Tumor Necrosis Factor Induces Enzymatic Changes in Liver Comparable to Those in Extrahepatic Cancer (43591)

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Abstract. **Human recombinant tumor necrosis factor was administered to rats in small doses to determine whether it causes changes in the activity of liver enzymes similar to those observed in cancer growing extrahepatically. Intraperitoneal injection of increasing doses of tumor necrosis factor (20–100 μg/kg/day for 5 days) resulted in a 20–50% decrease in hepatic alanine aminotransferase ($P \leq 0.05$), a 10–20% decrease in aspartate aminotransferase ($P \leq 0.04$), and a 50–200% increase in alkaline phosphatase ($P \leq 0.02$). The activity of hepatic 5′-nucleotidase was unchanged. In the serum, there was no significant change in the activity of any of the enzymes. Histologically, there was no damage detectable by light or electron microscopic examination of the liver, and no evidence of biliary obstruction. However, in frozen liver sections stained histochemically for alkaline phosphatase, there was a dramatic increase in the activity of this enzyme in hepatocytes, which was confined to the bile canaliculi. There was also a 3- to 9-fold increase in the mitotic activity of hepatocytes. Comparable changes have been reported in the tumor-free liver of animals with cancer.** [P.S.E.B.M. 1993, Vol 203]

Changes in the activity of liver enzymes have been observed in a variety of human and animal cancers growing extrahepatically (1–11). The changes involve cytoplasmic, mitochondrial, microsomal, lysosomal, peroxisomal, and membrane-associated enzymes, and frequently represent a reversion of the liver to a state of dedifferention (7, 9).

There is adequate evidence suggesting that metabolic alterations in the tumor-free organs of the host are caused by humoral factors released by the cancer or host cells (12). Early studies have shown that some of the changes in liver enzymes could be induced by injection of "toxohormone" into animals, a protein-like and heat-stable agent extracted from human tumor cells (1). More convincing evidence for a role of humoral factors came from experiments with parabiotic rats where subcutaneous implantation of a solid tumor in one member of the parabiotic pair caused significant changes in the activities of hepatic enzymes in both partners (13). Changes in the activities of hepatic enzymes have also been reported in the serum of patients with renal carcinoma (14–18) and malignant schwannoma (19) in the absence of metastasis to the liver or other organs. In these cases, the most noticeable change was frequently an increase in serum alkaline phosphatase (AP) activity, and occasionally in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity (18).

More recently, tumor necrosis factor/cachectin (TNF) was shown to induce metabolic changes in the host similar to those observed in the presence of cancer (20). In our laboratory, intraperitoneal injection of human recombinant TNF-α into rats at low doses (100 μg/kg/day for 5 day) resulted in a significant decrease in hepatic catalase activity similar to that observed in extrahepatic cancer and after toxohormone injection (1, 21). The decrease was accompanied by a significant reduction in the size and number of peroxisomes without any other hepatocellular changes detectable by elec-

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tron microscopy. At higher doses (250 μg/kg/day for 7–10 day), Tracey *et al.* (22) showed that histologically demonstrable liver damage occurred with proliferation of small bile ducts and significant inflammation.

The purpose of this study was to determine in the rat the effects of intraperitoneal injection of small doses of TNF on the activity of liver enzymes traditionally used as markers of hepatocellular damage (AST and ALT) and biliary obstruction (alkaline phosphatase [AP], γ-glutamyltransferase [GGT], and 5′-nucleotidase [5′NT]). The activity of these enzymes is frequently altered in cancer growing extrahepatically (2, 7), or is increased in the serum of cancer patients treated with recombinant TNF (23–26). The effects of TNF on these enzymes were studied in the liver, and in the kidney and serum for comparison. The liver was also examined by light and electron microscopy to determine whether any morphologic changes had occurred, and to correlate these changes with enzyme activity.

Animals and Methods

Animals. Male Sprague-Dawley rats (165 ± 15 g) were maintained on a 12:12-hr light:dark cycle and provided with water and chow *ad libitum*. The care and use of these animals conformed with the National Institutes of Health's current *Guide for the Care and Use of Laboratory Animals*, and the institutional Animal Care and Use Committee.

Experimental. Human recombinant TNF-α was generously donated by Asahi Chemical Industry America, Inc. (New York, NY) and contained a negligible amount of endotoxin as contaminant (2.33×10^6 units TNF/mg protein; 0.35 pg endotoxin/10^6 TNF; equivalent to 0.001 pg endotoxin/μg TNF) (21). Rats were injected intraperitoneally with 20–100 μg/kg/day of TNF for 5 days. Unless otherwise indicated, the total daily dose was divided into two equal doses which were injected at approximately 12-hr intervals. Six experiments were performed, each consisting of a group of five to six experimental rats injected intraperitoneally with TNF and a group of four to six control rats injected with the vehicle only (10% glycerol or phosphate-buffered saline containing 0.1% serum bovine albumin).

Experiments 1, 2, and 3 were designed to determine the effect of increasing doses of TNF (20, 50, and 100 μg/kg/days for 5 days, respectively) on the activity of hepatic enzymes.

Experiments 4 and 5 were prompted by reports that fasting affects the activity of liver enzymes (27). In Experiment 4, the experimental rats were injected with 50 μg/kg/day twice daily for 5 days, and both experimental and control rats were additionally fasted for 24 hr on Day 5. Experiment 5 was similar to Experiment 4 except that the total daily dose was divided into three doses of 33 μg each and injected at approximately 8-hr intervals, to evaluate the effect of more frequent TNF administration.

Experiment 6 was designed to investigate potential adverse effects of TNF on food consumption (28). The experiment was similar to Experiment 3 (in which the experimental rats were injected with 100 μg/kg/day for 5 days), but also included a group of control pair-fed rats that were given (and completely consumed) daily the same amount of food consumed on the previous day by their TNF-injected partners.

On Day 6 (or Day 7 for the pair-fed controls), all rats were anesthesized with ether and sacrificed by guillotine decapitation. Liver and kidneys were excised and homogenized in 0.2 *M* phosphate buffer, pH 7.4, as described previously (21). The homogenates were centrifuged at 30,000*g* for 30 min and the supernatant fluids were stored at −70°C until assayed (21). Whole blood was also collected immediately after decapitation from all the rats in Experiment 6, and the sera were separated by centrifugation and stored at −70°C.

Protein concentration in the tissue extract was determined by a biuret method adapted to the DACOS discrete analyzer (Coulter Electronics, Inc., Hialeah, FL). ALT (EC 2.6.1.2) and AST (EC 2.6.1.1) activities were determined at 37°C on a COBAS centrifugal analyzer (Roche Diagnostics, Nutley, NJ) using the Beckman optimized ALT and AST methods. 5′NT (EC 3.1.3.5) activity was determined at 37°C on a COBAS centrifugal analyzer by the Sigma method (Sigma Chemical Co., St. Louis, MO). AP (EC 3.1.3.1) activity was determined at 30°C on a Beckman DU-7 spectrophotometer (Beckman Instruments, Inc., Fullerton CA) using an in-house method with *p*-nitrophenylphosphate as substrate. GGT (EC 2.3.2.2) activity was determined at 37°C on an EKTACHEM 700 discrete analyzer (Eastman Kodak Co., Rochester, NY).

Liver slices from all the rats in Experiment 3 were fixed in 2% glutaraldehyde, postfixed in 1% osmium tetraoxide, dehydrated in a graded series of ethanol, and embedded in Maraglas resin. Thin sections were cut on a Reichert microtome (Warner-Lambert Technologies, Bloomington, MN) stained with lead and uranium, and examined in a Philips 201 electron microscope (Philips Electronic Instruments, Schaunburg, IL).

Liver sections were also prepared from all the rats in Experiments 3 and 6, and stained with hematoxylin and eosin for histologic examination by light microscopy. Mitotic activity was determined by averaging the mitotic count in 20 high-power fields. Frozen liver sections were also prepared and stained histochemically for AP as described by Hayhoe and Quaglino (29).

Statistical Analysis. All data were analyzed by the two-tailed unpaired Student's *t* test.

Results

Mean liver wet weight of the TNF-treated or pair-fed rats, represented as a percentage of the total body weight at the time of sacrifice, was not significantly different from that of the rats fed *ad libitum*. In Experiments 4 and 5, where the rats were fasted on day 5, the mean (±SD) liver weights of the TNF-injected rats (4.17 ± 0.27% and 4.22 ± 0.30% of body weight, respectively) were higher than those of the control rats (3.76 ± 0.28% and 3.72 ± 0.31% of body weight) by 11% ($P = 0.04$) and 13% ($P = 0.02$), respectively.

Data on total body weight and amount of food consumed by control and TNF-treated rats were reported previously (21).

Enzyme activities in normal rat liver and kidney are shown in Table I as the means of all the control rats fed *ad libitum*. The activities are reported in units per gram of wet tissue and are comparable to those reported by other investigators (30–33). Liver contained about seven times more ALT than the kidney, while the kidney contained much more AP and GGT. AST and 5′NT were in approximately similar amounts in both organs. It should be noted that the standard deviations for 5′NT were unusually high for both liver and kidney, and were equivalent to coefficients of variation of 55% and 81%, respectively. This was caused by the fact that the mean 5′NT activities varied significantly from experiment to experiment, which suggests that different amounts of this enzyme were being extracted. However the mean 5′NT activities within any one experiment showed much less variation (Fig. 3).

Table I. Enzyme Activities (Mean ± SD units/g tissue wet wt) in Normal Rat Liver

	n	Liver	Kidney
AP	18[a]	0.50 ± 0.13	18 ± 10
5′NT	18	3.3 ± 1.8	5.2 ± 4.2
ALT	18	44 ± 9	7.1 ± 2.4
AST	18	184 ± 23	151 ± 15
GGT	10[b]	<0.2	75 ± 21

[a] Number of control rats fed *ad libitum* in Experiments 1, 2, 3, and 6.
[b] Number of control rats fed *ad libitum* in Experiments 1 and 6.

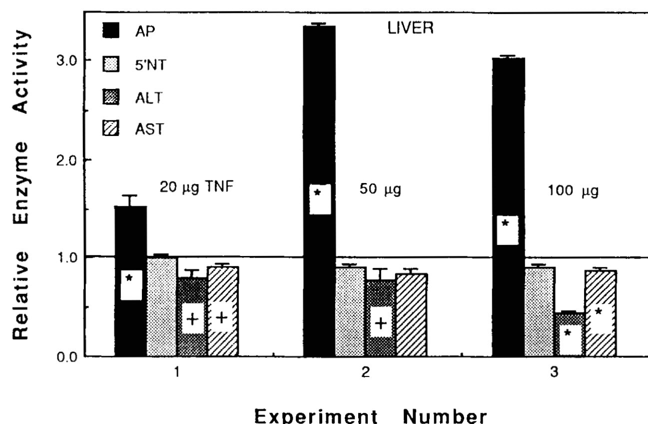

Figure 1. Effect of TNF on the activities of hepatic enzymes. In Experiments 1, 2, and 3, the experimental rats were injected with 20, 50, and 100 μg/kg/day, respectively, for 5 days. The number of experimental rats was 6, 6, and 6, respectively, and of control rats 6, 4, and 4, respectively. Error bars represent the SE. An asterisk indicates that the mean enzyme activities were significantly different from those of the control rats fed *ad libitum* at a P-level of 0.02 or less; the plus sign (+), indicates that mean activities were significantly different at P-levels of 0.03–0.05 (see Animals and Methods for additional details).

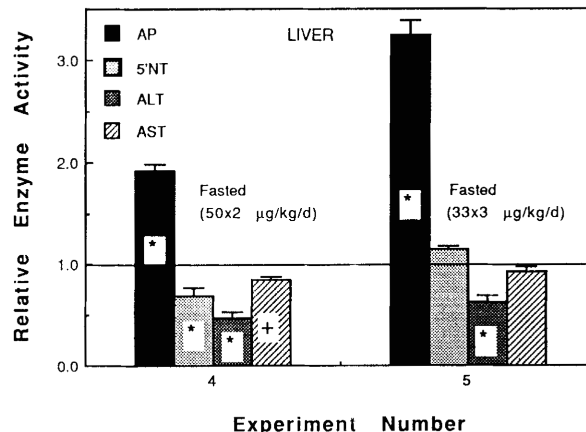

Figure 2. Effect of TNF and fasting on the activities of hepatic enzymes. In Experiments 4 and 5, the experimental rats were injected, respectively, with 50 μg/kg/day TNF twice daily and 33 μg/kg/day three times daily for 5 days. Experimental rats (n = 5 and 6, respectively) and control rats (n = 4 and 6, respectively) were also fasted for 24 hr on Day 5. Symbols are as described in the legend of Figure 1.

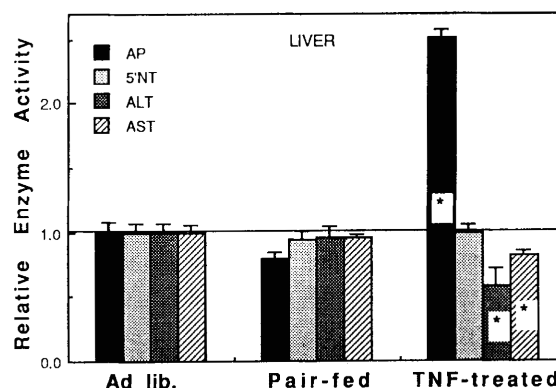

Figure 3. Comparison of the effect of TNF on the activity of hepatic enzymes in control rats fed *ad libitum* (n = 4), pair-fed rats (n = 6), and rats injected with 100 μg/kg/day of TNF for 5 days (n = 6). The asterisk indicates that the mean levels were significantly different from the control mean levels at P-levels of 0.01 or less.

The activities of AP, 5′NT, ALT, and AST in the liver of rats injected with increasing doses of TNF (Experiments 1–3) are shown in Figure 1 as multiples/fractions of the mean activities of the control rats arbitrarily set at 1. Activities were calculated in units per gram of protein extract, although essentially similar results were obtained when calculated per gram of wet tissue. The most significant changes were in the activi-

Table II. Enzyme Activities (Mean ± SD units/g extract protein) in Kidneys of Control Rats Fed *Ad Libitum*, Pair-Fed Rats, and TNF-Treated Rats[a]

	n	AP	5′ NT	ALT	AST	GGT
Control	4	202 ± 5	38 ± 3	69 ± 9	1023 ± 53	544 ± 105
Pair fed	6	119 ± 26[b]	34 ± 12	54 ± 5	993 ± 61	535 ± 109
TNF treated	6	133 ± 7[b]	44 ± 7	51 ± 3[b]	1092 ± 180	438 ± 111

[a] Rats were injected with 100 μg/kg/day of TNF (see Experiment 6 under Animals and Methods).
[b] Values were significantly lower than control ($P \le 0.002$).

Table III. Enzyme Activities (Mean ± SD units/liter) in Serum of Control Rats Fed *Ad Libitum*, Pair-Fed Rats, and TNF-Treated Rats

	n	AP	5′ NT	ALT	AST	GGT
Control	4	475 ± 65	45 ± 17	53 ± 7	101 ± 7	<10
Pair fed	6	416 ± 72	40 ± 11	57 ± 5	122 ± 22	<10
TNF-treated	6	482 ± 107	58 ± 18	54 ± 5	126 ± 25	<10

ties of AP and ALT. AP increased by 53% ($P = 0.02$) at 20 μg/kg of TNF, and by approximately 200% at 50 and 100 μg/kg/day of TNF ($P < 0.001$). On the other hand, ALT showed a gradual decrease, with the increase in TNF ranging from 21% to 54% ($P = 0.05$, 0.03, and < 0.02 at 20, 50, and 100 μg/kg/day of TNF, respectively). AST also showed a small decrease of 10–24%, which reached statistical significance only at the lowest and highest doses of TNF ($P = 0.04$ and < 0.02, respectively). 5′NT showed no significant change, while GGT was not detectable in normal rat liver (Table I).

Figure 2 shows the liver relative enzyme activities for Experiments 4 and 5, where the rats were injected with 100 μg/kg/day for 5 days and additionally fasted on Day 5. The results were comparable to those of the fed rats injected with the same dose of TNF (Experiment 3 in Fig. 1), except for 5′NT, which was significantly decreased only in Experiment 4. Administration of the daily dose of TNF in three instead of two equal injections (Experiment 5 versus 4) resulted in a significant ($P = 0.02$) additional increase in the relative activity of AP.

In Experiment 6, both pair-fed rats and rats fed *ad libitum* were used as control to determine whether the changes so far observed may have been caused by the transient decrease in body weight and/or food consumption that is known to be associated with TNF administration (21, 27). As shown in Figure 3, the liver enzymes of pair-fed control rats did not differ significantly from those of control rats fed *ad libitum*, while the TNF-injected (100 μg/kg/day) rats showed essentially the same changes seen in the previous experiments. Similar results were obtained previously in our laboratory for a number of other enzymes (21, 34).

The activity of the various enzymes showed some interesting changes in the kidneys, as illustrated by the results of Experiment 6 in Table II. Although the AP activities of the pair-fed and TNF-injected rats were similar, they were both significantly ($P \le 0.001$) lower (41% and 34%, respectively) than those of the control rats fed *ad libitum*. The reason for this decrease is not understood, but it is conceivable that kidney AP is very sensitive to changes in food consumption. ALT activity decreased in the TNF-injected and pair-fed control rats as compared with the control rats fed *ad libitum*, but the decrease was statistically significant only in the TNF-injected rats. GGT, which is present in large amounts in the kidney, showed a 20% decrease in activity; however, it was not statistically significant.

Table III shows the enzyme activities in serum from the rats in Experiment 6. Comparison of the mean activities of the controls fed *ad libitum*, and the pair-fed and TNF-injected rats showed no significant difference.

Hematoxylin and eosin-stained liver sections from the TNF-injected rats in Experiment 6 showed no histologic abnormalities. In particular, there was no evidence of cholestasis, bile duct proliferation, hepatocellular damage, or inflammation (Fig. 4A). Ultrastructurally, the bile canaliculi were normal in appearance, their lumen was patent, and the microvilli showed no abnormalities. The pericanalicular ectoplasm was normal in width and structure (Fig. 4B). Bile duct epithelial cells in the portal tracts also showed no abnormalities (not shown in Fig. 4). Histochemical staining of frozen liver sections from Experiment 6 for AP showed minimal canalicular activity in the hepatocytes of control rats fed *ad libitum* (Fig. 4C). In TNF-injected rats, there was a marked increase in AP activity in the hepatocytes; this increase was confined to the canalicular region and was observed uniformly in hepatocytes of all three zones of the liver acini (Fig. 4D).

Hematoxylin and eosin liver sections from TNF-injected rats showed a variable increase in the mitotic

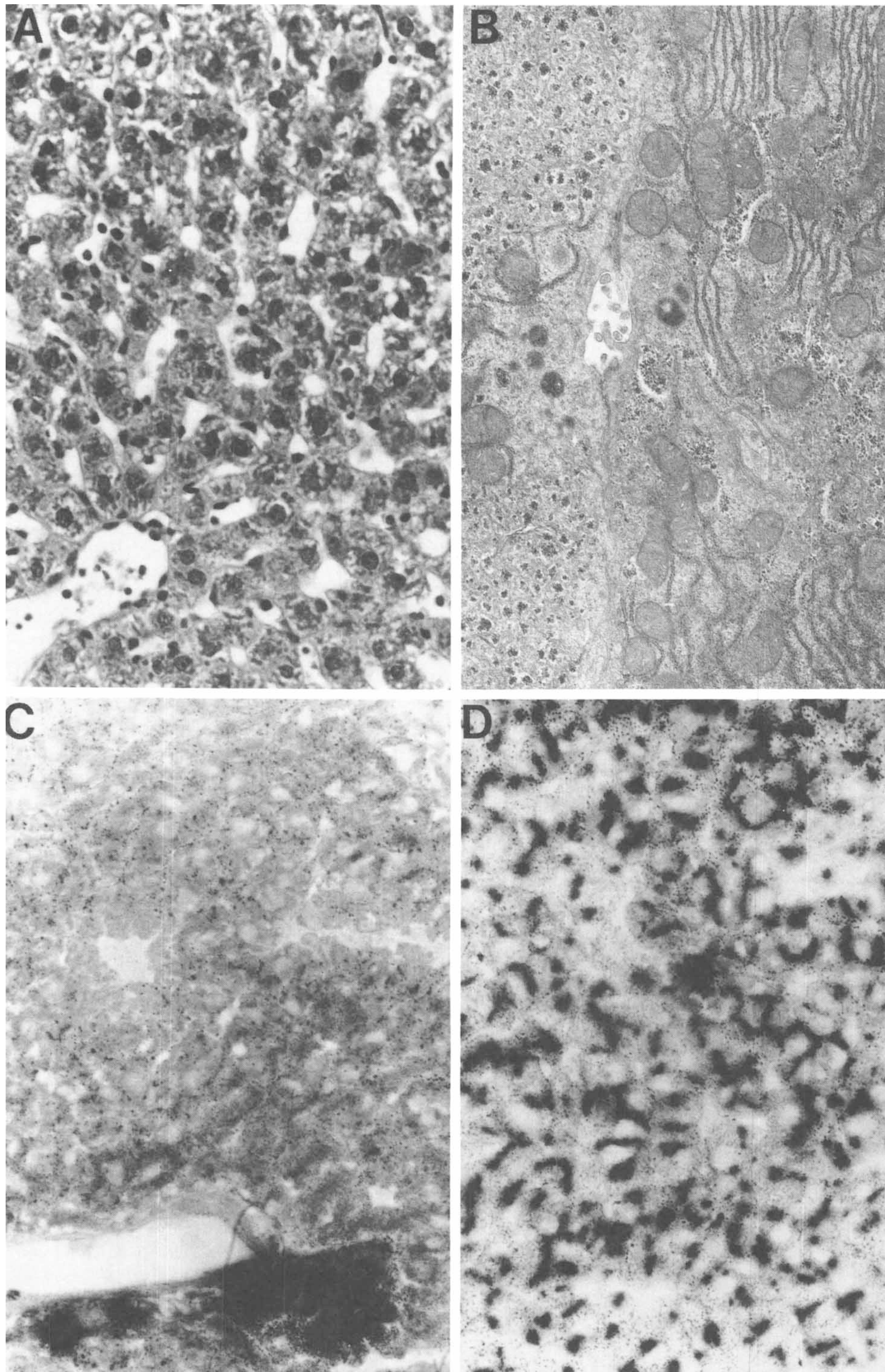

Figure 4. (A) Experiment 6, TNF-injected rat. The liver shows normal histologic appearance; a central vein is seen in the lower left corner (magnification ×150). (B) Experiment 3, TNF-injected rat. Electron photomicrograph shows normal bile canaliculus and absence of cholestasis (magnification ×11,000). (C) Experiment 6, control rat fed *ad libitum* Histochemical stain for AP shows minimal canalicular AP activity in hepatocytes. Note the strong AP activity in scattered inflammatory cells in portal space at the bottom (magnification ×150). (D) Experiment 6, TNF-injected rat. Markedly increased AP activity in the canalicular region of hepatocytes highlights the bile canalicular network (magnification ×150).

activity of hepatocytes over that observed in the control rats fed *ad libitum*. The mean mitotic count in 20 high-power fields was approximately 9-fold higher in Experiment 3 ($P = 0.0001$) and only 3-fold in Experiment 6 ($P = 0.2$).

Discussion

The results indicate that increasing doses of TNF injected intraperitoneally over a period of 5 days lead to decreases in ALT and AST, and to an increase in AP. Histologically, there was increased mitotic activity in the hepatocytes, and positive histochemistry in the canalicular regions for AP.

The decrease in ALT activity after TNF injection is reminiscent of the decrease observed in the liver in extrahepatic cancer (2, 7), and fetal liver (30). Herzfeld and Greengard (7, 9) suggested that the decrease in ALT in rats transplanted with the Walker 256 carcinoma may be part of a generalized pattern of liver enzyme dedifferentiation commonly observed in tumor-bearing animals. It is of interest to note that our finding of decreased hepatic ALT activity after TNF injection is consistent with the recent postulate by Siddiqui and Williams (35) that a similar decrease in extrahepatic cancer is mediated directly by TNF released from macrophages in the presence of the tumor. A decrease in the activity of the enzyme may result in decreased production of pyruvate from alanine. This is also consistent with our previous observation (34) that TNF decreases the activity of some of the enzymes of gluconeogenesis in the liver. The increase in hepatic AP activity after TNF administration has, to our knowledge, never been reported previously in the liver of TNF-treated or tumor-bearing animals. Using a clonal cell line of relatively undifferentiated mesenchymal cells, Ng *et al.* (36) showed transduction of the AP gene by retinoic acid as evidenced by an increase in AP mRNA synthesis. The increase was potentiated by TNF, although TNF alone showed no effect. It should be noted, however, that these cells contain AP of the bone type, and may behave differently from hepatocytes *in vivo*. The increase in hepatic AP that we observed may have been caused by TNF alone, or in cooperation with the retinoic acid that is normally present in mammalian liver.

In the serum, significant elevation in AP activity occurs in extrahepatic cancer (14–19) and after TNF administration (23–26). In patients with cancer, intravenous administration of recombinant TNF results in transient increases in serum bilirubin, and AP and GGT activity, all of which are indicators of cholestasis (23–26). Increase in serum AP activity has commonly been associated with cholestasis because of the demonstration in experimental animals and humans that synthesis of AP increases in the liver after ligation of the bile duct or biliary obstruction (37–40). The increased synthesis appears to be at the level of enhanced AP mRNA translation (40). Schlaeger *et al.* (41) also showed that ligation of the bile duct in the rat caused an increase in AP activity in the liver that was accompanied by increases in serum AP, 5′NT, and GGT activity.

In the present study, although there was no detectable increase in AP activity in the serum after the injection of relatively low doses of TNF in the rat, there was a significant increase in the canalicular region of the hepatocytes (Fig. 4) without any microscopic evidence of bile duct damage, obstruction, or proliferation. This increase may represent an early step in a sequence of events during which TNF may directly or indirectly stimulate the synthesis of AP. At higher concentrations, or longer presence in the circulation, TNF may also cause bile duct proliferation, inflammation, biliary obstruction, and a rise in serum AP (22, 42). The physiologic function of AP is not known, but it is believed that the enzyme somehow mediates bile transport across the canalicular membrane (43).

TNF administration to rats resulted in a 3- to 9-fold increase in the mitotic activity of hepatocytes. This observation is similar to that obtained by others, but the range of reported mitotic activity is quite large (44, 45). This is probably due to the short half-life of TNF (less than 20 min) (46) and magnifies the importance of frequency, route, and duration of TNF administration. Similar increases in mitotic activity have been observed in animals after transplantation of nonmetastasizing tumors (47–49).

Since TNF can induce the production of other cytokines (such as the production of interleukin 1 by macrophages), it is conceivable that the above changes in liver enzymes are induced by TNF independently, or in concert with other cytokines. We are currently engaged in determining whether interleukins 1 and 6 induce liver changes comparable to those induced by TNF, or whether each cytokine induces a characteristic pattern of enzymatic changes.

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1. Greenstein JP. Biochemistry of Cancer. New York: Academic Press, pp507–588, 1954.
2. Goodlad GAJ, Clark CM. Influence of the growth of the Walker 256 carcinoma on hepatic transaminases. Nature **195**:186–187, 1962.
3. Chung W, Roberts EH, Bauer JM. Some urea cycle enzymes and acyl-RNA synthetases of rat hepatomas. Cancer Res **27**:956–961, 1967.
4. Kato R, Takanaka A, Takahashi A, Onoda K. Drug metabolism in tumor-bearing rats: Activities of NADPH-linked electron transport and drug-metabolizing enzyme systems in liver microsomes of tumor-bearing rats. Jpn J Pharmacol **18**:224–244, 1968.

5. Wu C, Homburger HA. Responsiveness of enzymes in liver to growth of Novikoff hepatoma. Br J Cancer **23**:204–209, 1969.
6. Scherstén T, Wahlqvist L, Johansson LG. Lysosomal enzyme activity in liver tissue from patients with renal carcinoma. Cancer **23**:608–613, 1969.
7. Herzfeld A, Greengard O. The dedifferentiated pattern of enzymes in livers of tumor-bearing rats. Cancer Res **32**:1826–1832, 1972.
8. Scherstén T, Wahlqvist L, Jilderos B. Lysosomal enzyme activity in liver tissue, kidney tissue and tumor tissue from patients with renal carcinoma. Cancer **27**:278–283, 1972.
9. Herzfeld A, Greengard O. The effect of lymphoma and other neoplasms on hepatic and plasma enzymes of the tumor-bearing rat. Cancer Res **37**:231–238, 1977.
10. de Rosa G, Pitot HC. Alterations in enzymes of amino acid catabolism in livers of rats bearing the Morris 7800 hepatoma. Cancer Res **38**:950–954, 1978.
11. Sun AS, Cederbaum AI. Oxidoreductase activities in normal rat liver, tumor-bearing rat liver, and hepatoma HC-252. Cancer Res **40**:4677–4681, 1980.
12. Theologides A. Pathogenesis of anorexia and cachexia in cancer. Cancer Bull **34**:140–149, 1982.
13. Herzfeld A, Greengard O, Shields W. Tissue enzyme changes in parabiotic rats with subcutaneous lymphoma or fibrosarcoma. JNCI **60**:825–828, 1978.
14. Walsh PN, Kissane JM. Nonmetastatic hypernephroma with reversible hepatic dysfunction. Arch Intern Med **122**:214–222, 1968.
15. Utz DC, Warren MM, Gregg JA, Ludwig J. Reversible hepatic dysfunction associated with hypernephroma. Mayo Clin Proc **45**:161–169, 1970.
16. Ramos CV, Taylor HB. Hepatic dysfunction associated with renal carcinoma. Cancer **29**:1287–1292, 1972.
17. Axelsson U, Hägerstrand I, Zettervall O. Unusual pattern of alkaline phosphatase activity and renal carcinoma. Acta Med Scand **195**:223–225, 1974.
18. Lemmon WT, Holland PV, Holland JM. The hepatopathy of hypernephroma. Am J Surg **110**:487–491, 1965.
19. Henderson AR, Grace DM. Liver-originating isoenzymes of alkaline phosphatase in the serum: A paraneoplastic manifestation of a malignant schwannoma of the sciatic nerve. J Clin Pathol **29**:237–240, 1976.
20. Tracey KJ, Cerami A. Metabolic responses to cachectin/TNF. Ann NY Acad Sci **587**:325–331, 1990.
21. Yasmineh WG, Parkin JL, Caspers JI, Theologides A. Tumor necrosis factor/cachectin decreases catalase activity of rat liver. Cancer Res **51**:3990–3995, 1991.
22. Tracey KJ, Wei H, Manogue KR, Fong Y, Hesse DJ, Nguyen HT, Kuo GC, Beutler B, Cotran RS, Cerami A, Lowry S. Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. J Exp Med **167**:1211–1227, 1988.
23. Selby P, Hobbs S, Viner C, Jackson E, Jones A, Calvert AH, McElwain T, Fearon K, Humphreys J, Shiga T. Tumor necrosis factor in man: Clinical and biological observations. Br J Cancer **56**:803–808, 1987.
24. Sherman ML, Spriggs DR, Arthur KA, Imamura K, Frei E III, Kufe DW. Recombinant human tumor necrosis factor administered as a five-day continuous infusion in cancer patients: Phase I toxicity and effects on lipid metabolism. J Clin Oncol **6**:344–350, 1988.
25. Sheron N, Lau JYN, Daniels HM, Webster J, Eddelston ALWF, Alexander GJM, Williams R. Tumor necrosis factor to treat chronic hepatitis B virus infection. Lancet **336**:321–322, 1990.
26. Jones A, Selby PJ, Viner C, Hobbs S, Gore ME, McElwain TJ. Tumor necrosis factor, cholestatic jaundice, and chronic liver disease. Gut **31**:938–939, 1990.
27. Van Potter R, Gebert RA, Pitot HC. Enzyme levels in rats adapted to 36-hour fasting. Adv Enzyme Regul **14**:247–265, 1966.
28. Stovroff MC, Fraker DL, Swedenborg JA, Norton JA. Cachectin/tumor necrosis factor: A possible mediator of cancer anorexia in the rat. Cancer Res **48**:4567–4572, 1988.
29. Hayhoe TGJ, Quaglino D. Cytochemical demonstration and measurement of leukocyte alkaline phosphatase in normal and pathological states by a modified azo-dye coupling technique. Br J Haematol **4**:375–389, 1958.
30. Knox WE. Enzyme Patterns in Fetal, Adult and Neoplastic Rat Tissues. New York: S. Karger, pp296–306, 1967.
31. Szasz G. γ-Glutamyltranspeptidase. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis. New York: Academic Press, pp715–720, 1974.
32. Gerlach U, Hiby W. 5′-Nucleotidase. In: Bergmeyer HU, Ed. Methods of Enzymatic Analysis. New York: Academic Press, pp871–875, 1974.
33. Dixon M, Webb EC. Enzymes. New York: Academic Press, pp634–635, 1979.
34. Yasmineh WG, Theologides A. Effect of tumor necrosis factor on enzymes of gluconeogenesis. Proc Soc Exp Biol Med **199**:97–103, 1992.
35. Siddiqui RA, Williams JF. Tentative identification of the toxohormone of cancer cachexia: Roles of vasopressin, prostaglandin E2 and cachectin-TNF. Biochem Int **20**:787–797, 1990.
36. Ng KW, Hudson PJ, Power BE, Manji SS, Grummer PR, Martin TJ. Retinoic acid and tumor necrosis factor-α act in concert to control the level of alkaline phosphatase mRNA. J Mol Endocrinol **3**:57–64, 1989.
37. Aronsen KF, Hägerstrand I, Nordén JG. Enzyme studies in dogs with extra-hepatic biliary obstruction. Scand J Gastroenterol **3**:355–368, 1968.
38. Aronsen KF, Hägerstrand I, Nordén JG. Enzyme studies in man with biliary obstruction. Acta Pathol Microbiol Scand **80**:501–508, 1972.
39. Schmidt FW. Rationale for the use of enzyme determinations in the diagnosis of liver disease. In: Demers LM, Shaw LM, Eds. Evaluation of Liver Function. Baltimore: Urban and Schwarzenberg, pp51–57, 1978.
40. Seetharam S, Sussman NL, Komoda T, Alpers DH. The mechanism of elevated alkaline phosphatase activity after bile duct ligation in the rat. Hepatology **6**:374–380, 1986.
41. Schlaeger R, Haux P, Katterman R. Studies on the mechanism of increase in serum alkaline phosphatase activity: Significance of the hepatic bile acid concentration for the leakage of alkaline phosphatase from rat liver. Enzyme **28**:3–13, 1982.
42. Feingold RF, Barker ME, Jones AL, Grunfeled C. Localization of tumor necrosis factor-stimulated DNA synthesis in the liver. Hepatology **13**:773–779, 1991.
43. Kaplan MM. Serum alkaline phosphatase—Another piece is added to the puzzle. Hepatology **8**:526–528, 1986.
44. Mealy K, Wilmore DW. Tumor necrosis factor increases cell mass. Br J Surg **78**:331–333, 1991.
45. Beyer HS, Stanley M, Theologides A. Tumor necrosis factor-α increases hepatic DNA and RNA and hepatocyte mitosis. Biochem Int **22**:405–410, 1990.
46. Beutler BA, Milsark IW, Cerami A. Cachectin/tumor necrosis factor: Production, distribution and metabolic fate in vivo. J Immunol **135**:3972–3977, 1985.
47. Malgrem RA. Observations on a liver mitotic stimulant present in tumor tissue. Cancer Res **16**:232–236, 1956.
48. Baserga R, Kisieleski WE. Cell proliferation in tumor-bearing mice. Arch Pathol **7**:142–148, 1962.
49. Rev-Kury LH, Kury G, Friedell GH. Thymidine uptake in livers of tumor-bearing hamsters. Arch Pathol **82**:77–79, 1966.