EFFECT OF POLYCHLORINATED BIPHENYLS AND THIAMIN DEFICIENCY ON LIVER METABOLISM IN GROWING RATS

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Weanling rats were fed a thiamin-deficient diet containing 0, 10, or 500 ppm Aroclor 1254 (AR) ad libitum for 28 days. Control animals without and with AR at both levels received an oral thiamin supplement. Fasting blood glucose levels were determined at weekly intervals. The effects of AR, thiamin deficiency, and their interaction on liver cytosol and mitochondrial redox states and on cytoplasmic phosphorylation state as calculated from metabolite concentrations in freeze-clamped livers were determined. Liver lipid and various parameters of lipid synthesis, transport, and storage were also studied. AR treatment and thiamin deficiency resulted in enlarged livers, increased liver lipid, increased lipid synthesis, decreased plasma free fatty acids, and decreased carcass lipids. AR treatment and thiamin deficiency increased the oxidized state of the mitochondrial $NAD^+/NADH$ couples and similarly increased the cytoplasmic $NADP^+/NADPH$ couple associated with isocitrate dehydrogenase activity. These effects were more pronounced in AR-treated rats than in thiamin-deficient rats. The oxidation of the NADPH couple for fat synthesis could account for only a fraction of the NADPH utilized. It is suggested that the remainder was used by an induced drug metabolizing cytochrome enzyme system.

INTRODUCTION

Polychlorinated biphenyls, more commonly known as PCBs, are chlorinated hydrocarbons that have many industrial uses. Residues of these compounds have been found to be widely distributed in the environment (Fries, 1972). PCBs produced by the Monsanto Corporation, are marketed under the trade name Aroclor, followed by a 4-digit number identifying the compound mixture and the percent chlorine it contains. Aroclor 1254 (AR), the PCB mixture used in the present study, contains 54% chlorine.

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Journal of Toxicology an Environmental Health, 1:91–105, 1975
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Although much information has been published concerning the presence of PCBs throughout the environment, little work has been done to elucidate their biologic action. Previous reports from this laboratory have shown that AR lowers blood glucose by depressing gluconeogenesis (Mehlman et al., 1974, 1975). Hepatomegaly, fatty liver, and depressed food intake weight gain were also observed. Cold exposure and alloxan-induced diabetes appeared to accentuate the effect of AR on gluconeogenesis; however, AR appeared to have a sparing effect on ketone production. These studies suggested that the metabolic action of AR involves the pathways of carbohydrate and lipid metabolism. The present paper reports the results of two experiments concerned with the effects of AR on the hepatic metabolism of rats fed a high carbohydrate diet. Since gluconeogenesis and lipid metabolism are also affected by thiamin deficiency (Veech et al., 1973; Mehlman et al., 1971; Paquet et al., 1970) and since AR toxicity appears to mimic thiamin deficiency (Mehlman et al., 1975), this experimental variable was also included. Through the use of thiamin-deficient animals, it was hoped that the site of action of AR might be more clearly elucidated.

METHODS AND MATERIALS

Two experiments were conducted. In experiment 1, six groups, each containing 16 male weanling Sprague-Dawley rats (40–60 g), were fed a high carbohydrate-thiamin-deficient diet (Paquet et al., 1970) that contained 0, 10, or 500 ppm AR. The animals were housed individually in wire mesh cages in a temperature-humidity-controlled room having equal periods of light and dark. Control animals, fed diets without and with AR at both levels, were given daily oral supplements of a solution of thiamin hydrochloride (35 μg/100 g body weight). At the end of each week, fasting (16 hr without food) blood glucose levels (Saifer and Gerstenfeld, 1958) were determined in tail blood of five animals from each group. After 4 wk, six animals from each group were starved overnight, and the levels of intermediary metabolite concentrations were determined in freeze-clamped livers. The treatment of the tissues has been previously described, as has been the measurement of the metabolites and the calculation of pyridine nucleotide ratios and phosphorylation states (Veech et al., 1969; Krebs and Veech, 1970). The remaining 10 rats in each group were anesthetized with sodium amobarbital (60 mg/kg body weight), the thoracic cavity opened, and blood withdrawn by heart puncture. The sera were collected after centrifugation (4°C, 3,000 X g, 15 min) and used for the determination of glucose and immunoreactive insulin (Hales and Randle, 1963). The liver was excised, chilled, weighed, and used to determine liver lipid (AOAC, 1960), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) (Freedland, 1967), malic enzyme (ME, EC 1.1.1.40) (Freedland, 1967), and fatty acid synthetase (FA syn'tase) (Koh and Berdanier, 1974; Kumar et al., 1970).

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In experiment 2, six groups of 10 rats each were subjected to the same dietary treatment as in experiment 1. At the end of 4 wk, fatty acid synthesis was investigated in half the animals in each group by incorporating tritium according to the methods of Lowenstein (1971). The remaining animals in each group were decapitated and the neck blood collected to determine free fatty acids (Trout et al., 1960). The liver was excised, chilled, weighed, and used for the investigation of cholesterol synthesis (Koh and Berdanier, 1974). The gastrointestinal tract was emptied of its contents and returned to the carcass. The ingesta-free, liver-free carcass was used for the determination of carcass lipid (AOAC, 1960). Significant differences between the means for each parameter were determined using the Student *t* test for subgroup comparisons and a multifactorial analysis of variance for treatment comparisons (Steel and Torrie, 1960).

RESULTS

Rats fed the thiamin-deficient diet ate less than their controls given daily thiamin supplements (Table 1). Significant differences attributable to thiamin deficiency were observed after 3 wk of feeding. Food intake was not

TABLE 1. Serum Glucose Levels, Food Intakes, and Body Weights at Weekly Intervals in Thiamin-Supplemented and Thiamin-Deficient Rats Treated with Aroclor 1254

	Thiamin supplemented			Thiamin deficient		
	Aroclor 1254 (ppm)			Aroclor 1254 (ppm)		
	0	10	500	0	10	500
Food intake (g/day)						
Week 1	10 ± 1[a]	9 ± 1	8 ± 1	8 ± 1	8 ± 1	7 ± 1
Week 2	12 ± 1	10 ± 1	6 ± 1[b]	10 ± 1	4 ± 1[c]	6 ± 1[b]
Week 3	11 ± 1	12 ± 1	8 ± 1	8 ± 1[d]	8 ± 1[c]	8 ± 1
Body weight (g)						
Initial	52 ± 1	52 ± 1	52 ± 1	52 ± 1	52 ± 1	53 ± 1
Week 1	83 ± 1	83 ± 2	71 ± 2	83 ± 3	79 ± 2	68 ± 2[b]
Week 2	115 ± 2	115 ± 2	91 ± 3	92 ± 2[d]	90 ± 2[d]	73 ± 2[b,d]
Week 3	167 ± 3	170 ± 4	136 ± 5[b]	87 ± 3[d]	94 ± 3[d]	64 ± 2[b,d]
Final	191 ± 6	195 ± 7	116 ± 6[b]	83 ± 2[d]	93 ± 5[d]	60 ± 2[b,d]
Serum glucose (mg/100 ml)						
Week 1	93 ± 4[e]	78 ± 13	71 ± 6[b]	90 ± 2	80 ± 7	66 ± 9[b]
Week 2	92 ± 6	117 ± 14	105 ± 14	63 ± 2[d]	80 ± 5[d]	62 ± 5[d]
Week 3	118 ± 5	126 ± 5	80 ± 10[b]	87 ± 6[d]	81 ± 5[d]	54 ± 2[b,d]

[a] SEM for 16 rats.
[b] Differences attributable to 500 ppm Aroclor are significant ($p < 0.05$).
[c] Differences attributable to 10 ppm Aroclor are significant ($p < 0.05$).
[d] Differences attributable to thiamin deficiency are significant ($p < 0.05$).
[e] SEM for 5 rats.

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well correlated to body weight gain in AR-treated rats, indicating that AR decreased the efficiency of food utilization. Body weight gain was depressed by both thiamin deficiency and by the addition of AR to the diet. Thiamin-deficient animals weighed less than their supplemented controls, regardless of AR treatment. Animals fed the thiamin-deficient diet plus 500 ppm AR weighed the least of all the groups. In the thiamin-supplemented groups, no difference in body weight gain was observed when the diet contained 10 ppm AR, whereas animals fed the diet containing 500 ppm AR weighed significantly less than their controls.

At the end of the first week of experiment 1, no significant differences in fasting blood glucose attributable to either thiamin deficiency or to the inclusion of 10 ppm AR could be observed (Table 1). However, the addition of 500 ppm AR both with or without thiamin supplement to the diet resulted in lower fasting blood glucose levels. At the end of the second week, thiamin-supplemented animals fed the 10 ppm AR diet had higher fasting glucose levels than their thiamin-deficient counterparts. Thiamin deficiency resulted in lower blood glucose levels, indicating as previously reported (Veech et al., 1973) that gluconeogenesis was impaired. At the end of the third week, again, the deficient animals had lower fasting glucose levels; however, inclusion of 10 ppm AR had little effect. In contrast, 500 ppm AR significantly lowered the fasting glucose level in both the thiamin-deficient and thiamin-supplemented groups. This lowering of fasting blood glucose is probably due to an effect of AR on gluconeogenesis (Mehlman et al., 1974, 1975).

As previously reported (Veech et al., 1973), thiamin deficiency resulted in increased glucose, dihydroxyacetone phosphate, pyruvate, and lactate levels in the freeze-clamped livers from fasted rats (Table 2). At each level of AR treatment, glucose, pyruvate, and lactate levels were higher in the thiamin-deficient animals than in the corresponding thiamin-supplemented rats. Since the administration of AR itself tended to lower the liver concentration of glucose and pyruvate, it appears that thiamin deficiency had an "overriding" effect on the accumulation of these metabolites. In addition, it would appear that thiamin deficiency and AR treatment exerted an additive effect with regard to the elevation of liver lactate levels. In thiamin-supplemented animals, the addition of 500 ppm AR to the diet resulted in lower glucose, 3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate levels and higher α-glycerol phosphate and lactate levels. From these observations, it is apparent that in AR-fed, thiamin-supplemented animals the α-glycerol phosphate to 3-phosphoglycerate reaction is inhibited with the resultant accumulation of α-glycerol phosphate. Whether AR inhibits the α-glycerol phosphate to 3-phosphoglycerate reaction through an inhibition of the electron transport system is not known. Treatment of thiamin-deficient (but not thiamin-supplemented) animals with 500 ppm AR in the diet, in addition to the aforementioned effects, resulted in decreased glucose 6-phosphate and pyruvate levels and increased lactate levels.

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TABLE 2. Glycolytic Intermediates in Frozen-Clamped Livers of Thiamin-Supplemented or Thiamin-Deficient Rats Treated with Aroclor 1254

Metabolite	Thiamin supplemented			Thiamin deficient		
	Aroclor 1254 (ppm)			Aroclor 1254 (ppm)		
	0	10	500	0	10	500
Glucose	3.51 ± 0.15[a]	3.23 ± 0.09	2.57 ± 0.16[b]	4.42 ± 0.24[c]	3.82 ± 0.11[d]	3.00 ± 0.37[b]
Glucose 6-phosphate	0.055 ± 0.007	0.057 ± 0.005	0.041 ± 0.006	0.07 ± 0.01	0.045 ± 0.002[d]	0.042 ± 0.004[b]
Dihydroxyacetone phosphate	0.015 ± 0.001	0.018 ± 0.001	0.013 ± 0.001	0.023 ± 0.002[c]	0.014 ± 0.001[d]	0.016 ± 0.003
α-Glycerol phosphate	0.196 ± 0.008	0.174 ± 0.022	0.277 ± 0.031[b]	0.169 ± 0.009[c]	0.172 ± 0.018	0.513 ± 0.126[b,c]
3-Phosphoglycerate	0.147 ± 0.027	0.142 ± 0.013	0.061 ± 0.006[b]	0.210 ± 0.033	0.16 ± 0.031	0.088 ± 0.024[b]
2-Phosphoglycerate	0.020 ± 0.002	0.017 ± 0.001	0.006 ± 0.001[b]	0.025 ± 0.003	0.021 ± 0.003	0.012 ± 0.003[b,c]
Phosphoenolpyruvate	0.062 ± 0.011	0.059 ± 0.007	0.018 ± 0.002[b]	0.099 ± 0.019[c]	0.069 ± 0.015	0.037 ± 0.014[b]
Pyruvate	0.017 ± 0.001	0.016 ± 0.001	0.016 ± 0.001	0.033 ± 0.001[c]	0.033 ± 0.008[c]	0.024 ± 0.002[b,c]
Lactate	0.120 ± 0.014	0.142 ± 0.023	0.298 ± 0.027[b]	0.154 ± 0.012	0.233 ± 0.079[c]	0.461 ± 0.131[b,c]

Note: Values are expressed as μmol/g wet wt.
[a]SEM of 6 rats.
[b]Differences attributable to 500 ppm Aroclor are significant ($p < 0.05$).
[c]Differences attributable to thiamin deficiency are significant ($p < 0.05$).
[d]Differences attributable to 10 ppm Aroclor are significant ($p < 0.05$).

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TABLE 3. Tricarboxylic Acid Cycle, Transaminase, and Mitochondrial Metabolites in Frozen-Clamped Livers of Thiamin-Supplemented or Thiamin-Deficient Rats Treated with Aroclor 1254

	Thiamin supplemented			Thiamin deficient		
	Aroclor 1254 (ppm)			Aroclor 1254 (ppm)		
Metabolite	0	10	500	0	10	500
Malate	0.274 ± 0.010[a]	0.200 ± 0.026[b]	0.219 ± 0.023	0.219 ± 0.011	0.274 ± 0.052	0.245 ± 0.049
Citrate	0.498 ± 0.037	0.225 ± 0.013[b]	0.283 ± 0.019[c]	0.227 ± 0.014[d]	0.420 ± 0.061[b]	0.379 ± 0.060[c,d]
α-Ketoglutarate	0.038 ± 0.003	0.041 ± 0.007	0.160 ± 0.019[c]	0.193 ± 0.022[d]	0.293 ± 0.094[b,d]	0.385 ± 0.094[c,d]
Glutamate	1.78 ± 0.13	1.63 ± 0.033	2.44 ± 0.55[c]	4.55 ± 0.37[d]	4.05 ± 0.254[d]	1.70 ± 0.12[c,d]
NH_4^+	0.367 ± 0.030	0.558 ± 0.036[b]	0.391 ± 0.084	0.457 ± 0.022[d]	0.429 ± 0.022[d]	0.385 ± 0.058
Acetoacetate	0.884 ± 0.044	1.11 ± 0.03[b]	1.47 ± 0.12[c]	0.444 ± 0.075[d]	0.625 ± 0.166[d]	1.15 ± 0.27[c]
β-Hydroxybutyrate	2.61 ± 0.08	3.32 ± 1.29[b]	2.16 ± 0.16[c]	0.704 ± 0.144[d]	1.12 ± 0.33[b,d]	1.44 ± 0.70[c]

Note: Values are expressed as μmol/g wet wt.
[a] SEM for 6 rats.
[b] Differences attributable to 10 ppm Aroclor are significant ($p < 0.05$).
[c] Differences attributable to 500 ppm Aroclor are significant ($p < 0.05$).
[d] Differences attributable to thiamin deficiency are significant ($p < 0.05$).

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The concentration of tricarboxylic acid cycle and transamination intermediates and metabolites related to mitochondrial dehydrogenases are shown in Table 3. Thiamin deficiency resulted in lower citrate, acetoacetate, and β-hydroxybutyrate levels and higher α-ketoglutarate, glutamate, and ammonium ion levels. These changes in hepatic metabolism are classic symptoms of thiamin deficiency (Veech et al., 1973). When 10 ppm AR was added to the diet, citrate levels were lower in the thiamin-supplemented animals than in the non-AR-treated, thiamin-supplemented rats. The reverse was true in the thiamin-deficient rats; 10 ppm AR increased citrate levels, compared with the non-AR-treated, thiamin-deficient controls. The addition of 10 ppm AR also elevated NH_4^+, acetoacetate, and β-hydroxybutyrate concentrations in thiamin-supplemented rats. In thiamin-deficient rats, 10 ppm AR in the diet elevated α-ketoglutarate and β-hydroxybutyrate levels. When 500 ppm AR was added to the diet of the thiamin-supplemented rats, the differences in citrate levels observed with 10 ppm AR were again apparent, in addition to elevations in α-ketoglutarate, glutamate, and acetoacetate. Malate levels were unaffected by either thiamin or AR. In thiamin-deficient animals, the addition of 500 ppm AR to the diet increased citrate levels above those observed in deficient rats not fed AR and also increased α-ketoglutarate, acetoacetate, and β-hydroxybutyrate levels. Glutamate levels were lower in the thiamin-deficient rats fed 500 ppm AR than in non-AR-fed rats. These levels of glutamate were similar to those observed in the thiamin-supplemented, non-AR-fed control rats.

Levels of the various phosphate cofactors are presented in Table 4. Thiamin deficiency resulted in increased ATP concentrations and decreased AMP and P_i concentrations. The addition of 10 ppm AR to the diet had no effect on the concentrations of the phosphate cofactors in the thiamin-supplemented rats; in thiamin-deficient rats AR tended to reverse the effects of thiamin deficiency. When 500 ppm AR was added to the diet, it appeared that AR may have inhibited ATPase activity, since ATP concentrations were elevated and ADP, AMP, and P_i concentrations were decreased in both thiamin-supplemented and -deficient rats. The changes observed in phosphate cofactors suggest that AR and thiamin deficiency have additive effects on hepatic metabolism.

The calculated free redox and phosphorylation states in thiamin-deficient and -supplemented rats are presented in Table 5. Thiamin deficiency resulted in an increased ratio in all the redox couples calculated and tended to increase the ratio of [ATP] to [ADP] and $[HPO_4^{2-}]$. The addition of 10 ppm AR decreased the cytoplasmic $[NAD^+]/[NADH]$ ratio and simultaneously increased the mitochondrial $[NAD^+]/[NADH]$ ratio from glutamic dehydrogenase (GLDH) in thiamin-supplemented rats. The remaining ratios were not affected by the addition of 10 ppm AR in these rats. In thiamin-deficient rats, the addition of 10 ppm AR did not significantly lower the cytoplasmic $[NAD^+]/[NADH]$ ratio, but markedly increased the mitochondrial $[NAD^+]/[NADH]$ ratio from GLDH. The

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TABLE 4. Phosphate Cofactors in the Frozen-Clamped Livers of Thiamin-Supplemented and Thiamin-Deficient Rats Treated with Aroclor 1254

	Thiamin supplemented			Thiamin deficient		
	Aroclor 1254 (ppm)			Aroclor 1254 (ppm)		
Cofactor[a]	0	10	500	0	10	500
ATP	2.49 ± 0.10[b]	2.32 ± 0.14	2.71 ± 0.07[c]	2.78 ± 0.06[d]	2.50 ± 0.06[e]	3.00 ± 0.10[c,d]
ADP	1.46 ± 0.08	1.52 ± 0.09	1.13 ± 0.05[c]	1.42 ± 0.05	1.51 ± 0.07	1.14 ± 0.07[c]
AMP	0.420 ± 0.039	0.42 ± 0.027	0.279 ± 0.014[c]	0.347 ± 0.014[d]	0.410 ± 0.025	0.217 ± 0.011[c]
P_i	4.02 ± 0.22	4.31 ± 0.33	3.33 ± 0.14[c]	3.61 ± 0.08[d]	3.83 ± 0.14	3.37 ± 0.12[c]

Note: Values are expressed as μmol/g wet wt.

[a] Abbreviations used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; P_i, inorganic phosphorus.

[b] SEM of 6 rats.

[c] Differences attributable to 500 ppm Aroclor are significant ($p < 0.05$).

[d] Differences attributable to thiamin deficiency are significant ($p < 0.05$).

[e] Differences attributable to 10 ppm Aroclor are significant ($p < 0.05$).

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TABLE 5. Redox and Phosphorylation States in Livers from Thiamin-Supplemented and Thiamin-Deficient Rats Treated with Aroclor 1254

Mean ratio	Thiamin supplemented			Thiamin deficient		
	Aroclor 1254 (ppm)			Aroclor 1254 (ppm)		
	0	10	500	0	10	500
Cytoplasmic [NAD⁺]/[NADH]	1343 ± 122[a]	1187 ± 242	493 ± 61[b]	1626 ± 124[c]	1483 ± 181	751 ± 31[b,c]
Mitochondrial [NAD⁺]/[NADH] from GLDH	2.57 ± 0.27	3.47 ± 0.46[d]	9.77 ± 2.58[b]	3.98 ± 0.35[c]	7.68 ± 2.22[d]	21.55 ± 10.51[b,c]
Mitochondrial [NAD⁺]/[NADH] from HBDH	6.88 ± 0.30	7.25 ± 0.68	13.78 ± 0.34[b]	13.03 ± 0.54[c]	12.00 ± 0.84	21.21 ± 3.96[b,c]
Cytoplasmic [NADP⁺]/[NADPH] from ME	0.0027 ± 0.0002	0.0029 ± 0.0005	0.0025 ± 0.0003	0.0041 ± 0.0003[c]	0.0039 ± 0.0002	0.004 ± 0.0008
Cytoplasmic [NADP⁺]/[NADPH] from ICDH	0.0026 ± 0.0003	0.0027 ± 0.0004	0.0081 ± 0.0001[b]	0.0059 ± 0.0006[c]	0.0099 ± 0.0020	0.015 ± 0.002[b,c]
Cytoplasmic [ATP]/[ADP][HPO_4^{2-}]	739 ± 96	651 ± 110	1225 ± 95[b]	911 ± 57[c]	733 ± 59	1322 ± 72[b]

[a] SEM of 6 rats.
[b] Differences attributable to 500 ppm Aroclor are significant ($p < 0.05$).
[c] Differences attributable to thiamin deficiency are significant ($p < 0.05$).
[d] Differences attributable to 10 ppm Aroclor are significant ($p < 0.05$).

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remaining ratios were not affected by this dose of AR. The addition of 500 ppm AR markedly decreased the [NAD^+]/[NADH] ratio in the cytoplasm while increasing the [NAD^+]/[NADH] ratio from GLDH and from HBDH in the mitochondria. These increases were observed in both thiamin-deficient and -supplemented rats. No changes in the cytoplasmic [$NADP^+$]/[NADPH] from ME were observed in the 500 ppm AR-treated rats compared with their non-AR-treated controls; however, the [$NADP^+$]/[NADPH] ratio from isocitric dehydrogenase (ICDH) was greatly increased in rats fed 500 ppm AR in their diets as was the ratio of [ATP]/[ADP] [HPO_4^{2-}]. These data suggest that there is increase in PO_2 potential due to a decrease in the activity of energy utilizing processes or that the need for ATP is decreased or that the ability to utilize ATP is inhibited.

Serum glucose levels reflected both the effect of thiamin deficiency on glucose metabolism and AR treatment on gluconeogenesis (Table 6). Serum glucose levels were elevated in thiamin-deficient rats, but were decreased proportionately as the level of AR in the diet was increased. The high level of blood glucose in the thiamin-deficient rats probably represents an inability of these animals to metabolize the large amount of glucose coming from the diet. This impairment, however, must in some way be circumvented such that in both thiamin-deficient and -supplemented rats fed 500 ppm AR, the serum glucose levels were remarkably reduced. Serum insulin levels were not affected by thiamin deficiency per se or by AR treatment. Hepatomegaly, reported earlier (Mehlman et al., 1974, 1975) with AR treatment, was again observed. The livers were enlarged primarily due to the large accumulation of lipid. The increases in liver weight and in relative liver size with increased AR intake were similar in the thiamin-deficient and thiamin-supplemented rats. Thiamin deficiency resulted in an increase in liver lipid, but no increases in hepatic NADP-linked G6PD or ME activity or in FA syn'tase activity. Inclusion of 500 ppm AR greatly increased the liver lipid content in both the thiamin-supplemented and -deficient rats. This increase was accompanied by a large increase in G6PD and ME activity; no increase in FA syn'tase activity was observed.

When lipid synthesis was assessed by the incorporation of tritium into fatty acids (Table 7), no increase in fatty acid synthesis with thiamin deficiency was observed when the results are expressed as cpm/g liver. However, since the liver was larger in thiamin-deficient rats, more ^3H was incorporated into hepatic fatty acids of these rats compared with the control rats. In thiamin-supplemented rats fed 500 ppm AR, a significant increase in fatty acid synthesis was observed. This was true also for the thiamin-deficient rats, again indicating the possible additive effects of both AR and thiamin deficiency. This increase was approximately a threefold increase in synthesis in the liver, yet the plasma-free fatty acid level did not reflect this increase in synthesis. Free fatty acid levels were lower in the plasma of thiamin-deficient rats and in rats fed 500 ppm AR in the diet than in the thiamin-supplemented control rats. These results indicate that transport of

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TABLE 6. Effect of Thiamin Deficiency plus Aroclor 1254 on Various Serum and Lipid Components

	Thiamin supplemented			Thiamin deficient		
	Aroclor 1254 (ppm)			Aroclor 1254 (ppm)		
	0	10	500	0	10	500
Serum glucose (mg/100 ml)	154 ± 19[d]	146 ± 15	101 ± 15[e]	314 ± 23[f]	288 ± 26[f]	77 ± 10[e,f]
Serum insulin (μmol/ml)	34 ± 10	26 ± 11	27 ± 16	18 ± 3	25 ± 3	17 ± 3
Liver weight (g)	7.64 ± 0.25	9.07 ± 0.26[g]	17.0 ± 0.8[e]	3.65 ± 0.11[f]	5.75 ± 0.29[f,g]	10.1 ± 0.1[e,f]
RLS[a]	4.05 ± 0.10	4.68 ± 0.14	14.81 ± 0.63[e]	4.64 ± 0.12	6.23 ± 0.27[f,g]	17.03 ± 0.57[e,f]
Liver lipid (mg/g)	45.5 ± 1.1	51.1 ± 3.1	215.8 ± 7.0[e]	62.8 ± 11.3	83.4 ± 8.9[f,g]	140.6 ± 22.4[e,f]
Enzyme[b] activity (units[c]/100 g body wt)						
G6PD	10.2 ± 1.4	18.0 ± 3.8[g]	94.4 ± 10[e]	8.97 ± 1.63	31.6 ± 8.6[f,g]	124 ± 24[e]
ME	15.9 ± 0.6	20.9 ± 2.6[g]	110 ± 18[e]	11.3 ± 1.7	24.9 ± 3.3[g]	72 ± 9[e,f]
FA syn'tase	12.1 ± 0.6	16.6 ± 1.9[g]	12.5 ± 2.3	12.5 ± 1.2	20.2 ± 3.6[g]	17.5 ± 2.8[e]

[a] RLS = liver wt/body wt X 100.
[b] Abbreviations used: G6PD, glucose-6-phosphate dehydrogenase; ME, malic enzyme; FA syn'tase, fatty acid synthetase.
[c] One unit of enzyme activity is defined as that amount of enzyme that can produce 1 μmol/min of product under the conditions of the assay.
[d] SEM of 10 rats.
[e] Differences attributable to 500 ppm Aroclor are significant ($p < 0.05$).
[f] Differences attributable to thiamin deficiency are significant ($p < 0.05$).
[g] Differences attributable to 10 ppm Aroclor are significant ($p < 0.05$).

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TABLE 7. Parameters of Lipid Synthesis in Thiamin-Supplemented and Thiamin-Deficient Rats Treated with Aroclor 1254

	Thiamin supplemented			Thiamin deficient		
	Aroclor 1254 (ppm)			Aroclor 1254 (ppm)		
	0	10	500	0	10	500
Liver weight (g)	7.71 ± 0.69[b]	8.24 ± 31	15.08 ± 0.70[c]	4.15 ± 17[d]	5.67 ± 0.40[d,e]	11.67 ± 0.62[c,d]
RLS[a]	3.70 ± 0.01	4.26 ± 0.14[e]	13.82 ± 0.34[c]	4.16 ± 10[d]	6.38 ± 0.43[d,e]	16.15 ± 0.76[c,d]
^{3}H-fatty acids (cpm/g liver)	700 ± 250	860 ± 200	3000 ± 900[c]	1640 ± 830	3000 ± 1200[d,e]	6500 ± 1200[c,d]
Plasma fatty acids (μmol/ml)	0.97 ± 0.17	0.87 ± 0.12	0.64 ± 0.08[c]	0.58 ± 0.11[d]	0.58 ± 0.05[d,e]	0.59 ± 0.23
Acetate conversion to cholesterol (cpm/g liver)	250 ± 60	1340 ± 250[e]	4800 ± 110[c]	1400 ± 200[d]	2800 ± 100[d,e]	3200 ± 110[c,d]
Carcass lipid (mg/g)	82.2 ± 11.2	130 ± 9.8[e]	85.0 ± 9.3	44.6 ± 4.6[d]	32.4 ± 3.2[d,e]	43.1 ± 9.1[c,d]

[a] RLS = liver wt/body wt X 100.
[b] SEM of 5 rats.
[c] Differences attributable to 500 ppm Aroclor are significant ($p < 0.05$).
[d] Differences attributable to thiamin deficiency are significant ($p < 0.05$).
[e] Differences attributable to 10 ppm Aroclor are significant ($p < 0.05$).

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newly synthesized lipid must be impaired both by thiamin deficiency and by AR. The conversion of acetate to cholesterol was increased by thiamin deficiency and further increased by the inclusion of either 10 or 500 ppm AR in the diet. Residual carcass lipid was less in the thiamin-deficient rats than in the thiamin-supplemented rats and probably reflects the decreased transport of the hepatic lipids to the periphery for storage. It may also indicate defective peripheral lipid synthesis from carbohydrate precursors that are abundantly present in the thiamin-depleted rat.

DISCUSSION

In previous studies, the interpretation of the effects of AR on hepatic metabolism was complicated by concurrent hepatomegaly, decreased food intake, and decreased body weights. From the present experiments, it is clear that the increased liver size was due primarily to an increase in liver lipid. In turn, this increase in liver lipid was shown to be due in part to an increase in lipid synthesis and in part to a decrease in lipid transport from the liver. The low levels of plasma free fatty acids coupled with a decreased carcass lipid clearly implicate this defect in lipid transport from the liver. That lipid synthesis is a "favored" process can be seen in the increased levels of α-glycerol phosphate and the ratio of $[NADP^+]/[NADPH]$ in the cytosol of the liver, as well as increased activities of the NADP-linked G6PD and ME—the so-called "lipogenic" enzymes. Decreases in fasting blood glucose indicate an impairment by AR of the increase in gluconeogenesis normally expected in starved animals. In thiamin-deficient, nonstarved rats, however, serum glucose levels rose above those of the controls, indicating an impaired utilization of glucose from the diet. When 500 ppm AR was added to the diet, this treatment appeared to override the effect of thiamin deficiency such that these high levels fell below that expected in a nonstarved animal. This decrease in blood glucose levels of rats ingesting 500 ppm AR may have been due to a decreased gluconeogenesis or a rapid utilization of this glucose for lipid synthesis. Increased utilization of the glucose through an increase in insulin release or availability as suggested earlier (Mehlman et al., 1974, 1975) does not seem likely in view of the current results (Table 6). Decreased blood glucose levels with AR treatment due to a decreased food intake can also be ruled out since the food intakes of all three thiamin-deficient groups were identical during the last week of the experiment, yet blood glucose levels varied considerably.

The question of the rapid utilization of NADPH in both thiamin-deficient and AR-treated rats remains to be answered. AR and thiamin deficiency both increased the oxidized state of the mitochondrial NAD and NADP couples. The effect was much more pronounced with AR treatment than with thiamin deficiency. In the case of thiamin deficiency, the changes in the redox ratios resulted from blocks in pyruvate dehydrogenase, α-ketoglutarate dehydrogenase complex activity, and transketolase activity.

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Similar blocks were observed in AR-treated rats; however, the mechanism may be somewhat different, not because of an enzymic block at the thiamin-requiring transketolase step, but because of the very rapid utilization of NADPH.

While fat synthesis was increased in AR-treated rats, we cannot account for all of the utilization of NADPH through this process. The rate of NADPH utilization with fat synthesis is approximately 1.5 μmol/min/g. In the present experiment the addition of 500 ppm AR resulted in a NADPH utilization of approximately 10 μmol/min/g. Since NADPH utilization is increased with AR and is responsible for the oxidized NADP couple, this then implies the induction of another system such as cytochrome P-450 for utilizing NADPH. Induction of hepatic microsomal drug metabolizing enzymes such as cytochrome P-450 enzymes would be expected as one of the defenses against AR toxicity. These enzymes require NADPH for oxidation by the microsomal NADPH oxidase. Enhanced oxidase activity may generate H_2O_2 and, in turn, may contribute to liver injury. A similar mechanism has been proposed to explain the fatty liver observed with ethanol intoxication (Lieber and DeCarli, 1970).

From the current observations it may be suggested that the site of injury may be the membrane protein. It appears that the primary action of AR may involve membrane permeability or function rather than cytosolic or mitochondrial processes per se. This hypothesis is derived from the observations of large differences in the $[NAD^+]/[NADH]$ and $[NADP^+]/[NADPH]$ ratios in the mitochondria vs. the cytosolic ratios of these couples, indicating a disequilibrium in the usual balance in redox state between these cell fractions, and the discrepancy between liver lipid levels and lipid-synthesizing capability and the plasma lipids. Both discrepancies indicate that transport across membrane barriers was impaired. Whether the primary effect of AR involves an injury to the membrane, followed by compensatory alterations in cellular metabolism, or an effect on metabolism that results in a loss in membrane functionality remains to be elucidated.

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Received April 24, 1975
Accepted June 24, 1975

DSW 025788

STLCOPCB4009743