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PEROXISOME-ASSOCIATED ENZYMES AND SERUM LIPIDS IN TUMOUR-BEARING RATS TREATED WITH PEROXISOME-PROLIFERATING AGENTS

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Abstract—Xenobiotic induction of liver peroxisomes is associated with hypolipidemia. To test the involvement of the peroxisome proliferation with the hypolipidemia, male rats were inoculated in the groin with five different tumors: an aflatoxin-induced hepatoma, a lasiocarpine-induced hepatoma, an actinomycin-D-induced mesothelioma, a lasiocarpine-induced squamous cell carcinoma, and a methylnitrosourea-induced fibrosarcoma. After the tumours reached a suitable size, the rats were fed diets containing the peroxisome-proliferating hypolipidemic agents tibric acid (2-chloro-5-[3,5-dimethylpiperidinosulfonyl] benzoic acid) or Wy-14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid) for 2 weeks. Liver and tumor tissues were then assayed for the peroxisome-associated enzymes, catalase and carnitine acetyltransferase, and correlated with serum levels of triglyceride and cholesterol. The presence of the tumors caused a predictable decrease in liver catalase and a slight elevation of liver carnitine acetyltransferase. Serum cholesterol was elevated slightly, while serum triglyceride levels were elevated, unchanged, or decreased in the tumor-bearing rats maintained on control diet. Inclusion of the xenobiotics in the diet caused increases in liver weight, catalase, and carnitine acetyltransferase. Serum triglycerides were decreased in the three groups which were not already decreased, but a decrease in serum cholesterol was only found in one group after only one of the treatments. The latter finding demonstrates that peroxisomal enzyme induction can be dissociated from the decrease in serum cholesterol. The data were further evaluated by testing for correlations between the changes in these components, comparing changes within groups and between groups. These correlations indicate an inverse biological association between liver catalase and serum cholesterol and between liver carnitine acetyltransferase and serum triglyceride. The latter correlation was inverse only for comparisons between groups, suggesting that carnitine acetyltransferase activity is associated with serum triglycerides only during the perturbational state.

The induction of hepatic peroxisome proliferation by xenobiotics is associated with a decrease in serum cholesterol and/or triglycerides. This was described initially in rats treated with the hypolipidemic agent clofibrate [1, 2]. Subsequently it has been found to occur after treatment with several structural analogs of clofibrate, certain hypolipidemic agents not structurally related to clofibrate, several plasticizers with ester linkage to 2-ethylhexanol, and certain salicylates [3–9]. The association between peroxisome proliferation and hypolipidemia supported arguments for the involvement of peroxisomes in lipid metabolism [3], which were first based on the morphological associations between peroxisomes and lipid droplets [10]. Subsequent studies demonstrated that hepatic peroxisomes contained enzymes involved in the carnitine-dependent transfer [11] and β-oxidation [12] of fatty acyl CoA's. The activity of these enzymes is significantly increased during xenobiotic induction of peroxisomes [6, 9, 12–18].

Xenobiotic-induced proliferation of hepatic peroxisomes can produce up to a 10-fold increase in relative hepatic peroxisomal volume [18–23]. Peroxisomal enzymes, however, respond in three apparently different manners [15, 17, 18, 24, 25]. The enzymes involved in β-oxidation and carnitine-dependent transfer of fatty acyl CoA's are increased by an order of magnitude. Certain functional enzymes such as catalase, α-glycerolphosphate dehydrogenase, and glyoxylate aminotransferase increase 1- to 2-fold, while other specific oxidases generally do not respond to induction. These studies have helped establish the involvement of peroxisomes in lipid metabolism and have strengthened the argument for their involvement in the hypolipidemic response. The direct association between peroxisome proliferation and hypolipidemia has not been established, however.

In a previous study, we tested the abilities of peroxisomes, peroxisome-associated enzymes, and serum lipids to respond to peroxisome-proliferating hypolipidemic agents while the synthesis or activity of catalase was chemically inhibited with allylisopropylacetamide or aminotriazole, respectively [26]. We found that peroxisome proliferation, induction of carnitine acetyltransferase, and depression of serum lipids occurred in rats cotreated with either allylisopropylacetamide or aminotriazole and the inducing agents [26]. Similar findings have been reported recently in mice treated with aminotriazole [27]. This suggested that catalase is not required for

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the serum hypolipidemic response, but it did not rule out an association between the fatty acyl CoA metabolizing enzymes and hypolipidemia. Peroxisomes and their catalase content are decreased in tumor-bearing rats [28–32]. The mechanism for this alteration may well differ from the mechanism of the chemical inhibitors of catalase and therefore allows another avenue to study peroxisome proliferation in altered rats. While it has been found previously that peroxisomes and catalase increase in tumor-bearing rats treated with clofibrate [32], no reports are available on the response of the fatty acyl CoA metabolizing enzymes or serum lipids.

We have now studied the effects of Wy-14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid) and tibric acid (2-chloro-5-[3,5-dimethylpiperidinosulfonyl] benzoic acid) on tumor-bearing rats. In this study we examined the response of liver size, the activities of catalase and one of two fatty acyl CoA metabolizing enzymes, carnitine acetyltransferase, and serum triglyceride and cholesterol levels. Depression and induction of activity, the contrasting biological effects of the tumors and the chemicals, respectively offered a unique opportunity to test for correlations among the responses of the components assayed. In addition, the responses of the peroxisome-associated enzymes were also measured in the tumors. We found that, while the peroxisome proliferative response could be dissociated from the hypocholesterolemic response in the tumor-bearing rats, a strong correlation was found between liver catalase activity and serum cholesterol, and between liver carnitine acetyltransferase activity and serum triglycerides.

MATERIALS AND METHODS

Treatment of rats. Male weanling F-344 rats were purchased from Simonson Labs Inc. (Gilroy, CA). The rats were housed individually in stainless steel cages in environmentally controlled rooms with Purina Rat Chow and water provided *ad lib.* When rats were 40–60 g, tumors were transplanted into the left groin. The tumors used in these experiments were derived from chemically induced tumors as previously described. They included hepatomas induced by aflatoxin [33] and by lasiocarpine (referred to as lasiocarpine tumor [34]), a mesothelioma induced by actinomycin D [35], a squamous cell carcinoma induced by lasiocarpine (referred to as squamous cell carcinoma [34, 36]), and a fibrosarcoma induced by methylnitrosourea (MNU; M. S. Rao and J. K. Reddy, unpublished data). When the tumors had reached a desirable size (2–4 cm) 2–4 weeks after inoculation, the animals were provided with diets of ground chow containing 0.125% Wy-14,643 (courtesy of Dr. R. M. Tomarelli, Wyeth Laboratories Inc., Philadelphia, PA) or 0.125% tibric acid (courtesy of Dr. G. T. Holland, Pfizer Pharmaceuticals, Groton, CT). Control animals received ground chow. Treatment lasted for 14 days with withdrawal of food 16 hr prior to killing the animals. At the time of sacrifice, rats weighed 150–200 g and tumors were approximately 40 g in size.

Tissue preparation. Blood was withdrawn from the abdominal aorta (between 9:00 and 10:00 a.m.) while animals were under light ether anesthesia. The livers were then perfused *in situ* via the portal vein with ice-cold 0.9% NaCl, removed and weighed. The weighed sections of liver were placed in separate buffers for homogenate preparations as previously described [14]. Tumors were removed from tumor-bearing rats, necrotic material was dissected out, and the tissue was then handled as described for livers.

Biochemical assays. Catalase activity was measured spectrophotometrically as the rate of H_2O_2 utilization in the presence of homogenate as described by Luck [37]. Carnitine acetyltransferase was assayed as the binding of CoA to 5,5′-dithiobis-(2-nitrobenzoic acid) after its release from acetyl CoA in the presence of carnitine and homogenate as described by Markwell *et al.* [11]. Protein was determined from the respective homogenates using the method of Lowry *et al.* [38] with bovine serum albumin as standard. Enzyme activities were expressed as units/mg protein. Triglyceride and cholesterol were determined from serum samples using the micromethod of Azarnoff *et al.* [39].

Statistical methods. Significance of the difference between untreated tumor-bearing rats and control rats, and between treated rats and untreated rats with similar tumors was estimated using Student's two-tailed *t*-test. Correlation coefficients were calculated between the changes in liver weight, liver catalase, liver carnitine acetyltransferase, serum trigyceride and serum cholesterol. The set of coordinates for each correlation was based on the ranges afforded by the different tumor-bearing rats, with tests within groups: tumor presence vs tumor presence, and induction vs induction; and tests between groups: tumor presence vs induction, and induction vs tumor presence. For example, in the first case, a correlation of catalase vs cholesterol would include the coordinates: (control catalase, control cholesterol); (aflatoxin catalase, aflatoxin cholesterol); (lasiocarpine catalase, lasiocarpine cholesterol) . . . with the values expressed as a ratio of the respective control. Further discussion on this method and references to the equations used were described previously [40].

RESULTS

The presence of the tumors had a consistent effect on liver size and enzymes (Table 1), while a more variable response was found in serum lipids (Table 2). Relative liver weights (g liver/100 g body wt) were decreased in three of the five groups, ranging from 75% of control in rats bearing the aflatoxin-induced hepatoma to 102% in rats bearing the actinomycin-D-induced mesothelioma. Catalase activity was decreased significantly in all tumor-bearing rats except those bearing the squamous cell carcinoma. The decreases ranged from 44% of control in the actinomycin D group to 85% in the squamous cell carcinoma group. Carnitine acetyltransferase activity showed a tendency to be elevated in the tumor-bearing rats, ranging from 120 to 190% of control, but only the lasiocarpine group was significantly greater at 480% of control values (Table 1).

In all of the groups of tumor-bearing rats, the response of liver weight and enzymes to diets con-

Table 1. Rat liver weight and catalase and carnitine acetyltransferase activities in tumor-bearing rats treated with tibric acid (TA) or Wy-14,643 (Wy)*

Tumor type and treatment	Relative liver weight (g/100 g body wt)	Catalase (units/mg protein)	Carnitine acetyltransferase (units/mg protein)
Control	4.25 ± 0.18	44.2 ± 2.6	3.0 ± 0.4
+TA	7.99 ± 0.05†	94.4 ± 5.3†	189.8 ± 3.0†
+Wy	7.70 ± 0.15†	84.0 ± 3.1†	174.1 ± 5.4†
Aflatoxin	3.17 ± 0.24‡	26.9 ± 3.5‡	5.7 ± 2.2
+TA	6.03 ± 0.37†	58.6 ± 3.0†	96.9 ± 4.0†
+Wy	6.37 ± 0.14†	64.9 ± 4.9†	132.9 ± 4.6†
Lasiocarpine	3.52 ± 0.12‡	35.4 ± 1.5‡	14.3 ± 5.4‡
+TA	6.39 ± 0.84†	59.8 ± 3.0†	128.7 ± 25.4†
+Wy	6.59 ± 0.26†	77.1 ± 6.6†	148.0 ± 17.2†
Actinomycin D	4.35 ± 0.25	19.6 ± 3.6‡	4.0 ± 1.4
+TA	8.20 ± 0.18†	35.4 ± 6.7†	102.5 ± 16.4†
+Wy	7.67 ± 0.22†	59.4 ± 4.0†	145.7 ± 8.1†
Squamous cell	4.16 ± 0.13	37.6 ± 2.4	3.6 ± 1.1
+TA	7.86 ± 0.36†	75.4 ± 6.7†	123.4 ± 6.3†
+Wy	7.90 ± 0.27†	85.4 ± 6.8†	139.6 ± 5.6†
MNU	3.55 ± 0.17‡	29.8 ± 2.6‡	4.5 ± 1.4
+TA	6.30 ± 0.70†	86.8 ± 10.5†	139.4 ± 5.3†
+Wy	7.44 ± 0.32†	88.3 ± 16.1†	114.9 ± 6.3†

* Control and tumor-bearing male rats were treated with tibric acid or Wy-14,643 for 2 weeks. Livers were perfused, removed, weighed, and homogenized, and the enzymes were assayed on homogenate preparations as described under Methods. Values are the mean ± S.E.M. of four animals.

† Significant difference of treated from untreated rats bearing the same tumor type, $P < 0.05$.

‡ Significant difference of untreated tumor-bearing rats from control rats, $P < 0.05$.

taining Wy-14,643 or tibric acid was similar to the response in control rats (Table 1). Relative liver weight, catalase activity, and carnitine acetyltransferase activity were elevated significantly above the values for the untreated tumor-bearing controls. The extent of the inductions varied: relative liver weight, 181–210%; catalase activity, 169–329%; and carnitine acetyltransferase activity, 900–5800%. The largest increases were usually in animals in which the untreated tumor-bearing rats had the lowest values compared to controls. This resulted in similar end-point values in the induced animals (Table 1).

The serum cholesterol and triglyceride in the tumor-bearing rats were also altered from controls (Table 2). Serum cholesterol levels were consistently elevated, ranging from 105% of controls in the squamous cell group to a significant increase, 160% of controls, in the MNU group. Serum triglycerides were decreased significantly in the aflatoxin and lasiocarpine groups to 40 and 31% of controls, respectively, unchanged in the squamous cell group, and significantly elevated in the MNU group to 610% of control levels (Table 2). In general, the presence of the tumors elicited a range of responses, but these were all within a reasonable range of each other except for the response of liver carnitine acetyltransferase in rats bearing the lasiocarpine-induced hepatoma, and the response of serum triglycerides in rats bearing the MNU-induced fibrosarcoma.

The serum lipids in tumor-bearing rats were not affected consistently by inclusion of tibric acid or Wy-14,643 in the diets (Table 2). While serum cholesterol in non-tumor-bearing rats was reduced significantly to 74 and 72% of controls after treatment with tibric acid or Wy-14,643, respectively, only the squamous cell group treated with tibric acid had significantly decreased serum cholesterol. Squamous cell-bearing rats treated with Wy-14,643

Table 2. Serum triglyceride and cholesterol contents in tumor-bearing rats treated with tibric acid or Wy-14,643*

Tumor type and treatment	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)
Control	62.0 ± 5.3	73.7 ± 11.1
+TA	45.6 ± 3.6†	9.8 ± 1.2†
+Wy	44.9 ± 1.4†	17.2 ± 3.0†
Aflatoxin	85.8 ± 8.7	29.8 ± 3.9‡
+TA	84.8 ± 5.5	28.0 ± 5.2
+Wy	91.8 ± 6.5	24.5 ± 3.5
Lasiocarpine	82.0 ± 9.3	22.7 ± 6.3‡
+TA	62.7 ± 7.2	23.7 ± 5.5
+Wy		
Squamous cell	65.0 ± 2.1	70.3 ± 18.6
+TA	49.7 ± 3.7†	15.5 ± 3.3†
+Wy	73.6 ± 6.0	38.4 ± 3.5†
MNU	99.3 ± 4.7‡	448.7 ± 215.0‡
+TA	88.0 ± 4.4	65.6 ± 18.5†
+Wy	98.3 ± 7.0	75.0 ± 12.6†

* Serum was prepared from blood drawn from male rats described in Table 1 and assayed as described in Methods.

† Significant difference between treated rats and untreated rats bearing the same tumor type, $P < 0.05$.

‡ Significant difference between untreated tumor-bearing and control rats, $P < 0.05$.

Table 3. Catalase and carnitine acetyltransferase activities in tumors of tumor-bearing rats treated with tibric acid or Wy-14,643*

Tumor type and treatment	Tumor catalase (units/mg protein)	Tumor carnitine acetyltransferase (units/mg protein)
Aflatoxin	8.5 ± 3.8	2.9 ± 1.5
+TA	7.0 ± 0.8	27.0 ± 1.5†
+Wy	14.6 ± 1.9	81.8 ± 8.9†
Lasiocarpine	5.1 ± 1.1	4.8 ± 1.0
+TA	13.7 ± 4.2	27.7 ± 6.0†
+Wy	15.8 ± 1.8†	30.8 ± 10.6†
Actinomycin D	0.8 ± 0.1	3.2 ± 0.3
+TA	0.8 ± 0.2	7.0 ± 3.0
+Wy	1.2 ± 0.2	5.1 ± 1.0
Squamous cell	1.7 ± 0.3	2.2 ± 0.4
+TA	2.4 ± 0.7	6.7 ± 4.0
+Wy	2.2 ± 0.4	17.1 ± 3.1†
MNU	1.2 ± 0.3	7.9 ± 1.6
+TA	2.5 ± 0.4†	22.9 ± 7.9
+Wy	1.7 ± 2.1†	27.4 ± 4.3†

* Tumors were excised from the rats described in Table 1. The tissue was then prepared and enzymes were assayed as described in Methods.

† Significantly different from similar tumor untreated rat, $P < 0.05$.

actually had slightly elevated levels. The changes for all groups ranged from 76 to 113% of control (Table 2). The response of the serum triglycerides was more consistent. In rats bearing the squamous cell or MNU tumors, a significant decrease in serum triglycerides was found after treatment. No decreases were found in rats bearing the aflatoxin or lasiocarpine-induced hepatomas, but these values were already significantly lower than found in control rats.

The enzymatic activities of catalase and carnitine acetyltransferase were also measured in homogenates of tumor tissues from untreated and treated rats (Table 3). Catalase activities in the tumors from untreated rats were much lower than in liver, ranging from 0.8 to 8.5 units/mg protein. Carnitine acetyltransferase activities, however, were similar in tumor tissue from untreated rats and liver of control rats, ranging from 2.2 to 7.9 units/mg protein. After treatment of the tumor-bearing rats with tibric acid and Wy-14,643 for 2 weeks, both catalase and carnitine acetyltransferase activities were elevated in the tumor tissue (Table 3). Catalase activity was elevated significantly in the lasiocarpine-hepatoma after treatment with Wy-14,643 and in the MNU-fibrosarcoma after treatments with both tibric acid and Wy-14,643. Significant increases in carnitine acetyltransferase were found in the aflatoxin-hepatoma and the lasiocarpine-hepatoma after both treatments, and in the lasiocarpine-squamous cell carcinoma and the MNU-fibrosarcoma after treatments with Wy-14,643 (Table 3). These data demonstrate the ability of both liver-derived and extrahepatic derived tumor tissue to respond to treatments with these two peroxisome-proliferating hypolipidemic agents.

In these experiments, male rats were perturbed by two distinct stimuli, the presence of a tumor (five different groups) and treatment with a specific class of xenobiotics. Both stimuli produced a variable response in the five components measured: relative liver weight, liver catalase, liver carnitine acetyltransferase activity, serum triglycerides, and serum cholesterol. The relationship between the extent of the changes in these five components, as estimated by correlation coefficients, may provide valuable insight into biological relationships between the components. For the purposes of this study, we were most interested in the relationship between changes in the liver peroxisome-associated enzymes and the serum lipids.

The manner in which the correlation coefficients were determined is illustrated in Fig. 1. Values were grouped as either from untreated (control) tumor-bearing rats or induced tumor-bearing rats, with the latter group including the effects of both tibric acid and Wy-14,643. Values for each component (liver weight, ca.alase, etc.) were then expressed as ratios of the respective controls and used as coordinates for determination of the correlation coefficient. For each group of components compared (e.g. catalase vs cholesterol) this allowed for four groups of comparison. Two of the comparisons were within the groups: control-tumor-bearing rats vs control-tumor-bearing rats, and induced-tumor-bearing rats vs induced-tumor-bearing rats, and two of the comparisons between the groups: control-tumor-bearing rats vs induced-tumor-bearing rats, and induced-tumor-bearing rats vs control-tumor-bearing rats. As demonstrated in Fig. 1, this generally created a range of values for both the x and y axis which was suitable for correlation estimates (r). In addition, correlations

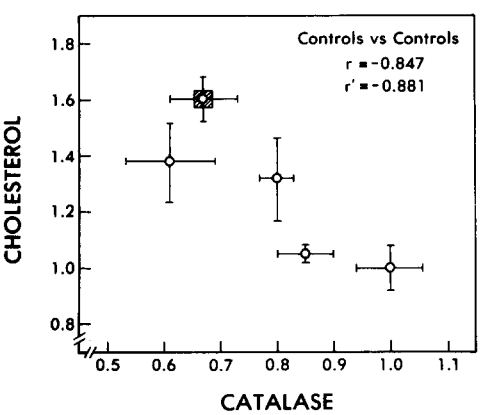

Fig. 1. Correlation between the relative changes in liver catalase activity and serum cholesterol content in rats bearing different tumor types. Values for liver catalase and serum cholesterol in untreated tumor-bearing rats were expressed relative to control values (Tables 1 and 2) and plotted as coordinates. The correlation coefficient (r) was determined from these coordinates, and the corrected correlation coefficient (r') was determined after deletion of the hatched-out coordinate. This is an example of a correlation within groups and illustrates the method used to the correlation coefficients presented in Table 4 and discussed in the text. The bars represent the relative S.E.M.

Table 4. Correlation between changes in catalase and cholesterol, and carnitine acetyltransferase (CAT) and triglyceride (TG), due to the presence of tumor or treatment of tumor-bearing rats with Wy-14,643 and tibric acid*

Tumor-bearing rats groups compared		Correlation coefficient	Rank†	Corrected correlation coefficient	Rank†	Coordinate(s) deleted‡
Catalase vs	Cholesterol					
Controls	Controls	−0.847§	1	−0.881§	2	MNU
Induced	Induced	0.518	1	0.779§	2	MNU
Controls	Induced	−0.646§	2	−0.908§	1	Sq. cell
Induced	Controls	0.708§	1	0.892§	2	Lasio
CAT vs Triglyceride						
Controls	Controls	−0.306	2	−0.806§	2	MNU
Induced	Induced	−0.722§	1	−0.914§	1	MNU
Controls	Induced	0.725§	2	0.760§	2	MNU
Induced	Controls	0.052	5	0.922§	2	MNU

* Values from Tables 1 and 2 were expressed as the ratio of their respective controls and used as coordinates to determine the correlation coefficient as described under Fig. 1.

† When similar groups were compared (controls vs controls, induced vs induced) four correlations were derived. When induced groups were compared to controls (or vice versa) five correlations were derived. Highest to lowest correlations of the group (catalase vs CAT, relative liver weight, etc.) were ranked 1–5.

‡ For determination of the corrected correlation coefficient the set of coordinates listed was deleted from calculations. When induced groups were used, the coordinates for the tibric acid and Wy-14,643 treatment were both deleted.

§ Correlation coefficient is significantly different from zero, $P < 0.05$.

were also performed after deletion of one set of coordinates (r'). This often led to a significant improvement in the correlative coefficient. Most often, the deleted coordinate arose from a set of values which was altered in an uncharacteristic fashion such as the serum lipid values in rats bearing the MNU tumors.

The results presented here (Fig. 1 and Table 4) are limited to the correlations between changes in catalase and cholesterol, and between carnitine acetyltransferase and triglyceride, as a total of forty-five different correlations were made when all five components were compared. Two points were noticed in the other comparisons which will be mentioned briefly. When the changes in control-tumor-bearing rats vs control-tumor-bearing rats were compared, the corrected correlations (r') between the changes in relative liver weight and the other four components were quite high. Positive correlation with changes in liver catalase ($r' = 0.899$) and serum triglyceride ($r' = 0.930$), and negative correlation with liver carnitine acetyltransferase ($r' = -0.900$) and serum cholesterol ($r' = -0.987$), were found. In contrast, comparisons of relative liver weight changes with the other components in control vs induced, etc., were not as high. In addition, quite high correlations were found when the change in a component in control-tumor-bearing was compared to the change in the same component in induced-tumor-bearing rats (e.g. catalase control vs catalase induced, $r' = -0.850$; CAT control vs CAT induced, $r' = -0.876$; TG control vs TG induced, $r' = -0.944$). The high negative correlations found here are consistent with our observation that those values depressed the most by the presence of tumor were relatively induced the most. The exception to this rule was the response of cholesterol (cholesterol control vs cholesterol induced, $r' = 0.702$).

Overall, the best correlations were found between the changes in catalase vs cholesterol and carnitine acetyltransferase vs triglyceride. These values are presented in Table 4. Comparison of catalase in control vs cholesterol in control, and catalase in control vs cholesterol in induced-tumor-bearing rats, lead to negative correlations ($r' = -0.881$ and -0.908 respectively). When catalase in induced was compared to cholesterol in induced or control tumor-bearing rats, however, the correlations were positive ($r' = 0.779$ and 0.892 respectively). In part, this positive correlation arose from the compensatory greater relative induction of catalase in tumor-bearing rats. If the actual concentration of catalase is compared to the relative change in cholesterol, negative correlations were found in both cases ($r' = -0.487$ and -0.855 respectively). The correlations determined were also ranked against the other correlations (e.g. catalase vs cholesterol, catalase vs catalase, etc.) for each group of comparisons. In all cases, the correlations between changes in catalase and cholesterol were the closest or second closest in unity (Table 4).

When the changes in carnitine acetyltransferase were compared to changes in triglyceride, the corrected correlations also were always closest or second closest in unity (Table 4). In these comparisons, however, the correlations were negative when comparing within groups: control vs control or induced vs induced ($r' = -0.806$ and -0.914 respectively) and positive when comparing between groups: control vs induced or induced vs control ($r' = 0.760$ and 0.922 respectively). The polarity of these correlations could not be changed by using the actual rather than relative values of carnitine acetyltransferase ($r' = 0.760$ and 0.552 respectively). This suggests that while the responses of the relationship between catalase and cholesterol to the two stimuli were similar, the responses of the relationship between carnitine acetyltransferase and triglyceride were not.

DISCUSSION

The results of this study clearly indicate that under certain circumstances the induction of peroxisome-associated enzymes can be dissociated from the decrease in serum cholesterol. The response of serum triglyceride, however, cannot be completely dissociated from peroxisomal-enzyme induction by these studies. In the two sets of tumor-bearing rats which did not show a decrease in serum triglyceride, the triglycerides were already reduced further than the xenobiotic treatment would normally reduce them.

These findings are in accord with the current concept of peroxisomes and their involvement in lipid metabolism. In particular, two sets of enzymes which mediate the oxidation [12] and carnitine-dependent transfer [11] of fatty acyl groups have been localized, at least in part, within peroxisomes. Both enzymes are induced approximately one order of magnitude by peroxisome proliferating agents [13–18]. This finding itself suggests that both enzyme systems are equally important for peroxisomal fatty acid metabolism and that this regulation of the two enzymes may be linked. As these two enzymes have fatty acids as substrates, carnitine acetyltransferases and acyl CoA β-oxidation [11, 12] would be expected to affect triglyceride synthesis in a more direct fashion than cholesterol synthesis. A recent report has indicated that peroxisomes may contain activity for the breakdown of cholesterol [41]. Unfortunately, induction of this activity was not measured previously [41], or in this study. The relationship between the peroxisomal fatty acyl CoA metabolizing enzymes and hypolipidemia has also been suggested from other studies.

As a further test of the relationship between the peroxisome-associated enzymes and serum lipids, we made use of the variable responses of these components to test for correlations between them. This methodology, of course, cannot provide direct evidence for a relationship, but it does provide some valuable insight into the biological relationship between catalase and serum cholesterol, and carnitine acetyltransferase and serum triglyceride. When the actual as well as relative response of catalase was compared to serum cholesterol, we found high negative correlatives. This inverse response of serum cholesterol and liver catalase activity is in agreement with previous studies on the lowering effect of injected catalase on serum cholesterol [42] and the decreased serum cholesterol in mice with an unstable form of catalase [43]. This correlation suggests that while catalase induction is not necessary for [26, 41], nor does not necessarily produce (this report), a decrease in serum cholesterol, there is an underlying biological association between the two.

When the response of carnitine acetyltransferase was compared to serum triglyceride, high negative correlations were found for comparisons within groups. When comparisons were made within groups, in the presence of tumors or in induced tumor-bearing rats, the serum triglyceride responded in the opposite direction of liver carnitine acetyltransferase. This is in excellent agreement with other studies on the involvement of peroxisomal fatty acyl CoA metabolizing enzymes with serum triglyceride levels. This suggests that in the response to tumor presence or to the xenobiotic treatment, a direct relationship between increased carnitine acetyltransferase activity and decreased serum triglycerides has occurred. It is interesting that when comparisons were made between groups, control vs induced and vice versa, the correlations were high, but were positive. At this time we have no explanation for this positive correlation found when comparisons were made between groups. The regulation of serum triglyceride, as well as cholesterol, levels is a complicated event, however, and carnitine acetyltransferase and the other peroxisome-associated fatty acyl CoA metabolizing enzymes may only affect triglycerides during unusual physiological responses.

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