TABLE III. Distribution of I^{125} Labelled Normal Globulin and I^{131} Labelled Anti-Rat Brain Globulin After Intrathecal Injection into Same Rat.

Date	No. of rats	Interval (hr)	% of dose per gram of tissue per 100 g rat Blood	Brain	Liver	
2/15/63	11	18	1.23 ± .14*	.68 ± .18	.51 ± .12	Normal
			.61 ± .13	.35 ± .11	.46 ± .10	Anti
			2.02	1.94	1.14	Ratio N/A

* Numbers after ± sign are standard deviation. When compared at the .05 level using the student "t", the differences between the means were signficant for brain and blood, but not for liver.

localization of antiserum than of normal serum in brain would seem to correlate with other experimental work in which it was found that it was possible to damage organs such as kidney, pancreas, colon and thyroid (1-5) with their corresponding antisera but that similar damage could not be produced by anti-brain sera(6). It would appear that the brain has a more effective barrier or a more rapid clearing mechanism for antibody.

Summary. (1) Anti-rat brain globulin labelled with I^{131} does not localize in brain after either intravenous or intrathecal injections. (2) Normal rabbit I^{131} globulin seems to localize more in brain than anti-rat brain globulin labelled with I^{131}. (3) When I^{125} labelled normal globulin and I^{131} labelled antiserum globulin were injected into the same rats, the ratio of amount of antiserum globulin in rat brain after 18 hours was half the amount of normal globulin present. (4) time between injection and sacrifice did not change the ratio. (5) The antiserum does not localize in the liver.

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Relation Between Blood Level of Corticoids and Their Inhibiting Effect on the Hypophyseal Stress Response. (28442)

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The negative feed-back action of adrenocortical hormones on the corticotropic function of the pituitary gland is generally thought to be effected by changes in blood corticoid levels. This implies that an inverse relationship would exist between concentration of corticoids present in the blood and amount of ACTH produced by the adenohypophysis in response to stress. Recently Yates *et al.*(1) have reemphasized this correlation in stating that the increments in plasma corticoid concentration produced by noxious stimuli "are dependent in a predict-

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able and formal manner upon the pre-existing corticosterone concentration."

Subcutaneous administration of corticoids in massive dosages has often been employed as a method of suppressing the pituitary-adrenal response to stress(2,3,4). The resulting pituitary inhibition appears to last for many hours, and depends on the amount of corticoids injected and the intensity of the applied noxious stimulus(2). However, it has never actually been demonstrated that rate of inhibition is directly related to amount of circulating cortical hormones.

Accordingly, the present study was undertaken to determine the degree of pituitary-adrenal activation as well as the existing plasma corticosterone concentration in rats which had been stressed at various time intervals after corticosterone administration.

Method. Corticosterone,* dissolved in ethanol and diluted with saline, was injected subcutaneously at a dose level of 50 mg/kg into 2 groups of female albino rats. Two groups of control rats were injected with saline containing the same amount of ethanol. Fifteen minutes before decapitation one of each of the 2 corticosterone-treated and the 2 saline-treated groups was transferred to another cage and brought from the rat room to another room, a procedure which ordinarily activates the pituitary-adrenal axis. The remaining 2 groups were taken with a minimum of handling in the rat room and quickly decapitated. The time interval between corticosterone injection and decapitation varied from 30 minutes to 40 hours in a series of otherwise identical experiments.

After decapitation, blood was collected from the trunk vessels in heparinized tubes and centrifuged; the adrenals were removed, cleaned and weighed. The plasma content of corticosterone and rate of corticoid production by the excised adrenals *in vitro* were determined by the method of van der Vies *et al.*(5). In this way indices for corticosterone plasma level and for rate of adrenocortical activity could be obtained in the same animal.

Results and comment. The results are summarized in Table I.

TABLE I. *In vitro* Corticoidogenesis and Plasma Corticosterone Levels in Stressed and Non-Stressed Rats, Decapitated at Different Time Intervals After s.c. Injection of 50 mg/kg Corticosterone or Saline.

Decapitation after (hr)	Saline-injected				Corticosterone-injected			
	In vitro cort prod*		Plasma cort level†		*In vitro* cort prod		Plasma cort level	
	Pre-stress 1	Post-stress 2	Pre-stress 3	Post-stress 4	Pre-stress 5	Post-stress 6	Pre-stress 7	Post-stress 8
½	8.8 ± 2.2‡	27.4 ± 1.2	16.8 ± 3.5	42.4 ± 2.2	6.3 ± .5	21.6 ± 1.3	245.0 ± 17.3	261.1 ± 9.5
1	16.7 ± 2.8	27.9 ± .7	12.3 ± 4.6	37.3 ± 5.8	7.7 ± .4	14.0 ± 3.5	102.7 ± 25.6	113.2 ± 31.8
2	13.2 ± 2.8	21.4 ± 4.7	15.3 ± 2.8	23.3 ± 5.6	7.1 ± .5	5.8 ± .7	84.9 ± 11.6	65.6 ± 3.9
4	14.8 ± 4.3	22.6 ± 4.5	23.1 ± 5.8	41.8 ± 3.6	5.1 ± .7	5.5 ± .5	54.7 ± 9.8	63.0 ± 3.2
10	4.9 ± .3	23.1 ± .5	4.4 ± .1	44.3 ± 2.5	4.3 ± .1	6.0 ± 1.4	11.1 ± 1.3	10.4 ± 2.7
16	11.5 ± 3.1	30.8 ± 2.1	5.2 ± 2.5	33.1 ± 1.8	9.8 ± 2.0	15.1 ± 3.4	8.1 ± 3.2	18.2 ± 4.8
24	15.3 ± 3.5	24.5 ± .5	12.8 ± 2.9	36.9 ± 1.0	12.8 ± .7	17.6 ± 3.3	11.9 ± 2.7	17.6 ± 4.0
40	11.5 ± 2.6	31.1 ± 2.5	10.5 ± 4.0	40.8 ± 3.3	15.2 ± 2.6	26.5 ± 3.8	12.5 ± 6.5	34.9 ± 4.2

* In μg/100 mg adrenal/hr. † In μg/100 ml plasma. ‡ Each figure represents mean of 5 animals ± S.E.

* Generously supplied by Organon Ltd., Holland.

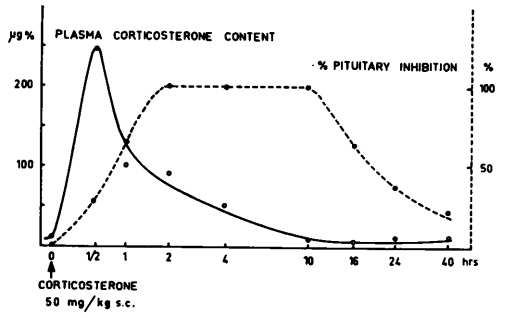

FIG. 1. Changes in plasma corticosterone levels and in rate of pituitary inhibition after subcutaneous injection of 50 mg/kg corticosterone.

Saline-injected groups. In the initial (pre-stress) levels of the *in vitro* corticoidogenesis and plasma corticosterone some fluctuation can be noted among the independently performed experiments (columns 1 and 3). This is due to differences in unspecific noxious stimulation and daily variations. In all experiments the transfer to a strange environment caused significant elevations in plasma corticosterone concentration and *in vitro* corticoidogenesis (columns 2 and 4), which seem to be rather independent of the initial values.

Corticosterone-injected groups. The blocking action of this corticoid eliminated the effects of minor unspecific stimuli, so that the pre-stress rate of *in vitro* corticoid production remained at the basal level (about 6 µg/100 mg/hr) as long as the inhibition existed (column 5). The stress-induced activation of the corticoidogenesis appeared to be depressed by corticosterone treatment during a limited period of time (column 6). Within 2 hours adrenocortical activity was completely inhibited; it remained so for about 8 hours and then gradually returned.

The plasma corticosterone concentration increased rapidly after corticosterone administration, reaching its peak within 30 minutes, and then fell towards physiological values during the next 4-6 hours (column 7). The post-stress corticosterone levels became significantly higher than the pre-stress levels only after 16 hours (column 8).

It is apparent from these data that pituitary-adrenal inhibition by corticosterone does not parallel the plasma corticosterone concentration. Although 30 minutes after injection the plasma corticosterone content has reached a peak and already started to decrease, it takes 2 hours before the inhibition becomes complete. Moreover, the blockade continues to exist after the plasma corticosterone levels have returned to normal. This is also illustrated in Fig. 1, in which percentage inhibition and plasma corticosterone content are given as a function of time. If basal corticoid production (6 µg/100 mg/hr) is considered as the zero value, then the increments in the rate of corticoid production above this level in the stressed corticosterone-treated groups (column 6) can be expressed as a percentage of the increments in the corresponding stressed saline-treated groups (column 2). The percentage inhibition is then given by

$$100 - \frac{(\text{increment groups column 6}) \times 100}{\text{increment groups column 2}}$$

The dissociation between the course of pituitary inhibition and that of the plasma corticosterone levels indicates that there is no direct relationship between the existing blood corticoid levels and rate of adrenocortical activity. This is in agreement with recent indications that the pituitary stress response is not directly related to physiological changes in the amount of circulating adrenocortical hormones(6,7). Rather, it might suggest that the inhibition is dependent upon an accumulation of the hormone by some structure which controls pituitary-adrenal activity.

Summary. Plasma corticosterone concentrations and adrenal corticoid production *in vitro* are determined in rats which have been subjected to stress at different time intervals after subcutaneous injection of a high dose of corticosterone. The inhibiting effect of this injection on the pituitary-adrenal response to stress in these rats appears not to run parallel with the induced elevation in plasma corticosterone levels. It is concluded that pituitary impairment by corticoid administration is not correlated with the existing blood level of corticoids.

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Effect of Thalidomide Treatment of Hens on Embryonic Development and Fertility.* (28443)

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While thalidomide has been associated with an increased number of malformations in human embryos(1), only a few studies have reported malformations in other mammals. Malformations have been reported in rabbits (2,3,4) and in rats(5). Lack of malformations or a significant increase in resorption of embryos has been noted by Sellers(4), Christie(6) and Felisati(3) in thalidomide-treated rats, mice and rabbits. Dalderup(7) suggested abnormalities of dentition might occur. Lucey(8) observed no live births in 44 treated monkeys while there were 11 live births in 57 untreated monkeys. Kemper(9) found 20% gross malformations in embryos from hen's eggs injected with thalidomide and delayed development of the germ in eggs injected before incubation. Kemper and Berger(10) also noted blood changes in 10-week-old chicks given thalidomide daily by stomach tube for 28 to 35 days. A pronounced anemia, with blockage of granulopoiesis believed to be connected with folic acid metabolism, was noted from the 10th day on. Verrett and McLaughlin(11) injected eggs with 5 mg/egg of a thalidomide suspension in alcohol and found 80% hatched with no gross limb malformations. Some embryos that failed to hatch were malformed. Thalidomide in dioxane gave increased incidence of embryonic limb malformation, although no malformations were caused by the same dose of dioxane alone. No report has been found as to the effect on embryos when hens were fed thalidomide. In daily feeding of hens, the drug might be expected to accumulate in the preformed yolk and thus affect the embryo. Experiments to test this supposition have been carried out.

Experimental. Pre-drug treatment of hens. Thirty Heisdorf and Nelson strain, single-comb White Leghorn hens, hatched in March, 1962, were range reared until 7½ months of age when they were housed in laying cages. They had been treated for worms with piperazine citrate 9 days before the experimental period began. These hens were inseminated with pooled semen from 6 Cornell, random-bred White Leghorn males, 7½ months of age, 2 days before the start of the 10-day pre-drug egg collection period, and every 7th or 8th day thereafter. Enough semen was used to give good fertility for 14 days. Eggs collected daily for 10 days before drug treatment began were incubated and examined for infertile eggs and malformed embryos as described below. Three hens had low egg production and were discarded.

Drug treatment of hens. In all experiments, thalidomide was fed in gelatin capsules, by mouth, in the late afternoon. Eggs were collected daily from the start of the drug treatment. All eggs were stored at 8°C and incubated at 5-day intervals. Eggs were candled after 5 days of incubation. All eggs appearing to be infertile were opened and

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