HAMSTER FEMALE PROTEIN

A Divergent Acute Phase Protein in Male and Female Syrian Hamsters

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Acute phase proteins are serum constituents that change in concentration after injury. This acute phase reaction may result in decreased serum levels (albumin), or more frequently, a 50–300% increased serum level (C_3 or haptoglobin) (1, 2). Human C-reactive protein (CRP)[1] is the classic acute phase reactant, its level increasing as much as 500-fold after injury (for review see reference 3). The beneficial effects of CRP in the inflammatory response are unknown although CRP can initiate or modulate a variety of biological events (4). Furthermore, CRP has evolved conservatively, insofar as homologous proteins with similar functional attributes have been found in many other species: the stable preservation of this protein during evolution implies some biological importance (5). In general, CRP homologs are present at relatively low serum levels, and sex of the species has little influence on normal content or acute phase changes after injury. A serum protein homologous to CRP has been described in the Syrian hamster. This protein, called female protein (FP) (6), has structural and functional features in common with CRP (7). However, FP is an unusual homolog because it is a prominent serum protein in females (1–2 mg/ml), and because its expression is sex limited, normal males having low serum levels (6).

In the present report, the capacity of FP to respond as an acute phase protein was examined in male and female Syrian hamsters. The results indicated a divergent sex-limited acute phase response, characterized by increased FP serum levels in males and a transient decrease in females. When male/female FP levels were inversed by hormonal manipulation, a corresponding change in acute phase response also occurred, so that the pattern of response was related to high or low serum FP concentration rather than gender per se. Studies with ^{125}I-FP indicated a similar catabolism of FP in both sexes under normal conditions and during acute phase changes; therefore, the variations in FP levels primarily reflect differences in rate of FP synthesis rather than degradation.

Materials and Methods

Animals. Random bred and inbred (LCH) Syrian hamsters were obtained from the Rocky Mountain Laboratories' hamster colony. Animals were fed Purina laboratory chow and water ad lib. Hamsters injected with ^{125}I-FP received 0.1% KI in drinking water before and during the experiment.

Antisera and Protein Purification. Rabbit antisera specific for FP and its use for ring diffusion quantification were previously described (6, 7). Hamster serum albumin was quantified by ring

[1] *Abbreviations used in this paper:* AP, amyloid P component; CRP, C-reactive protein; FP, female protein; PAGE, polyacrylamide gel electrophoresis; PC, phosphorylcholine; $T_{1/2}$, half-life; T_0, 5 min after injection.

diffusion in specific antibody-agar. Electrophoretic mobility of FP either as isolated protein or in whole serum was compared by polyacrylamide gel electrophoresis (PAGE) or immunofixation (8), respectively.

FP was isolated by passing hamster sera over a phosphorylcholine (PC)-conjugated Sepharose column in the presence of 0.5 mm Ca^{++} as previously described (7). The FP that eluted with 10^{-3} M PC in the same buffer was free of contaminants as determined by gel diffusion analysis and PAGE.

Surgical Procedures. Male hamsters under pentabarbital anesthesia were castrated by excision of the testicle after ligation of the spermatic cord.

Drugs. Hamsters were injected intramuscularly with 1.5 mg cortisone acetate in aqueous suspensions (Upjohn Co., Kalamzoo, MI). Female hamsters received testosterone either in aqueous suspension (Robinson Laboratory, San Francisco, CA) 2.5 mg three times weekly, or depotestosterone propionate (Repotest; Burns-Biotec Laboratories, Omaha, NB) 2.5 mg/wk. A similar depression of FP levels was detected with both preparations, so that depotestosterone was used in long-term experiments and continued during the acute phase test interval. Turpentine (steam distilled; Phipps Products Corporation, Boston, MA) was passed through Millex-HA 0.45 μm filter (Millipore Corp., Bedford, MA) and a total of 0.5 ml was injected intramuscularly into the rear legs of hamsters. Hamsters were also injected subcutaneously with 0.5 ml of 1% croton oil (Sigma Chemical Co., St. Louis, MO) in liquid paraffin.

Acute Phase Experiments. Hamsters, usually in groups of 10, were bled under ether anesthesia from the retroorbital area before challenge and at appropriate intervals thereafter. The typical experiment incorporated control groups that were plasma bled only or that received a 0.5-ml injection of saline rather than a noxious stimulus. To establish the pattern of fluctuation of FP levels in normal females, hamsters in groups of 10 were bled either daily or at 6-h intervals for 1 wk. After a 1 wk rest, the original bleeding schedule was started again for 1 wk. Using this protocol, FP levels were charted for individual animals over a 3–6 wk interval.

Radioactive Labeling. Purified FP (1 mg) was labeled with ^{125}I (1 mCi) using Iodogen (Pierce Chemical Co., Rockford, IL) (0.010 mg) at 4°C for 10 min with subsequent dialysis. This procedure resulted in a specific activity of 0.3–0.4 mCi/mg with little detectable denaturation as (*a*) >95% of the label was precipitable by sequential reaction with specific rabbit anti-FP and an equivalent amount of guinea pig anti-rabbit Ig; (*b*) the ^{125}I-FP was 7S by 10–37% sucrose density gradient ultracentrifuge analysis; and (*c*) >95% of the label was specifically absorbed to PC-Sepharose in the presence of Ca^{++}. The half-life ($T_{1/2}$) data were obtained using ^{125}I-FP that, after labeling and dialysis, was passed over a small PC-Sepharose column equilibrated with 0.1 M Tris HCl buffer, 0.5 mM $CaCl_2$, pH 7.0. The column was washed with the same buffer until negligible counts per minute were in passthrough and then the ^{125}I-FP was eluted with 0.1 M EDTA Tris HCl, pH 7.0, and dialyzed against phosphate-buffered saline (PBS). >95% of the ^{125}I put on the column was contained in the PC eluate in 4 different preparations.

Metabolic Studies. Hamsters under pentobarbital anesthesia were injected intravenously into a rear leg vein exposed by cutting the skin. Retroorbital plasma samples were obtained at 5 min (T_0) and various intervals thereafter and counts per minute in 50 λ plasma was determined in a well counter. Paired groups of male and female hamsters received a known amount of ^{125}I-FP (usually ~10 μc ^{125}I per hamster, representing 15–25 μg FP) in 0.25 or 0.5 ml PBS. Similar results were obtained using smaller amounts (2–5 μg) of ^{125}I-FP. The integrity of the ^{125}I-FP in vivo was shown by specific anti-FP precipitation of ^{125}I in four sera obtained at 12 h (all >87%) and four sera taken at 45 h (all >85%).

Metabolic parameters were calculated according to Waldmann and Strober (9). The 5 min plasma sample was used to calculate plasma volume and serum FP concentration at T_0. A semi-log plot of a fraction of remaining plasma ^{125}I at various intervals after injection was resolved into exponential functions (10). The terminal linear slope (slope b_1) of ^{125}I-FP disappearance found after 15–20 h represents $T_{1/2}$ of plasma ^{125}I after extravascular equilibration. When extrapolated to T_0, the intercept (C_1) represents an estimate of the body pool of ^{125}I-FP which is intravascular. The more rapid, nonlinear early disappearance of ^{125}I-FP was resolved into another exponential (slope b_2) by curve peeling, and its intercept (C_2) was used to

calculate the fraction of intravascular pool catabolized per day or fractional catabolic rate (FRC) by the formula, $FRC = [1/(C_1/b_1) + (C_2/b_2)]$ where $b_1 = 0.693/T_{1/2}$.

Results

Characteristics of FP in Serum From Normal Male Hamsters. Male hamsters normally have easily detectable serum FP (up to 0.5 mg/ml) only for a transient interval from 15 to 30 d of age (6). These serum levels subsequently decline to a level that is difficult to detect by simple gel diffusion. However, adult males do have FP in serum, albeit at low levels, that can be quantified by hemagglutination inhibition or ring diffusion assay with appropriate dilution of antisera. Using these assays, serum FP was found to be within the range of 4–20 μg/ml in 74% of 50 males at 90 d of age; the other 26% were equally distributed between 20 and 45 μg/ml. These low serum levels of 10–15 μg/ml in adult males presumably reflect a testosterone suppression, as previous studies have shown that FP promptly increases in male serum after castration or DES treatment (6). Because FP in normal males was quantitatively so different from that in females, it was critical to establish that FP in male serum was the same as that in females. Accordingly, male FP was examined using serum from normal males and also from males with elevated FP levels such as found after castration, diethylstilbestrol treatment or turpentine injections. FP from male serum was found to be identical to female FP by the following criteria: (*a*) male FP produced a precipitin line that fused identically with female FP on simple gel diffusion when tested with various rabbit antisera specific for FP isolated from females; (*b*) male FP was electrophoretically similar to female FP when analyzed either by agarose electrophoresis of whole serum with anti-FP immunofixation, or by PAGE of isolated FP; (*c*) male FP was similar in size to female FP (pentamer and monomer) (7); and (*d*) male FP was selectively bound to PC-Sepharose and eluted with free PC as previously shown with female FP (7).

Variability of FP Concentration in Serum of Normal Females. Serum FP levels in normal females have previously been noted to range from 0.5 to 3.0 mg/ml (6, 7) and within a group of 10 will vary 20–30% ± mean value. This variation in FP serum levels was greater than that found with other hamster serum proteins such as immunoglobulin and B_1C (11). To evaluate these individual differences, the kinetics of FP concentration was examined in individual females by obtaining daily plasma samples for 7, 14, or 21 d. The results of three experiments of 10 females each indicated that this normal spread of FP level was due to individual fluctuation in concentration rather than constant individual differences in FP level. Thus, although mean concentration of an experimental group remained relatively stable, the FP levels of 20–30% of females at one time were randomly increasing or decreasing over an interval of 3–4 d. However, FP concentration appeared to fluctuate in all females at one time or another without any obvious periodicity. Changes of 0.6–0.8 mg FP/ml were not uncommon and were not related to estrus cycle. Also, plasma samples obtained at 6 h intervals indicated that FP changes were not related to time of day. Animals caged alone or in groups showed a similar fluctuation and no synchronism was detected among cage-mates. These experiments suggest an unusual homeostatic control of FP characterized by periodic increases or decreases of serum level.

Changes in FP During Acute Phase Response. Turpentine (0.5 ml) injected intramuscularly was used as an acute phase stimulant in male and female hamsters and

changes in FP levels were measured from subsequent serial plasma samples. As shown in Fig. 1, the mean plasma FP in a group of 10 male hamsters 48 h after one injection of turpentine had increased from 15 to 95 μg/ml. FP levels remained elevated for a prolonged interval (3 wk) and three additional injections of turpentine (days 1, 2, 3) did not result in higher FP levels. Saline injections or bleeding per se had no significant effect on FP levels as seen in the control groups. For comparative purposes, serum albumin concentration was also monitored (Fig. 1) and showed a prompt decrease after turpentine. A similar acute phase increase of FP was seen after injection of croton oil (not shown). Elevated FP levels also were detected in male hamsters with transplanted tumors such as GD36 (12), an Ig-secreting, SV40-derived lymphoma (13); average FP concentration in 19 male hamsters with this tumor was 0.31 mg/ml (range 0.01–1.18). Similarly, modest increases of FP (0.04–0.08) were detected in serum of male hamsters after inoculation with complete Freund's adjuvant.

In contrast to results in the male, the acute phase reaction to turpentine injection in female hamsters consisted of a transient decrease in FP levels. After one injection of turpentine, FP characteristically decreased ~50% within 24 h (Fig. 2) and returned to normal range after 4–6 d. Similar results were seen after injection of croton oil (not shown). Injection of saline or bleeding alone (control female) had no significant effect on FP levels. Serum albumin levels in female hamsters were depressed by turpentine injection similar to males and remained low for at least 3 wk (seen in Fig. 5).

Additional injections of turpentine (days 1, 2, 3) did not further depress FP levels but did prolong the duration of the depression (Fig. 2). In three separate experiments,

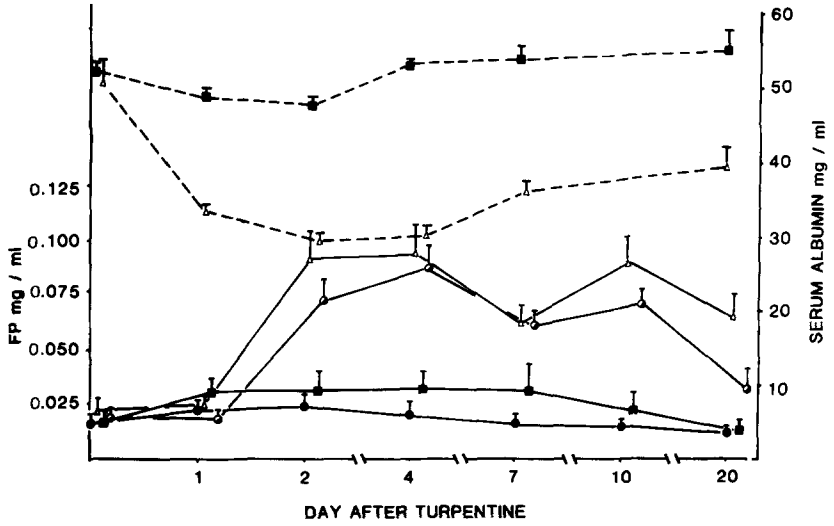

FIG. 1. Effect of turpentine on serum FP in male hamsters. Male hamsters in groups of 10 were injected once on day 0 with saline (■) or 0.5 ml turpentine i.m. (△). Another group received additional turpentine injections on days 1, 2, 3 (◑). FP (——) levels were determined on these hamsters and a normal group (●) and the mean concentration plotted (bar, +1 SEM). Serum albumin (---) concentrations also were determined in the hamsters injected with saline or turpentine on day 0 and the mean concentration plotted (bar, +1 SEM).

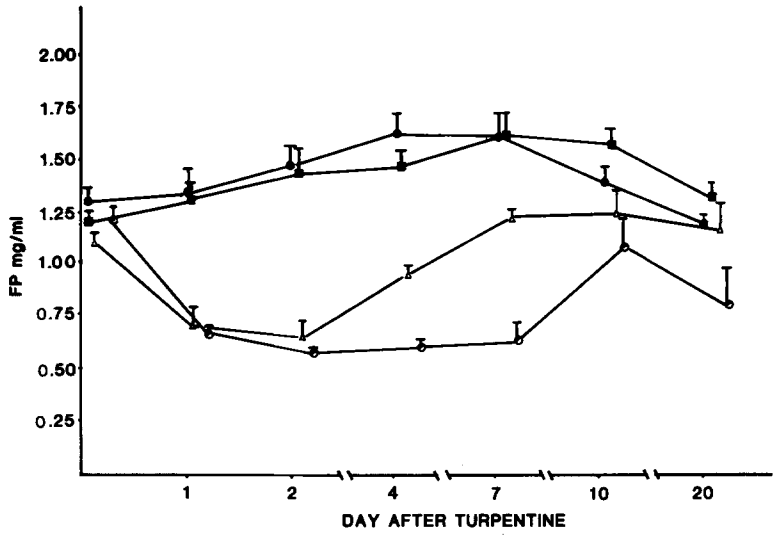

FIG. 2. Effect of turpentine on serum FP in female hamsters. Experiments are similar to those in Fig. 1 except using female hamsters that, in groups of 10 each, were injected once with saline on day 0 (■), turpentine (day 0) (△), or four times with turpentine (days 0, 1, 2, 3) (◑). Along with control group (●), FP levels were determined at various intervals and the mean level plotted (bar, +1 SEM). Serum albumin change (not shown) in group receiving one injection turpentine was similar to that found in male hamsters in Fig. 1 (see Fig. 5).

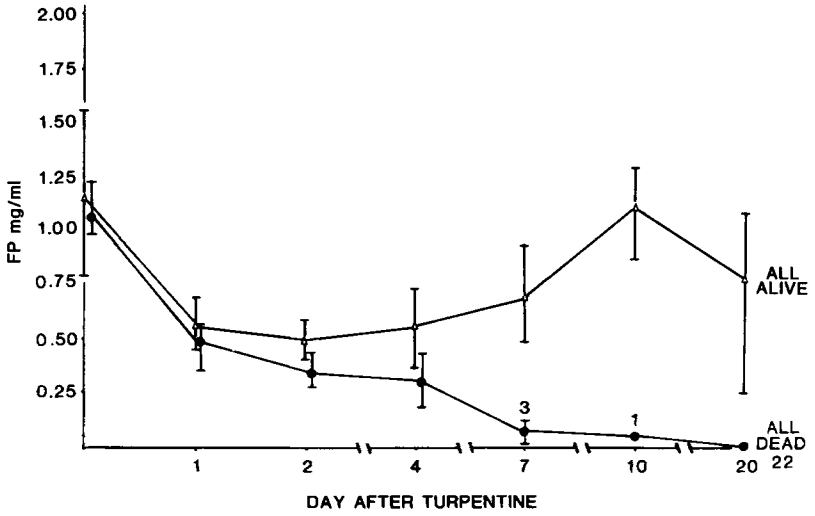

FIG. 3. FP levels in 10 female hamsters (seven survivors [△] vs. three nonsurvivors [●]) injected four times with turpentine. Same group as presented in Fig. 2, except mean FP concentration (and absolute range) in three dying hamsters is compared with the FP levels found in seven survivors.

10–30% of the hamsters receiving this treatment (turpentine, days 0, 1, 2, 3) expired during the 3 wk period of the experiment and their FP levels were noted retrospectively to be exceptionally low for a few days before death. When compared with survivors, the FP of nonsurvivors segregated in a distinct low range. For example, Fig. 3 shows the same group that received four injections of turpentine as presented in Fig. 2 and compares mean FP levels (and absolute range) in the seven survivors with the FP

levels in the three animals that expired by day 22. Death was characteristically preceded by very low (<100 μg/ml or 10% of normal) levels of FP. On the other hand, serum albumin levels were similar in the survivor and nonsurvivor groups (not shown).

Effect of Cortisone on FP in Females. Hamster FP is an unusual homolog of CRP because serum levels of FP are sensitive to steroid sex hormone control. The question arises whether the release of corticosteroids during inflammation could be responsible for acute phase change of FP in female hamsters injected with turpentine. To evaluate this potential steroid involvement, cortisone was administered to normal and injured females in an attempt to mimic and enhance, respectively, the acute phase changes of serum FP concentration. In groups of 10, female hamsters were injected with cortisone acetate (1.5 mg daily), turpentine (day 0), or both. The change of serum FP in these three groups is shown in Fig. 4. The administration of cortisone alone did cause a progressive decrease in FP levels to 0.07 mg/ml by day 11; however, the FP decrease in 24 h was not as great as seen after turpentine alone. In the group receiving both

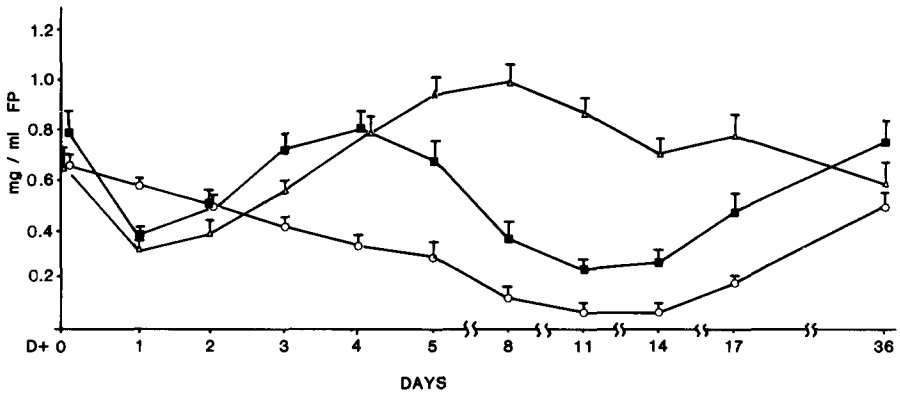

FIG. 4. Effect of cortisone acetate (1.5 mg/d, day 0–11) on serum FP of normal and turpentine-injected (0.5 ml) animals. Female hamsters in groups of 10 received cortisone alone (○), turpentine alone (△) or both treatments (■). Change in plasma FP concentration was determined and mean value plotted (bar, +1 SEM).

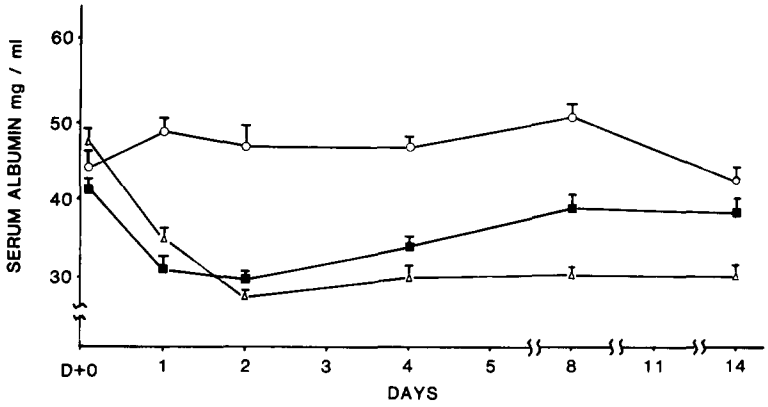

FIG. 5. Effect of cortisone on serum albumin concentration. Groups of animals are same as in Fig. 4, showing that cortisone did not affect normal albumin levels, although it did diminish the prolonged depression of albumin after turpentine. Mean value plotted (bar, +1 SEM).

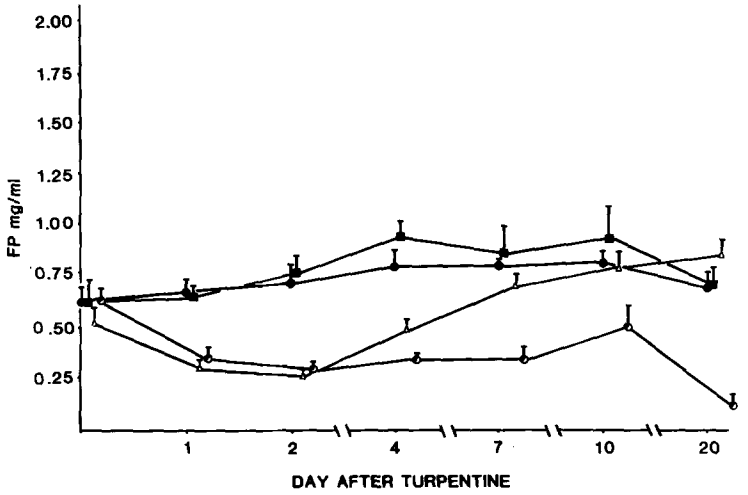

FIG. 6. Effect of turpentine injection (0.5 ml) on FP concentration in plasma of castrated male hamsters. Previously castrated male hamsters in groups of 10 were injected on day 0 with saline (■) or turpentine (△), or with turpentine on days 0, 1, 2, and 3 (◑). Plasma FP concentration was determined in these groups and in a control group (●) of castrated males and mean value was plotted (bar, +1 SEM).

cortisone and turpentine, administration of daily cortisone did not appreciably affect the acute phase decrease of FP. Furthermore, FP in this group returned to normal level (recovery phase) even with cortisone, so that the depressing effect of the cortisone actually was first evident about day 5. The lack of cortisone inhibition on the recovery phase of FP suggests that this increase to normal level represents a rather dynamic recovery, which is less susceptible to cortisone inhibition than normal synthesis. Although the results of this experiment do not rule out a steroid mediator in FP response to inflammation, it is apparent that exogenous steroid, even when given as a large single dose (not shown), did not reproduce the acute phase response. Furthermore, exogenous cortisone did not enhance the FP acute phase response which actually countered and delayed the depressing effect of cortisone. Serum albumin levels (Fig. 5) were not depressed by cortisone alone and a similar decrease in albumin occurred after turpentine with or without cortisone. However, daily cortisone did shorten the characteristically prolonged depression of serum albumin.

Acute Phase Reaction of FP in Males and Females with High and Low FP Levels, Respectively. During the acute phase response, FP levels increased in males and decreased in females. Is change in concentration intrinsically related to gender or is it related to the low and high FP levels in normal males and females? The results of the following studies indicated that the pattern of acute phase response correlated with FP levels at the time of challenge rather than sex per se. This was shown in experiments with castrated male hamsters which develop high FP levels (0.5–0.6 mg/ml) (6). The response to turpentine injection was tested in 10 males 6 wk after castration when FP concentration had stabilized at levels about 50-fold greater than normal. As seen in Fig. 6, the acute phase response of castrated males was very similar to that seen in normal females with transient depression of FP from 0.53 to 0.26 mg/ml (day 2). Intact males, treated with diethylstilbestrol, also develop high serum FP levels (6), and show a similar acute phase decrease after turpentine injection (not shown).

In a corollary experiment, females with depressed FP levels were challenged with turpentine. Exogenous testosterone will eradicate the elevated FP levels which develop in castrated males (6) and also will depress FP in normal females. Thus, a group of 10 normal female hamsters (mean FP of 0.75 mg/ml) was treated with 2.5 mg depotestosterone/wk, and 10 wk later their mean FP was 0.14 mg/ml (range 0.07–0.23). Separated into two groups of five each, they were injected with turpentine or saline (Fig. 7 A). The female acute phase response of FP now consisted primarily of a delayed but substantial net increase of FP with mean peak levels on day 12 of 0.53 mg/ml. Longer testosterone treatment (21 wk) of 10 female hamsters resulted in mean FP of 0.03 mg/ml (range 0.01–0.06). The response of this group to turpentine was almost identical to the normal male response (peak 0.110 by day 3) (Fig. 7 B). Thus, the opposing acute phase change of FP in normal male and female hamsters appeared to be a function of serum levels at time of challenge and hormonal alteration of FP levels also resulted in a commensurate change in the acute phase response.

Catabolism of ^{125}I-FP. Normal female hamster serum contains 50–100-fold more FP than normal male serum. This situation could be the result of sex differences either in FP synthesis (female > male) or in FP catabolism (male > female). To determine the

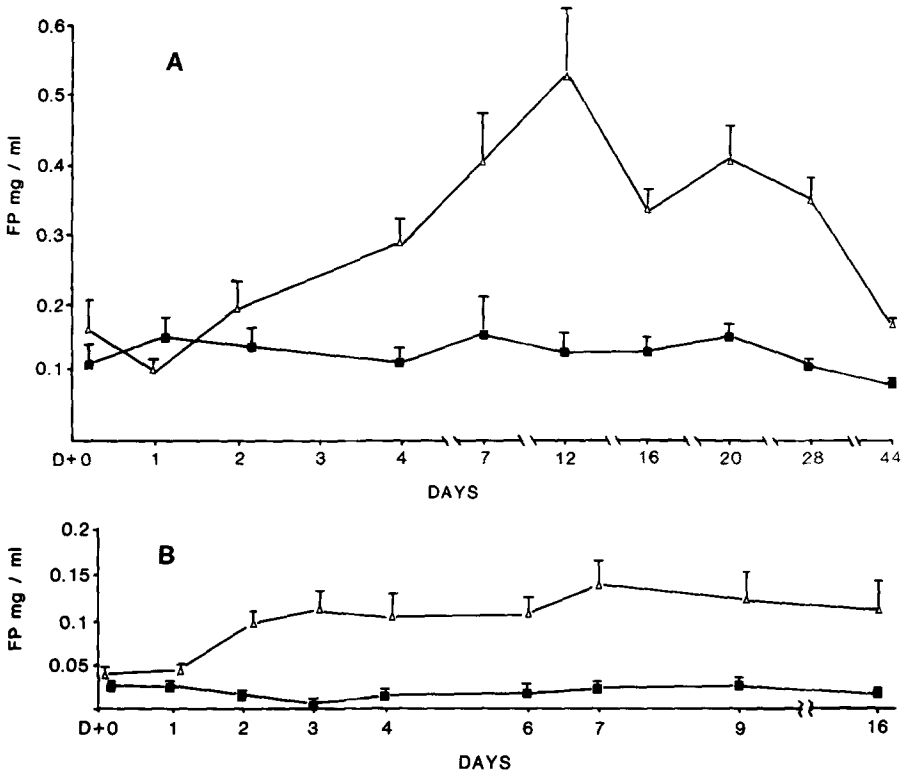

FIG. 7. Effect of turpentine on FP in female hamsters suppressed with testosterone. Female hamsters were treated with depotestosterone propionate (2.5 mg/wk) for 10 wk (*A*) or 21 wk (*B*). In groups of five each, they were injected with 0.5 mg turpentine (△) or saline (■) on day 0. Mean FP values (bar, +1 SEM) were plotted. Even after partial testosterone suppression (*A*), a marked acute phase increase in FP level was detected. FP acute phase response in fully suppressed females (*B*) was very similar to that of normal males.

metabolism of FP, the catabolism of ^{125}I-FP was studied in paired groups of normal male and female hamsters. The results with four different ^{125}I-FP preparations were similar and showed a comparable $T_{1/2}$ (slope b_1) in 15 males (9.5–14 h) and 15 females (12–16 h). Further calculations of ^{125}I-FP catabolism for the total curve (two exponenential functions) in eight hamsters indicated a mean FRC/h slightly faster in males (0.129) vs. females (0.073) due to an enhanced extravascular distribution in males (unpublished results). However, the overall FP catabolism (FRC × total circulating FP) was 34-fold greater in the females (0.335 mg FP/h) vs. males (0.010 mg FP/h). These results indicate that sex differences in FP serum concentration were primarily due to a greater synthesis in females. Calculation of FP catabolism per 24 h revealed that an impressive amount (80.4 mg/kg) of FP was synthesized per day in normal female hamsters.

As previously shown, challenge of females with intramuscular turpentine characteristically results in a 50% decrease in serum FP concentration. To determine if a change of catabolism of FP (i.e., some consumptive process) was responsible for this acute phase reaction, ^{125}I-FP was injected on day 0 into females (groups of three each) that had received intramuscular turpentine on days −2, −1, and 0. Accordingly, the $T_{1/2}$ of ^{125}I-FP could be measured during various phases of response including the initial decrease (day 0), after the decrease (day −1), and during recovery increase (day −2). When compared with a normal control group, the catabolism of plasma ^{125}I-FP in all groups was similar and not accelerated during initial drop nor prolonged during recovery increase. Similar results were obtained in three other experiments and indicate that the variations in serum levels during acute phase were a reflection of changes in synthetic rate. Also, examination of the turpentine injection site and other organs did not demonstrate any sequestration of the radioactive label.

Similarly, the catabolism of the same ^{125}I-FP preparation was evaluated in male hamsters (groups of three each) with increasing FP levels (injected with turpentine on day 0, −1) or already elevated FP levels (injected with turpentine on day −2). The terminal linear slope (b_1) of ^{125}I-FP in all groups was similar to that of normal control ($T_{1/2}$, 10.2–11.7 h). Calculation of net catabolism from the total curve (FRC × total circulating FP) revealed an increased rate of synthesis (0.06 mg FP/h) during acute phase elevation of FP (normal control group, 0.014 mg FP/h).

Discussion

In addition to CRP, hamster FP is closely related to another human protein called amyloid P component (AP). Human CRP and AP share extensive homology of their amino acid sequence, have a similar subunit assembly, and the same pentameric morphology by electron microscopy (14, 15). However, human serum AP is not an acute phase protein (16) but is a constituent in normal basement membrane (17) and characteristically is found in deposits of amyloid from which it was first isolated (18, 19). Proteins homologous with human CRP and AP have been described in a variety of vertebrates including mammals, birds, amphibians and fish (20, 21; reviewed in 22). A conservative evolution within this family of proteins (called pentraxins) (14) has facilitated the identification of these homologous proteins in that many functional and structural features have been preserved (22). As a member of this stably preserved family of proteins, hamster FP is an unusual homolog because it is a prominent normal serum protein in females and is under sex hormone control (6). Furthermore,

FP has the PC-binding specificity (Ca^{++} dependent) characteristic of CRP yet structurally FP is more similar to AP (80% NH_2 terminus identity) (7). As an acute phase reactant, FP displays equally unique features that, to our knowledge, have not been described in pentraxins or any acute phase protein. That is, the FP concentration after injury changes in opposite directions depending on gender of the hamster. This divergent acute phase response emphasizes the 50- to 100-fold physiologic difference between the sexes when FP synthesis and serum concentration are compared. Because catabolism of ^{125}I-FP was not significantly perturbed during the acute phase reaction, the acute phase changes in FP concentration apparently reflected changes in the rate of FP synthesis; that is, a transient decrease in females vs. a sustained increase in males. Various mediators have been implicated in the mechanism to trigger synthesis of CRP after injury (23–25). The question arises whether the divergent FP response was a result of (*a*) a contrasting mediator release after injury, i.e., a female inhibitor agent vs. the male stimulatory factor, or (*b*) release of the same mediator which could trigger opposite responses according to the available receptor at the site of synthesis. Preliminary attempts to define an acute phase serum factor with stimulatory (male) or inhibitory (female) activity on FP synthesis have been unsuccessful.

It is clear, however, that the acute phase response was not inherently related to gender per se, since an acute phase change characteristic of the opposite sex was observed when the relative concentration of FP was inversed by hormonal treatment. Therefore, the acute phase response pattern correlated with serum concentration of FP at time of challenge. This result suggested that sex hormone treatment also reversed the net effect of the "mediator" involved in the acute phase response. This may not represent two separate hormone effects, however, since an inhibitory acute phase stimulus somehow may be intrinsically related to ongoing high FP synthesis and a stimulatory response with low FP synthesis. However, it was noted that females with even a partial suppression of serum FP by testosterone (Fig. 7 A), showed a net increase of FP levels after injury. Therefore, testosterone may effect some reversal of the acute phase response before a complete repression of FP synthesis.

The FP response to injury was not diametrically opposite in males and females. In contrast to the prolonged elevation of FP in male acute phase, the female depression was finite and transient with return to normal levels. Although further injury (additional injections of turpentine) did not further depress FP levels, it did delay the onset of recovery to normal levels. In contrast, this recovery phase was not affected by concomitant cortisone injections which normally depressed FP levels in females. Therefore, the restoration of normal FP levels appeared to represent a very active phase of synthesis. Indeed, a progressive fall in females of FP to very low levels (except for hormonally induced depression) has usually been associated with impending death. This has been observed with lethality from turpentine injection, x-irradiation, pneumococcal septicemia, and wet tail disease (unpublished). Perhaps substantial serum FP levels are a necessary ingredient for viability of normal females. On the other hand, depressed synthesis may be an especially valid indicator of extreme illness. FP synthesis in normal females does appear to be under delicate control as fluctuation of serum levels was characteristic and simple laparotomy induced a typical acute phase decrease (unpublished). Perhaps synthesis of FP in females is directly inhibited by nonspecific toxic elements rather than by some specific inhibitory humoral signal. A hypothesis to unify the male-female dichotomy would postulate that such a

nonspecific toxic effect of injury caused the depression in females with their recovery phase actually corresponding to the acute phase increase of FP of males. However, in contrast to the male, the normal female acute phase response did not usually result in a net increase of serum FP levels.

Fluctuations in rate of FP synthesis probably are responsible for normal changes in FP levels in females; catabolism of ^{125}I-FP was similar in a number of individual females. With decreased synthesis, the rapid half-life of FP would result in the abrupt fall of serum levels observed in normal females. From the standpoint of synthesis, FP was indeed a major protein, as the 80 mg/kg catabolized (i.e., synthesized) per day in females was comparable to that calculated for IgG_2 (26), the major immunoglobulin of hamsters (11). However, exogenous testosterone could suppress production of FP in the female as it could in the castrated male hamster (6). Therefore, both genders share a comparable testosterone-sensitive mechanism to control synthesis of an apparently identical FP.

When compared with CRP, the mechanism of control for FP in acute phase must be more complex because of the overall regulation by sex hormones. However, this sex hormone control of FP also permits a unique situation in which the acute phase reaction can be manipulated and indeed reversed by appropriate treatment. The unusual characteristics of this pentraxin in normal and injured male and female hamsters are particularly intriguing because this protein is so similar functionally and structurally to CRP and AP. Whether FP fulfills a basic biological function similar to other pentraxins or has unique properties peculiar to the male and female Syrian hamster, the bizarre attributes of this protein may provide a new perspective on the physiological role of these ancient proteins.

Summary

Normal adult male hamsters have low levels (10–20 μg) of female protein (FP) in serum which increase approximately fivefold during an acute phase response. In contrast, normal females have 50- to 100-fold higher serum levels and the acute phase reaction consists of a transient decrease in FP (~50%), followed by a return to normal levels even under adverse conditions such as cortisone treatment (which by itself has a depressing effect on FP levels in normal females). The acute phase response was not inherently associated with gender, as the pattern of response could be changed to that of the opposite sex by appropriate hormonal manipulation. That is, castrated or diethylstilbestrol-treated males with high FP levels showed a female-type response whereas testosterone-treated females with low FP levels showed a male-type response. Studies on catabolism of ^{125}I-FP showed a similar rapid half-life ($T_{1/2}$, 9–16 h) in normal males and females and indicated that the sex difference in serum concentration was due to greater synthesis of FP in females. The divergent acute phase reaction of serum FP was related directly to changes in the FP synthetic rate (increased in males, decreased in females). As an indicator of serious pathology, a decrease of FP to very low levels in females was associated frequently with impending death.

The authors are grateful to Dr. T. A. Waldmann who graciously contributed many helpful criticisms on the metabolic studies.

Received for publication 13 December 1982 and in revised form 17 January 1983.

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