in the extracts. The amounts of hexa- and octachloronaphthalene recovered were totaled and the % absorption of each was calculated.

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1. Hyflo Super-Cel obtained from Johns-Manville Products Corporation, Baltimore, Maryland.

Experiments with feces to which known amounts of the chlorinated naphthalenes were added, showed that from 99 to 100% of both the compounds could be recovered by this method.

Conjugation Studies

Conjugation studies involving urine and feces were conducted much in the same manner as were the absorption experiments, the only major difference being the addition of an hydrolysis step. In brief, urine and fecal extracts were divided into two halves. The chlorinated naphthalene in one half was determined directly, while the other half was hydrolyzed before determination. If conjugated products were being formed, the amount of chlorinated naphthalene determined after hydrolysis should have shown an increase over that determined before. The exact procedure follows:

Two adult stock rats weighing approximately 400 grams each were placed in separate cages, and total urine and feces were collected for three twenty-four periods prior to feeding diets containing hexa- and octachloronaphthalene. Urine was collected on filter paper sheets placed in the bottom pan of the cage, while feces were collected on cheese cloth suspended under the cages. The papers containing the urine were shredded and extracted for four hours with 100 mls. of petroleum ether in Soxhlet extractors. The feces were pulverized and extracted for twenty-four hours with 35 mls. of petroleum ether in Bailey-Walker extractors. After cooling and filtering any precipitate which formed, the extracts were divided into two halves and each evaporated to dryness under reduced pressure. One of each of the urine and fecal extract residues were taken up in 5 mls. of absolute methyl alcohol, 10 mls. of 2.5N H_2SO_4 were added, and the solutions were boiled for three hours under reflux. After hydrolysis, 5 mls. of distilled water were added to each, and the solutions extracted with 25 mls. of petroleum ether. The ether layers were drawn off, evaporated to dryness and taken up in 4 mls. of petroleum ether. These solutions were chromatographed as previously described, the eluate fractions were made up to 10 mls. and their optical densities were determined.

The residues from the remaining urine and fecal extracts were taken up in 4 mls. of petroleum ether, chromatographed as before, and their eluate fractions made up to 10 mls. Their optical densities then were determined.

The non-hydrolyzed and hydrolyzed values for three successive twenty-four hour periods prior to feeding diets containing hexa- and octachloronaphthalene were averaged, and the average values used as blanks for the hydrolyzed and non-hydrolyzed extracts.

After the third collection, one rat was given a diet consisting of 5 mgs. of hexachloronaphthalene in 50 gms. of stock feed, and the other rat, a diet containing the same amount of octachloronaphthalene in 50 gms. of stock feed. The urine and feces were collected and analyzed in the same manner as were the blanks. By subtracting the blank readings from those obtained after feeding the chlorinated naphthalenes, and referring to the respective standard curves, the amounts of conjugated and free compounds were calculated for each collection period.

Experiments designed to determine the effect, if any, of refluxing alcoholic solutions of hexa- and octachloronaphthalene with 2.5N H_2SO_4 showed that 100% recovery of the compounds could be achieved by the method described.

III. EXPERIMENTAL RESULTS

Tissue Studies

Eight rats were used in the rat tissue studies proper, the total number of liver slices amounting to sixty-six, and the kidney slices to sixteen. The % uptake of hexachloronaphthalene in three hours was calculated for each slice and from these values, the uptake in ugs. per mg. of tissue was determined. Table 2 shows a typical tissue run for a rat liver and kidney. The uptakes for the organs of each animal were averaged and these values are shown in tables 3 and 4.

Liver and kidney tissue from three freshly slaughtered calves was used in the calf studies. The % uptakes and uptakes in ugs. per mg. of tissue in three hours were calculated for each animal. These values are shown in tables 5 and 6. An evaluation of the data readily shows that both rat and calf liver have the capacities for hexachloronaphthalene uptake, that for the calf being three and one-half times that for the rat. Both rat and calf kidney show no uptake of hexachloronaphthalene.

As a check on the reliability of the calf data, a kinetic experiment was made. This involved calf liver which had been stored for twenty-four hours by freezing. During incubation, samples were taken at one, two, three and four hour intervals and the hexachloronaphthalene uptake determined as previously described. Table 7 shows the uptakes for each time interval. No reading for the four hour sample could be obtained due to the disintegration of the slices after three hours incubation. The fact that uptake increased with increasing time indicates that the calf tissue results are reliable.

TABLE 2

A TYPICAL TISSUE RUN FOR A RAT LIVER AND A KIDNEY

Slice #	Wgt. in mgs.	Initial ugs. of Hexa	O.D. After Run	% Uptake
LIVER:				
1	200	0(blk.)	.083	0.0
2	"	16.4*	.120	7.5
3	"	16.4	.123	0.0
4	"	0.0	.079	0.0
5	"	16.4	.122	2.5
6	"	16.4	.119	10.0
7	"	16.4	.122	2.5
8	"	16.4	.120	7.5
9	"	0.0	.084	0.0
10	"	16.4	.120	7.5
11	"	16.4	.123	0.0
12	"	16.4	.119	10.0
13	"	16.4	.122	2.5
14	"	16.4	.121	5.0
Avgs. for 11 slices			.121	5.0
Avgs. for 3 blank slices			.083	0.0
O.D. of Hexachloronaphthalene			.038	
Theoretical O.D. for 16.4 ugs.			.040	
% recovery			95.0%	
% uptake				5.0
KIDNEY:				
1	100	0(blk.)	.001	0.0
2	"	16.4	.042	0.0
3	"	0.0	.001	0.0
4	"	16.4	.040	0.0
5	"	0.0	.001	0.0
6	"	16.4	.041	0.0
7	"	16.4	.042	0.0
Avgs. for 4 slices			.041	0.0
Avgs. for 3 blank slices			.001	0.0
O.D. of Hexachloronaphthalene			.040	
Theoretical O.D. for 16.4 ugs.			.040	
% recovery			100.0%	
% uptake				0.0

* Five ml. aliquot of alcohol extract = 16.4 ugs.

TABLE 3

UPTAKE OF HEXACHLORONAPHTHALENE BY RAT LIVER TISSUE

	Rat	# of Slices	Weight	Avg. % Uptake	Avg. Uptake ug./mg. Tissue
	1	8	220 mgs.	6.66	1.20 x 10^{-2}
	2	8	180 "	4.22	0.94 x "
	3	11	200 "	6.66	1.42 x "
	4	11	200 "	5.00	1.00 x "
	5	11	200 "	10.50	2.17 x "
	6	7	200 "	5.83	1.17 x "
	7	5	200 "	0.00	0.00
	8	5	210 "	7.15	1.36 x "
Totals	8	66		46.02	9.23 x "
Avgs. for 8 rats			201 mgs.	5.75	1.15 x 10^{-2}

TABLE 4

UPTAKE OF HEXACHLORONAPHTHALENE BY RAT KIDNEY TISSUE

	Rat	# of Slices	Weight	Avg. % Uptake	Avg. Uptake ug./mg. Tissue
	1	4	100 mgs.	0.00	0.00
	2	6	100 "	0.84	0.33 x 10^{-2}
	3	6	100 "	0.84	0.33 x "
Totals	3	16		1.68	0.66 x "
Avgs. for 3 rats			100 mgs.	0.56	0.22 x 10^{-2}

TABLE 5

UPTAKE OF HEXACHLORONAPHTHALENE BY CALF LIVER TISSUE

	Calf	# of Slices	Weight	Avg. % Uptake	Avg. Uptake ug./mg. Tissue
	1	8	200 mgs.	21.22	4.32×10^{-2}
	2	8	200 "	18.75	3.95 x "
	3	4	200 "	16.25	3.32 x "
	4	4	200 "	24.95	4.34 x "
Totals	4	24		81.17	15.93 x "
Avgs. for 4 calves			200 mgs.	20.29	3.98×10^{-2}

TABLE 6

UPTAKE OF HEXACHLORONAPHTHALENE BY CALF KIDNEY TISSUE

Calf	# of Slices	Weight	Avg. % Uptake	Avg. Uptake ug./mg. Tissue
1	10	200 mgs.	0.71	0.18×10^{-2}

TABLE 7

KINETIC UPTAKE OF HEXACHLORONAPHTHALENE
BY CALF LIVER TISSUE

Hour	# of Slices	Weight	Avg. % Uptake	Avg. Uptake ug./mg. Tissue
1	4	230 mgs.	15.00	2.61×10^{-2}
2	4	230 "	20.10	3.50 x "
3	4	230 "	24.90	4.34 x "
4	4	230 "	---	-------

Balance Studies

Feces were collected every twenty-four hours until no more of the chlorinated naphthalenes could be detected in the extracts. Table 8 shows a typical absorption run. Each rat was put through the experiment twice, the second run taking place two days after the last traces of the chlorinated naphthalenes disappeared from the extracts. The recovery in ugs. and %, for each twenty-four hour period are shown for each compound in table 9. From these data, it is readily seen that octachloronaphthalene is absorbed to a lesser extent than is hexachloronaphthalene. Figure 3, a plot showing the cumulative % excretion totals for hexa- and octachloronaphthalene for each twenty-four hour period, illustrates this fact more clearly.

Conjugation Studies

Urine and feces were collected over twenty-four hour periods until no more of the hydrolyzed and non-hydrolyzed compounds could be detected in the extracts. Each run was duplicated using the same animal, the second trial taking place on the third day after the last traces of chlorinated naphthalenes disappeared from the previous trial's extracts. The recoveries of conjugated and free compounds, and the total % recoveries for feces are shown in table 10.

TABLE 8

A TYPICAL ABSORPTION RUN

Collection Period (each = 24 hrs.)	Hexa O.D. at 320 mu.	Hexa Recovery in ugs.	Octa O.D. at 320 mu.	Octa Recovery in ugs.
Blk.	.026	0.0	.015	0.0
Blk.	.026	0.0	.015	0.0
Blk.	.026	0.0	.015	0.0
Avg. =	.026	Avg. =	.015	
1	.072	16.8	.160	80.0
2	.281	102.0	.560	300.0
3	.421	158.0	.473	252.0
4	.296	108.0	.251	130.0
5	.026	0.0	.044	16.0
6	.025	0.0	.014	0.0
Total Recovery in ugs.		384.8		778.0

TABLE 9

RELATIVE ABSORPTIONS OF HEXA- AND OCTACHLORONAPHTHALENE
OF 5 MGS. FED TO ALBINO RATS

Collection Period (each = 24 hrs.)	Recovery in ugs. for Each Period			
	Trial I		Trial II	
	Hexa	Octa	Hexa	Octa
1	16.8	80.0	16.8	92.0
2	102.0	300.0	168.0	200.0
3	158.0	252.0	106.0	264.0
4	108.0	130.0	84.0	184.0
5	0.0	16.0	46.0	80.0
6	0.0	0.0	16.0	48.0
Totals 6 (144 hrs.)	384.8	778.0	436.8	868.0
Total % Recovered of 5,000 ugs. fed	7.68	15.57	8.72	17.75
Total % Absorbed of 5,000 ugs. fed	92.32	84.43	91.28	82.64

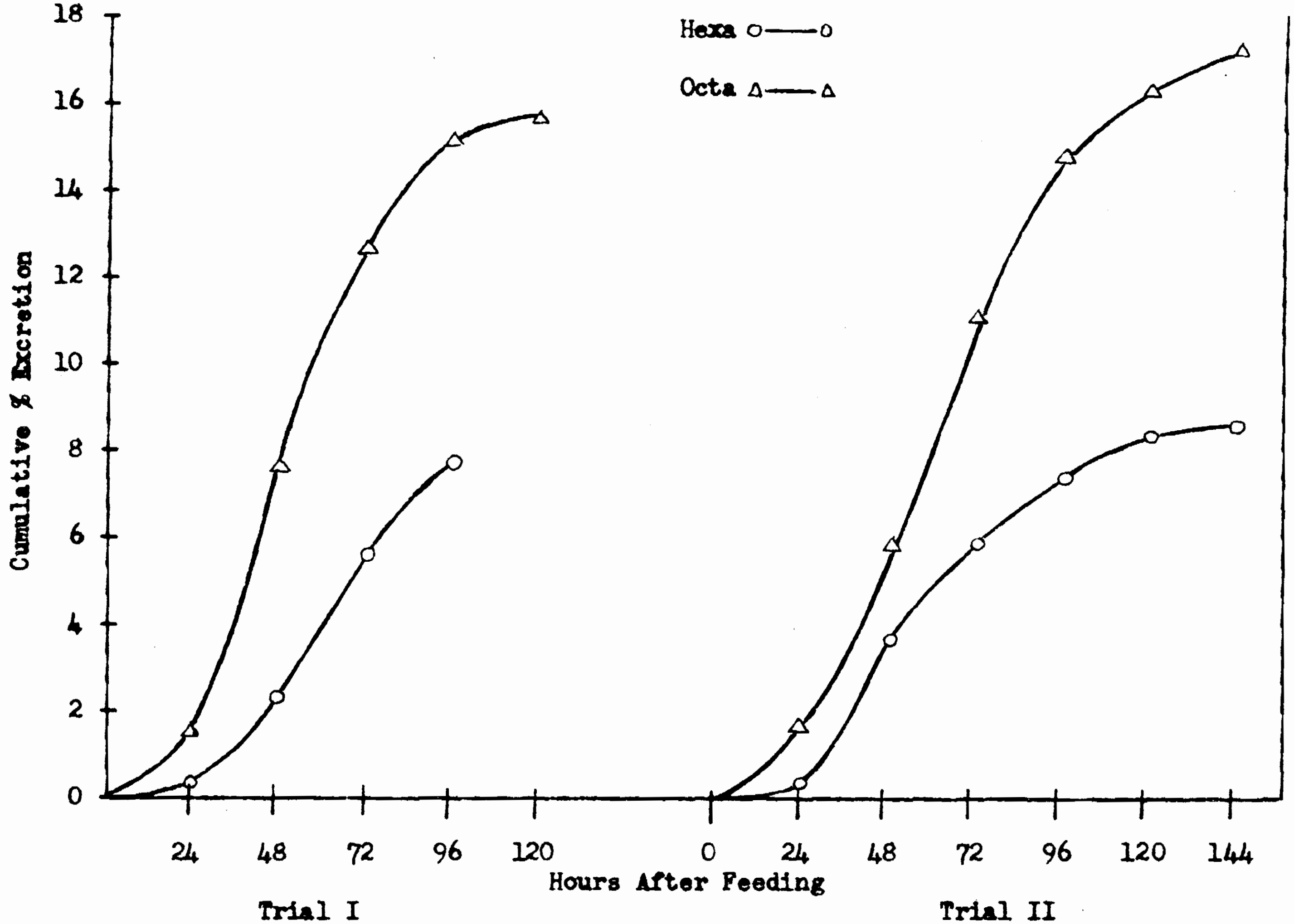

FIGURE 3. PERCENT EXCRETIONS OF HEXA- AND OCTACHLORONAPHTHALENE

TABLE 10

AMOUNTS OF FREE AND CONJUGATED HEXA- AND OCTACHLORONAPHTHALENE RECOVERED IN RAT FECES AFTER FEEDING 5 MGS. EACH

	Trial I				Trial II			
	Hexa		Octa		Hexa		Octa	
Coll. Period (24 hrs.)	Recov. in ugs. Free	Recov. in ugs. Conj.	Recov. in ugs. Free	Recov. in ugs. Conj.	Recov. in ugs. Free	Recov. in ugs. Conj.	Recov. in ugs. Free	Recov. in ugs. Conj.
1	2	24	0	98	20	6	70	69
2	28	69	49	220	56	41	136	93
3	44	86	91	132	78	127	74	193
4	34	18	106	89	21	91	39	128
5	2	14	50	54	0	19	11	132
6	0	0	0	17	0	0	0	77
7	0	0	0	0	0	0	0	8
Total Recovery	110	225	286	610	175	328	330	700
Diff. due to conj.	115		324		153		370	
% conj.	51.1%		53.1%		46.7%		52.8%	
Total % of 5,000 ugs. fed recov. as conj.	4.6%		13.0%		6.1%		15.0%	

No evidences of chlorinated naphthalenes, free or conjugated, appeared in the urine, while the fecal data give definite evidences of conjugation.

In addition to this study, a rat weighing approximately 400 grams was given 15 mgs. of hexachloronaphthalene in corn oil by subcutaneous injection, and the feces were collected and analyzed as before. Here again, evidences of conjugation appeared in the fecal extracts. The amount of conjugation per gram of feces for a seven day trial was approximately 10.9 ugs.

CHAPTER III

DISCUSSION OF RESULTS

The discussion is divided into three sections, each concerned with one of the studies conducted.

I. TISSUE STUDIES

From the tissue studies, three facts are evident. First, both rat and calf liver show uptake of hexachloronaphthalene; second, the uptake by calf liver is three and one-half times that by the rat; and third, no uptake is shown by either rat or calf kidney. Liver then, would seem to be an active metabolizing organ of hexachloronaphthalene for both the rat and calf. If the ability to detoxify and metabolize is correlated with the amount of uptake, it would seem that the rat is not able to detoxify or metabolize hexachloronaphthalene to any great extent. The small amount of conjugate appearing in the feces would seem to bear out this statement. The calf on the other hand, shows a greater amount of liver uptake than does the rat, and, therefore, may be able to detoxify a greater amount of the compound. In this respect, it is interesting to note that in the calf, an accumulation of fat in the liver is not usually encountered in chloronaphthalene poisoning (9), while in the rat, fatty liver production is the most noticeable result of chlorinated naphthalene poisoning. The lesser ability to metabolize on the part of the rat, therefore, may account for the greater apparent liver damage. It would seem then, that the difference in uptakes by the rat and the calf does not imply a difference in metabolic pathways for these two species, but rather an increased capacity for hexachloronaphthalene metabolism on the part of the calf liver.

An interesting fact regarding the nearly negative uptake of hexachloronaphthalene by calf kidney is that in a number of calves autopsied after death from chloronaphthalene poisoning, marked kidney damage was seen. It is possible, of course, that such kidney lesions were secondary effects of liver damage, but the amount of damage would seem to indicate otherwise.

In the tissue studies conducted, only one level of hexachloronaphthalene was used. Further studies with different levels of the entire series of chlorinated naphthalenes may yield more information on uptake by liver and kidney.

II. BALANCE STUDIES

From an evaluation of the balance studies, it can be concluded that the relative absorptions of the highly chlorinated naphthalenes can play at least one role in the toxicities of these compounds. The data bear out the previous suggestions of Bell (4), and Engel and Hash (10).

III. CONJUGATION STUDIES

The data from this study definitely indicate the formation of hydrolyzable, conjugated products as a means of metabolism of hexa- and octachloronaphthalene. The most positive evidence lies in the fact that conjugates appeared in the fecal extracts of the rat subcutaneously injected with 15 mgs. of hexachloronaphthalene. That conjugated products appeared in the feces and not in the urine as do the metabolized products of naphthalene itself, suggests a difference in the modes of metabolism of naphthalene and its chlorinated derivatives. A similar non-appearance of chlorinated, organic compounds in the urine of dogs fed highly chlorinated naphthalenes was reported by Cleary, Maier and Hitchings (7).

The study also shows that the amounts of conjugated products eliminated in the feces are approximately the same for both hexa- and octachloronaphthalene, about 50% of the total recovery after hydrolysis. The difference in the total amounts recovered of the 5 mgs. of each fed, is due to the lesser amount of absorption of the octachloro compound.

The experiments conducted lead to a number of interesting questions regarding chlorinated naphthalene metabolism and mode of action. Those concerning liver and kidney uptake by the rat and calf have been mentioned already. The most noteworthy, however, refers to the fate of the chlorinated naphthalenes absorbed, but not eliminated as conjugates. The relative absorptions of both hexa- and octachloronaphthalene are large in comparison to the amounts of metabolized products eliminated in the feces. What then is the fate of the rest? One possible explanation involves the storage of the unmetabolized compounds after absorption in some body organ, or more logically, in the animal's fat deposits. Certainly, investigations regarding this question are called for.

Furthermore, an investigation of the relative absorptions and conjugations of the entire series of chlorinated naphthalenes may give much information on the metabolism of these compounds. Again, further investigations as to the nature of the conjugated products formed during metabolism undoubtedly would yield valuable information concerning the metabolism of the highly chlorinated naphthalenes.

Concerning future studies, the possible use of isotopic tracers would provide a convenient means of making further investigations, particularly with regard to the question of storage.

Finally, an evaluation of the data as a whole makes it possible to theorize on a probable scheme for the elimination of metabolized chlorinated naphthalenes. From the information at hand, it does not seem illogical to assume that the compounds in question, once absorbed into the animal's blood stream, are carried to the liver where they either are metabolized or sent into storage. The metabolized compounds, conjugates, are passed into the intestines and eliminated in the feces. It is believed that the bile functions in the transport of the metabolized products from the liver to the intestines. The fact that in the calf at least, quite extensive lesions of the gall bladder and common bile duct are seen after chronic chlorinated naphthalene poisoning, indicates that the bile does function in some phase of the metabolism of these compounds. A second indication is that in the calf, the gall bladder may possibly participate in the storage of the chlorinated naphthalenes, both metabolized and unmetabolized. With respect to bile function, it is interesting to speculate on whether the presence of a gall bladder, and, therefore, stored bile, in the calf, may account for the increased uptake and metabolism of hexachloronaphthalene by this animal over that by the rat, an animal having no gall bladder.

It is evident that much work yet remains to be done on the metabolism of the chlorinated naphthalenes, a problem offering many questions of unusual interest.

CHAPTER IV

SUMMARY

Experiments were undertaken to study, *in vitro* and *in vivo*, the metabolism of highly chlorinated naphthalenes by the albino rat and the calf. Three studies were made. The first involved the determination of the amount of uptake of hexachloronaphthalene by rat liver and kidney tissue. Similar studies on calf liver and kidney also were made. Secondly, balance studies were conducted, comparing the relative amounts of hexa- and octachloronaphthalene absorbed after feeding these compounds to rats. Finally, experiments were undertaken to investigate the possible formation of conjugated products as a means of chlorinated naphthalene metabolism.

From the studies, a number of conclusions were derived.

1. In the rat and calf, liver seems to be an active metabolizing or detoxifying organ as evidenced by uptake of hexachloronaphthalene by liver of both these animals. Kidney on the other hand showed no uptake in either animal. Calf liver has a greater capacity for hexachloronaphthalene uptake than does rat liver tissue.

2. Absorbability is at least one factor determining the relative toxicities of the highly chlorinated naphthalenes.

3. The formation of conjugated products, eliminated in the feces, provides at least one means of chlorinated naphthalene metabolism.

4. Finally, a possible scheme for the elimination of metabolized chlorinated naphthalenes was presented.

CHAPTER V

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CHAPTER VI

VITA

Brendan Edward Joyce was born on January 25, 1933, the son of Professor and Mrs. Patrick A. Joyce of North Bergen, New Jersey. He attended Robert Fulton School and Saint Michael's High School, and was graduated from Manhattan College, New York, in June, 1953, with a Bachelor of Science Degree in chemistry. While at Manhattan College, he served as secretary of the Glee Club, and was active in the American Chemical Society Student Affiliate, and in intra-mural sports. After working as a chemist for the Sylvania Electric Products, Inc., Tungsten and Chemical Division, he entered the Virginia Polytechnic Institute, Blacksburg, Virginia, in September of 1953, to begin study for the Master of Science Degree in biochemistry. While at V. P. I., he was Choir Director of Saint Mary's Church, Blacksburg, and is a member of the Phi Lambda Upsilon and Phi Sigma Fraternities.

Brendan Edward Joyce