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Received October 5, 1959. P.S.E.B.M., 1960, v103.

Serum Proteins of Rats after Partial Hepatectomy.* (25559)

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The assumption that the remarkable increase in mitotic activity associated with some stages of restoration of liver tissue after partial hepatectomy of rats is mediated by humoral factors has been supported by findings obtained after injection of serum from hepatectomized animals(1-3) and by results of parabiosis experiments(4-6). Changes in plasma and serum proteins of rats after partial hepatectomy have been investigated(7-11). During our studies on effects of injections of serum and serum fractions on mitotic activity and liver regeneration (to be presented later), we found it desirable to obtain base values correlating changes in mitotic activity with those of serum proteins following partial hepatectomy.

Methods. Rats of inbred Lewis strain, with average age of 68 days, were used. Weight of male animals was 212 ± 43 g, of females 182 ± 23 g. Blood specimens were obtained by cardiac puncture with needles and syringes coated with "Siliclad," to minimize hemolysis. Serum paper electrophoresis was carried out in Spinco model R instrument using 0.09 M veronal buffer at pH 8.65. Runs were carried out at room temperature with serum quantities of 0.013 to 0.015 ml and continued until satisfactory resolution of components was achieved. Average distance between distant ends of gamma globulin and albumin approximated 13 cm, requiring, as a rule, application of a potential difference of 120 volts for approximately 21 hours. Total protein was determined by digestion of an aliquot of serum and subsequent Nesslerization(12). At time of sacrifice, suitable tissue blocks were fixed in buffered formalin from which 6 μ thick sections were stained with Harris hematoxylin-eosin. In these slides mitotic activity was estimated by the method of Chalkley(13). Nuclei of liver cells were used as points of reference, and mitoses encountered as "hits" from scanning 500 nuclei were recorded for each section of liver tissue.

Results. In addition to 10 controls, 100 animals, evenly divided between males and females, were placed into 10 groups of 10 animals (5 males and 5 females), tested at one of 10 time intervals, the earliest being 6 hours after operation, the others as indicated in Fig. 1. Six animals of each group were subjected to partial hepatectomy according to procedure of Higgins and Anderson(14) while the remaining 4 were subjected to sham operations, *i.e.*, laparotomy and exposure of liver followed by closure of wound. Operations were carried out under ether anesthesia. With few exceptions surgery as well as sacrifice took place between 9 and 11 a.m., to avoid any fluctuations due to diurnal changes of mitotic activity(15). Animals were sacrificed at stated time intervals immediately after blood was obtained by cardiac puncture. Total body weights and weights of livers were recorded. After coagulation of blood specimens, serums were obtained by centrifugation for 5 minutes

* Supported by grant from Ill. Div. of Am. Cancer Soc. and Helen Ecker Cancer Soc.

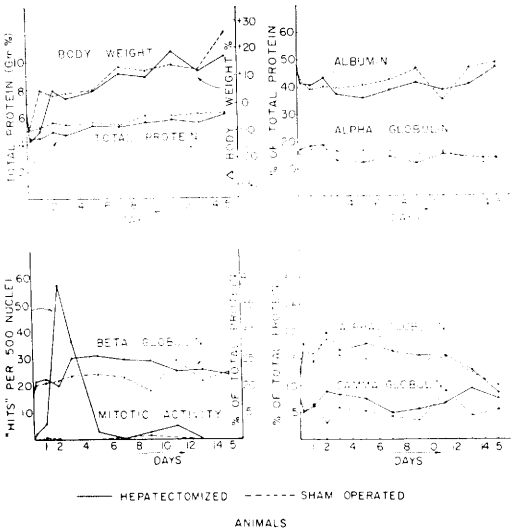

FIG. 1. Analytical data from hepatectomized and sham-operated rats. Each point represents mean of 6 partially hepatectomized or 4 sham-operated rats. Each of these groups comprised equal numbers of male and female animals.

at 600 x g. Preliminary hemoglobin determinations were carried out on all serum specimens; those containing more than 60 mg/100 ml were rejected and additional animals were tested. Determination of serum bilirubin indicated, as expected, moderate impairment of liver function after hepatectomy or sham-operation, with complete recovery not occurring during experimental period of 2 weeks.

Fig. 1 shows results of assays of total protein and protein fractions, as well as changes in body weight, and mitotic activity. The drop in body weight resulted most probably from dehydration. It was less pronounced in sham-operated animals and animals in both groups regained their original weights during observations of 2 weeks. Total serum protein values dropped immediately after hepatectomy and, to a lesser extent, also after sham operation.

The following comments may be made regarding changes in individual protein fractions:

(1) *Albumin.* This fraction reached its lowest concentration about 5 days after hepatectomy or sham operation and subsequently tended to rise toward normal levels. In general, the behavior of albumin paralleled approximately that of total serum protein. The changes were more pronounced after hepatectomy than after sham operation.

(2) *Globulins.* In hepatectomized as well as in sham-operated animals there was a rise in globulins, with hepatectomized animals affected to a greater degree. However, at least in the initial first 2 days this might have been due in part to changes in total blood volume. The most important changes were observed in the beta-globulin fraction. In hepatectomized animals this fraction tended to increase up to 60% beyond the values in controls, being most pronounced between third and ninth days after operation.

In sham-operated animals these changes were similar but of lesser magnitude. Other changes included an occasional separation of alpha$_2$-globulin fraction into 2 subfractions. This separation was not connected with presence of hemoglobin. In sham-operated animals a similar phenomenon was less pronounced.

Although changes in globulins tended to become less apparent toward end of experimental 2 weeks, their return to normal was not completed during this period.

In accord with well established data in the literature, mitotic activity reached its maximum between second and third day after hepatectomy and gradually fell to low levels between 5th and 7th day. A slight mitotic activity exceeding that occasionally found in sham-operated rats was observed in hepatectomized animals through the 11th day.

Changes in serum proteins described, on the whole, conformed to those reported previously (7-11), although in some instances differences in methodology make direct comparison difficult. Moreover, since these studies were designed to serve different purposes, comparison of changes in hepatectomized and sham-operated rats is possible in only one of them (7). On the other hand, detailed analysis of serum protein fractions during a prolonged period after hepatectomy is available only in another of these studies (11), but without consideration of sham-operated animals. In this study the most prominent change observed was a rise in beta-globulins which followed a similar pattern as in our work. It should be emphasized that in our own work, with the exception

of mitotic activity, all changes observed—drop of body weight, total protein level and albumin, rise in globulin fractions, especially of beta-globulin—were present not only in hepatectomized but also in sham-operated rats with the former showing more pronounced changes than the latter. This makes it likely that these changes may, at least in part, be associated not only with liver regeneration but also with recovery from trauma and growth processes, such as those initiated by laparotomy incision.

Summary. Changes in serum proteins and mitotic activity in the liver of Lewis rats were studied after partial hepatectomy or sham operation, at intervals of 6 hours to 15 days. A rise of beta-globulin fraction roughly paralleled the rise in mitotic activity. Serum protein changes in sham-operated animals showed a similar trend but were less pronounced.

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Received October 13, 1959. P.S.E.B.M., 1960, v103.

Effects of Reserpine on Adrenal Responses to Nicotine.* (25560)

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Small doses of nicotine injected into the thoracic aorta stimulate the adrenal medulla resulting in marked increases in levels of circulating catechol amines along with corresponding increases in heart contractile force (1,2). Our experiments were designed to study effects of previous reserpine treatment on (a) responsiveness of the adrenal medulla to chemical stimulation and (b) reactivity of the cardiovascular system to endogenously released catechol amines.

Methods. All experiments were performed on open-chest bilaterally vagotomized dogs. A total of 10 control and 25 reserpinized dogs were injected intra-aortically with test doses of 10 and 20 μg/kg of nicotine (administered as .01% solution of the alkaloid). Control dogs were anesthetized with 30 mg/kg pentobarbital sodium intravenously while reserpinized dogs, more sensitive to the anesthesia, were given only 15 to 20 mg/kg. Both groups were given supplemental doses as needed to maintain surgical state of anesthesia. Reserpine† was given intramuscularly and daily dosages varied from .05 to 1 mg/kg of body weight for 1 to 7 days. Plasma levels of epinephrine and norepinephrine were determined by modification(3) of the fluorimetric method of Weil-Malherbe and Bone(4). Blood samples were taken from indwelling polyethylene catheter placed in right femoral artery. The chest was opened by mid-line incision, and heart contractile force measured by suturing a strain gauge arch(5,6) directly to the surface

* This investigation was supported by grants from Nat. Heart Inst. and South Carolina Heart Assn.

† Serpasil generously supplied by CIBA Pharmaceutical Products, Summit, N. J.