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α-Fetoprotein as a Marker for Hepatic Regeneration in the Dog[1]

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A radioimmunoassay for human α-fetoprotein (AFP) was utilized to quantitate AFP following partial liver resection in the dog. Nine dogs were studied; seven underwent 70% hepatectomy and two received sham operations. Serum AFP, alkaline phosphatase, glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvate transaminase (SGPT), total bilirubin, and galactose elimination capacity (GEC), a quantitative index of hepatic function, were measured preoperatively and at regular intervals postoperatively. Following 70% hepatectomy, AFP was first noted to be increased from the preoperative level (94 ± 7 SEM ng AFP activity/ml) on the fourth postoperative day (556 ± 75; $P < 0.05$) with a peak value being reached between Days 8 and 12 (1008 ± 111; $P < 0.025$). AFP then gradually decreased, returning to normal by Day 24 (116 ± 12; $P > 0.5$). These changes in AFP concentration parallel, in a slightly delayed fashion, hepatic proliferative activity as measured by DNA synthesis following hepatic resection in the dog. No alteration in AFP concentration was seen in the control animals. Elevations in SGOT, SGPT, and alkaline phosphatase were observed in all dogs following liver resection from Day 2 through 26. In contrast to AFP, changes in the serum concentrations of these enzymes were highly variable in both magnitude and duration. GEC was not significantly altered following liver resection in any dog. The results indicate that AFP is superior to liver enzymes and GEC as a marker for hepatic regeneration in the dog.

INTRODUCTION

α-Fetoprotein (AFP) is one of many macromolecules synthesized by hepatocytes and secreted into the bloodstream. AFP is found at greatly elevated levels during fetal life, but decreases to barely detectable concentrations in the perinatal period. Recurrence of elevated AFP levels frequently occurs during adult life in patients with hepatocellular carcinoma, testicular teratocarcinoma, and a variety of liver diseases including hepatitis and cirrhosis [9, 17, 21]. Transient increases in serum AFP concentrations have also been found in rats and mice following either partial hepatectomy or administration of hepatotoxins [3, 18]. Studies utilizing primary liver cell culture indicate that regenerating hepatocytes are a principal source of this increased AFP production [8]. These results suggest that AFP might serve as a quantitative monitor of hepatic regeneration. The objective of the present study was to assess the efficacy of AFP as a marker of hepatocyte proliferation following 70% partial hepatectomy in the dog.

MATERIALS AND METHODS

Animals. Eight adult male mongrel dogs, 20 to 30 kg, were housed in individual runs and received *ad lib.* water and kennel rations for the duration of the study. The exact age of each dog could not be documented; however, weekly monitoring of the body weight of each animal did not reveal any significant change during the 4- to 8-week time course of the experiment. Following intravenous

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pentobarbital anesthesia (25 mg/kg), seven dogs underwent 70% partial hepatectomy according to the method of Sigel [19] and two dogs received a sham laparotomy. All animals were maintained with intravenous 5% dextrose solution during and for 24 hr following surgery. Excised liver lobes were perfused with normal saline and weighed. Preoperative liver weight was estimated by assuming the resected portion represented 70% of liver mass. Each dog was sacrificed between 3 and 8 weeks postoperatively and the residual liver was weighed.

Quantitation of AFP. Anti-AFP was prepared by injecting rabbits with AFP fractions obtained from human cord serum purified with DEAE-cellulose (Bio-Rad, Richmond, Calif.) and Con A Sepharose (Pharmacia, Piscataway, N. J.). Subsequent absorption with normal human serum produced a monospecific antiserum to human AFP. Further purification of cord serum AFP using Sepharose 4B coupled with anti-human serum yielded AFP that gave a single band on polyacrylamide gel electrophoresis. This product was labeled with ^{125}I using the lactoperoxidase method [11] and used in a double-antibody radioimmunoassay.

Peripheral venous blood for AFP analysis was drawn preoperatively and on alternate postoperative days. Samples for AFP determination were centrifuged and stored at −10°C. Whole or diluted serum (0.05 ml) was added to a test tube containing 0.1 ml of 5% rabbit serum in 0.075 *M* Na_2HPO_4 (pH 7.4)–.075 *M* NaCl (PBS) and 0.01 ml of diluted anti-AFP. After incubation at 37°C for 4 hr, 0.01 ml of ^{125}I-AFP was added and the tube incubated overnight (16 hr) at room temperature. The next day, 0.01 ml of goat anti-rabbit IgG antiserum was added and the tubes incubated for 30 min at 37°C and 1 hr at 4°C. After washing with 0.4 ml PBS, the pellet was counted in a Beckman Biogamma counter. Canine AFP concentrations were calculated using a standard curve prepared with human AFP reference serum obtained from the World Health Organization. Inter–intraassay variation was less than 10%.

Liver chemistries. On postoperative Days 1, 2, 4, 6, 8, 12, 16, 20, and 24 blood was drawn in a clot tube for glutamic-oxaloacetic transaminase (SGOT), glutamic-pyruvate transaminase (SGPT), alkaline phosphatase, and total bilirubin. The enzymes and bilirubin were determined with a Technicon SMAC Autoanalyzer in the central clinical laboratory.

Galactose elimination capacity. Galactose elimination capacity (GEC), which estimates the liver's maximal capacity for metabolism of galactose, was measured preoperatively and 2, 4, 8, 12, and 26 days following partial hepatectomy in three dogs and one control. After obtaining a blank blood sample, galactose was intravenously administered as a bolus dose (0.5 mg/kg body wt) in a sterile 30% solution. Heparinized blood samples were drawn at 5-min intervals for 60 min and serum galactose concentrations determined with the Worthington Galactostat Kit. GEC was calculated by the method of Tygstrup [23].

RESULTS

The partial immunological identity shared by canine and human AFP as reported by Nishi and Hirai [14, 15] was also demonstrated by us in double diffusion studies and by radioimmunoassay inhibition curves. The continuous precipitin band that developed between the antibody and wells containing purified human AFP and pooled newborn puppy serum provided visual proof of complex formation between anti-human AFP and canine AFP, while the spur that formed at the junction of the canine and human precipitin bands indicated that the immunological identity of the AFP from the two species is not complete (Fig. 1). Logarithmic plots of inhibition curves prepared from these same samples and from the serum of an adult dog 1 week following 70% partial hepatectomy indicated that the radioimmunoassay was sensitive to canine as well as human AFP. The slope of the inhibition curves in Fig. 2 illustrates the lower affinity of anti-human AFP for the canine

AFP found in dog serum. This discrepancy in inhibition curve slope most likely resulted in an underestimation of absolute AFP concentration in dog serum, since all AFP concentrations were expressed relative to the human AFP standard curve.

Figure 3 reveals the alterations in serum AFP which occurred following 70% partial hepatectomy. AFP became significantly increased above the preoperative level (94 ± 7 ng AFP activity/ml, mean ± SE) by the fourth postoperative day (556 ± 75 ng AFP activity/ml, $P < 0.05$). Peak values (1008 ± 122 ng AFP activity/ml, $P < 0.025$) were reached on postoperative Days 8 through 12 and ranged from 300 to 1980 ng AFP activity/ml in individual dogs. Return to preoperative AFP levels occurred by postoperative Day 24. Included in Fig. 3 is the time course of DNA synthesis following 70% partial hepatectomy in the dog as previously measured by Sigel and coworkers [20]. Changes in AFP activity parallel, in a slightly delayed fashion, hepatic regeneration as monitored by DNA synthesis. In contrast to the partially hepatectomized animals, no statistically significant alteration in serum AFP concentration could be detected following sham operation.

The three hepatic enzymes significantly increased within 24 hr after liver resection

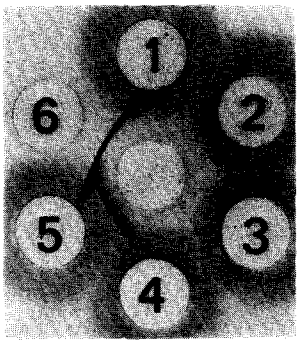

FIG. 1. Analysis of AFP from canine and human sources by Ouchterlony double diffusion in agar stained with Coomassie blue. The center well contains anti-human AFP and the surrounding wells contain pooled serum from normal humans (1), adult dogs (4), newborn dogs 2–3 weeks old (5), and AFP purified from human cord serum (6).

(Fig. 4). Although peak values for SGOT, alkaline phosphatase, and SGPT occurred on postoperative Days 1, 20, and 24 respectively, all three enzymes remained elevated throughout the entire 28-day postoperative period. Serum bilirubin significantly increased on postoperative Day 1 but returned to normal by Day 8. Only minimal changes in these variables could be detected following sham laparotomy.

GEC showed a tendency to increase rather than decrease subsequent to partial hepatectomy, but at no time was the post-

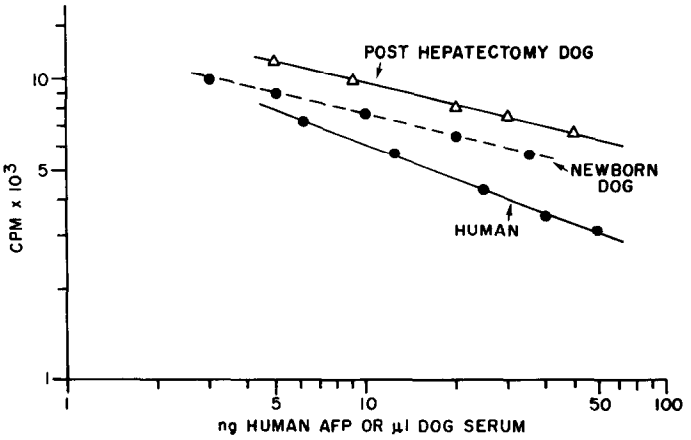

FIG. 2. AFP radioimmunoassay inhibition curves determined with aliquots of pooled newborn dog serum, serum from an adult dog 7 days following partial hepatectomy, and AFP purified from human cord serum.

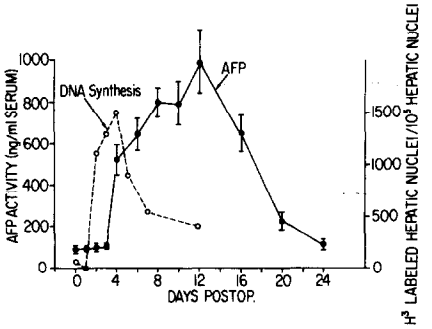

FIG. 3. Changes in serum AFP (mean ± SE) observed in seven dogs following 70% partial hepatectomy compared to the corresponding alterations in hepatic DNA synthesis observed by Sigel *et al.* [20] under conditions similar to our own.

operative GEC significantly different from the preoperative value (Fig. 5).

Restitution of hepatic mass was nearly complete by 21 days following partial hepatectomy. Liver weights of dogs sacrificed between 3 and 8 weeks postoperatively ranged from 84 to 111% of the estimated preoperative weights (Table 1).

DISCUSSION

The most significant finding of this investigation is that AFP is produced in increased amounts during the course of normal liver regeneration in the dog. Although hepatic DNA synthesis was not assessed in this experiment, its time course following 70% partial hepatectomy in the dog has been well defined by Sigel [20] and recently by Francavilla [6] using methods and conditions very similar to those used in this study. These investigators found peak DNA synthesis activity at 3 and 4 days following partial hepatectomy with elevations above control levels persisting to postoperative Day 12. In our study, maximum serum AFP activity was present between postoperative Days 8 and 12 and gradually returned to preoperative values

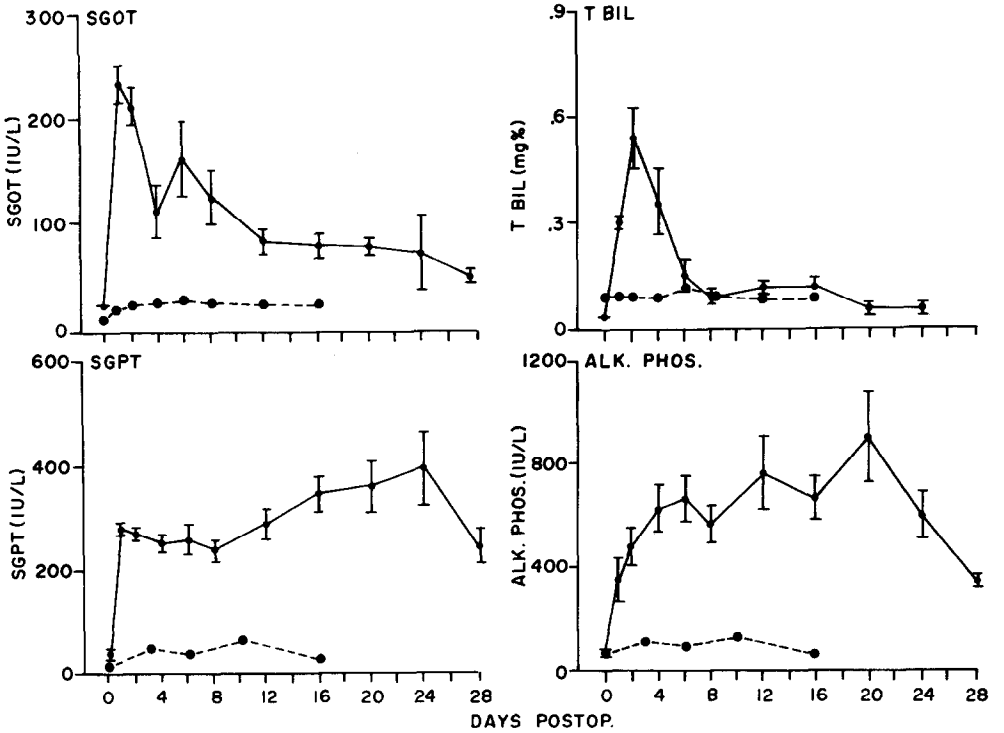

FIG. 4. SGOT, total bilirubin, SGPT, and alkaline phosphatase (mean ± SE) at various intervals following either 70% partial hepatectomy in seven dogs (solid lines) or sham laparotomy in two dogs (broken lines).

by Day 24 (Fig. 3). Thus the pattern of serum AFP activity following partial hepatectomy in the dogs tends to parallel hepatocyte proliferation in a delayed fashion, with a lag period of 5–8 days.

A kinetically similar pattern of AFP production and hepatocyte DNA synthesis is seen following two-thirds partial hepatectomy in the rat [16]. In this species, maximum DNA synthesis occurs at 24 hr following partial hepatectomy and serum AFP activity peaks 2 days later. The magnitude of the AFP response seems to be related to the age of the animal, with younger rats exhibiting higher AFP levels [12]. This may partially explain the wide variation in peak AFP response seen in dogs in our study, since it was impossible to document the precise age of each mongrel used.

Increased serum AFP has been reported in children who have undergone hepatic resection [10]. However, Alpert and Fuller [11] failed to find elevated AFP levels in 11 adult patients following extensive liver resection. In their study the majority of serum samples were obtained during the first 2 postoperative weeks. Although the exact time course of hepatic DNA synthesis is not known for man, it is known to be slower than that of the rat or dog. It is conceivable that the maximal AFP response following partial hepatectomy in man could occur after 2 weeks. Further studies are required to determine if serum AFP activity will serve as a sensitive marker of hepatic regeneration in man.

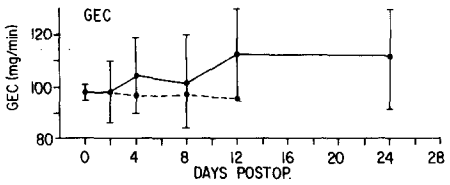

FIG. 5. GEC values (mean ± SE) at various intervals following either 70% hepatectomy in three dogs (solid lines) or sham laparotomy in one dog (broken lines).

Following partial liver resection, increased levels of SGOT and SGPT probably reflect hepatocellular injury [2]. It has been suggested that elevations in alkaline phosphatase represent increased synthesis by bile duct epithelium [5] or regenerating parenchymal cells [22]. In the present study, significant elevations of each of these enzymes preceded DNA synthesis by 24 hr and persisted through the fourth postoperative week. In contrast to these conventional indices of liver function, increased levels of AFP were not apparent until 2 days after the initiation of regeneration and were coincident with the period of rapid liver growth. Therefore, increases in AFP concentration appear to parallel the time course of hepatic regeneration in the dog more closely than elevations in SGOT, SGPT, or alkaline phosphatase.

TABLE 1

COMPARISON OF ESTIMATED PREOPERATIVE AND RESIDUAL WET LIVER WEIGHTS

Time of death postoperative (weeks)	Dog No.	Cause of death	Estimated preoperative wet liver wt (g)	Residual dry liver wt (g)	Percentage of preoperative wt
2	1607	Sacrificed	605	442	73
3	1614	Sacrificed	710	621	87
4	1434	Sacrificed	727	639	88
5	1417	Sacrificed	531	445	84
7	1420	Sacrificed	714	790	111
7	1427	Sacrificed	879	843	96
8	1445	Sacrificed	743	675	91
4	1523 (Control)	Sacrificed	—	721	100
8	1561 (Control)	Sacrificed	—	640	100

The maximal capacity of the liver to metabolize galactose (GEC) has been reported to provide a quantitative means of assessing liver function in rat and man [7, 13]. However, no significant differences were observed in pre- and postoperative GECs in dogs that underwent either a sham operation or partial hepatectomy. In fact, there was a tendency for GEC values to increase rather than decrease following liver resection. A probable explanation for this observation is an increase in galactose metabolizing enzymes in residual hepatocytes similar to that seen by Bauer and associates following partial hepatectomy in the rat [4].

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