INFLUENCE OF DIETARY FACTORS AND SEX ON THE TOXICITY OF CARBON TETRACHLORIDE IN RATS*

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Halogenated hydrocarbons such as chloroform, carbon tetrachloride, dichloroethane, trichloroethylene and similar solvents are toxic compounds with affinity for the liver. The degree of their hepatotoxic action appears to be determined to a large extent by the lipid content of the liver. In accordance with this assumption, diet rich in fat increases the susceptibility of hepatic tissue whereas relatively high intake of carbohydrate or protein exerts a protective influence on the liver (1).

In the first papers dealing with the relation of foodstuffs to the toxicity of chloroform (1 *c*, *d*) and carbon tetrachloride (2), diet rich in protein has been regarded as inferior in protective effect to a carbohydrate diet. In contrast, more recent investigations have put protein definitely above carbohydrate with respect to the protective effect against chloroform (1 *e*, 3) or dichloroethane (1 *f*). The protective effect of protein was related to (4) its lipotropic activity, which in turn is determined by its content of methionine. Lipotropic factors are also instrumental in the prevention of purely dietary hepatic injury (4). Here methionine may be replaced by cystine *plus* choline. Cystine, which in itself has antilipotropic activity, given alone ameliorates dietary hepatic necrosis and aggravates dietary cirrhosis of the liver (4). In this connection, it is of interest that Miller and Whipple have asserted that methionine, cystine plus choline and, in a minor degree, cystine alone have a protective effect on the production of chloroform necrosis of the liver in dogs (5). The beneficial effect of methionine as supplement to a diet low in protein and high in fat also could be demonstrated in rats exposed to dichloroethane (1 *f*). Neither in dietary nor in toxic hepatic injury does methionine act by virtue of its lipotropic quality alone. Choline, a more potent lipotropic factor, cannot replace methionine.

The results obtained with chloroform and dichloroethane were not duplicated with carbon tetrachloride. In confirmation of previous claims (2), even in

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recent studies (6), carbohydrate appeared to be superior to protein in the protection of dogs exposed to carbon tetrachloride. This seemingly different reaction to carbon tetrachloride on the one hand, and to chloroform and dichloroethane on the other, required further investigation. Such was the purpose of the present paper.

Materials and Methods

Groups of young rats, approximately 100 gm. in weight, of both sexes, were placed on six different dietary regimens. The diets were fed *ad libitum*, with a record kept of the daily food consumption. The composition of the rations varied in the relative amount of protein (casein), fat (crisco), and carbohydrate (sucrose). When the diets used (Table I) were analyzed in terms of total calories and calories supplied by protein, fat, and carbohydrate, they fell into two distinct divisions: (1) High fat and low carbohydrate group (diets I, V, and VI) with a total caloric intake of 559 calories per 100 gm. of food. (2) Low fat and high carbohydrate group with a total caloric intake of 394 and 434 (average 419 calories) per 100 gm. (diets II, III, and IV). In each of the two divisions the amount of protein was the main variant. Methionine[1] was added to one group in each. This resulted in six different diets. Whereas the relative composition of the rations based on weight-percentage would have indicated much greater variations in the intake of fat (from 10 to 35 per cent) than in that of carbohydrate (from 43 to 78 per cent), the caloric composition showed much closer figures; *i.e.*, from 21 to 56.5 per cent for fat and from 31 to 69 per cent for carbohydrate. Reference to a high or low fat ration implies, by necessity, a low or high carbohydrate diet respectively.

TABLE I

Composition of the Diets

Diet constituent	Group					
	I	II	III	IV*	V	VI*
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Casein	18	18	8	8	8	8
Crisco	35	10	10	10	35	35
Sucrose	43	68	78	78	53	53
Salt mixture	4	4	4	4	4	4
Caloric composition	**I**	**II**	**III and IV**		**V and VI**	
	per cent	*per cent*	*per cent*		*per cent*	
Protein	13	16.5	8		5.5	
Fat	56	21.0	23		56.5	
Carbohydrate	31	62.5	69		38.0	

* Plus daily supplement of 50 mg. of *dl*-methionine.

All animals received a daily supplement of B vitamins, by mouth: thiamine 25 micrograms, riboflavin 40 micrograms, pyridoxine 25 micrograms, and calcium pantothenate 100 micro-

[1] *dl*-Methionine was supplied by Wyeth Incorporated.

grams. Once weekly they received 5 drops of oleum percomorph (6250 I.U. of vitamin A and 900 I.U. of vitamin D) and 1 mg. of α-tocopherol. Rats that received the methionine were given the 50 mg. in 2 ml. of water, down the throat by means of a syringe. Three weeks after the rats were placed on these various diets, the exposure to carbon tetrachloride was begun. This continued throughout the 5 month period, 7 hours a day, 5 days a week. During the day, while in the gas chamber, the animals received only water. Food and vitamin supplements were placed in the individual cages for overnight consumption. The concentration of carbon tetrachloride[2] in the air of the gas chamber was maintained at a level of approximately 300 p. p. m.

The exposures were made in a galvanized iron cylinder fitted with a plate glass cover. Air-carbon tetrachloride mixture was pulled through the chamber at the rate of 350 liters per minute. The mixing was accomplished by drawing a small fraction of the air through a weighed amount of carbon tetrachloride in a constant leveling flask and diluting it with the rest of the incoming air in a mixing device attached to the chamber. The average daily concentrations of carbon tetrachloride were calculated from the amount of it volatilized during the period of exposure. Standardization of the apparatus in previous experiments showed that concentrations determined in this manner are within $\pm$ 5 per cent of the concentrations obtained by direct chemical analysis of the vapors. The chemical analyses in these previous experiments were made by passing the carbon tetrachloride mixture over a heated platinum wire and determining released chloride. These experiments also indicated that mixing was thorough throughout the chamber.

As animals died, they were replaced. At the end of the 5 month (22 weeks) period, all animals, originals and replacements, were killed. Only animals which survived 5 months and were killed, or animals which died before the end of the experimental period of 5 months, were considered for the final analysis of the findings obtained. Rats which were killed before the end of the experimental period of 5 months were discarded. Every experimental animal was autopsied; the liver, kidney, heart, and lungs were removed, preserved in 4 per cent formaldehyde solution made isotonic with NaCl, and histologically examined.

FINDINGS

Even before the histological investigation was concluded, the number of fatalities, weight changes, and food consumption of the various groups gave some indication as to the effect of diet (methionine in particular), and to some extent also to that of sex, in alleviating or aggravating the toxic effect of carbon tetrachloride. In Tables II and III are presented data on changes of body weight and monthly food consumption in the various groups. Tables IV to IX contain all the individual data on death and survival, together with the results of the histological examination. In Tables II and III are included only those rats with which the experiments were started, without later replacements. In Tables IV to IX, replacements (marked with A or B after their numbers) were also considered, provided they died before the end of the experimental period of 150 days. Table X gives the summary of all histological data from Tables IV to IX, together with the number of rats surviving 5 months, divided in each group according to sex.

[2] Through the kindness of Dr. P. A. Neal carbon tetrachloride (Merck) was analyzed at the National Institute of Health, Bethesda, Maryland, and placed at our disposal.

The analysis of all pertinent findings, as given in Tables II to X, and based on statistical significance (by virtue of obvious differences or chi-square test), is consistent with the conclusion that lowering protein and increasing fat content both tend to aggravate the toxicity of carbon tetrachloride. The rats of group II, receiving what would be considered a normal diet (with high protein, low fat, and high carbohydrate), showed a steady increase in weight, consumed

TABLE II

Average Weight of the Rats

Group	Diet	Sex	At start	1st month	2nd month	3rd month	4th month	5th month
			gm.	gm.	gm.	gm.	gm.	gm.
I	Casein 18 Crisco 35 Sucrose 43	9 ♂ 6 ♀	105 101	122 (9)* 112 (6)	130 (8) 109 (6)	154 (7) 136 (5)	165 (5) 155 (4)	145 (2) 150 (4)
II	Casein 18 Crisco 10 Sucrose 68	8 ♂ 7 ♀	102 62	148 (8) 104 (7)	172 (8) 127 (7)	182 (8) 149 (7)	198 (8) 163 (6)	206 (8) 165 (6)
III	Casein 8 Crisco 10 Sucrose 78	8 ♂ 7 ♀	138 119	131 (8) 118 (7)	144 (6) 119 (6)	152 (6) 125 (5)	166 (5) 142 (4)	176 (3) 135 (1)
IV	III + 50 mg. methionine daily	8 ♂ 7 ♀	138 117	150 (8) 120 (7)	172 (6) 131 (7)	180 (6) 136 (7)	197 (5) 143 (7)	204 (5) 139 (7)
V	Casein 8 Crisco 35 Sucrose 53	8 ♂ 7 ♀	149 116	136 (8) 107 (7)	136 (1) 102 (7)	130 (1) 92 (6)	107 (1) 88 (3)	— (0) — (0)
VI	V + 50 mg. methionine daily	8 ♂ 7 ♀	135 102	142 (8) 117 (7)	135 (8) 110 (7)	144 (7) 130 (4)	137 (7) 131 (4)	131 (5) 133 (4)

* Number in parentheses indicates number of rats alive.

more food than those of other groups, and had the least number of fatalities, only one out of the 15 rats dying before the end of 5 months. The harmful effect of a high fat:carbohydrate ratio with unchanged high protein intake (group I) is shown by the decrease in the survival rate: only 6 out of 17 animals survived the test. In group III the protein intake is lowered and the $\frac{\text{Fat}}{\text{Carbohydrate}}$ ratio is kept on a low level. In this group only 4 out of 16 experimental animals survived 5 months. The additional supplement of methionine to this diet low in casein and fat increased the number of survivors to 12 out of 16 (group IV). This is evidence that methionine was chiefly respon-

sible for the protective effect of casein, or at least in the present experiments methionine added to a diet low in casein behaved as a diet rich in casein. In this connection it is of special interest (see Tables II and III) that gain in weight and food intake improved considerably when methionine was added to the diet low in casein, both reaching figures almost identical with those observed in group II (high casein—low fat diet). When, in addition to low protein, the

TABLE III

Average Monthly Food Consumption

Group	Diet	No. of rats	1st month	2nd month	3rd month	4th month	5th month
			gm.	gm.	gm.	gm.	gm.
I	Casein 18	9 ♂	173 (9)*	190 (8)	226 (7)	222 (5)	213 (2)
	Crisco 35	6 ♀	142 (6)	153 (6)	167 (5)	206 (4)	195 (4)
	Sucrose 43						
II	Casein 18	8 ♂	296 (8)	269 (8)	269 (8)	289 (8)	296 (8)
	Crisco 10	7 ♀	213 (7)	261 (7)	233 (7)	239 (6)	260 (6)
	Sucrose 68						
III	Casein 8	8 ♂	225 (8)	223 (6)	245 (6)	231 (5)	205 (3)
	Crisco 10	7 ♀	205 (7)	197 (6)	183 (5)	177 (4)	124 (1)
	Sucrose 78						
IV	III + 50 mg. methionine	8 ♂	234 (8)	240 (6)	262 (6)	271 (5)	308 (5)
	daily	7 ♀	186 (7)	215 (7)	219 (7)	245 (7)	246 (7)
V	Casein 8	8 ♂	200 (8)	166 (1)	221 (1)	194 (1)	— (0)
	Crisco 35	7 ♀	152 (7)	164 (7)	147 (6)	117 (3)	— (0)
	Sucrose 53						
VI	V + 50 mg. methionine	8 ♂	189 (8)	195 (8)	205 (7)	226 (7)	224 (5)
	daily	7 ♀	163 (7)	164 (7)	146 (4)	196 (4)	209 (4)

* Number in parentheses indicates number of rats alive.

fat content was increased, as in group V, the rats showed the most pronounced susceptibility to exposure to carbon tetrachloride. None of the 22 rats fed this diet of 8 per cent casein and 35 per cent crisco survived for a period of 150 days. A supplement of methionine to this diet, as in group VI, permitted 9 out of 15 animals to live up to 150 days. Rats in group V (high fat—low protein) lost weight steadily, with an average loss of 30 gm. at the end of the 4th month. With a supplement of methionine to this diet (group VI) the males maintained their weight and the females gained an average of 31 gm.

Suggestive evidence for influence of sex on survival was presented in group I (Table IV), group IV (Table VII), and group V (Table VIII). In these groups

TABLE IV

Group I

Casein 18, crisco 35, sucrose 43, salt mixture 4.

Rat No.	Sex	Survival	Liver			Kidney	Lungs
			Fatty infiltration	Injury (parenchymatous)	Cirrhosis		
97	♂	Died 7th wk.	..... Not examined				
3	♂	" 11th "	++ to +++	+ (h.d.)	+	—	—
97A	♂	" 13th "	+ to ++	—	+ to ++*	N.n.	Interst. pn.
7	♂	" 15th "	++	—	++ to ++++	N.n.	—
73	♂	" 18th "	++	—	++	N.n.	Interst. pn.
5	♂	" 19th "	++	+ (r.n.)	++ to +++	N.n.	—
9	♂	" 19th "	++	+ (f.n.)	+++ to ++++	N.n.	—
75	♂	" 21st "	++ to +++	—	+++ to ++++	N.n.	—
1	♂	Killed 22nd "	+ to ++	+ (h.d.)	+ to +++	—	—
11	♂	" 22nd "	+ to ++	+++ (h.d.)	++	N.n.	—
8A	♀	Died 4th "	++	—	—	N.n.	—
8	♀	" 14th "	+++	—	+ to ++	—	Hemorrhage
74	♀	" 18th "	++	—	+ to ++*	N.n.	Interst. pn.
2	♀	Killed 22nd "	++ to +++	—	+ to ++	—	—
4	♀	" 22nd "	++ to +++	—	+ to +++	—	—
6	♀	" 22nd "	++ to +++	+++ (h.d.)	+ to ++	—	—
10	♀	" 22nd "	++ to +++	+ (h.d.)	++ to +++	N.n.	—

* Also typical proliferation of bile ducts.

h.d., hydropic degeneration (periportal). N.n., necrotizing nephrosis. Interst. pn., interstitial pneumonia. r.n., residual necrosis. f.n., focal necrosis.

TABLE V

Group II

Casein 18, crisco 10, sucrose 68, salt mixture 4.

Rat No.	Sex	Survival	Liver			Kidney	Lungs
			Fatty infiltration	Injury (parenchymatous)	Cirrhosis		
37	♂	Killed 22nd wk.	+ to ++	—	+	—	—
39	♂	" 22nd "	++ to +++	—	+ to ++	—	—
41	♂	" 22nd "	++ to +++	—	+ to ++	—	Atelect.
43	♂	" 22nd "	+ to ++	+ (h.d.)	+	—	—
45	♂	" 22nd "	+ to ++	+ (h.d.)	—	—	Atelect.
47	♂	" 22nd "	+	—	+	—	—
85	♂	" 22nd "	++ to +++	—	+	—	Atelect.
87	♂	" 22nd "	++	—	—	—	Atelect.
38	♀	Died 14th "	++	—	—	N.n.	Bronchopn. with abscess
40	♀	Killed 22nd "	++ to +++	—	+	—	Atelect.
42	♀	" 22nd "	++	—	—	—	—
44	♀	" 22nd "	++	—	—	—	Atelect.
46	♀	" 22nd "	+ to ++	—	+	—	—
48	♀	" 22nd "	++ to +++	—	++	—	Atelect.
80	♀	" 22nd "	++	—	—	—	—

TABLE VI

Group III

Casein 8, crisco 10, sucrose 78, salt mixture 4.

Rat No.	Sex	Survival	Liver			Kidney	Lungs
			Fatty infiltration	Injury (parenchymatous)	Cirrhosis		
19A	♂	Died 1st wk.	+ (r.n.)	++ (h.d.)	—	—	—
79	♂	" 5th "	Not examined				
19	♂	" 7th "	++	++	—	N.n.	Edema
23	♂	" 13th "	++	++++ (h.d.)	—	N.n.	Atelect.
77	♂	" 18th "	+++	—	+ to ++	N.n.	—
15	♂	" 19th "	+++	—	+ to ++	N.n.	—
13	♂	Killed 22nd "	++	—	± to +	—	—
17	♂	" 22nd "	+++	—	+ to +++	—	—
21	♂	" 22nd "	++	—	—	—	Bronchopn.
24	♀	Died 3rd "	+++	—	+	—	—
18	♀	" 9th "	+++	—	—	N.n.	—
14	♀	" 17th "	+++	—	++	—	—
20	♀	" 18th "	+++	—	—	N.n.	—
76	♀	" 19th "	++	—	—	N.n.	—
22	♀	" 19th "	+ to +++	—	—	N.n.	—
16	♀	Killed 22nd "	+++	—	+ to ++	—	—

TABLE VII

Group IV

Casein 8, crisco 10, sucrose 78, salt mixture 4 plus methionine.

Rat No.	Sex	Survival	Liver			Kidney	Lungs
			Fatty infiltration	Injury (parenchymatous)	Cirrhosis		
31	♂	Died 8th wk.	++++	—	—	—	—
35	♂	" 8th "	—	—	—	—	Atelect.
35A	♂	" 8th "	—	—	—	—	—
83	♂	" 8th "	++	—	+++	—	—
25	♂	Killed 22nd "	++	++ (h.d.)	+ to ++	—	—
27	♂	" 22nd "	++ to +++	—	+	—	—
29	♂	" 22nd "	++	+ (h.d.)	++	—	Atelect.
33	♂	" 22nd "	++ to +++	—	+	—	—
81	♂	" 22nd "	+++ to ++++	—	—	—	Atelect.
26	♀	Killed 22nd "	++ to +++	—	+	—	—
28	♀	" 22nd "	++ to +++	+ (h.d.)	± to +	—	—
30	♀	" 22nd "	+++	—	++	—	—
32	♀	" 22nd "	+++	+ (h.d.)	+ to ++	—	—
34	♀	" 22nd "	++ to +++	—	+	—	—
36	♀	" 22nd "	++	—	+	—	—
78	♀	" 22nd "	++	—	—	—	Atelect.

TABLE VIII

Group V

Casein 8, crisco 35, sucrose 53, salt mixture 4.

Rat No.	Sex	Survival	Liver			Kidney	Lungs
			Fatty infiltration	Injury (parenchymatous)	Cirrhosis		
53A	♂	Died 1st wk.	..... Not examined				
49A	♂	" 4th "	+	—	—	N.n.	Pleuritis
49	♂	" 7th "	+++	—	—	N.n.	—
53	♂	" 7th "	++	—	—	N.n.	Interst. pn.
57	♂	" 7th "	+++	—	—	N.n.	—
55	♂	" 8th "	++	—	—	N.n.	—
59	♂	" 8th "	+++	—	—	N.n.	—
59A	♂	" 8th "	+	++ (h.d.)	—	N.n.	Edema
51	♂	" 9th "	++ to +++	—	—	—	—
91	♂	" 9th "	±	—	—	N.n.	Bronchopn.
53B	♂	" 13th "	+++	—	—	N.n.	Interst. pn.
91A	♂	" 14th "	+++ to ++++	—	+	N.n.	—
57A	♂	" 15th "	+ to ++	++++ (h.d.)	+++ to ++++	N.n.	Interst. pn.
89	♂	" 18th "	++ to +++	—	—	N.n.	—
60A	♀	" 9th "	++	—	+	N.n.	Interst. pn.
60	♀	" 12th "	+ to ++	—	± to ++	—	—
56	♀	" 15th "	++	—	—	N.n.	Bronchiectasis
82	♀	" 15th "	—	—	+	—	—
54	♀	" 16th "	+ to ++	++ (r.n.)	+++	N.n.	Interst. pn.
52	♀	" 17th "	+	—	+	—	—
50	♀	" 19th "	±	—	++	—	—
58	♀	" 20th "	+	—	++ to +++	N.n.	—

TABLE IX

Group VI

Casein 8, crisco 35, sucrose 53, salt mixture 4 with methionine.

Rat No.	Sex	Survival	Liver			Kidney	Lungs
			Fatty infiltration	Injury (parenchymatous)	Cirrhosis		
65	♂	Died 12th wk.	+	—	—	—	—
67	♂	" 16th "	±	—	++ to +++	N.n.	Interst. pn.
61	♂	" 19th "	+++	—	+++ to ++++	N.n.	Interst. pn.
63	♂	Killed 22nd "	++	—	++++	—	Atelect.
69	♂	" 22nd "	+	—	++ to +++	—	—
71	♂	" 22nd "	++ to +++	—	+	—	—
93	♂	" 22nd "	++ to +++	—	+	—	—
95	♂	" 22nd "	+ to ++	—	+ to ++	—	—
70	♀	Died 10th "	+	—	—	—	—
70A	♀	" 11th "	++	—	—	N.n.	—
66	♀	" 12th "	±	—	+	—	—
62	♀	" 12th "	—	—	+	—	Bronchopn. with abscess
64	♀	Killed 22nd "	++	—	+	—	—
68	♀	" 22nd "	++ to +++	—	+ to +++	—	Atelect.
72	♀	" 22nd "	+++	—	+	—	—
84	♀	" 22nd "	+ to ++	—	+ to ++	N.n.	—

female rats showed better survival rate than males of the same groups. For instance, in group V (Table VIII) 10 out of 14 males died during the first 9 weeks of the experiment; while of 8 females of the same group, only 1 died.

With regard to the histological examination of liver, kidneys, and lungs all pertinent data are recorded in Tables IV to X. No pathological changes were found in the heart muscle of any of the experimental animals. The histological

TABLE X

Group	Diet	Sex	No. of rats in group*	No. of rats surviving 150 days	Liver					Kidney (necrotizing nephrosis)	Lungs (Atelectasis edema pneumonia)
					Necrosis (including hydropic degeneration)		Cirrhosis				
					Without cirrhosis	With cirrhosis	0	+	++ to ++++		
I	Casein 18 Crisco 35 Sucrose 43	♂ ♀	9 (8) 7	2 4	0 0	5 2	0 1	1 0	7 6	7 3	2 2
II	Casein 18 Crisco 10 Sucrose 68	♂ ♀	8 7	8 6	1 0	1 0	2 4	4 2	2 1	0 1	4 1
III	Casein 8 Crisco 10 Sucrose 78	♂ ♀	9 (8) 7	3 1	3 0	0 0	4 4	1 1	3 2	4 4	3 0
IV	The same as for III, plus methionine (50 mg. daily)	♂ ♀	9 7	5 7	0 0	2 2	4 1	2 4	3 2	0 0	3 1
V	Casein 8 Crisco 35 Sucrose 53	♂ ♀	14 (13) 8	0 0	1 0	1 1	11 1	1 3	1 4	12 4	6 3
VI	The same as for V, plus methionine (50 mg. daily)	♂ ♀	8 8	5 4	0 0	0 0	1 2	2 4	5 2	2 2	3 2

* Figures in parenthesis represent number of rats in the particular group which were examined microscopically.

manifestations of injury caused by exposure to carbon tetrachloride were to a large extent identical with those seen in dietary injury of the liver and kidneys (4), such as fat infiltration, focal and residual necrosis, portal cirrhosis of the liver, and necrotizing, mainly cortical, nephrosis. A striking difference, however, was the pronounced periportal hydropic degeneration which was encountered in the liver of several rats in most of the groups. This change resembles the effect of inhalation of chloroform on the liver.

For completeness of the histologic report the presence and extent of fat infiltration have been recorded in Tables IV and IX. Such data, however, are

not a valid substitute for quantitative chemical analysis of fat deposition, and may have only minor qualitative significance. In consequence, they were omitted from Table X which contains the summary of all pertinent findings. For the statistical analysis of hepatic injury in the various groups only necrosis and cirrhosis have been considered.

Ceroid was found only in traces and then only in a few of the cirrhotic livers. This was probably due, not to the fact that the cirrhosis was of toxic origin, but to the absence of cod liver oil in the dietary forms used. Without cod liver oil in the ration, even pure dietary cirrhosis of the liver will show no, or only a small amount of, ceroid (4, 7).

Hydropic degeneration, central and midzonal, was described by Cameron and Karunaratne (8) in the livers of rats receiving carbon tetrachloride by injection, in contrast to the periportal distribution of the hydropic degeneration in the present series of animals exposed to carbon tetrachloride by inhalation. Hydropic degeneration has also been found in the liver of dogs exposed to trichloroethylene (9).

Whereas a high casein diet with low, or even high, fat content lends protection against dietary hepatic and renal injury (4), yet no complete protection was achieved in animals fed such diet forms and exposed to carbon tetrachloride. Some prevention of pathological changes in the liver and almost complete protection of the kidneys were apparent in group II, with high casein—low fat—high carbohydrate (Table V). With increasing fat intake, protection broke down almost completely (group I, Table IV). Necrosis, cirrhosis of the liver, and nephrosis were more marked in males than in females in this group.

On a diet low in casein and fat (group III) the histological changes were more pronounced than in rats fed a diet high in casein and low in fat (group II), necrotizing nephrosis being practically absent in the latter and well represented in group III. Males in group III appeared to show more severe hepatic manifestations than females in the same group. Addition of methionine (group IV) increased the survival rate, suppressed the nephrotic changes completely, but had only slight if any beneficial influence on the hepatic injury.

On a ration commonly employed in the production of dietary hepatic injury (low casein—high fat) the very high mortality of rats exposed to carbon tetrachloride appeared to be based more on pathologic changes in the kidneys than in the liver. Necrotizing nephrosis was an almost constant finding in the males (in 12 out of 13 microscopically examined animals), but was encountered only in 4 out of 8 females (Table VIII). The concentration of carbon tetrachloride seemed to be too low for intensive necrotizing injury of the liver; and, for the manifestation of fibrotic changes, most of the experimental rats (group V) did not survive for a sufficiently long period of time (8). It is of especial interest that, with added methionine, the deleterious effect of the low casein—high fat diet was considerably alleviated (group VI, Table IX). Necrotizing nephrosis

became a rare finding, with distinctly prolonged survival of the experimental animals. In contrast, cirrhosis, especially in males, was only slightly, if at all, benefited by methionine. In females, the protective effect of methionine on the liver was more definite. The hepatic changes in both sexes were definitely milder in group VI (Table IX), with low casein–high fat and methionine, than in group I (Table IV) with high casein–high fat.

DISCUSSION

In broad generalization, the experiments with carbon tetrachloride here reported confirm the observations in the literature on the toxicity of chloroform (1 *e*, 3, 5) and dichloroethane (1 *f*) in relation to various dietary factors. Lowering of the protein (casein) intake exerts the most deleterious influence. High fat intake with correspondingly low carbohydrate intake also increases the susceptibility of rats to carbon tetrachloride. Harmful effect of fat means beneficial effect of carbohydrate and it appears to be impossible to decide which of these dietary constituents has the direct influence: fat or carbohydrate. Low protein and high fat, singly or in combination, shorten the survival period of the experimental animals and are conducive to the production of necrosis and cirrhosis of the liver, and to the development of necrotizing nephrosis. Conversely, lowering of fat intake and increasing protein counteract, to some extent, the toxic manifestations of exposure to carbon tetrachloride.

Detoxification achieved by high protein intake can also be accomplished by substitution of methionine for protein.

Throughout the experiments, dietary factors showed more definite correlation with the renal than with the hepatic changes. Cirrhosis was present even in animals fed a diet high in protein, or a diet low in protein but supplemented with methionine. Under the same experimental conditions, necrotizing nephrosis was practically eliminated. It is of interest that methionine in the dose used (50 mg. daily), added to a basal diet low in protein and *high* in fat (group VI), had a more pronounced prophylactic effect with regard to prevention of renal and hepatic changes and, at least in males, even to survival, than a diet rich in protein. This result is in good accord with observations on the lipotropic effect of free methionine *versus* bound methionine (protein) in growing and mature animals. Free methionine proved to be more effective than an equal amount of bound methionine (in form of protein) when used in growing animals (10 *a*, *e*). This finding was explained by the assumption that the amount of dietary methionine available for lipotropic action is limited by the amount used in growth.

In contrast to the above observations free methionine when added to a low protein and *low* fat diet was not so effective as a high protein (bound methionine)–low fat diet (group IV, Table VIII). At the same time rats of this group receiving diet supplemented with methionine showed a very satisfactory

growth rate. It is conceivable, therefore, that a larger portion of free methionine was used by animals of this group for growth, than by rats receiving low protein–high fat diet and supplements of free methionine.

The pathologic changes in the lungs were apparently independent of dietary factors.

The combination of hepatic and renal injury, known as the "hepatorenal syndrome," is a common occurrence in rats in which the relevant changes are elicited by purely dietary means (4). Exposure to carbon tetrachloride represents only an aggravating factor which makes the dietary management of the "syndrome," especially in its hepatic manifestations, more difficult than in ordinary dietary injury.

In applying the findings of the present experiments to the condition of carbon tetrachloride poisoning in man, supplements of methionine and of methionine-containing protein appear to be the most suitable dietary directions, especially with regard to the renal changes. Nephrosis is often the leading symptom in acute or more protracted exposure to carbon tetrachloride in man (11). Its possible management by dietary means is worthy of consideration.

In several groups included in the present study, especially in those with a high fat content, sex appeared to be of special influence on susceptibility to carbon tetrachloride. This influence manifested itself in several ways and, taking one given group, was often limited to one or two but not to all specific manifestations of lowered resistance to carbon tetrachloride. These observations gain in their statistical significance only by the fact that when present they were found in the various groups always in the same direction: male rats showed in general lower survival rate, higher incidence of renal changes and—to a lesser extent—of hepatic changes than female rats.

These observations are in good accord with the protective effect of estrone, supplied in the form of pellets implanted in the spleen of female spayed rats, on the production of dietary cirrhosis of the liver (12).

Griffith (13) noticed greater difficulty in producing "choline deficiency," with hemorrhagic cortical necrosis of the kidneys, in female rats less than 30 days of age, in contrast to male rats of the same age group. Weichselbaum (14) reported much higher incidence of death (due presumably to hemorrhagic hepatic necrosis) in male rats fed a diet low in sulfur-containing amino acids (cystine and methionine) than in female rats kept under identical experimental conditions. After oral administration of chloroform, Eschenbrenner (15) found necrotizing nephrosis in male mice but not in females. The same author attempted (16) to correlate this observation on susceptibility of chloroform necrosis to special morphological characteristics and sex differences of the mouse kidney. Normal females and castrated males showed no kidney necrosis after ingestion of chloroform, and a large percentage of Bowman's capsules in their kidneys were lined with squamous cells. Conversely, normal males, and castrated males treated with testosterone, responded with necrotizing nephrosis to ingestion of chloroform and a large percentage of Bowman's capsules in their kidneys

were lined with cuboid cells. Necrosis when present was limited to portions of the proximal and distal convoluted tubules and was not observed in Bowman's capsules or in the upper portions of the proximal convoluted tubules.

Dietary factors in the prevention of renal changes caused by chloroform in mice were apparently not investigated by Eschenbrenner.

The influence of sex on hepatorenal syndrome of dietary or toxic origin is suggestive enough to stimulate further study of the interrelation of sex hormones and dietary factors.

SUMMARY

Six groups of rats on different diets were exposed to the inhalation of carbon tetrachloride (about 300 p. p. m.) for 150 days. Food intake and changes in weight were followed throughout the experiment. Animals fed a diet low in protein showed greater susceptibility than rats on a diet high in protein. Methionine was a good substitute for protein (casein) in the diet. Increase in fat intake with correspondingly lower carbohydrate intake exerted a harmful effect, especially evident in combination with a low protein diet. In this change of the fat:carbohydrate ratio, whether the increased fat or the lowered carbohydrate is the specific factor must remain unanswered at the present time.

Necrotizing nephrosis was the presenting sign of the intoxication caused by carbon tetrachloride, in addition to hepatic changes, such as hydropic degeneration, necrosis, and cirrhosis. Dietary factors (methionine and methionine-containing protein, as well as low fat intake) more consistently prevented renal injury than cirrhosis of the liver.

Under identical dietary conditions, especially with higher fat intake, male rats appeared to evince greater susceptibility to carbon tetrachloride than female rats. The significance of this observation and its wider applicability has been discussed.

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