Effects of Cortisol and Adrenalectomy on Induction of Interferon by Endotoxin.* (32021)

BOSKO POSTIC, CATHERINE DEANGELIS, MARY K. BREINIG, AND MONTO HO
Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa.

There is substantial evidence that bacterial endotoxins and viruses induce the formation of interferon in intact animals by different pathways(see 1). Thus, in rabbits, actinomycin D inhibited the production of virus-induced interferon (VII) while endotoxin-induced interferon (EII) was not affected(2). The formation of EII was not inhibited by puromycin, while VII and protein synthesis were markedly suppressed(3). Further, the production of EII was shown to be relatively unchanged following variation of body temperature while VII production was markedly affected(4). While it is known that adrenal corticosteroids inhibit VII formation in embryonated eggs, intact mice, and in cell cultures(5,6,7), no information on the effect of cortisol on EII formation is available. Studies in this area were undertaken as a contribution to the understanding of the mechanism for the induction of interferon by endotoxin and its distinction from VII.

Materials and methods. As in previous work(8,4), endotoxin-induced interferon (EII) was produced as follows: sera from albino rabbits weighing 1 kg were obtained following intravenous (i.v.) inoculation of 10 μg of endotoxin from *Escherichia coli* 0113 extracted according to the Boivin method (kindly supplied by Dr. A. I. Braude). Virus-induced interferon (VII) was stimulated by injecting rabbits intravenously with 0.5 ml of stock suspensions containing 5×10^9 PFU of Newcastle disease virus (NDV, Herts strain kindly supplied by Dr. Samuel Baron). To assure virus inactivation, all serum samples from virus-inoculated animals were heated at 56°C for 15 minutes. For titration of interferon activity, serum samples were assayed on rabbit embryo primary cell cultures by reduction of the cytopathic effect of vesicular stomatatis virus (VSV). Interferon titers were expressed in the reciprocals of the highest serum dilution inhibiting the cytopathic effect of VSV in 50% of the culture tubes(8). Normal rabbit serum was previously shown to be free of any interferon activity(8).

Studies with cortisol were done as follows: Two milliliters of a solution containing 250 mg of cortisol (Upjohn) or an appropriate dilution in phosphate buffered saline were injected intravenously into rabbits 2 hours before the injection of an inducer of interferon (endotoxin or NDV).

Surgical removal of the adrenals was done under aseptic conditions through a mid-line abdominal incision while the rabbits were under nembutal ether anesthesia. Control animals were similarly operated on except for the removal of the adrenals. To induce interferon, adrenalectomized and control rabbits received endotoxin intravenously 7 days after surgery.

Results. The effect of cortisol on EII is presented in Table I. As observed previously (8), the appearance of interferons after 10 μg of endotoxin reached its peak at 2 hours. It is also evident that cortisol at doses of 1 to 5 mg or more inhibited EII. No more in-

TABLE I. Effect of Cortisol on Endotoxin-Induced Interferon (EII).

		Interferon titers (geometric means)	
mg*	No. rabbits	2 hr†	4 hr
0	10	64	12
1	4	37	8
5	6	8	2
25	4	3	2
250	4	4	2

* Cortisol was inoculated intravenously 2 hr before (25 and 250 mg doses) or simultaneously (1 and 5 mg doses) with endotoxin.

† Two and 4 hr after inoculation of 10 μg endotoxin serum samples were obtained. Each rabbit provided one sample for each time period.

* This investigation was supported by N.I.H. grant AI-02953.

TABLE II. Effect of Cortisol on Virus-Induced Interferon (VII).

mg*	Dose	No. rabbits	Interferon titers (geometric means) 2 hr	4 hr	7 hr
0	0	10	111	512	119
25	1	4	169	891	315
250	2	4	45	223	338
250	3	7	34‡	73‡	37
250	3†	4	147	447	ND

* Single doses of cortisol were given 2 hr, double doses 24 and 2 hr, and triple doses were given 48, 24, and 2 hr before 5×10^9 PFU of NDV i.v.

† After 3 daily doses of cortisol, 48 hr elapsed before NDV was inoculated.

‡ $p < 0.05$; where p = probability by the Wilcoxson rank sum test that the titers of cortisol pretreated rabbits and controls were from the same population.

ND = Not done.

hibition was attained with much larger doses, and the time of appearance of EII was not affected by the steroid.

For comparison, the effect of cortisol on VII is presented in Table II. It is evident that in contrast to EII, 25 and 250 mg of the steroid had no inhibitory effect on interferon production following inoculations with NDV. VII was only inhibited by 250 mg administered 3 times. The steroid was effective for less than 48 hours, as animals rested for 48 hours after 3 injections of steroid produced interferon normally in response to NDV.

To ascertain whether the observed inhibition of EII and VII production was due to the cortisol content of the sera assayed or to an interferon-inactivating factor produced as a result of administration of cortisol, the following experiments were carried out: A sample of undiluted serum EII was divided in 3 sets. The first two were mixed respectively with equal volumes of serum drawn from a rabbit 2 and 4 hours after an inoculation of 250 mg cortisol. The third set was mixed with normal rabbit serum. The interferon content of these 3 mixtures was compared and no difference in titers was found. The same result was obtained when samples of serum EII were substituted for VII. These results indicated that circulating cortisol or the serum of cortisol treated rabbits had no direct inactivating effect on interferon.

The possibility that cortisol itself induced interferon was tested by drawing serum samples from rabbits 2, 4, and 7 hours following the injection 250 mg of cortisol. These samples did not inhibit VSV as measured by the assay for interferon.

The possible effect of endogenous corticosteroids on EII was next examined by measuring the interferon response of adrenalectomized rabbits (Table III). It is clear that when endotoxin was used to induce interferon, the animals whose adrenals were removed produced more than 10 times the amount of interferon than the controls with intact adrenals. On the other hand, when adrenalectomized animals were given 25 mg cortisol daily, interferon production was suppressed to a level below that of controls. Apparently the enhancement of EII levels following adrenalectomy may be explained by the elimination of endogenous corticosteroids, and 25 mg of cortisol daily represented a pharmacological dose which more than compensated for the effects of adrenalectomy.

The mechanism by which cortisol reduced the production of EII was next considered. We tested whether or not the steroid may have produced a state of "tolerance" to interferon induction similar to what is observed after an inoculation of endotoxin in rabbits or mice(9,10). Rabbits were divided in 3 groups (Table IV). The first group received endotoxin on 2 successive days. The second group was pretreated with cortisol before

TABLE III. Effect of Adrenalectomy on EII.

Adrenalectomy	Cortisol postoperatively	Serum interferon titers* Individual	Geometric means
Yes	None	90, 362, 512, 512, 512, 512, 512	388
Yes	25 mg daily	3, 6, 11	6
No	None	16, 23, 23, 23, 32, 45, 64	28

* Values refer to individual rabbits. Sera were sampled on the seventh postoperative day and 2 hr following 10 μg of endotoxin i.v.

TABLE IV. Tolerance to Interferon Induction in Rabbits Receiving Cortisol.

Group	Pre-treatment	Day 1 Time (hr) 0	Day 1 Time (hr) 2	Day 2 Time (hr) 24	Day 2 Time (hr) 26
1	None	Endotoxin	48, 64*	Endotoxin	1, 2
2	Cortisol†	"	1, 1, 2, 4, 4	"	1, 1, 1, 1, 4
3	"	None	<1, <1	"	6, 12, 12, 48, 32, 16

* Serum interferon titers of individual rabbits.
† 250 mg given intravenously 24 and 2 hr before time 0.

receiving endotoxin on 2 successive days. The third group received endotoxin 48 hours after the last dose of 2 daily inoculations of cortisol.

The observations on Group 1 show the usual "tolerance" phenomenon; *i.e.*, 24 hours after one dose of endotoxin, animals did not respond to a second dose by producing interferon. Pretreatment with cortisol did not affect this hyporeactivity despite the fact that interferon induced by the first dose of endotoxin was reduced by the steroid (Group 2). The effect of the steroid, as in Table II, was short-lived; *i.e.*, animals were normally responsive 24 hours after cortisol treatment (Group 3). In contrast, the duration of tolerance is at least 3 days(9). It is concluded from these data that cortisol does not affect the development of the "tolerant" state, and that its action cannot be equated with the development of tolerance since its effect is much shorter. However, as the mechanisms of both phenomena are poorly understood, we cannot preclude the possibility that they may be related.

Discussion. It is already well documented that adrenal corticosteroids decrease the production of virus-induced interferon in the intact animal. Rytel and Kilbourne(7) reported that optimal inhibition of interferon was obtained with about 150 mg/kg of cortisol. Comparably high doses were also used by Mendelson and Glasgow(6) and Lieberman *et al*(11). Our experience with 1 kg rabbits was that the amount of cortisol required (2 to 3 doses of 250 mg) seemed to be of an even greater magnitude. At 25 mg, there was even a potentiation of VII production (Table II), although this increase was not statistically significant, and we cannot say that it was real. Nevertheless, the possibility exists that under certain conditions, steroids may actually potentiate VII production. This is supported by the recent work of Mendelson and Finland(12) who found that steroids enhanced interferon production by peritoneal leucocytes of mice. In contrast to this relative resistance of VII to suppression by cortisol, we found that the production of EII was inhibited by 1 to 5 mg. This difference in sensitivity suggests that the mechanisms of inhibition by steroids may be different. It has been suggested that the effect of cortisol on VII production may be on the basis of its inhibition of protein synthesis(5), but it is unlikely that cortisol acts on the production of EII in this manner, since its production in mice and rabbits does not require protein synthesis(13,3). One could speculate that cortisol acts on interferon release at the site of the cell membrane or by a change in the electrolyte milieu(14). Another alternative is that the combination of cortisol and endotoxin also produced complex pathophysiological reactions which may be inhibitory to EII production. One such reaction is bilateral renal necrosis or a generalized Shwartzman phenomenon in young rabbits(15). It is unlikely that this would inhibit EII since the experiments reported here were terminated not later than 4 hours after the inoculation of endotoxin. Another evidence suggested that the inhibition of EII was due to the direct effect of corticosteroids, since a marked enhancement of EII production was observed in adrenalectomized animals and was readily reversed by the administration of cortisol. This significant result indicates that even physiological amounts of circulating corticoids are sufficient to depress the release of EII. No comparable suppression of VII has been observed in adrenalectomized rabbits (unpublished data). Finally, the inhibition of EII

by cortisol as well as its potentiation in adrenalectomized animals is paralleled by the known general antiendotoxic effect of cortisol (15), and the dramatic increased susceptibility of adrenalectomized animals to endotoxin death(16).

Summary. The administration of 1 to 5 mg of cortisol to 1 kg rabbits markedly suppressed interferon production by *E. coli* endotoxin. Single doses of cortisol, up to 25 mg, did not decrease circulating interferon levels following inoculations with Newcastle disease virus. This type of interferon was only partially suppressed with multiple injections of 250 mg. Thus, administered cortisol inhibited more readily the production of endotoxin-induced interferon than the virus-induced counterpart. Adrenalectomy markedly potentiated the production of interferon by endotoxin. Adrenalectomized rabbits inoculated with endotoxin produced serum interferon to a mean titer of 1:388, while the mean titer in control animals was 1:28, suggesting that endogenous steroids suppress the interferon esponse to endotoxin.

Mrs. Linda Carcione offered excellent technical assistance. One of us (Miss Catherine DeAngelis) was a recipient of a summer research fellowship under NIH grant 5416-05.

1. Finter, N. B., ed., 1966, Interferons, North-Holland Publishing Co.

2. Ho, M., Kono, Y., Proc. Nat. Acad. Sci., 1965, v53, 220.

3. Ke, Y. H., Singer, S. H., Postic, B., Ho, M., Proc. Soc. Exp. Biol. & Med., 1966, v121, 181.

4. Postic, B., DeAngelis, C., Breinig, M. K., Ho, M., J. Bact., 1966, v91, 1277.

5. Kilbourne, E. D., Smart, K. M., Pokorny, B. A., Nature, 1961, v190, 650.

6. Mendelson, J., Glasgow, L. A., J. Immunol., 1966, v96, 345.

7. Rytel, M. W., Kilbourne, E. D., J. Exp. Med., 1966, v123, 767.

8. Ho, M., Science, 1964, v146, 1472.

9. Ho, M., Kono, Y., Breinig, M. K., Proc. Soc. Exp. Biol. & Med., 1965, v119, 1227.

10. Youngner, J. S., Stinebring, W. R., Nature, 1965, v208, 456.

11. Lieberman, M., Polikoff, R., Pascale, A. M., Proc. Soc. Exp. Biol. & Med., 1966, v122, 484.

12. Mendelson, J., Finland, M., ibid., 1966, v123, 98.

13. Youngner, S., Stinebring, W. R., Taube, S. F., Virology, 1965, v27, 541.

14. Thorn, G. W., Jenkins, D., Laidlaw, J. C., Goetz, F. C., Dingman, J. F., Arons, W. L., Streeten, D. H. P., McCracken, B. H., N. Eng. J. Med., 1953, v248, 284.

15. Thomas, L., Bull. N. Y. Acad. Med., 1955, v31, 485.

16. Kass, E. H., Finland, M., Adv. Int. Med., 1958, v9, 45.

17. Chedid, L., Parant, M. Boyer, F., Skarnes, R. C., in Bacterial Endotoxins, M. Landy & W. Braun, ed., 1964, p500.

Received January 9, 1967. P.S.E.B.M., 1967, v125.

Comparative Viral Sensitivity of Bovine and Avian Cells Before and After Storage in Liquid Nitrogen.* (32022)

PADMAN S. SARMA AND FRED EDWARDS (Introduced by R. J. Huebner)
Microbiological Associates, Inc., Bethesda, Md.

Dimethyl sulfoxide (DMSO) has been found useful for minimizing the loss of cell viability on freezing and storage in the frozen state and is gaining wide acceptance for routine storage of mammalian and avian cells for virus studies (1-8). The procedure enables one to have on hand pre-tested cell stocks suitable for routine or investigational use and assures the safety of such cultures by preventing loss by accidental microbial contamination or by chromosomal mutation which frequently occur when cultures are repeatedly transferred *in vitro*. Many commercial manufacturers of tissue cultures depend on frozen cell stocks for production of various types of primary, diploid, and heteroploid cultures.

Although cultures derived from frozen cell stocks are in wide use, adequate information

* This work was supported by National Cancer Institute Field Studies, Contract PH 43-64-941, Nat. Inst. Health, Bethesda, Md.