which is described, the chromosome numbers did not revert to higher numbers, and the large teleocentric chromosome persisted in practically all cells during one year of cultivation (present time). Many chromosome aberrations decreased in frequency to the level observed in uninfected (control) FL cells. The irreversible changes, which were correlated with alterations of *in vitro* and *in vivo* behavior of the cells, are referred to as reverse transformation or retransformation.

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Received April 25, 1967. P.S.E.B.M., 1967, v126.

Variation in Adrenal Cortical Hormones Within Physiologic Ranges, Stress and Interferon Production in Mice.* (32370)

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The relationship of adrenal corticosteroid administration and of stress to the clinical course of virus infections and to interferon production has not been established(1,2,3). Corticosteroid treatment did not appear to affect the clinical course of poliomyelitis(4) or mumps(5) but did appear to predispose to relapses of infectious hepatitis(6). Stress or substantial doses of adrenal steroids rendered normally resistant adult mice susceptible to Coxsackie B virus(7,8,9). ACTH and the stress of overcrowding were both noted to reactivate rabies virus in guinea pigs (10,11). Under certain conditions, various stresses increased susceptibility of mice to vesicular stomatitis virus and herpes simplex virus(12,13,14). However, intact mice inoculated after a second 3-hour daily period of sound stress were more *resistant* to vesicular stomatitis virus, though adrenalectomized mice inoculated on the second day of stress showed increased mortality from encephalitis (14). *Decreased* susceptibility to poliomyelitis was found in monkeys subjected to 24 hours of avoidance stress(15). In humans, delayed recovery from infectious mononucleosis was correlated with deficient ego strength (16), and from influenza correlated with depression(17).

Several studies report suppression of interferon production *in vivo* and *in vitro* by adrenal cortical steroid hormones in high doses(18,19,20,21,22). Cortisone in doses of 0.5 to 10.0 mg/mouse administered 24 hours prior to Newcastle disease virus (NDV) injection reduced the interferon titer 6 hours after injection 2½ to 8-fold(23). The administration of 1 to 5 mg of hydrocortisone to 1 kg rabbits markedly suppressed interferon production by *E. coli* endotoxin, but single doses of hydrocortisone up to 250 mg did not decrease circulating interferon levels following inoculation with Sindbis virus or NDV(24). Adrenalectomy markedly poten-

* This work was conducted under research funds of the Veterans Administration and USPHS grants. Miss Suzanne Donahue and Mrs. Gertrude Adler provided valuable technical assistance. We are grateful to Helena Kraemer, Ph.D., Stanford Univ. School of Medicine and to the Western Regional Research Support Center, Veterans Admin., Sepulveda, Calif. for statistical help.

tiated the production of endotoxin-induced but not virus-induced interferon in rabbits (24). One study reports no effect on interferon production of mouse leukocytes infected *in vitro* by the addition of cortisol *in vitro* (25). However, *enhanced* interferon production was noted in peritoneal cell explants from mice treated with 5 my cortisol *in vivo* (25).

It has been postulated that a suppressed interferon response is responsible for stress-induced increases in susceptibility to virus diseases(26). High intensity sound stres reduced interferon production in mice 7 to 9 days after intranasal injection of vesicular stomatitis virus(26). Jensen and Rasmussen subjected 6-week-old female Swiss-Webster mice to non-specific central nervous system mediated stress in an avoidance-learning shuttle box apparatus(27). Mice were challenged with 5.6×10^7 plaque forming units of Newcastle disease virus, and interferon assay was performed on sera pooled from 2-3 mice using a mouse embryo tissue culture-vesicular stomatitis virus test system. Animals were bled 4 hours after injection, and plaques were counted after 48-hour incubation. They report that mice subjected to 4-6 hours of daily stress for from 4 days to 4 weeks had an average interferon titer of 1:3964 compared to 1:5404 in controls. This was statistically significant in a non-parametric test. If, however, mice were injected 5-6 hours after termination of stress or a day after 1-4 weeks of stress, no significant differences in interferon response were noted. The greatest suppression of interferon was found in mice stressed only on the day of challenge, these animals not yet having learned to avoid the electric shock. No suppression of interferon production was noted in animals challenged prior to stress. Guinea pigs subjected to various experimental traumatic insults (X-ray and U-V irradiation, induced hypersensitivity, gravity shock, or thermal injury) produced less interferon and showed more clinical disease after inoculation with herpes simplex virus by various routes(28).

This study relates interferon levels induced in individual adult male mice by intravenous inoculation with Newcastle disease virus to plasma adrenal corticosterone levels in the same animals. Plasma concentration of corticosterone, ranging from minimal in adrenalectomized animals through high physiologic levels as a result of ACTH stimulation to somewhat above maximal physiologic ranges as a result of administration of exogenous corticosterone, were studied. In addition, the effect on interferon production of the stress of randomized electric shock preceded by a warning buzzer administered during a 5-hour period prior to inoculation of virus and of stress subsequent to inoculation with NDV was determined, in conjunction with measurement of plasma corticosterone.

Methods. Adult Swiss-Webster male mice of approximately 30 g were injected by tail vein with approximately 10^8 plaque-forming units of NDV in 0.2 cc in all experiments. Maximal interferon response was observed to occur 6 hours after injection.

Animals were bled 6 hours after inoculation in all experiments. Assay for interferon was by the plaque inhibition method employing L cell monolayers growing in a 60×150 mm plastic tissue plate. Forty to 50 plaque-forming units of bovine vesicular stomatitis virus served as the challenge. Interferon containing fluids were incubated with the monolayers 16 to 24 hours prior to challenge. Plaques were counted 48 hours after challenge, and interferon titers were expressed as the dilution inhibiting development of 50% of the plaques. An interferon reference of known titer was included in all assays, and any required corrections were made to compensate for differences in interferon sensitivity of individual assays. Each assay included animals under each of the current experimental conditions and controls. Plasma corticosteroid assays were done by the micro method of Glick, Redlich, and Levine on a volume of 50 lambda of plasma(29). All experiments were conducted so that specimens were obtained at approximately the same hour of the day (late afternoon) to preclude the possibility of steroid level differences due to circadian variations. Animals were acclimated to the experimental laboratory to minimize stress effects of novelty. Animals

were bled from the retro-orbital plexus using a lightly heparinized pipette.

All results were evaluated by computer, employing an analysis of variance design. In spite of relatively wide variability in individual interferon titers within each experimental group, significant differences were obtained by t-test.

Experimental findings. Forty-six control mice inoculated with NDV had a mean interferon titer of 1:20,200 (Table I). The mean plasma corticosterone level of these same animals 6 hours after inoculation was 47.9 μg/ml. The mean plasma corticosterone level of 11 mice injected with sterile saline and bled 6 hours later was only 22.5 μg/ml. Thus, it appears that the virus itself results in a significant adrenocortical response and, therefore, can be considered a potent stressor *per se.*

TABLE I. Intact Mice.

	No. of animals	Mean interferon titer (1:)	Mean corticosteroid (μg/ml)
Saline controls	11		22.6
NDV controls	46	20,200	47.9
Post-stressed	23	21,700	54.4
(Immobilization post-stressed)	11	23,800	59.9
Pre-stressed	21	35,600	53.1

Twenty-three animals were subjected to stress from the time of inoculation of virus to the time of bleeding 6 hours later (Table I). Stress consisted of 12-volt electric shock administered to the feet by a grid (with alternating electrification of component bars to prevent avoidance) for 30 seconds preceded by 1 minute by a warning buzzer. Stress periods were given at random intervals to minimize adaptation. Stress averaged 4-5 episodes/hour, and amperage was slightly increased during the course of the experimental period. These "post-stressed" animals responded to NDV with a mean interferon titer of 1:21,700, virtually identical to control animals. These findings are in agreement with those of Chang and Rasmussen(26), who found interferon production not influenced by stress administered subsequent to inoculation with virus. Post-stressed animals had a mean plasma corticosterone level not significantly different from controls.

Eleven mice subjected to another type of stress subsequent to inoculation, immobilization in wire, mesh did not differ in their interferon responses from animals stressed by buzzer-electric shock (Table I). This finding is also consistent with Jensen and Rasmussen's report of the similarity of shock, confinement, immobilization and avoidance conditioning as stresses (personal communication).

Twenty-one mice were subjected to the shock stress procedure already described for 5 hours prior to inoculation with NDV with no stress administered subsequent to inoculation (Table I). In contrast to the findings of the others, these animals showed a significantly ($P = <.05$) *increased* titer of interferon averaging 1:35,600.

Mean plasma corticosteroid level of pre-stressed animals did not significantly differ from controls or the post-stressed groups. Thus, it is clear that exogenous stress does not add a significant increment to the adrenal response beyond that induced by the virus injection.

To evaluate further the relationship of adrenal cortical hormones to interferon production and to seek a possible non-adrenal mediated stress influence on interferon synthesis, adrenalectomized animals subjected to 5 hours of buzzer-shock stress prior to inoculation with NDV were compared with NDV challenged non-stressed adrenalectomized controls. Steroid levels of all animals were determined, and any animal with a serum corticosterone of greater than 7 μg/ml was eliminated as having been incompletely adrenalectomized. Adrenalectomized animals received normal saline to drink. Two additional groups of mice (adrenalectomized pre-stressed and adrenalectomized non-stressed) were compared, each group having received corticosterone replacement therapy consisting of 1 mg of corticosterone/100 mg of body weight injected subcutaneously daily between adrenalectomy and experiment (usually 2 days), with the last injection given on the morning of the experiment. There were no significant differences in the mean interferon titers among

TABLE II. Adrenalectomized Mice.

	No. of animals	Mean interferon titer (1:)	Mean corticosteroid (μg/ml)
No steroid replacement, no stress	28	43,600	3.6
Steroid replacement, no stress	20	43,500	13.5
No steroid replacement, stress	22	42,000	4.9
Steroid replacement, stress	22	42,200	12.2

the 4 groups: adrenalectomized-stressed; adrenalectomized-non-stressed; adrenalectomized with steroid replacement-stressed; adrenalectomized with steroid replacement-non-stressed (Table II). Mean corticosteroid levels in the replacement therapy groups were somewhat below those in non-virus-injected animals and far below those in intact animals challenged with virus.

If one compares all adrenalectomized mice, whether or not steroid replacement was given, with all non-adrenalectomized animals, it is apparent that the adrenalectomized animals respond to NDV with significantly ($P<.01$) greater interferon production (Table III). Thus, adrenalectomy is accompanied by an *increased* interferon response to NDV. This finding is consistent with that of Postic and coworkers with endotoxin induced interferon in rabbits, though they found no such augmentation of interferon level after inoculation of viruses in adrenalectomized animals(24). It is clear that partial replacement of corticosterone in adrenalectomized animals in this experiment had no effect on the enhancement of interferon production induced by adrenalectomy.

Seven intact mice were given 3 units of ACTH intramuscularly 1 hour before inoculation with NDV and 3 units 3 hours after inoculation (and thus 3 hours prior to bleeding) to relate interferon production to maximal physiologic stimulation of adrenal cortical hormone output. The relatively high serum corticosterone levels, somewhat greater than the adrenal response induced by virus alone or virus plus stress, had no significant effect on interferon production (Table IV). Administration of 1 mg of corticosterone acetate in sesame seed oil subcutaneously 1 hour before inoculation with NDV and another milligram 3 hours after inoculation, leading to a serum corticosteroid level nearly triple that induced by virus alone and almost double that occurring with ACTH stimulation, did not significantly alter interferon response (Table IV). Thus, contrary to other *in vivo* studies using larger doses of hormone, our administration of corticosteroid did not suppress interferon production.

To elucidate further the relationship between adrenal corticosteroid level and interferon response, a correlation coefficient was calculated between these two values using the total of 250 animals from all experiments (completely and partially adrenalectomized with and without steroid replacement, intact with corticosterone or ACTH administration). The correlation coefficient was —0.15; thus, the null hypothesis that this correlation is zero can be rejected at the 5% level of confidence. This inverse relationship between interferon response and adrenal hormone level seems primarily a result of the high interferon levels in adrenalectomized animals; the correlation coefficient between plasma steroid and interferon levels in 166 mice with a steroid level of 39.9 μg/ml or less was —0.12. There is a negligible positive correlation coefficient (0.05) between steroid and inter-

TABLE III. Adrenalectomized *vs* Intact Mice.

	No. of animals	Mean interferon titer (1:)	Mean corticosteroid (μg/ml)
All adrenalectomized mice (including those with replacement therapy)	92	45,200	8.6
All adrenalectomized mice without replacement therapy	50	42,900	4.2
All non-adrenalectomized mice	158	25,200	74.5

TABLE IV. Treated Intact Mice.

	No. of animals	Mean interferon titer (1:)	Mean corticosteroid (μg/ml)
ACTH	7	20,600	71.6
Corticosterone	9	19,100	139.9

feron in the 84 mice with a steroid level of 40 μg/ml or more.

Inoculation with interferon-inducing virus strongly stimulates adrenal cortical hormone secretion. Thus, it is not surprising that ACTH, which does not add a significant increment in adrenal response, or even low doses of corticosterone have no effect on interferon production. The findings of other studies on adrenal steroid suppression of interferon synthesis seem clearly related to the very high, non-physiologic amounts of hormone used. Cortisone (0.5 mg/mouse) reported moderately to reduce interferon response to NDV (23) is probably functionally equivalent to considerably more than the milligram of corticosterone we utilized. Implants of cortisol into the median eminence block adrenocortical activity; whereas, corticosterone implants have no effect(30). Moreover, physiologic action of hormones not normally present in significant amounts in a given species may be greater than that of the naturally-occurring related hormone. Early studies of the immunosuppressive effect of steroids in rabbits gave misleading impressions because of the potency of cortisone and hydrocortisone in suppressing antibody in contrast to corticosterone, the major naturally occurring adrenal glucorticoid in that species(31).

It appears that very low levels of circulating adrenal cortical hormones are accompanied by somewhat enhanced interferon production by unknown mechanisms. It is conceivable that absence of adrenal medullary hormones may relate to the increased interferon production in adrenalectomized animals. The increase in interferon synthesis at low levels of circulating corticosteroid may have to do with our finding of *enhanced* interferon response in animals subjected to stress prior to inoculation with virus. The preceding stress may produce relative adrenal exhaustion, so that virus does not initially induce a vigorous adrenal response. However, we observed comparable adrenal response at the only time we performed such measurement, 6 hours after inoculation, at which point adrenal recovery may have taken place. Such biphasic adrenal response has been observed in other situations(32). Our results are compatible with Jensen and Rasmussen's observation of increased resistance to virus infection after 2 daily periods of sound stress(14), and with Marsh and others' finding of decreased susceptibility to poliomyelitis after 24 hours of stress(15). Reasons for the discrepancy between the report of suppressed interferon production by stress prior to the injection of mice with NDV(27) and our observation of enhanced interferon production under similar circumstances are unclear. Experimental details appear roughly comparable; however, the animals were supplied from a different sources; their sex was different; virus strains were different; the assay was not identical; and pools of sera rather than single animal sera were utilized in the other studies. The actual degree of suppression noted by these investigators was not great; yet, we have no single satisfactory explanation of the discrepancy between the results.

Summary. Both stress and pharmacologic levels of adrenal cortical hormones have been reported to suppress the synthesis of interferon. We measured interferon in mice 6 hours after intravenous injection of NDV and related interferon titers to plasma corticosterone levels. The stress of repeated random electric shocks preceded by a warning buzzer during interferon production did not alter the response from that of controls; such stress administered for 5 hours prior to virus inoculation significantly enhanced interferon production. Administration of ACTH did not alter interferon response. Corticosterone administration adequate to produce high circulating steroid levels just greater than maximal attainable physiologic levels also did not alter response. All adrenalectomized mice had relatively higher interferon titers; however, neither prior stress nor partial corticosterone replacement therapy in any combination significantly altered interferon production. Administration of the virus itself

produced an adrenal response in intact animals not subjected to other exogenous stress as great as that in animals subjected to exogenous stress prior or subsequent to injection of virus.

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Received April 25, 1967. P.S.E.B.M., 1967, v126.

Kinetics of Strontium and Calcium Skeletal Metabolism in the Rat.* (32371)

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While the preferential absorption of Ca as compared to Sr across the gastro-intestinal tract and in renal excretion is well established(1), differential handling of Sr and Ca by the skeletal system, *in vivo,* has not been clearly demonstrated, although it is suspected that it exists, on the basis of the differing properties of these two elements. In a study employing peritoneal lavage in nephrectomized rats, the differential removal of ^{85}Sr and ^{45}Ca from the rat was shown to be due mainly to renal function and not to differential handling of the two elements by the skeleton(1a). In two studies employing compartmental analysis of data derived from double tracer studies in elderly human subjects, slight differences were found in a number of parameters of skeletal metabolism calculated from ^{85}Sr data when compared to those derived from ^{47}Ca data(1b,2). Because

* Research supported by U. S. Atomic Energy Commission.