

Plasma Protein Biosynthesis Following Laparotomy and Partial Hepatectomy¹ (35064)

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(Introduced by A. Kurt Weiss)

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Majumdar *et al.* (1) have reported on the rate of synthesis of plasma albumin and fibrinogen following acute stress (Celite injection) and partial hepatectomy. They observed a marked increase in the rate of synthesis of these proteins following both procedures and showed that the transient increased synthesis following stress was adrenal dependent, whereas that following partial hepatectomy was not. Their studies were limited to albumin and fibrinogen and to the first 20 hr after stress or partial hepatectomy.

Two considerations make it of interest to extend these studies beyond 24 hr and to include observations of the seromuroid fraction in addition to albumin and fibrinogen. First, previous investigation (2) into the effects of injury on the seromuroid fraction has shown that its rate of synthesis was markedly stimulated by stress (laparotomy) 24 hr following surgery, and that the synthetic rate was still significantly elevated at 48 hr. Thus, it was of interest to determine the duration of the effects of stress on the rate of plasma protein biosynthesis. Second, it has been clearly established (3, 4) that the first wave of cell division does not occur until at least 24 hr following partial hepatectomy. The effects of rapid cell division on the rate of plasma protein biosynthesis as a function of time of liver regeneration have not been reported. The present paper reports the effects of stress (laparotomy) and partial hepatectomy on the rate of synthesis of albumin, fibrinogen, and the seromuroid frac-

tion between 1 and 14 days following surgery.

Materials and Methods. Male, albino rats were purchased from the Holtzman Company, Madison, Wisconsin. The animals received food and water *ad libitum* and were used when they weighed 200–225 g. Glycine-1-¹⁴C (6.35 mCi/mmole) was obtained from New England Nuclear Corporation. Heparin (20,000 U/ml) was purchased from the Upjohn Company, Kalamazoo, Michigan and bovine thrombin (5,000 U/vial) from Parke, Davis and Company, Detroit, Michigan.

Partial hepatectomies, involving removal of about 70% of the liver (left lateral and median lobes) were performed according to the procedure of Higgins and Anderson (5). Laparotomies (sham hepatectomies) involved all the steps in partial hepatectomy including manipulation of the liver, but stopped short of ligation and removal of the liver lobes.

For measurement of the rates of synthesis of the albumin, fibrinogen, and seromuroid fractions, glycine-1-¹⁴C was injected intravenously *via* the jugular vein (6) at a dosage level of 2.5 μ Ci/100 g of body weight. Blood was obtained 2 hr later by cardiac puncture using ether anesthesia. The blood was mixed rapidly with 0.1 ml (2000 U) of heparin and the plasma was obtained by centrifugation. Immediately following cardiac bleeding the animals were sacrificed and the livers were removed, rinsed, blotted, and weighed.

Fibrinogen was converted to fibrin by a modification of the method of Miller *et al.*

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(7). Five-tenths ml of plasma, 20 ml of saline, 0.5 ml of 2.5% protamine sulfate, 1.0 ml of 2.5% CaCl_2 , and 0.05 ml of thrombin were mixed, in that order, in a 50-ml polypropylene centrifuge tube. The clot was allowed to form overnight in the cold and was then collected by centrifugation. The fibrin was washed with three 20-ml portions of 0.9% saline and finally dissolved in 4 ml of 1 *N* NaOH. One-ml portions were placed in scintillation vials for counting (8) and 0.25-ml aliquots were used for determination of protein content by the method of Lowry *et al.* (9).

The remainder of the plasma was allowed to clot and the serum obtained was used for the determination of specific activities of the albumin and seromuroid fractions as previously described (8). Specific activities were expressed as dpm/mg of protein. The intravascular concentration of total proteins, seromuroid, and albumin, as well as the electrophoretic distribution of the serum proteins were determined as described previously (8).

Values for the relative rate of synthesis of each fraction were obtained from the following formula:

$$\text{Relative synthetic rate (RSR)} = \frac{\text{dpm incorporated/100 ml/2 hr/g of liver (expt)}}{\text{dpm incorporated/100 ml/2 hr/g of liver (control)}}$$

where dpm incorporated/100 ml = dpm/mg of protein \times mg of protein/100 ml.

Results. The relative synthetic rates of the various plasma protein fractions as a function of time following laparotomy and partial hepatectomy are shown in Fig. 1-3.

Albumin. Laparotomy alone had no effect on the RSR of albumin at any time interval measured following surgery. This is in agreement with the previous reports of Neuhaus *et al.* (8) and Koj (10). An early transient, adrenal-dependent increase in albumin synthesis has been observed by Majumdar *et al.* (1), and Balegno and Neuhaus (11) which peaked at about 8 hr and returned to normal by 16 to 18 hr. The earliest time interval tested in the present study was 24 hr following surgery, thus the early adrenal-dependent stimulation was not observed.

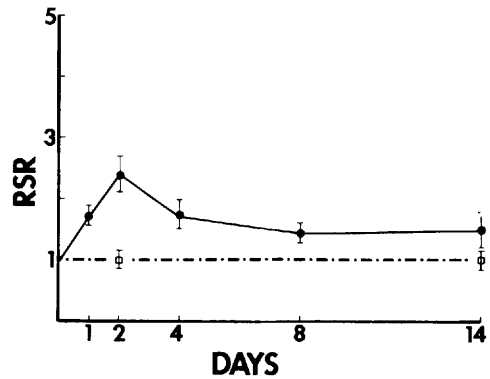


FIG. 1. Relative synthetic rates (RSR) of albumin as a function of time following laparotomy and partial hepatectomy. Values are expressed as means \pm SEM and were calculated as described in the text. Each point represents at least 6 rats; (●—), partial hepatectomy; four control rats were assayed at each time point. The control value obtained from day to day did not vary significantly, therefore, all values were combined and averaged. Thus, (□- -), represent the combined mean \pm SEM of the nonoperated controls.

Partial hepatectomy, on the other hand, caused an increase in the RSR of albumin which reached a maximum of about 2.5 at 2 days (Fig. 1). This was followed by a moder-

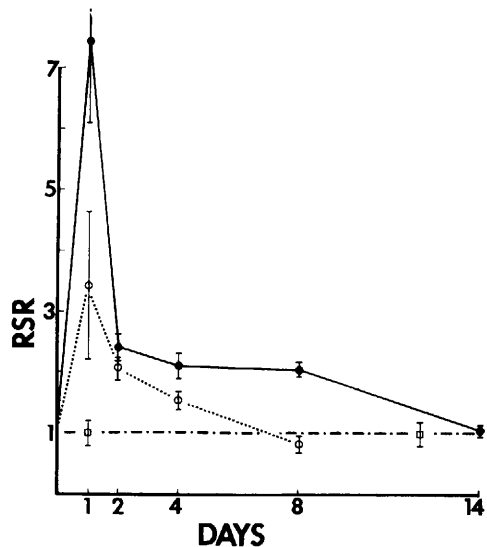


FIG. 2. Relative synthetic rate (RSR) of fibrinogen as a function of time following laparotomy and partial hepatectomy; (○- -), laparotomy; (●—), partial hepatectomy; (□- -), nonoperated controls. For details see legend to Fig. 1.

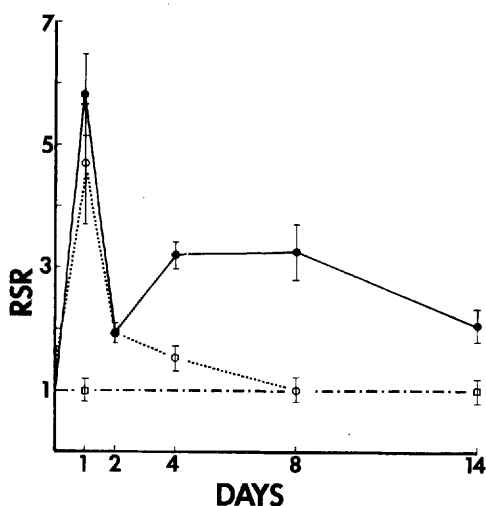


FIG. 3. Relative synthetic rate (RSR) of the seromuroid fraction as a function of time following laparotomy and partial hepatectomy: (○- -), laparotomy; (●- -), partial hepatectomy; (□- -), nonoperated controls. For details see legend to Fig. 1.

ate decrease to 1.75 at 4 days which remained relatively constant over the following 10 days. The RSR of albumin was still significantly above normal at 14 days.

Fibrinogen. The RSR of fibrinogen as a function of time following surgery is shown in Fig. 2. Laparotomy caused a 3–4-fold increase that reached a peak at 24 hr followed by a rapid decrease. The RSR was back to normal by 8 days.

Partial hepatectomy caused a response in the RSR of approximately 7 times the control level at 24 hr, dropping to 2.5 at 48 hr. The RSR remained at this level at 4 and 8 days and declined to normal by 14 days.

Seromuroid fraction. The response of the seromuroid fraction (Fig. 3) following laparotomy resembled that of fibrinogen and following partial hepatectomy showed a biphasic response. After an initial 5- to 6-fold increase, the RSR declined to approximately 2 times the normal. This was followed by a sustained secondary response that remained elevated at 3 to 3.5 times normal at 4 and 8 days and then slowly declined. The RSR was still significantly elevated at 14 days. The initial response was of the magnitude and

timing observed previously (8).

Discussion. The RSR values reported here are based upon the assumption that no significant changes occurred in the hepatic glycine pool as a result of laparotomy or partial hepatectomy. Christensen *et al.* (12) could find no changes in liver glycine concentrations following partial hepatectomy, and Ferrari and Harkness (13) found no changes in the glycine content of liver at 6 and 24 hr after partial hepatectomy. Roberts and Simonsen (14) carried out extensive investigations on free amino acids in liver following laparotomy and partial hepatectomy and concluded that laparotomy had no significant effect on any of the amino acids. Partial hepatectomy resulted in increases of certain of the amino acids at 12 hr and at 2 and 6 days, but glycine was not affected. Thus, it has been assumed that the increases observed in the RSR values in this study are predominantly reflecting an increased rate of protein synthesis and are not the result of a significant decrease in the glycine pool.

Although the experimental design used by Majumdar *et al.* (1) and ourselves was quite similar, we obtained a different interpretation of the data as regards fibrinogen biosynthesis following stress (laparotomy) because of the different manner in which the results were expressed. Majumdar and co-workers found that at 20 hr following stress the specific activities of albumin and fibrinogen had returned to control levels and thus concluded that their synthetic rates had returned to normal by this time. However, determination of specific activities alone fail to account for changes in the circulating pool sizes of various plasma proteins brought about by stress or partial hepatectomy. Twenty-four hr after stress, and even sooner, the circulating pool sizes of fibrinogen and the seromuroid fraction are increased twofold or more and the albumin levels are decreased by 20–25% (15, 16). Thus, the expression of the data as specific activities alone can be misleading as to the true rate of plasma protein synthesis under these conditions. Therefore, we have preferred to express our data in terms of the relative synthetic rate which takes into ac-

count changes in plasma protein pool size concentrations. Koj (10) also has used a similar method to express his data for the same reasons. When expressed in this manner we find that although the rate of synthesis of albumin is normal, that of fibrinogen has increased severalfold at 24 hr. In agreement with Majumdar and co-workers, it was found that partial hepatectomy stimulated the synthesis of both albumin and fibrinogen by 24 hr.

The duration of the effect of laparotomy on the synthesis of fibrinogen and the seromucoid fraction was approximately 4–6 days, whereas that following partial hepatectomy was at least 14 days. This, coupled with the fact that albumin synthesis is affected by partial hepatectomy but not by laparotomy, indicates that separate mechanisms are indeed involved in the stimulatory effects of the two treatments, as suggested by Majumdar *et al.* (1).

An interesting finding of this investigation was the biphasic nature of the response of the seromucoid fraction following partial hepatectomy. It is suggested that the initial phase following partial hepatectomy is more directly associated with the initial stress of surgery and the secondary response reflects the proliferative phase of the regenerating liver.

Summary. Partial hepatectomy resulted in an increase in the relative synthetic rates (RSR) of albumin, fibrinogen, and the seromucoid fraction which extended over a period of at least 14 days following the operation. The response of the seromucoid fraction showed a biphasic pattern; an early marked increase closely related to the initial stress and a less marked secondary response. The response of albumin was less dramatic than that of the other fractions and showed no evidence of a biphasic character.

Laparotomy caused an increase in the RSR of the seromucoid and fibrinogen fractions

but had no effect on albumin synthesis. The patterns of response for fibrinogen and the seromucoid fraction following laparotomy were different from those following partial hepatectomy; RSR values were back to normal by 8 days and the secondary wave of seromucoid synthesis was absent.

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