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Negligible Role of Adrenal Hormones in Regulation of DNA Synthesis in Livers of Partially Hepatectomized Rats (40546)

ONNO T. TERPSTRA, RONALD A. MALT, AND NANCY L. R. BUCHER

Surgical Services, Shriners Burns Institute and Massachusetts General Hospital, and the John Collins Warren Laboratories of the Huntington Memorial Hospital and Department of Surgery of Harvard University, at the Massachusetts General Hospital, Boston, Massachusetts 02114

Although widely studied, the role of adrenal hormones in regulating liver growth remains uncertain (reviewed in (1–3)). In most instances ACTH and corticosteroids, usually administered in pharmacologic doses, are reported to promote hypertrophy of both normal and regenerating livers, but to have a generally suppressive influence upon hepatocyte proliferation (hyperplasia) in both hepatic regeneration and normal developmental growth. Adrenalectomy, on the other hand, is found to stimulate cell proliferation in normal or regenerating livers according to some investigators, but not others.

In a recent reexamination of the problem, Desser-Wiest investigated serum levels of corticosterone (the principal adrenal corticosteroid in the rat) in relation to cell proliferation in livers of normal, adrenalectomized, and partially hepatectomized rats (4–7). The results, interpreted as showing an inverse relation between levels of active (i.e., non-protein-bound) corticosteroids and DNA synthesis, suggested that liver growth is regulated by the concentration of the active fraction of these hormones in the blood (5, 6). Additional support for this concept might have been derived from studies in partially hepatectomized rats deprived of adrenal corticosteroids by prior adrenalectomy. It was found, however, that although adrenalectomy caused corticosterone levels to fall to near zero for a day or two, they gradually returned to normal in about 2 weeks. This restitution did not occur when the animals were both adrenalectomized and orchidectomized, leading to the assumption that male gonadal cells were able to compensate for the adrenal loss (7). (Possible regeneration of adrenal tissue, as an alternative explanation, was not rigorously excluded.) Under the experimental conditions employed, an excessively high mortality rate precluded investigation of hepatic regeneration in partially hepatectomized rats that were previously adrenalectomized and orchidectomized (4).

In the present study, in an attempt to resolve some of the continuing uncertainties, we have examined the rate of incorporation of [^{3}H]thymidine into hepatic DNA as an index of regenerative activity in partially hepatectomized rats 1 week after combined adrenalectomy–orchidectomy, with and without replacement treatment with corticosteroids. The 1-week delay was introduced in order to permit recovery from the effects of the surgical stress attendant upon the first operation, and decay of endogenous corticosteroids. Serum concentrations of corticosterone were negligible in these animals.

Materials and methods. Animals. Male Sprague–Dawley rats, (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) 6 to 7 weeks old and weighing 200–230 g, were randomly assigned to control or experimental groups. The diurnal light-and-dark frequency was 12 hr, and room temperature and humidity were kept constant. Experimental and control animals were kept together in metal wire cages in groups of six and fed Purina Laboratory Rat Chow *ad libitum* before and after operations. Adrenalectomized and sham-adrenalectomized animals were allowed a choice of NaCl solution or tap water to drink. After partial hepatectomy 20% dextrose was added to the NaCl solution; animals that were partially hepatectomized but not subjected to adrenalectomy or sham adrenalectomy received only tap water.

All animals were injected subcutaneously with 5 ml of a solution of 0.9% NaCl and 10% dextrose at the time of hepatectomy and 5, 17, 29, and 41 hr afterwards, unless otherwise stated, to prevent acute postoperative hypoglycemia.

Surgical procedures. All procedures, except

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the postoperative injections, were carried out under light ether anesthesia between 2 and 4 PM. Bilateral adrenalectomies were performed through a single dorsal midline skin incision and bilateral flank incisions through the underlying muscles. Special care was taken to avoid injury to the adrenal capsule; after removal of the gland, absence of tears was confirmed under 10× magnification. (Tears would have indicated the possibility of peritoneal implants, capable of regenerating sufficient cortical tissue to restore function.) In "sham-adrenalectomy" the adrenals were exposed through identical incisions but were not excised.

Bilateral orchidectomies were carried out through a small midline incision in the lower abdomen in all adrenalectomized and sham-adrenalectomized animals.

Partial hepatectomy consisted of a standard two-thirds removal of the liver (8).

For continuous intravenous infusion of corticosterone an indwelling polyethylene cannula (Clay Adams PE-50) was placed in the right external jugular vein and connected to a syringe pump via a swivel, which permitted the animals to move about freely (9).

Corticosteroids. Cortisone acetate (Cortone Acetate Saline Suspension: Merck, Sharpe, and Dohme) suspended in saline solution was given intramuscularly in a dosage of 0.25 mg per 100 g body wt just before hepatectomy and again 17 hr later. Controls received 0.5 ml saline solution at the same intervals. Corticosterone was donated by the Upjohn Company, Kalamazoo, Michigan. We prepared a solution by adding 4 mg to 1 ml of absolute ethanol, sonicating intermittently for a few seconds until dissolved, then adding it to 250 ml of the balanced electrolyte mixture used for infusion as previously described. The infusion was started immediately prior to the hepatectomy and was continued until the time of sacrifice 30 hr later. Each rat received 0.25 mg corticosterone per 100 g body wt per 24 hr in 17 ml of the electrolyte solution. Controls received the same amount of electrolyte solution without corticosterone.

Analytical techniques. Rats were sacrificed at 0, 16, 22, 30, and 48 hr after the hepatectomy by cervical dislocation. In some animals aortic blood was withdrawn by puncture and centrifuged at 5000 rpm for 5 min; sera were stored at −20° until analysis for corticosteroid content (10).

One hour before death each rat was given an injection of 50 μCi of [*methyl*-^{3}H]thymidine (6.7 Ci/mmole, New England Nuclear Corp., Boston, Mass.) through the penile vein under light ether anesthesia. The liver was excised and slices were fixed for histological study. The remaining liver was frozen in liquid nitrogen and stored at −20° until subsequent extraction of DNA, which was quantified by the diphenylamine procedure (11) and assayed for radioactivity in a liquid scintillation spectrometer (12). Preliminary observations showed the numbers of labeled nuclei in radioautographs to be in general conformity with DNA specific activity measurements, which were adopted as the preferred method of analysis.

Statistics. Significance was tested by Student's *t* test for unpaired data (13). All data are given as mean value ± SEM.

Results. That adrenalectomies were physiologically complete was supported by the failure of five adrenalectomized–orchidectomized rats to survive for even a week (mean survival time 5.4 ± 1.1 days), whereas five similar animals given 1% NaCl solution to drink were all alive after 30 days. It is well known that survival of adrenalectomized animals is lengthened by a diet rich in sodium and low in potassium (14).

To promote survival of adrenalectomized–orchidectomized rats that were subjected 1 week later to partial hepatectomy, we added glucose to the NaCl solution provided for drinking and, in addition, injected the animals subcutaneously with glucose–saline solution at the time of hepatectomy and at intervals thereafter. In the absence of glucose approximately 80% of the rats died with convulsions within 24 hr after the hepatectomy. Administration of glucose caused the mortality to fall to 18%.

Corticosterone levels were assayed in sera of randomly chosen animals at various intervals after the partial hepatectomy. In seven of the animals that were adrenalectomized and orchidectomized 1 week in advance of the hepatic resection the corticosterone levels were below the lower limit of the method (<0.1 μg/100 ml serum), whereas in three sham-adrenalectomized and orchidectomized

animals that were similarly hepatectomized the levels were 7.0 ± 2.1 μg/100 ml.

The rate of [^{3}H]thymidine incorporation into the DNA of intact livers was not appreciably altered by adrenalectomy–orchidectomy performed 1 week earlier, as shown by comparison of the 0-hr values in Fig. 1. The rates in partially hepatectomized animals undergoing hepatic regeneration 1 week after adrenalectomy and orchidectomy also differed insignificantly from those in correspondingly hepatectomized control rats that had had a sham-adrenalectomy and orchidectomy, as seen by comparison of rates of DNA labeling in the experimental and control groups at intervals up to 48 hr in Fig. 1.

The course of [^{3}H]thymidine incorporation both in the experimental and sham-adrenalectomized control rats in Fig. 1 differed slightly, however, from the pattern characteristic of regenerating livers in normal rats, which is shown in Fig. 2 (solid lines). In these normal animals the rate of incorporation was maximal at 22 hr after partial hepatectomy and fell abruptly by 30 hr in good agreement with our previouly published data (2, 15). Both the adrenalectomized–orchidectomized and the sham-adrenalectomized–orchidectomized rats in Fig. 1, although following a course initially similar to normal, failed to exhibit the typical drop at 30 hr. Figure 2 shows that additional control rats, partially hepatectomized *without previous surgery* and given saline–dextrose injections according to the schedule employed in Fig. 1, yielded a curve almost superimposable upon the control curve in Fig. 1 (compare dashed line curves in Figs. 1 and 2); the rate in these saline-injected but otherwise intact rats at 30

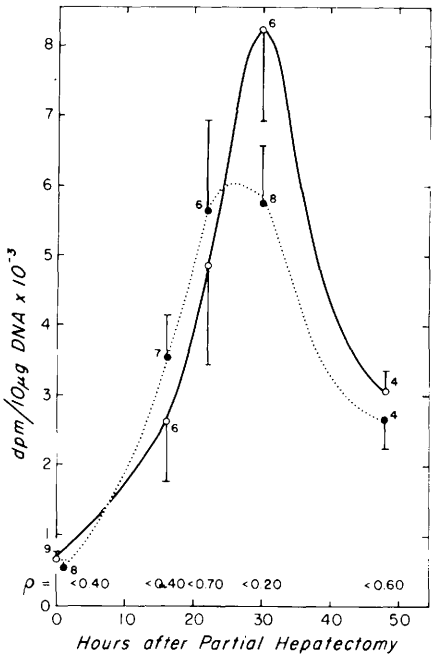

FIG. 1. Effect of adrenalectomy on [^{3}H]thymidine incorporation into hepatic DNA following partial hepatectomy. Rats were adrenalectomized and orchidectomized (solid lines) or sham adrenalectomized and orchidectomized (dotted lines) 1 week prior to partial hepatectomy. All animals had a choice of NaCl or tap water to drink until hepatectomy when 20% dextrose was added to the NaCl solution. All also received 5 ml of 0.9% NaCl plus 10% dextrose by subcutaneous injection at the time of hepatectomy and 5, 17, 29, and 41 hr afterward or until sacrificed. Vertical lines show SEM, the numbers are rats per point. *P* values (student's *t* test) for comparison of experimental and control values for each time point are shown at bottom; for the 30- vs 22-hr points in the experimental group, $P < 0.20$.

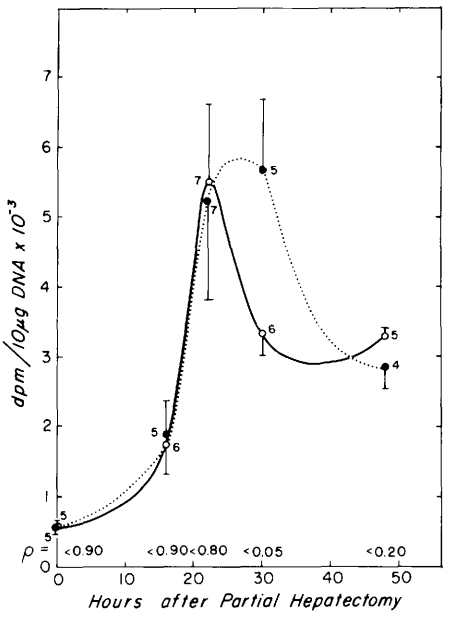

FIG. 2. Effect of saline–dextrose injections on [^{3}H]-thymidine incorporation into hepatic DNA or normal control rats following partial hepatectomy. Normal rats were partially hepatectomized and given injections of saline–dextrose as in Fig. 1 (dotted lines). They received only tap water to drink. Other normal rats were partially hepatectomized, but not injected (solid lines).

hr in Fig. 2 was 1.7 times higher than the noninjected control value ($P < 0.05$). Hence, the prolonged elevation of DNA-labeling rate observed in the rats in Fig. 1 appeared attributable to the saline–dextrose injections, rather than to the surgical procedures preceding the hepatectomy.

In an attempt to compensate for the loss of endogenous adrenal cortical hormones, we injected near physiologic doses of cortisone acetate intramuscularly into hepatectomized rats that had been adrenalectomized and orchidectomized 1 week earlier; the rate of DNA labeling at 30 hr was similar to that in rats that had undergone sham adrenalectomy and orchidectomy but received placebo injections instead (Table I). We also continuously infused adrenalectomized–orchidectomized and sham-adrenalectomized–orchidectomized animals intravenously with corticosterone for 30 hr after partial hepatectomy. All animals received subcutaneous saline–dextrose injections as before. Again no significant difference in DNA labeling was found (Table I).

Discussion. Our study supports the view that adrenal hormones have a relatively unimportant role, if any, in the physiologic regulation of regenerative liver growth.

That the adrenalectomized–orchidectomized rats employed in the present study had negligible endogenous supplies of adrenal cortical hormones was attested by the near undetectable concentrations of these substances in their serum as well as the 100% animal mortality when sodium chloride was withdrawn from the drinking water.

In previous studies the mortality in adrenalectomized rats that were partially hepatectomized 1 to 2 weeks afterward was 40–50% (6, 16); in adrenalectomized–orchidectomized rats survival was almost nil (4). We found that this prohibitive mortality could be dramatically reduced by repeatedly injecting saline–dextrose solution. Although the course of regeneration was slightly altered by this treatment, our experiments with control animals (Fig. 2) showed that the modestly prolonged period of increased DNA synthesis that occurred was due to the injections per se, and not to the absence of steroid hormones. Moreover, the occurrence of this prolongation in both sham and adrenalectomized–orchidectomized rats implied that it could not be due to release of endogenous corticosteroids resulting from the "stress" of repeated injections in fully conscious animals.

In agreement with a similar study by Seidman *et al.* (16), involving adrenalectomized and sham-adrenalectomized rats, we found quite similar [^{3}H]thymidine incorporation rates in 7-day sham-adrenalectomized–orchidectomized and 7-day adrenalectomized–orchidectomized rats with intact livers. Thus, despite the small temporary increase in proliferative activity 1 or 2 days after adrenalectomy reported by others (5, 6, 17), maintenance of the very slow proliferative activity characteristic of normal adult rat liver seems not to be dependent upon the presence of corticosteroids.

Following partial hepatectomy the rates of DNA labeling in adrenalectomized–orchidectomized rats and sham-adrenalectomized–orchidectomized controls differed little; hence deprivation of adrenal hormones does not appear to exert an important influence upon the course of hepatic regeneration.

TABLE I. EFFECT OF CORTICOSTEROID ADMINISTRATION ON RESPONSE OF HEPATIC DNA SYNTHESIS 30 hr AFTER PARTIAL HEPATECTOMY IN SHAM OR ADRENALECTOMIZED–ORCHIDECTOMIZED ANIMALS.[a]

	DNA specific activity (dpm/10 μg DNA)				
Treatment	No. of rats	Adrenalectomy and orchidectomy	No. of rats	Sham adrenalectomy and orchidectomy	*P* value
Cortisone acetate im[b]	8	6027 ± 709			<0.50
Placebo			5	4989 ± 1124	
Continuous intravenous corticosterone[c]	6	5460 ± 673	5	5064 ± 808	<0.70

[a] All rats received subcutaneous saline–dextrose injections as in Figs. 1 and 2.
[b] 0.25 mg/100 g body wt at time of hepatectomy and again 17 hr later.
[c] 0.25 mg/100 g body wt/24 hr.

This conclusion also agrees with the prior study of Seidman *et al.* (16), in which rats were adrenalectomized 5 to 7 days prior to partial hepatectomy, but in which only a single time point was examined (16). The divergent finding by Hemingway (18), that regeneration was enhanced by prior adrenalectomy, was obtained only in animals adrenalectomized within 48 hr in advance of the hepatectomy; the enhancement did not occur when adrenalectomy preceded partial hepatectomy by 7 days (18). It seems likely that factors associated with surgical stress rather than loss of adrenal hormones were responsible for the early enhancement observed by Hemingway, because stressful procedures such as laparotomy, intraperitoneal injection of an irritant, sham adrenalectomy, or adrenalectomy itself intensify the regenerative process when carried out within several days before the hepatic resection (19, 20).

In view of the general agreement of our results with those of Hemingway (18) and Seidman *et al.* (16), superimposition of orchidectomy upon adrenalectomy seems to have had little influence upon the outcome of our experiments. It was included for the sake of completeness, because of the suggestion that gonadal tissue might compensate for adrenal deficiency (7).

Administration of near physiologic doses (21, 22) of adrenal corticosteroids (0.25–0.5 mg/100 g body wt/day), which might serve either as partial replacement for the deficiency in the adrenalectomized–orchidectomized animals or as supplementation of endogenous levels in the sham-adrenalectomized–orchidectomized group, resulted in no detectable inhibitory or stimulatory influence upon DNA labeling in 30-hr regenerating livers, regardless of whether given by repeated injection or continuous infusion (Table I). Loeb and his associates (23) found that as little as 0.2 mg of cortisone acetate in divided doses twice daily for 6 days (which they considered equivalent to 0.5 mg of corticosterone/100 g body wt/day) significantly reduced hepatic DNA synthesis, as evidenced by diminished net gain in DNA content, in livers of young growing rats; on the other hand, much larger doses were required for even moderate depression of regenerative growth following partial hepatectomy (24). Our negative results with small doses are consistent with these observations. It remains unsettled whether the extreme resistance of hepatocytes to corticosteroids during regenerative compared to developmental growth demonstrated by Loeb's group (24) indicates regulation of those two forms of growth by different physiological mechanisms, or merely reflects differing intensity of stimulation.

In the recent series of studies on the role of the adrenal in liver growth, mentioned earlier, Desser-Wiest (4–7) has proposed that hepatic cell proliferation in normal and regenerating livers is regulated by the concentration of physiologically active—i.e., free as opposed to protein-bound—corticosteroids. The ability of free corticosterone to stimulate liver tyrosine transaminase was employed as an index of free-hormone availability; the somewhat inverse relations of DNA synthesis to transaminase activity and to corticosterone-binding capacity of serum were adduced as support for the concept. A postulated competition for hepatocyte corticosteroid receptor sites by progesterone was proposed to explain the rise in hepatic mitosis during pregnancy and invoked as additional evidence for corticosteroids as negative growth regulators (25).

It appears that adrenal cortical hormones—at least in pharmacologic doses—have an inhibitory influence upon proliferative liver growth, and they may possibly function as growth regulators during normal development. The weight of evidence from our own and other laboratories, however, indicates that when liver cell proliferation is intensively stimulated, as by partial hepatectomy, deletion of these hormones makes little difference and hence their role as physiologic regulators of the regenerative growth process seems to be of relatively minor importance.

Summary. Rats were depleted of adrenal corticosteroids by adrenalectomy and orchidectomy, as evidenced by determination of blood levels 1 week later, and by failure of the animals to survive without supplementary NaCl. When 7-day adrenalectomized–orchidectomized rats were partially hepatectomized, incorporation of [^{3}H]thymidine into hepatic DNA, indicative of rate of liver regeneration, differed insignificantly from the

rate in similarly treated controls, and diverged at only one time point (30 hr) from the rate in regenerating livers of normal rats. The divergence was due to the saline–dextrose injections, not to adrenalectomy–orchidectomy. Administration of adrenal cortical hormones in near physiologic doses did not alter [^{3}H]thymidine incorporation in experimental or control rats. The evidence does not support an important role for these hormones as physiologic regulators of liver growth during hepatic regeneration.

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1. Bucher, N. L. R., Inter. Rev. Cytol. **15,** 245 (1963).
2. Bucher, N. L. R., and Malt, R. A., "Regeneration of Liver and Kidney." Little, Brown, Boston (1971).
3. Schulte-Hermann, R., Crit. Rev. Toxicol. **3,** 79 (1974).
4. Desser-Wiest, L., Wiener Klin. Wchnschr. **86,** 390 (1974).
5. Desser-Wiest, L., J. Endocrinol. **60,** 315 (1974).
6. Desser Wiest, L., Cell Tissue Kinet. **8,** 1 (1975).
7. Desser-Wiest, L., Österreichische Ztschr. Onkol. **3,** 70 (1976).
8. Higgins, G. M., and Anderson, R. M., AMA Arch. Pathol. **12,** 186 (1931).
9. Bucher, N. L. R., and Swaffield, M. N., Cancer Res. **3,** 3189 (1973).
10. Murphy, B. E. P., J. Clin. Endocrinol. Metabol. **27,** 973 (1967).
11. Burton, K., Biochem. J. **62,** 315 (1956).
12. Hinrichs, H. R., Peterson, R. O., and Baserga, R., Arch. Pathol. **78,** 245 (1964).
13. Colton, T., "Statistics in Medicine," Little, Brown, Boston (1974).
14. Farris, E. J., and Griffith, J. Q., "The Rat in Laboratory Investigation." Lippincott, Philadelphia (1949).
15. Bucher, N. L. R., and Swaffield, M. N., *in* "Advances in Enzyme Regulation" (G. Weber, ed.), Vol. 13, p. 281. Pergamon Press, New York (1975).
16. Seidman, I., Teebor, G. W., and Becker, F. F., Cancer Res. **27,** 1620 (1967).
17. Horvath, E., and Kovacs, K., Ztschr. gesamt. exp. Med. **127,** 236 (1956).
18. Hemingway, J. T., Nature (London) **185,** 106 (1960).
19. Šimek, J., Erbenová, Z., Deml, F., and Dvořáčková, I., Experientia **24,** 1166 (1968).
20. Moolten, F. L., Oakman, N. J., and Bucher, N. L. R., Cancer Res. **30,** 2353 (1970).
21. Reichlin, S., and Brown, J. G., Amer. J. Physiol. **199,** 217 (1960).
22. Yates, F. E., and Urquhart, J., Physiol. Rev. **42,** 359 (1962).
23. Henderson, C., Fischel, R. E., and Loeb, J. N., Endocrinology **88,** 1471 (1971).
24. Castellano, T. J., Schiffman, R. L., Jacob, M. C., and Loeb, J. N., Endocrinology **102,** 1107 (1978).
25. Desser-Wiest, L., and Desser, H., J. Endocrinol. **72,** 407 (1977).

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