Effects of Cortisone on Thymidine Incorporation by Various Nonlymphoid Tissues of the Weanling Rat[1] (37353)

JOHN N. LOEB AND LUCY L. YEUNG
(Introduced by S. E. Bradley)

Department of Medicine, Columbia University College of Physicians and Surgeons, New York City, New York 10032

Effects of the glucocorticoid hormones on growth and cell division have been of interest ever since the observation of Ingle over 30 yr ago that the administration of these compounds to normal rats results in a marked suppression of somatic growth (1). Subsequent studies have shown that the administration of these hormones results in a decrease in the rate of growth or even an actual involution of many of the individual somatic tissues (2–6), and more recently it has become possible to demonstrate a similar inhibition of growth and cell division in a number of mammalian cell lines in tissue culture (7–11). Most recently it has been shown that even very small doses of glucocorticoid hormone produce a prompt suppression of DNA synthesis (12) and fall in DNA polymerase activity (13) in the livers of weanling rats, that the doses required for these effects correspond to little if any more than the normal daily endogenous output of corticosterone (12, 13), and that both effects are rapidly reversible upon discontinuation of hormone treatment.

In view of the demonstration that liver is exquisitely sensitive to the growth-inhibitng effects of glucocorticoids it becomes of interest to examine effects of hormone administration on DNA synthesis in other tissues as well and, more specifically, to compare the effects of hormone administration on tissues with "stable" cell populations, where DNA synthesis occurs primarily in association with new cell accretion ("growth"), with effects on other tissues where DNA synthesis instead reflects largely cell renewal ("turnover"). A selective suppression of DNA synthesis in the former tissues, if such indeed could be demonstrated, would be of particular interest and moreover might provide a useful model for an investigation of the biochemical basis for the well known suppressive effects of these compounds on somatic growth. The following studies examine the acute effects of cortisone administration upon thymidine incorporation by a variety of tissues in the growing rat. The results to be presented demonstrate that DNA synthesis in different tissues shows a remarkable range in its sensitivity to suppression by glucocorticoid hormone and that despite this range the relative responses of various tissues are extremely consistent from experiment to experiment. The pattern of response in the different tissues examined thus far suggests the generalization that thymidine incorporation is particularly susceptible to suppression in those tissues in which DNA synthesis reflects new cell accretion rather than cell renewal.

Materials. All studies were performed with normal male Sherman (Columbia strain) rats of the weights indicated below. Thymidine-methyl-^{3}H (20 mCi/μmole) was purchased from the New England Nuclear Corp., and cortisone acetate was obtained from Merck, Sharp & Dohme.

Incorporation of radioactive thymidine into DNA. Rats which had been begun on injections of either cortisone acetate or normal saline 24 hr previously were injected be-

[1] This work was supported in part by U.S. Public Health Service Grants HD-05506 and AM-05397 and by the Perkin Memorial Fund of the Presbyterian Hospital in the City of New York. Dr. Loeb is the recipient of an Irma T. Hirschl Career Scientist Award.

tween 9 and 10 AM with ^{3}H-thymidine as indicated below. Beginning 4 hr after the administration of radioactive thymidine, control and cortisone-treated animals were killed alternately over a 2-hr period, and the organs excised and treated as described below. Livers, brains, kidneys, spleens, testes, hearts, and gastrocnemius muscles were excised, rinsed in 0.25 *M* sucrose solution, weighed, wrapped in aluminum foil, frozen over dry ice, and stored at —20°. After opening the upper gastrointestinal tract with a longitudinal incision and rinsing out its contents with tap water, mucosal scrapings from the glandular stomach and from a 5-cm segment of jejunum taken from the junction of the first and second quarters of the small intestine were transferred to glass tubes, frozen rapidly, and stored at —20°. Homogenates of heart (1:12), gastrocnemius muscle (1:10), liver (1:4), spleen (1:16), brain (1:3), testis (1:6), and kidney (1:4) were subsequently prepared from thawed tissue samples and 0.25 *M* sucrose solution by means of hand-driven Dounce-type homogenizers (Blaessig Glass Specialties, Rochester, NY); each mucosal sample was similarly homogenized in 3 ml of 0.25 *M* sucrose. In order to determine the radioactivity present in DNA, 2-ml aliquots of each homogenate were treated with 2 ml of 10% trichloroacetic acid ("TCA") at 0° in conical centrifuge tubes, all subsequent steps also being carried out at 0° unless otherwise specified. After 10 min the resulting precipitates were centrifuged down, washed twice with 3 ml cold 5% TCA, drained, and resuspended in 2 ml water. Two milliliters of 0.6 *M* KOH was then added, and the resulting mixtures (containing 0.3 *M* KOH) were incubated at 37° as described by Fleck and Munro (14) in order to hydrolyze any contaminating RNA. After 1 hr the tubes were chilled in ice and 0.5 ml of "100%" (w/v) TCA was added to each in order to precipitate DNA and protein. After 10 min the precipitates were centrifuged down and the supernatants containing the RNA hydrolysis products discarded. The pellets were then washed once with 3 ml 5% TCA and twice with 5 ml 95% ethanol. The resulting pellets were heated with occasional stirring at 90° for 15 min to hydrolyze the DNA (15) and allowed to cool to room temperature. The precipitates were spun down at room temperature, and 0.5 ml of each supernatant was added to 10 ml of Bray's solution (16). Radioactivity was determined by means of a Packard Tri-Carb scintillation spectrometer; counting efficiency in different experiments ranged between 11 and 14%.

The concentration of DNA hydrolysis products was determined by the method of Giles and Myers after the addition of perchloric acid to each of the TCA extracts to a final concentration of 10% (17).

Results. An initial series of experiments examined the effects of a large dose of cortisone upon the incorporation of radioactive thymidine by a variety of different tissues. Weanling rats weighing 55–65 g received either a single subcutaneous injection of 5 mg of cortisone acetate or two divided doses of 2.5 mg each, and the incorporation of ^{3}H-thymidine into DNA (20 μCi per rat by intraperitoneal injection) was determined 24 hr after the initial dose of hormone as described under "Methods." Table I shows the results of a typical experiment, and Fig. 1 summarizes the results of a series of four such studies. For convenience in comparison of the effects on different organs the latter figure expresses all specific activity values as per cent of control. It is evident that the effects of a single (large) dose of cortisone vary widely from tissue to tissue but that the effects upon any given tissue are consistently reproducible: It is seen that (a) the administration of a large dose of cortisone results within a single day in a profound and consistent suppression of radioactive thymidine incorporation in liver, heart, kidney, brain, and gastric mucosa; and (b) in contrast, the same dose of hormone in the same animals has no effect on thymidine incorporation by jejunal mucosa, testis, or spleen.

In view of the observed wide range in suppressibility of thymidine incorporation by cortisone treatment, and because of the previously demonstrated exquisite sensitivity of liver DNA synthesis to suppression by even very small doses of glucocorticoid hormone (12), it was of interest to examine the effects

TABLE I. Effects of a Large Dose of Cortisone on the Incorporation of ^{3}H-Thymidine into the DNA of Different Tissues in the Weanling Rat.[a]

Tissue	dpm per μg DNA[b]		% Inhibition by cortisone	p
	Control	Cortisone		
Liver	104 ± 8	9 ± 3	91%	<0.001
Heart	23 ± 5	3 ± 1	87%	<0.001
Kidney	32 ± 4	4 ± 2	88%	<0.001
Brain	1.9 ± 0.4	0.9 ± 0.3	53%	<0.1
Gastric mucosa	83 ± 6	13 ± 4	84%	<0.001
Jejunal mucosa	58 ± 14	47 ± 7	19%	>0.5 (NS)
Testis	50 ± 6	49 ± 10	2%	>0.5 (NS)
Spleen	166 ± 22	111 ± 21	33%	>0.1 (NS)

[a] Rats were begun on injections of either cortisone acetate (8 animals) or isotonic saline (9 animals) 24 hr before the administration of ^{3}H-thymidine as described under "Methods." Hormone was administered in two divided doses of 2.5 mg each, 8 hr apart.

[b] Mean values ± 1 SE.

of a much smaller dose of cortisone on thymidine incorporation by these same tissues. The dose chosen was 0.5 mg cortisone per rat, a dose which previously had been shown to be sufficient to result in a marked suppression of both somatic growth and liver DNA synthesis (12). Again, as in the experiments employing the larger dose (5 mg per rat, see above), thymidine incorporation was examined after only a single day of hormone treatment. Figure 2 presents the results and shows that even at this smaller dose (1/10 the dose of hormone used in the previous experiments) thymidine incorporation is profoundly suppressed in liver, heart, skeletal muscle, and kidney. It is evident that suppression at this low dose is virtually as complete as it was at the higher dose (see Fig. 1), and thymidine incorporation by heart, skeletal muscle, and kidney in indeed found to have the same exquisite sensitivity to suppression by cortisone treatment as has previously been reported for liver (12).

Discussion. The foregoing results show that the administration of even small doses of glucocorticoid hormone produces a profound suppression of thymidine incorporation in a wide range of rapidly growing somatic tissues of the weanling rat. While in this sense these observations are reminiscent of the well-known effects of the glucocorticoid hormones on a variety of lymphoid tissues, it is important to emphasize that what has been observed in the "cortisone-sensitive" tissues examined in the present studies is only a suppression of thymidine incorporation *per se*. In contrast to the situation in lymphoid tissues, where the administration of glucocorticoid hormone results in a rapid lysis of preformed cells, and hence where mechanisms other than a simple suppression of DNA synthesis must be invoked to explain their rapid involution, there is no evidence to suggest that the administration of cortisone results in an actual involution of any of the cortisone-sensitive tissues examined here. Indeed in one of these latter tissues (liver) a previous study has already shown that even very large doses of hormone, amounting to some 50 times that necessary to inhibit new DNA synthesis, fail to produce any appreciable degradation of pre-existing liver DNA (12).

In contrast to our observations in liver, kidney, heart, and skeletal muscle, thymidine incorporation by gastric and jejunal mucosa, spleen, and testis was found to be completely unaffected by low doses of cortisone. The finding in spleen, although perhaps superficially paradoxical for a "lymphoid tissue," is consistent with earlier observations in mice by Stevens *et al.*, who noted that although the administration of cortisone resulted in a rapid decrease in splenic weight, even very large doses of hormone were without significant effect on the incorporation of thymidine into DNA (18). These authors attributed the

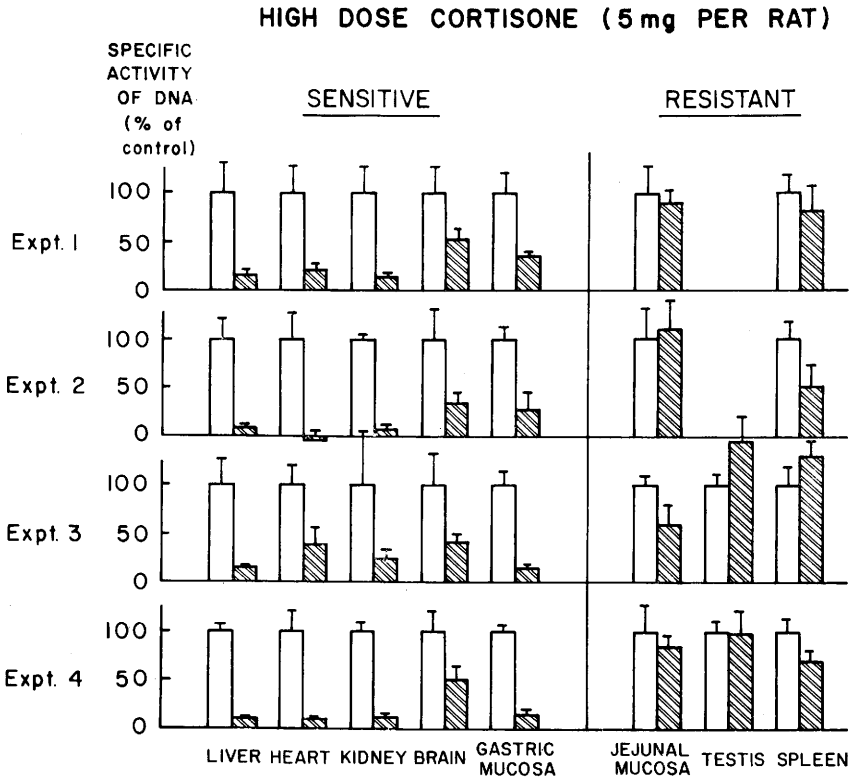

FIG. 1. Effects of a high dose of cortisone (5 mg) on thymidine incorporation by various tissues of the weanling rat. Rats were begun on cortisone acetate 24 hr before the administration of ^{3}H-thymidine as described under "Methods." In Expts 1 and 2, hormone was administered as a saline suspension in a single dose of 5 mg; in Expts 3 and 4, it was given in two divided doses of 2.5 mg each, 8 hr apart. Control animals received injections of identical volumes of 0.9% NaCl. There were 4–9 animals in each group; values shown are means ± 1 standard error.

decrease in weight to the well-known lympholytic effect of the glucocorticoids (see above) and the persistence of incorporation to the fact that the rodent spleen, in addition to being a lymphoid organ, is a major site of red blood cell production (see, *e.g.*, discussion in Ref. (19)). Consistent too with this interpretation was their observation that after the administration of radioactive thymidine the specfic activity of splenic DNA was some 5-fold greater than that of DNA in thymus and lymph nodes (18, 19). As expected, the levels of incorporation by brain in the present studies are exceedingly low (see Table I) and presumably principally reflect DNA synthesis by nonneuronal elements (20).

The results of the preceding experiments show that thymidine incorporation by various different tissues exhibits an enormous range in its sensitivity to acute suppression by cortisone administration. The observation of principal interest, however, is not the wide range of sensitivity *per se* but rather the finding that at low doses of hormone the tissues studied can be divided quite clearly into two distinct groups: those in which thymidine incorporation is markedly suppressed (liver, kidney, heart, and skeletal muscle), and those in which thymidine incorporation is completely unaffected (gastric and jejunal mucosa, spleen, and testis). An additional example of a tissue presumably belonging to this latter group is bone marrow, where numerous studies by others have shown that low doses of hormone fail to result in any appreciable depression of erythropoiesis (21–24).

The pattern of response in the various tissues examined thus far suggests the generalization that thymidine incorporation is

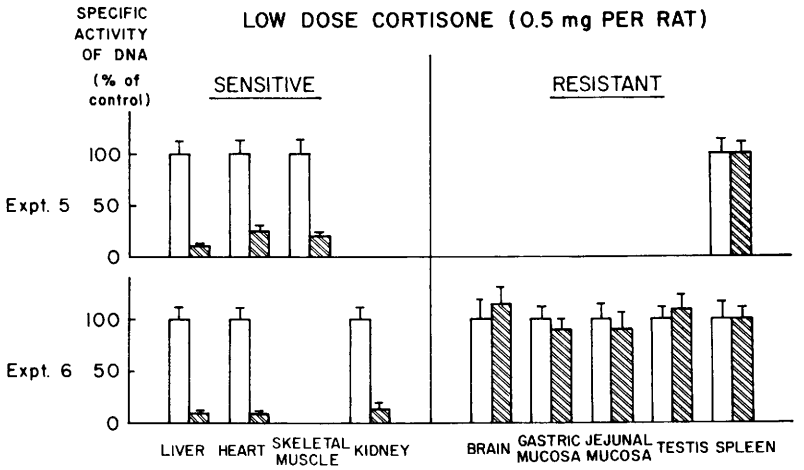

FIG. 2. Effects of a low dose of cortisone (0.5 mg) on thymidine incorporation by various tissues of the weanling rat. Rats were begun on cortisone acetate 24 hr before the administration of ^{3}H-thymidine as described under "Methods." Hormone was administered as a saline suspension in two divided doses of 0.25 mg each, 8 hr apart (*i.e.*, the dose was 1/10 that used in the experiments summarized in Fig. 1). Control animals received injections of identical volumes of 0.9% NaCl. There were 9 animals in each of the above groups; values shown are means ± 1 standard error.

particularly susceptible to suppression in those tissues which are composed of stable cell populations and in which DNA synthesis is primarily a concomitant of new cell accretion ("growth") rather than one of cell renewal. The fact that the doses of hormone required for the suppression of thymidine incorporation in the sensitive tissues are identical to those required for a generalized suppression of somatic growth (12) moreover suggests that the phenomenon of cortisone-induced inhibition of DNA synthesis in some of these individual tissues may provide a convenient model for studies on biochemical mechanisms underlying the well-known suppression of growth which occurs both in experimental animals and in children (25, 26). On the basis of the observations reported here, it will be of interest not only to examine the cortisone sensitivity of a still wider range of growing tissues but to explore possible mechanisms by which the administration of glucocorticoid hormone results in a rapid suppression of DNA synthesis in individual sensitive tissues. One such study in liver has recently shown that the suppression of thymidine incorporation is accompanied by a profound fall in DNA polymerase activity (13), but it is not yet clear whether the decrease in DNA synthesis can be attributed solely to the decrease in enzyme level. Additional studies, including a detailed examination of the relative time-courses of changes in thymidine incorporation and polymerase activity, are currently in progress.

Summary. Acute effects of both high and low doses of cortisone upon the incorporation of radioactive thymidine have been examined in a variety of non-lymphoid tissues of the weanling rat. It is shown (a) that thymidine incorporation by different tissues varies over an enormous range in its sensitivity to suppression by cortisone administration, and (b) that at low doses of hormone all the tissues studied are clearly separable into two distinct classes: those in which cortisone produces a profound suppression of thymidine incorporation, and those which are completely unresponsive to the hormone. The pattern of response observed thus far suggests the generalization that thymidine incorporation is particularly susceptible to suppression in tissues which are composed of stable cell populations and in which DNA synthesis is primarily a concomitant of new cell accretion ("growth") rather than one of cell renewal.

The phenomenon of cortisone-induced suppression of thymidine incorporation in individual tissues sensitve to low doses of hormone may provde a convenient model for studies of biochemical mechanisms underlying the well-known suppression of somatic growth which occurs at comparable doses both in experimental animals and in children.

1. Ingle, D. J., Endocrinology **29**, 649 (1941).
2. Ingle, D. J., Proc. Soc. Exp. Biol. Med. **44**, 174 (1940).
3. Dougherty, T. F., Physiol. Rev. **32**, 379 (1952).
4. Tappan, D. V., Boutwell, R. K., and Booth, B., Proc. Soc. Exp. Biol. Med. **97**, 52 (1958).
5. Bullogh, W. S., Biol. Rev. **37**, 307 (1962).
6. Winick, M., and Coscia, A., Pediat. Res. **2**, 451 (1968).
7. Grossfeld, H., and Ragan, C., Proc. Soc. Exp. Biol. Med. **86**, 63 (1954).
8. Holden, M., and Adams, L. B., Proc. Soc. Exp. Biol. Med. **95**, 364 (1957).
9. Cox, R. P., and MacLeod, C. M., J. Gen. Physiol. **45**, 439 (1962).
10. Gabourel, J. C., and Aronow, L., J. Pharmacol. Exp. Ther. **136**, 213 (1962).
11. Pratt, W. B., and Aronow, L., J. Biol. Chem. **241**, 5244 (1966).
12. Henderson, I. C., Fischel, R. E., and Loeb, J. N., Endocrinology **88**, 1471 (1971).
13. Henderson, I. C., and Loeb, J. N., Nature (London) **228**, 556 (1970).
14. Fleck, A., and Munro, H. N., Biochim. Biophys. Acta **55**, 571 (1962).
15. Schneider, W. C., *in* "Methods in Enzymology" (S. P. Colowick and N. O. Kaplan, eds.), Vol. III, p. 680. Academic Press, New York (1957).
16. Bray, G. A., Anal. Biochem. **1**, 279 (1960).
17. Giles, K. W., and Myers, A., Nature (London) **206**, 93 (1965).
18. Stevens, W., Colessides, C., and Dougherty, T. F., Endocrinology **78**, 600 (1966).
19. Stevens, W., Colessides, C., and Dougherty, T. F., Endocrinology **76**, 1100 (1965).
20. Messier, B., and Leblond, C. P., Amer. J. Anat. **106**, 247 (1960).
21. Quittner, H., Wald, N., Sussman, L. N., and Antopol, W., Blood **6**, 513 (1951).
22. Yoffey, J. M., Ancill, R. J., Holt, J. A. G., Owen-Smith, B., and Herdan, G., J. Anat. **88**, 115 (1954).
23. Fruhman, G. J., and Gordon, A. S., Proc. Soc. Exp. Biol. Med. **88**, 130 (1955).
24. Fruhman, G. J., and Gordon, A. S., Endocrinology **57**, 711 (1955).
25. Blodgett, F. M., Burgin, L., Iezzoni, D., Gribetz, D., and Talbot, N. B., New Engl. J. Med. **254**, 636 (1956).
26. Falliers, C. J., Tan, L. S., Szentivanyi, J., Jorgensen, J. R., and Bukantz, S. C., Amer. J. Dis. Children **105**, 127 (1963).

Received Dec. 5, 1972. P.S.E.B.M., 1973, Vol. 143.