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DIETARY MODIFICATION OF METABOLISM AND TOXICITY OF CHEMICAL SUBSTANCES—WITH SPECIAL REFERENCE TO CARBOHYDRATE

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Abstract—Rats were fed various test diets only on the day before sacrifice or every day for 3 weeks prior to sacrifice in order to assess the effects of protein (casein), fat (a mixture of olive and corn oils) and carbohydrate (sucrose) on the liver mixed-function oxidase activity. The activity was determined by measuring metabolic rates of 8 volatile hydrocarbons, i.e., benzene, toluene, styrene, chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethylene, and trichloroethylene. Contrary to the general belief, it was found that carbohydrate, not protein or fat, regulates the metabolism of these hydrocarbons: a diet which was deficient in carbohydrate remarkably enhanced the metabolism irrespective of protein and fat contents in the diet. This conclusion was confirmed by employing two types of diet, one in which the carbohydrate was replaced by an isocaloric amount of protein or fat (thus keeping total calories of each diet constant) and the other in which the carbohydrate content was varied with protein and fat contents fixed (total calories of each diet differed from others according to the carbohydrate content). In accordance with this, dietary carbohydrate intake also exerted a remarkable influence on the hepatotoxicity of carbon tetrachloride which needs to be metabolically activated to become cytotoxic: the smaller the intake, the more severe the liver injury.

Microsomal mixed-function oxidases (microsomal MFO) are a primary group of enzymes involved in the biotransformation of chemicals to biologically inert or highly reactive metabolites. Environmental factors which affect MFO activity, therefore, are likely to alter the toxicity of chemicals. Concerning nutrition, a well-known MFO modifier, the effect has so far been investigated mainly in relation to dietary protein, and the general conclusion has been that it is the nutrient protein which regulates MFO activity, i.e., a high (low)-protein diet increases (decreases) the activity [1–5]. This conclusion has been reached, however, by employing high (low)-protein diets which were prepared by replacing a known quantity of carbohydrate (protein) in the diet with an equal amount of protein (carbohydrate). The high-protein diet, therefore, was nothing other than a diet which was deficient in carbohydrate, the low-protein diet, on the other hand, being a diet containing excessive amounts of carbohydrate. Thus, it was very difficult to determine exactly which nutrient, protein or carbohydrate, really affected the enzymes. In fact, several investigators reported that diets rich in carbohydrates suppressed the enzyme activity [6–8].

In the present paper, the individual effects of protein, fat and carbohydrate on the metabolism of eight aromatic or chlorinated hydrocarbons have been separately assessed, and the results have been discussed in relation to toxicity.

MATERIALS AND METHODS

Animals. Male rats of Wistar strain were used throughout. They were housed individually in stainless-steel wire-bottom cages in an air-conditioned room of 18–22° with artificial lighting from 6 a.m. to 6 p.m. They were fed pellet food (Nippon Clea CE-2) and water *ad lib.* until they reached a weight of 250 g on average. The rats were then switched to a nutritionally adequate liquid diet, the composition of which is shown in Table 1 (we designate this diet as the basal diet.). The diet was prepared according to the method described by DeCarli and Lieber [9] with the modification that we used sucrose in place of dextrin–maltose as the carbohydrate.†

They were fed 80 ml of the basal diet (1 kcal/ml) placed in a large test tube (100 ml) with L-shape outlet daily at 4 p.m. After three weeks on the diet, they were given various test diets in order to assess the nutritional effects on the liver MFO. The liquid diet feeding had a great advantage in bringing daily caloric intake under control. The rats, fed at 4 p.m., consumed the diet, basal or test, by 10 p.m. on the same day.

Metabolism study. For enzyme assay, rats were usually killed at 10 a.m. by decapitation. A 10% (w/v) crude homogenate of the liver was prepared with a 1.15% KCl–0.01 M phosphate buffer, pH 7.4, in a glass homogenizer. It was then homogenized at 10,000 *g* for 10 min at 0°. One milliliter of the super-

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† In a preliminary experiment, we compared sucrose with starch regarding the effect on MFO. One group of rats were fed a diet containing sucrose and the other a diet containing corn starch. After three weeks on the diets, they were killed, the liver removed, and the metabolic rates of eight hydrocarbons, the microsomal protein content, and the cytochrome P-450 level measured. No significant differences were noted between the two groups in any of these measurements. Hence, we used sucrose as the carbohydrate throughout the subsequent experiments.

Table 1. Composition of the basal diet*

Components†	Content‡
Casein (sodium salt)	3.47 g
Oil	2.79 g
Olive oil	2.16 g
Corn oil	0.63 g
Sucrose	9.72 g
L-Cystine	41 mg
DL-Methionine	25 mg
Ethyl linoleate	212 mg
Vitamin mixture	407 mg
DL-α-Tocopherol	2 mg
Mineral mixture	813 mg
Carrhagheenan	212 mg

* This diet was designated as Diet A.

† These were all purchased from Oriental Yeast Co. Ltd., Tokyo.

‡ The value shows an amount of each nutrient taken by a rat a day. An appropriate amount of water was added to the mixture of these components, which was then mixed thoroughly, to make a liquid diet of 80 ml.

natant was used as the enzyme source. Five microliters of each of eight liquid hydrocarbons were dissolved in 100 ml of distilled water, and 0.1 ml (carbon tetrachloride) or 0.2 ml (the other hydrocarbons) of this solution was used as the substrate for enzymatic reaction. The enzyme and the substrate were incubated with a cofactor solution (2 ml containing 0.5 μmoles NADP, 10 μmoles glucose 6-phosphate, and 25 μmoles $MgCl_2$ in 0.1 M phosphate buffer, pH 7.4) in an air-tight incubation vessel for 10 min at 37°. The enzyme activity was assessed by measuring the disappearance rate of substrate as reported elsewhere [10].

The substrates studied, i.e., benzene, toluene, styrene, chloroform, carbon tetrachloride, 1,2-dichloroethane, 1,1-dichloroethylene, and trichloroethylene, were all of reagent grade purchased from Nakarai Chemicals (Kyoto, Japan). NADP and glucose 6-phosphate were obtained from Boehringer (Mannheim, West Germany).

Liver microsomal protein and cytochrome P-450. A part of the 10,000 *g* supernatant was further centrifuged at 105,000 *g* for 60 min to obtain microsomal fraction. The microsomal protein content was measured according to the method of Lowry *et al.* [11] as modified by Miller [12]. The amount of cytochrome P-450 was estimated according to the spectrophotometric method of Omura and Sato [13].

Carbon tetrachloride-induced hepatotoxicity. Rats that had received various test diets at 4 p.m. on the preceding day were exposed to 400 ppm of carbon tetrachloride for 4 hr (8 to 12 a.m.) in a dynamic air-flow chamber as reported previously [14]. On the day of exposure, they were given 80 ml of tap-water in place of daily food.

Immediately before exposure and 0, 4, 8 and 20 hr after exposure, blood samples were taken into hematocrit capillary tubes from a cut in the tail, which were then centrifuged at 15,000 *g* for 5 min to obtain blood serum. Activities of serum glutamic–oxaloacetic transaminase (GOT) and glutamic–pyruvic transaminase (GPT) were measured as indices of liver damage using UV Test Reagents of Boehringer Mannheim. Immediately after the last blood sampling was over (20 hr after exposure), the rats were killed by decapitation, the liver excised, and the microsomal protein and cytochrome P-450 contents measured as described above.

RESULTS

Effects of caloric restriction. Rats that had been given 80 ml of the basal diet every day for not less than 3 weeks were submitted to caloric restriction on the day before enzyme assay. Five groups of rats were each given 0, 20, 40, 60, and 80 ml of the diet at 4 p.m. together with 80, 60, 40, 20, and 0 ml of drinking water, respectively. They were killed at 10 a.m. on the next day, and the liver MFO activity was determined by measuring metabolic rates of toluene and trichloroethylene (Fig. 1). The enzyme activity increased almost linearly with decreasing food intake. For instance, the activity of rats that had fasted overnight (therefore consuming 0 calories) was 3 to 4 times as high as the activity of rats that had consumed daily food (80 kcal).

Effects of protein, fat and carbohydrate. After 3-week maintenance on the basal diet, rats were fed various test diets either on the day before sacrifice only (1-day feeding) or every day for another 3 weeks prior to sacrifice (3-week feeding) in order to assess acute or chronic effects of each diet on liver MFO. Each of the test diets differed from the others only in contents of three nutrients, protein, fat and carbohydrate. These diets were prepared by varying the content of a nutrient in the basal diet at the expense of one of the other two nutrients without changing the total calories.

The results of 1-day feeding experiment are shown in Table 2. The two groups of diets in which sucrose contents were varied at the expense of oil (Diet A, B, C and D) and casein (A, E and F) both increased the metabolic rates of all the hydrocarbons with the sucrose content being decreased. Particularly, an increase caused by Diet D, a sucrose-free diet, was noteworthy: the extent of increase was almost comparable to that caused by 1-day complete food deprivation. On the other hand, those diets which contained the same amount of sucrose (A, G and H)

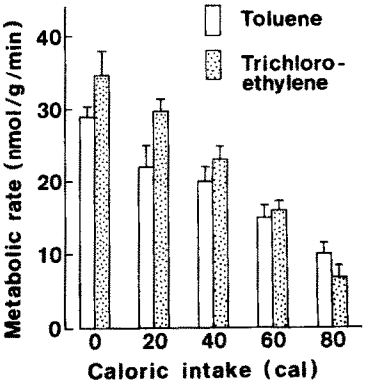

Fig. 1. Accelerated metabolism of toluene and trichloroethylene due to caloric restriction (mean ± S.D. for five rats).

Table 2. Metabolic rates of hydrocarbons after 1-day or 3-week feeding of various test diets

Diet	Casein (g)	Oil (g)	Sucrose (g)		Metabolic rates (nmoles/g liver/min)* Benzene	Toluene	Styrene	Chloroform	Carbon tetrachloride	1,2-Dichloroethane	1,1-Dichloroethylene	Trichloroethylene
A	3.47	2.79	9.72	1-day	7.9 ± 0.3	11.9 ± 0.5	20.4 ± 2.1	11.1 ± 1.6	1.22 ± 0.32	14.9 ± 3.2	15.3 ± 2.8	12.6 ± 2.7
				3-week	9.5 ± 1.2	14.5 ± 1.4	23.1 ± 1.7	14.6 ± 2.4	1.03 ± 0.24	17.2 ± 1.5	15.3 ± 3.2	13.4 ± 1.0
B	3.47	1.60	12.40	1-day	5.9 ± 1.9	8.4 ± 2.1	20.5 ± 2.2	8.1 ± 2.0	0.92 ± 0.22	9.8 ± 2.8	12.1 ± 3.6	9.1 ± 2.9
				3-week	8.4 ± 1.2	11.2 ± 2.2	21.5 ± 0.7	11.3 ± 1.8	0.71 ± 0.21	9.7 ± 5.4	13.1 ± 0.9	10.8 ± 1.1
C	3.47	5.15	4.40	1-day	14.5 ± 2.8†	16.5 ± 2.2†	23.9 ± 3.3	20.1 ± 2.4†	2.42 ± 0.37†	23.4 ± 3.3†	21.9 ± 3.7†	16.8 ± 2.9
				3-week	13.0 ± 2.0†	15.3 ± 1.2†	22.7 ± 2.2	22.8 ± 3.3†	1.73 ± 0.48†	25.4 ± 3.4†	20.4 ± 1.7†	17.8 ± 0.9†
D	3.47	7.11	0	1-day	20.7 ± 4.8†	23.9 ± 3.9†	30.6 ± 4.3†	28.2 ± 3.3†	3.77 ± 0.33†	38.7 ± 5.3†	35.5 ± 6.0†	28.7 ± 7.5†
				3-week	22.4 ± 3.7†	27.0 ± 4.8†	31.9 ± 1.2†	32.8 ± 3.9†	3.61 ± 0.39†	40.5 ± 3.4†	34.0 ± 2.0†	31.5 ± 2.5†
E	0.79	2.79	12.40	1-day	7.3 ± 1.5	9.5 ± 2.9	20.5 ± 1.0	9.0 ± 1.4	0.94 ± 0.36	14.1 ± 2.5	13.3 ± 3.1	11.2 ± 2.4
				3-week	7.9 ± 2.8	9.4 ± 2.4†	16.8 ± 2.8†	9.8 ± 1.7†	0.73 ± 0.21	13.2 ± 3.0	12.9 ± 2.3	10.1 ± 0.6†
F	8.78	2.79	4.40	1-day	11.8 ± 1.5†	14.5 ± 2.0†	22.1 ± 3.2	16.6 ± 2.5†	1.96 ± 0.34†	21.8 ± 4.3†	23.4 ± 5.1†	17.9 ± 2.0†
				3-week	10.9 ± 1.8	14.8 ± 0.9	25.4 ± 3.4	16.9 ± 3.7	2.13 ± 0.70†	19.0 ± 0.3	18.7 ± 1.9	15.7 ± 3.3
G	8.78	0.43	9.72	1-day	7.8 ± 2.0	13.5 ± 2.7	22.3 ± 0.9	12.9 ± 0.5	1.40 ± 0.48	16.6 ± 0.3	13.4 ± 2.8	12.5 ± 2.3
				3-week	7.9 ± 0.9	11.2 ± 2.0	21.0 ± 1.8	12.7 ± 1.2	1.45 ± 0.26	14.3 ± 3.3	13.1 ± 2.2	12.3 ± 0.8
H	0	4.33	9.72	1-day	8.0 ± 2.1	11.1 ± 2.7	18.7 ± 1.6	11.2 ± 2.8	1.72 ± 0.31	16.0 ± 5.3	13.1 ± 3.9	10.2 ± 1.3
				3-week	8.1 ± 1.4	11.3 ± 1.7	7.6 ± 1.9†	11.7 ± 1.9	1.18 ± 0.61	14.2 ± 1.8	12.2 ± 5.7	12.5 ± 1.4
1-day fast					26.5 ± 0.3†	28.0 ± 3.0†	34.1 ± 3.3†	37.4 ± 3.3†	4.02 ± 0.28†	49.2 ± 0.8†	43.1 ± 3.4†	37.8 ± 3.2†

* Values represent the mean ± S.D. for five rats.
† Significantly different ($P < 0.05$) from Diet A (the basal diet).

Table 3. Microsomal protein and cytochrome P-450 contents in liver after 1-day or 3-week feeding of various test diets*

Diet		Liver wt (g)	Liver/body (w/w, %)	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmoles/mg protein)
A	1-day	11.6 ± 0.8	3.94 ± 0.12	21.7 ± 1.9	0.96 ± 0.07
	3-week	11.5 ± 0.2	3.68 ± 0.18	20.4 ± 1.4	0.99 ± 0.05
B	1-day	12.3 ± 0.8	4.13 ± 0.09	21.0 ± 1.4	0.88 ± 0.04
	3-week	13.1 ± 0.1†	3.90 ± 0.05	21.0 ± 2.5	1.00 ± 0.01
C	1-day	10.6 ± 0.4	3.57 ± 0.11†	22.7 ± 2.8	0.97 ± 0.07
	3-week	11.0 ± 0.2	3.43 ± 0.11†	19.0 ± 2.4	1.06 ± 0.05
D	1-day	9.5 ± 0.9†	3.27 ± 0.26†	24.1 ± 2.0	0.91 ± 0.07
	3-week	10.1 ± 0.6†	3.31 ± 0.02†	19.7 ± 0.8	1.07 ± 0.06
E	1-day	11.2 ± 0.1	3.63 ± 0.13	19.4 ± 0.9	0.96 ± 0.10
	3-week	11.4 ± 0.2	3.74 ± 0.03	16.9 ± 1.8	1.06 ± 0.05
F	1-day	12.0 ± 0.7	3.89 ± 0.15	21.6 ± 2.2	0.89 ± 0.04
	3-week	12.6 ± 0.5†	4.04 ± 0.20†	18.5 ± 1.1	0.95 ± 0.05
G	1-day	13.3 ± 0.6†	4.31 ± 0.10†	22.2 ± 2.2	0.88 ± 0.05
	3-week	13.6 ± 0.7†	4.19 ± 0.10†	18.2 ± 2.3	0.85 ± 0.06†
H	1-day	10.5 ± 0.6	3.51 ± 0.13†	20.9 ± 1.4	0.90 ± 0.04
	3-week	7.7 ± 0.9†	3.21 ± 0.26†	14.0 ± 0.9†	0.80 ± 0.03†
1-day fast		8.3 ± 0.1†	2.93 ± 0.12†	23.5 ± 0.7	0.97 ± 0.03

* Values represent the mean ± S.D. for five rats.
† Significantly different ($P < 0.05$) from Diet A (the basal diet).

caused no significant change in the metabolism of hydrocarbons, although the contents of both casein and oil varied to an extreme degree. This finding indicates that protein produces only a negligible influence on the enzyme activity. The general tendency in the alteration of enzyme activity was almost the same whether the activity was computed against unit liver weight or unit quantity of microsomal protein.

Three-week feeding on test diets affected the enzymes almost in the same way as the 1-day feeding with a few exceptions as follows: the prolonged feeding on a casein-free diet (Diet H) caused a much more drastic decrease in body weight, in liver weight, in microsomal protein content, and in cytochrome P-450 level than the overnight feeding on the same diet (Table 3). Nevertheless, no significant difference in the metabolism of hydrocarbons other than styrene was noted between the two feeding conditions (Table 2). The metabolism of styrene was exceptionally liable to the detrimental effect of long-term protein deficiency.

At first sight, however, the data listed in Table 2 may give an impression that the enzyme activity would increase with increasing fat content in a diet, because our test diets were such that the lower (higher) the carbohydrate content was, the higher (lower) the fat content (Diet A, B, C and D). It was worth trying to discover, therefore, whether the deficiency in carbohydrate or the affluence in fat really exerted an influence on the enzyme activity. Accordingly, another experiment was performed by employing a new series of test diets, which contained various amounts of carbohydrate or fat independently of the other. This time, therefore, the total caloric intake a day was varied (unlike the first experiment) (Table 4). When rats were fed diets with varying amounts of sucrose (Diet I, J, K and L) on the day before sacrifice, the enzyme activity increased almost linearly with decreasing sucrose content. Diet L, a carbohydrate-free diet in which sucrose was completely removed with casein and fat contents unchanged, increased the activity almost to the same extent as caused by Diet D, a carbohydrate-free but fat-rich diet in which sucrose was totally replaced by an isocaloric amount of oil. On the other hand, when rats were fed those diets each of which differed from others only in fat content (Diet M, N, O and P), no significant change was noted in the enzyme activity. These results indicate that the increase in enzyme activity caused by the low-carbohydrate, high-fat diet (Diet C or D) is attributable not to an extra amount of oil added but to a diminished amount of sucrose in the diet. It can be concluded, therefore, that liver MFO activity is exclusively dependent on dietary carbohydrate intake irrespective of protein and/or fat intake(s).

One-day feeding on a carbohydrate-free diet (Diet D or L) resulted in an increase in the quantity of microsomal protein per unit weight of liver without causing any significant change in the cytochrome P-450 content (Tables 3 and 5). It remains unknown, however, whether the diet really increased the protein content either by stimulating its synthesis or inhibiting its degradation, or the increase was merely a seeming one produced by a marked loss of liver weight due to the diet.

Hepatotoxicity of carbon tetrachloride (CCl_4). As is shown in Table 6, the severity of CCl_4-induced liver damage was closely correlated with the amount of carbohydrate intake: the smaller the intake was, the more severe the liver injury. This tendency was confirmed with both series of diets, the one with the same total calories (Diet A, B, C and D) and the other with varying total calories (I, J, K and L). The carbohydrate-free diets containing other nutrients rather profusely (Diet D and L) caused very severe liver damage: extent of the severity was almost comparable to that caused by 1-day total food deprivation. This potentiation effect of low-carbohydrate

Table 4. Effects of carbohydrate and fat intakes on the metabolism of hydrocarbons

Diet	Casein (g)	Oil (g)	Sucrose (g)	Benzene	Toluene	Styrene	Chloroform	Carbon tetrachloride	1,2-Dichloroethane	1,1-Dichloroethylene	Trichloroethylene
I	3.47	2.79	12.40	7.0 ± 1.0	9.8 ± 1.4	18.5 ± 3.4	8.8 ± 1.1	0.66 ± 0.50	11.5 ± 3.7	11.6 ± 1.0	7.9 ± 1.9
J‡	3.47	2.79	9.72	7.6 ± 1.7	10.4 ± 1.0	18.5 ± 3.3	10.0 ± 1.5	1.20 ± 0.45	13.7 ± 2.0	12.9 ± 1.6	10.6 ± 1.2
K	3.47	2.79	4.40	12.8 ± 1.8†	15.0 ± 1.8†	23.1 ± 0.9†	16.1 ± 2.4†	1.89 ± 0.54	21.7 ± 2.4†	20.7 ± 3.0†	16.0 ± 2.7†
L	3.47	2.79	0	21.9 ± 0.5†	24.1 ± 0.5†	32.2 ± 2.3†	29.2 ± 2.2†	3.10 ± 0.70†	39.9 ± 0.8†	37.4 ± 1.7†	28.4 ± 2.6†
M	3.47	0	9.72	7.8 ± 1.8	10.5 ± 1.3	21.4 ± 2.0	10.5 ± 2.7	0.96 ± 0.21	11.7 ± 0.7	14.2 ± 4.4	7.9 ± 2.5
N	3.47	1.60	9.72	6.4 ± 1.1	10.5 ± 2.5	18.0 ± 3.8	10.8 ± 1.2	1.08 ± 0.14	12.9 ± 4.8	12.6 ± 2.7	8.7 ± 1.3
O	3.47	5.15	9.72	6.2 ± 1.7	11.6 ± 0.9	17.4 ± 1.8	7.8 ± 1.7	1.39 ± 0.23	11.1 ± 3.2	12.3 ± 2.2	10.1 ± 1.6
P	3.47	7.11	9.72	6.3 ± 1.6	9.9 ± 0.4	16.7 ± 0.5	7.6 ± 0.3	1.31 ± 0.34	14.4 ± 3.4	14.1 ± 0.4	11.1 ± 2.8

* Values represent the mean ± S.D. for five rats.
† Significantly different ($P < 0.05$) from Diet J.
‡ Diet J is identical with Diet A in Table 2, i.e. J is a basal diet, too.

diets was already apparent at the end of exposure and became much more evident with elapse of post-exposure time.

In addition, carbon tetrachloride exposed to rats that had taken carbohydrate-deficient diets caused striking decreases in the microsomal protein and cytochrome P-450 contents, another finding which demonstrates the enhanced hepatotoxicity due to diminished carbohydrate intake.

DISCUSSION

Concerning nutritional modification of metabolism and toxicity of chemical substances, it has generally been believed that it is a nutrient protein which regulates the metabolism and therefore the toxicity [1–5, 15, 16]. Our present findings that a low-carbohydrate diet produces an enhanced MFO activity does not seem to be in conflict with the widely held belief that a high-protein diet enhances the activity, since there is no substantial difference between the two diets both in protein and carbohydrate contents. What it really means, however, is quite different, in that it is not protein but carbohydrate which really exerts an influence on the enzyme activity. It can be said that protein and fat intakes produce only negligible effects on the activity compared to those remarkable effects caused by carbohydrate intake.

One-day food deprivation has been known to accelerate metabolism of a variety of hydrocarbons [17] and alter susceptibility of animals to some hepatotoxic agents such as carbon tetrachloride, chloroform, 1,1-dichloroethylene, etc. [18–23]. As described in the present report, a carbohydrate-free diet which contained both protein and fat rather abundantly accelerated the metabolism of various hydrocarbons including these hepatotoxic hydrocarbons, almost to such a remarkable extent as caused by 1-day total food deprivation (Tables 2 and 4). This clearly indicates that the enhanced enzyme activity due to 1-day fasting is associated with the lack of the very nutrient, carbohydrate, and also indicates that neither protein nor fat may be substituted for carbohydrate as far as the enzyme activity is concerned.

Several hepatotoxic agents such as carbon tetrachloride have to be converted by liver MFO to highly reactive intermediates to become cytotoxic [24–26]. Dietary modification of CCl_4 hepatotoxicity has so far been investigated with special preference for protein. It has been reported that a high-protein diet inflicted severe liver damage on rats through acceleration of CCl_4 metabolism, whereas a low-protein diet decelerated the metabolism and consequently protected them from the damage [1, 2, 15]. As already mentioned, high- and low-protein diets are synonymous with low- and high-carbohydrate diets, respectively. It is evident from our present investigation that carbohydrate, not protein, affects the metabolism and toxicity of carbon tetrachloride. In fact, diets rich in carbohydrates were reported as early as 1940s to be highly protective against CCl_4-induced hepatic damage [27, 28]. The underlying mechanism, however, has hitherto remained unknown. Our investigation presents a probable

Table 5. Effects of carbohydrate and fat intakes on the microsomal protein and cytochrome P-450 contents in liver*

Diet	Liver wt (g)	Liver/body (w/w, %)	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmoles/mg protein)
I	12.2 ± 0.3	3.97 ± 0.06	21.5 ± 1.1	0.95 ± 0.11
J‡	11.8 ± 0.7	3.87 ± 0.07	22.9 ± 0.9	0.92 ± 0.04
K	10.8 ± 0.4†	3.62 ± 0.13†	22.5 ± 0.7	0.92 ± 0.07
L	9.2 ± 0.4†	3.12 ± 0.08†	25.3 ± 0.9†	0.95 ± 0.07
M	11.4 ± 0.5	3.73 ± 0.17	23.1 ± 0.7	0.91 ± 0.06
N	11.9 ± 0.9	3.77 ± 0.16	21.3 ± 1.5	0.90 ± 0.07
O	12.1 ± 0.5	3.97 ± 0.11	21.2 ± 0.5	0.91 ± 0.04
P	12.0 ± 0.2	3.72 ± 0.20	20.7 ± 1.0	0.90 ± 0.06

* Values represent the mean ± S.D. for five rats.
† Significantly different ($P < 0.05$) from Diet J.
‡ Diet J is identical with Diet A in Table 2, i.e., J is a basal diet, too.

Table 6. Release of transaminases into serum following a 400-ppm × 4-hr exposure to carbon tetrachloride

Diet		Immediately before exposure	GOT* (IU/l) Hours after exposure 0	4	8	20
A‡	Exposed	32 ± 6	51 ± 10	101 ± 39	119 ± 51	186 ± 48
	Non-exposed	31 ± 3	36 ± 5	36 ± 9	35 ± 4	39 ± 3
B	Exposed	32 ± 2	33 ± 8	53 ± 14	61 ± 17	56 ± 10†
	Non-exposed	35 ± 5	35 ± 8	37 ± 8	35 ± 3	37 ± 6
C	Exposed	37 ± 3	63 ± 5†	174 ± 46†	222 ± 61†	872 ± 443†
	Non-exposed	32 ± 6	30 ± 5	39 ± 11	39 ± 9	42 ± 8
D	Exposed	39 ± 8	107 ± 12†	337 ± 75†	555 ± 116†	7373 + 844†
	Non-exposed	38 ± 6	36 ± 3	43 ± 10	42 ± 3	43 ± 6
I	Exposed	35 ± 6	33 ± 7	42 ± 2†	43 ± 5†	42 ± 7†
	Non-exposed	33 ± 4	30 ± 3	33 ± 5	30 ± 10	36 ± 3
J‡	Exposed	32 ± 6	44 ± 7	100 ± 19	105 ± 19	146 ± 18
	Non-exposed	33 ± 5	28 ± 4	30 ± 5	35 ± 3	36 ± 3
K	Exposed	32 ± 6	44 ± 7	186 ± 27†	234 ± 13†	840 ± 70†
	Non-exposed	38 ± 8	36 ± 7	36 ± 6	40 ± 12	46 ± 14
L	Exposed	32 ± 4	92 ± 7†	372 ± 57†	595 ± 109†	6019 ± 567†
	Non-exposed	41 ± 9	36 ± 3	34 ± 4	34 ± 4	40 ± 5
1-day fast						
	Exposed	40 ± 9	96 ± 8†	284 ± 35†	439 ± 63†	6133 ± 828†
	Non-exposed	31 ± 9	39 ± 4	40 ± 5	43 ± 6	43 ± 4

		Immediately before exposure	GPT* (IU/l) Hours after exposure 0	4	8	20
A‡	Exposed	11 ± 1	17 ± 3	48 ± 28	53 ± 23	88 ± 33
	Non-exposed	11 ± 3	10 ± 1	11 ± 2	9 ± 3	12 ± 3
B	Exposed	14 ± 5	15 ± 2	26 ± 3	26 ± 5	22 ± 7†
	Non-exposed	11 ± 1	11 ± 2	13 ± 5	11 ± 3	12 ± 2
C	Exposed	14 ± 5	24 ± 6†	82 ± 29†	110 ± 31†	334 ± 199†
	Non-exposed	13 ± 2	10 ± 1	14 ± 3	10 ± 1	11 ± 3
D	Exposed	16 ± 2	35 ± 8†	150 ± 29†	217 ± 69†	1092 ± 376†
	Non-exposed	14 ± 3	12 ± 5	18 ± 6	13 ± 4	15 ± 7
I	Exposed	12 ± 3	15 ± 2	19 ± 4†	19 ± 6†	17 ± 6†
	Non-exposed	13 ± 2	16 ± 4	13 ± 1	11 ± 4	14 ± 4
J‡	Exposed	11 ± 1	16 ± 4	45 ± 12	59 ± 6	73 ± 10
	Non-exposed	11 ± 2	10 ± 2	14 ± 5	14 ± 7	11 ± 2
K	Exposed	12 ± 2	26 ± 5†	95 ± 18†	104 ± 28†	269 ± 71†
	Non-exposed	11 ± 1	12 ± 1	12 ± 4	14 ± 1	13 ± 1
L	Exposed	11 ± 2	36 ± 10†	146 ± 11†	198 ± 36†	1032 ± 274†
	Non-exposed	15 ± 2	11 ± 2	11 ± 5	13 ± 5	11 ± 2
1-day fast						
	Exposed	12 ± 2	37 ± 10†	141 ± 11†	198 ± 65†	1294 ± 457†
	Non-exposed	15 ± 3	13 ± 1	12 ± 4	11 ± 1	16 ± 4

* Values represent the mean ± S.D. for five rats.
† Significantly different ($P < 0.05$) from Diets A and J.
‡ The basal diet.

Table 7. Microsomal protein and cytochrome P-450 contents following a 400-ppm × 4-hr exposure to carbon tetrachloride*

Diet		Liver wt (g)	Liver/body (w/w, %)	Microsomal protein (mg/g liver)	Cytochrome P-450 (nmoles/mg protein)
A‡	Exposed	9.0 ± 0.4	3.32 ± 0.18	22.6 ± 2.7	0.60 ± 0.08
	Non-exposed	7.8 ± 0.4	2.90 ± 0.17	26.3 ± 2.5	0.82 ± 0.14
B	Exposed	8.8 ± 0.2	3.22 ± 0.11	23.8 ± 1.7	0.65 ± 0.10
	Non-exposed	8.0 ± 0.4	3.06 ± 0.10	26.6 ± 1.6	0.88 ± 0.09
C	Exposed	9.0 ± 0.4	3.39 ± 0.08	21.1 ± 0.9	0.44 ± 0.09†
	Non-exposed	7.9 ± 0.4	2.98 ± 0.14	24.5 ± 1.3	0.88 ± 0.03
D	Exposed	9.1 ± 0.2	3.45 ± 0.05	17.9 ± 1.3†	0.28 ± 0.04†
	Non-exposed	7.7 ± 0.6	2.95 ± 0.12	26.6 ± 3.7	0.90 ± 0.12
I	Exposed	9.9 ± 0.7	3.15 ± 0.16	22.2 ± 1.5	0.60 ± 0.02
	Non-exposed	9.1 ± 0.2	2.92 ± 0.07	22.5 ± 1.2	0.90 ± 0.03
J‡	Exposed	9.5 ± 0.2	3.09 ± 0.11	20.7 ± 0.5	0.58 ± 0.06
	Non-exposed	8.5 ± 0.4	2.75 ± 0.12	24.2 ± 1.0	0.89 ± 0.06
K	Exposed	9.6 ± 0.2	3.06 ± 0.03	19.2 ± 2.1	0.42 ± 0.06†
	Non-exposed	8.3 ± 0.3	2.73 ± 0.10	23.9 ± 1.7	0.96 ± 0.04
L	Exposed	9.4 ± 0.4	3.12 ± 0.03	18.7 ± 1.7	0.28 ± 0.02†
	Non-exposed	8.3 ± 0.3	2.70 ± 0.15	23.8 ± 0.3	0.95 ± 0.06
1-day fast	Exposed	8.6 ± 0.4	3.42 ± 0.13	15.2 ± 1.3†	0.21 ± 0.03†
	Non-exposed	6.5 ± 0.4†	2.60 ± 0.06	25.5 ± 1.6	0.83 ± 0.03

* Values represent the mean ± S.D. for five rats.
† Significantly different ($P < 0.05$) from diets A and J.
‡ The basal diet.

explanation for this: a diet rich in carbohydrates protects animals from liver injury by decelerating the transformation of CCl_4 to highly toxic intermediates.

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