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Seromucoid and Albumin Syntheses After Uninephrectomy and Partial Hepatectomy in the Rat (38220)

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(Introduced by K. C. Hsu)

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Partial hepatectomy (PHX) is followed by an elevation in the rate of synthesis of the seromucoid fraction of plasma protein and this elevation can be differentiated from that caused by laparotomy alone (1). Chandler and Snider (2) observed that the increase in seromucoid production was biphasic. They suggested that the initial peak was due to surgical stress and that the second peak was specifically related to the regenerative response of the liver. Elevated seromucoid levels have also been reported in other proliferative states such as neoplasia (3) and pregnancy (4). Uninephrectomy (UNX) and PHX are generally analogous in that both operations stimulate rapid growth in residual organ tissue. It was of interest, then, to determine whether renal compensatory hypertrophy is accompanied by serum protein changes comparable to those found during liver regeneration. This point has only been partially investigated by Darcy (5) who showed that an immunologically specific plasma glycoprotein (seromucoid) was equally increased after UNX or laparotomy.

The present work was undertaken in order to determine whether changes in seromucoid synthesis parallel renal hypertrophy. As a comparison, albumin was also investigated. In addition, since the liver is the source of these plasma proteins (6), the effect of PHX upon UNX was also studied.

Materials and Methods. Animal procedures. Male Furth/Wistar rats (Microbiological Associates, Baltimore) were treated as described below. In Experiment 1, 200–250 g rats were used. Rats were left-UNXed, sham operated (SO) or unoperated (C) as previously described (7). Five to 6 rats of each group were killed 1, 2, 10 days after operation. Blood samples were obtained by heart puncture into heparinized glassware. A sample was collected for microhematocrit determination and plasma was made from the rest. In Experiment 2, 100–125 g rats were used. One group was UNXed and a second group was PHXed by ligation and removal of the median lobe. This approximated 30% of the whole liver. Another group was both left-UNXed and PHXed through the same incision. The fourth group was SO. Two to 4 rats of each group were sacrificed 1, 2, 3 and 7 days following surgery and blood collected as before. In a third experiment, using 150–200 g rats, 5 UNX and 5 SO were sacrificed 48 hr after surgery. All rats were sacrificed between 11:00 and 13:00 to minimize possible diurnal variations (8).

Plasma Protein Synthesis. In Experiments 1 and 2, 2 hr before sacrifice, glycine-1-^{14}C, 46.5 mCi/mmole, 7.5 μCi/100 g, was injected ip (Schwarz, Orangeburg, New York). In Experiment 3, leucine-3,4-^{3}H, 2 Ci/mmole (Schwarz), 38 μCi/100 g was used. Total plasma protein was collected by the method of Chandler and Neuhaus (9), albumin by the method of Majumdar *et al.* (1) and the seromucoid by the method of Neuhaus *et al.* (10), each fraction from a separate aliquot of original plasma. The final precipitates were dissolved in *N* NaOH with heating to 50° for 1 hr. Aliquots were either counted in a Beckman LS-200 Scintillation spectrometer as Cab-O-Sil gels (7)

or were used for protein determinations by biuret (11).

Tissue Hyperplasia. In Experiment 2, thymidine-methyl-^{3}H, 10 Ci/mmole, 20 μCi/100 g (Schwarz) was injected ip along with the glycine described above. At sacrifice, the livers and kidneys were blotted and weighed. Weighed samples were quick-frozen in solid CO_2-acetone and stored at $-70°$. DNA was extracted from thawed organs and quantified according to Burton (12). Aliquots were counted for ^{3}H activity.

Results. Total plasma protein. While both SO and UNX rats had increased uptake of label into total plasma protein compared to C rats (between 75 and 100% on day 1), there was no consistently significant difference between them in this respect.

Seromucoid. After UNX, the seromucoid fraction represented an increasing percent of both total protein content and of the radioactivity in that protein (Figs. 1 and 2). This increase reached a maximum on the second postoperative day and was significantly different from SO rats with both precursors (Figs. 1, 2 and Table I). It then dropped precipitously. PHX rats did not show relative elevated seromucoid radioactivity levels until the third day and this increased portion of total plasma protein was maintained for at least 1 week (Fig. 2). Rats, both UNX and PHX, had composite responses, i.e., second day peaks and then continued elevations.

Albumin. In Fig. 3, it can be seen that after UNX, plasma albumin radioactivity was unchanged relative to total protein on the first day but after SO, it was significantly lower. Subsequent changes in albumin were not significantly different. This observation was repeated in Experiment 2 (Fig. 2) in which UNX rat albumin radioactivities were significantly higher than the other operative procedures on day 1 only.

Interaction of UNX and PHX. Dually operated rats had depressed kidney DNA synthesis compared to UNX alone and depressed liver DNA synthesis compared to PHX alone (Fig. 4). At no time, however, did these doubly operated rats have significantly less restoration of liver or kidney mass compared to their respective singly operated groups (Fig. 5). In Figs. 4 and 5, the SO group was used as a base line to simplify the figures. This was possible because SO has relatively little effect upon

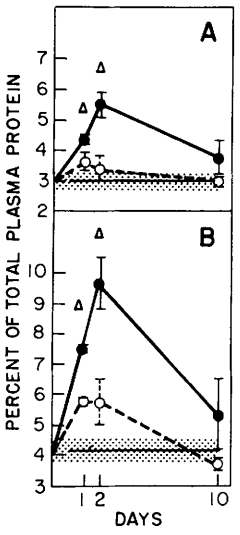

FIG. 1. Seromucoid as a percent of total plasma protein. A. Mg seromucoid per ml plasma/mg total protein per ml plasma. B. DPM (seromucoid) per ml plasma/DPM (total protein) per ml plasma. (2 hr after glycine-^{14}C injection). ●——● UNX; ○— — —○ SO; Vertical lines = ±Standard Error; Shaded area = C ± S.E., △ = significant difference between UNX and SO (Exp. 1).

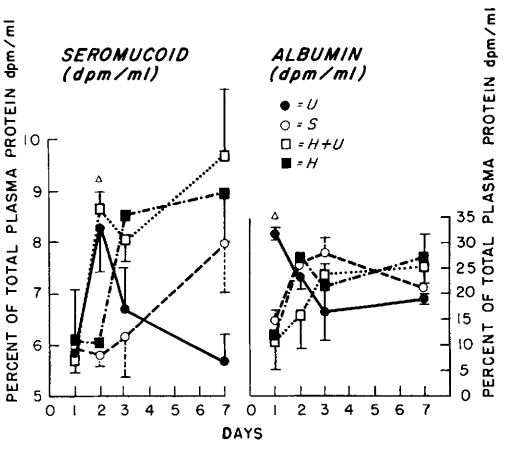

FIG. 2. Seromucoid and albumin radioactivities as percentages of total plasma protein radioactivity (Experiment 2). Symbols as in Fig. 1. ■—·—■ PHX; □·····□ PHX + UNX.

TABLE I. Seromucoid Content and Radioactivity in Experiment 3.[a]

Group	Content		Radioactivity	
	mg/ml[b]	%[c]	DPM/ml[b]	%[d]
SO[e]	2.94 ± 0.20	3.15 ± 0.29	35800 ± 3200	4.96 ± 0.22
	$P < 0.025$	$P < 0.05$	$P < 0.05$	$P < 0.05$
UNX[e]	3.95 ± 0.27	4.18 ± 0.29	57800 ± 8100	6.48 ± 0.55

[a] 2 days after operation, 2 hr after leu-3,4-^{3}H, 38 μCi/100 g, ip.
[b] Plasma.
[c] Percentage of seromucoid in total plasma protein.
[d] Percentage of seromucoid radioactivity in total plasma protein radioactivity.
[e] SO = sham operated, UNX = uninephrectomized; 5 rats in each group.

these parameters (7).

Discussion. The method of reporting changes in seromucoid production used here was designed to avoid the effect of changes in either precursor or protein pools. Presumably, all liver derived plasma proteins (6) are formed from the same amino acid pool. Therefore, differences in the proportional uptake of label into different plasma proteins should be independent of such pool sizes. The effects of possible changes in hemoconcentration are also eliminated by this method of calculation. Koj (13) has used similar methods to compare plasma protein synthetic rates after stress. The similar 48 hr response with two different precursors (Figs. 1, 2 and Table I) also tends to support the validity of the result. The data are calculated in terms of mg or DPM per ml. The latter figure is independent of plasma protein pool. The usefulness of this approach has been discussed (2, 13). The actual content of seromucoid rose in parallel with the radioactivity (Fig. 1, Table I). This has been reported after laparotomy (10). These consistent increases would not have been emphasized had the calculation been made on a DPM/mg basis (9).

The differences in response between SO and UNX rats could have been due to different degrees of stress as shown by the significant differences in body wt changes (Table II). Hematocrits were not as greatly effected. However, the yet greater stress of PHX did not produce a similar response.

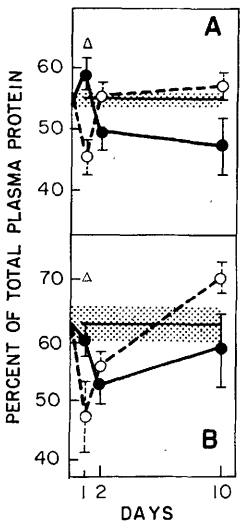

FIG. 3. Plasma albumin as a percent of total protein. Same format as in Fig. 1.

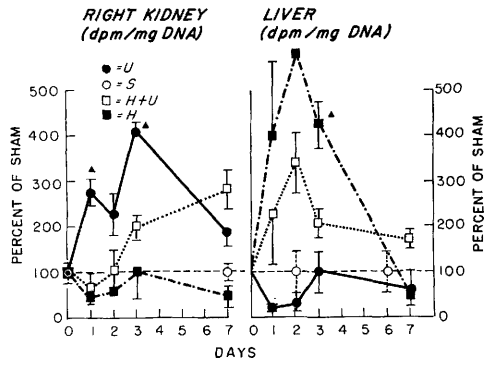

FIG. 4. Uptake of ^{3}H from thymidine-methyl-^{3}H into kidney and liver DNA as a percent of SO values. Symbols as in Fig. 3. ▲ = significant differences between singly and doubly operated rats (calculated as DPM/mg DNA).

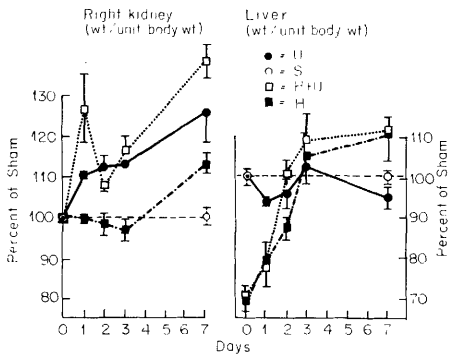

FIG. 5. Restoration of kidney and liver mass after UNX or PHX or both as a percent of SO values (calculated as g/100 g body wt). Symbols as in Fig. 4.

Majumdar *et al.* (1) have shown that neither serum albumin nor fibrinogen synthesis responds proportionally in rats subjected to insults of different severities. It is not known whether seromucoid synthesis is equally insensitive to the level of trauma *per se*.

The modest seromucoid peak at 24 hr after SO was observed by Chandler and Neuhaus (9) who reported their results in the same form as is used here. The response differs markedly from the much greater elevation and 48 hr peak after UNX. This argues for a specific response. In turn, the UNX response is different and independent from the post-PHX response as shown by the response of the doubly operated rats. The limited duration of the seromucoid peak precedes the peak of DNA synthesis by 24 hr (Fig. 4) but the proliferative response of the kidney continued beyond the time limits of seromucoid elevation (14) (Fig. 4). Conversely, after PHX the seromucoid synthetic rate remains elevated after both hepatic hyperplasia and hypertrophy are substantially completed (Figs. 4 and 5). Moreover, the rats both UNX and PHX showed inhibition of hyperplasia compared to singly operated animals but did not show any difference in seromucoid response. These data do not suggest a direct relationship between hyperplasia and elevated seromucoid production but that both are specific responses to new physiological conditions.

Albumin synthesis is unaffected by laparotomy at 24 hr while PHX has only a modest effect on this plasma protein (2). The greater responses of other proteins such as fibrinogen or seromucoid (2) significantly reduce the percentage uptake into albumin (Figs. 2 and 3). After UNX the relative labeling of albumin is maintained for the first day. This may reflect an initial and transient increase in albumin anabolism as a result of increased blood loss in UNX animals (14). Whether or not transient elevations in plasma proteins in the renoprival rat are nonspecific stimuli of renal hypertrophy as suggested by Royce (15) remains conjectural.

When both renal and hepatic tissue were

TABLE II. Body Wt Changes and Hematocrits 2 Days After Sham Operation (SO) or Uninephrectomy (UNX).

Experiment	△ Body Weight			Hematocrit		
	C[a]	SO	UNX	C	SO	UNX
1	+7.7 ± 1.9 g[d]	+4.0 ± 2.3 g	−7.8 ± 1.1 g	46 ± 1%	42 ± 1%	40 ± 2%
	n.s.[b]	$P < 0.001$		n.s.	n.s.	
2[c]		−2.0 ± 2.0	−6.5 ± 2.6		41 ± 1	33 ± 5
		n.s.			n.s.	
3		+4.6 ± 2.0	−1.8 ± 0.6		48 ± 1	47 ± 2
		$P < 0.025$			n.s.	

[a] C = unoperated controls.

[b] Not significant, significance between adjacent groups.

[c] Partially hepatectomized rats in this experiment lost 7.5 ± 6.5 g and dually operated rats lost 15.3 ± 1.3 g.

[d] Standard error of the mean.

removed from the same animal there was only limited interference with either regeneration. This inhibition was specifically directed at the hyperplastic response and not at all to the gross recovery of lost tissue weight. Since the days between 3 and 7 were not examined, it is possible that the inhibition took the form of extending the peak period as well as flattening it. This would have resulted in similar total DNA synthesis. However, if the inhibition of thymidine uptake was absolute rather than temporal, then a dissociation of hyperplasia from hypertrophy has been achieved. This has been observed after Dactinomycin administration to UNX rats (16). We have earlier reported that an implanted renal tumor inhibited renal compensatory hypertrophy (17). The present study would indicate that simple competition for growth factors by competing hypertrophies does not explain the previous observation.

Summary. After UNX, seromucoid synthesis represents an increasing proportion of total plasma protein synthesis. The increase reaches a sharp peak at 48 hr and rapidly diminishes. This pattern is distinct from those produced by either simple laparotomy or PHX. The seromucoid response is apparently not related to the renal hyperplastic response but is more likely a direct result of the UNX itself. After SO or PHX, relative albumin label drops. This is not observed for the first day after UNX. This may be the result of a transient increase in albumin synthesis. When UNX and PHX are done in the same animal, there is some inhibition of the respective hyperplasias but no interference with the expected restoration of lost tissue.

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