

## The Effect of Massive Doses of Cortisone on the Peripheral Blood and Bone Marrow of the Mouse

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**D**URING THE COURSE of studying the effects of massive doses of cortisone on mice, it was noted that a pronounced lymphoid hypoplasia and a lymphopenia in the peripheral blood occurred.<sup>1</sup> In view of these findings a study of the total blood eosinophils, lymphocytes and granulocytes and hemoglobin was made. The first two of these blood elements have been shown to decrease in number after the administration of adrenal cortical substances.<sup>2, 3</sup> To gain more information about the nature of these hematologic changes, the response of the bone marrow was studied at the same time.

### METHODS

Mature male mice\* were paired by weight into two groups: the treated group received 2.5 mg. cortisone† subcutaneously while the controls received 0.1 cc. of saline, cortisone vehicle or no injection at all. Two methods were used (neck bleeding and tail bleeding) to study the peripheral blood.

*Neck method:* Paired animals (treated and control) were sacrificed at various intervals after injection by a cut in the upper cervical region. One to 3 large drops of blood were allowed to fall on a slide and were used for counts and smears. Blood counts were done by standard methods, eosinophile counts were determined by Randolph's method,<sup>4</sup> 100 consecutive white blood cells were identified in the counts of smears stained with Jenner-Giemsa stain, and the hemoglobin was determined with the Haden-Hausser chamber.

*Tail method:* Care was taken to accustom the animals to handling and to isolate aggressive members from any group of mice to eliminate extraneous shock factors. To obtain blood, the mouse was secured in a towel, the tail was immersed in warm water (45 to 50 C.) for fifteen to thirty seconds, dried, and compressed at its base. A small longitudinal puncture was made into a tail vein with the tip of a sharp No. 11 Bard-Parker blade and the blood taken directly for counts and smears. In most cases the procedure consumed less than four minutes. Only animals which met the following minimal requirements were used: total eosinophile count 140/mm<sup>3</sup>, total lymphocyte count 9,500/mm<sup>3</sup>, differential count 50 per cent lymphocytes, hemoglobin 14.0 Gm. Blood counts were made at two, four, eight, twelve and twenty-four hours and two, three, five and nine days after injection so that no mouse was bled more than twice within four hours and not more than four times. Under these conditions the hemoglobin never fell below 13.0 Gm.

*Bone marrow:* Studies were done on 22 treated and 15 control mice. Marrow was obtained, after decapitation, from the right femur. The bone was dissected clean and the lower third of the femur was compressed with a fine forceps. Thick pink marrow was expressed from the broken upper end of the shaft and transferred to a glass slide. A drop of

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\* RAP strain (Rockland Farms, New City, N. Y.) weighing 25-30 Gm.

† Cortone (Merck) 1.0 cc. = 25 mg.

human serum was added as diluent and mixed thoroughly with the marrow. Thin smears were made and stained with Jenner-Giemsa. Five hundred consecutive cells were identified in the differential count and the percentages calculated. The classification used was a modification of those used in rats by Vogel<sup>5</sup> and Cameron and Watson.<sup>6</sup> The general architecture and cellular composition of the sternum and left hind leg was studied in paraffin sections made from tissues which were fixed in 10 per cent formalin and decalcified. The peripheral blood of 11 treated and 9 control mice was also studied by the tail method.

## RESULTS

### *Peripheral Blood Studies*

A marked fall in both lymphocytes and eosinophils occurred in blood obtained by the neck method at intervals from two hours through nine days after the injection of cortisone (figs. 1 and 2). As can be seen from these figures, several of

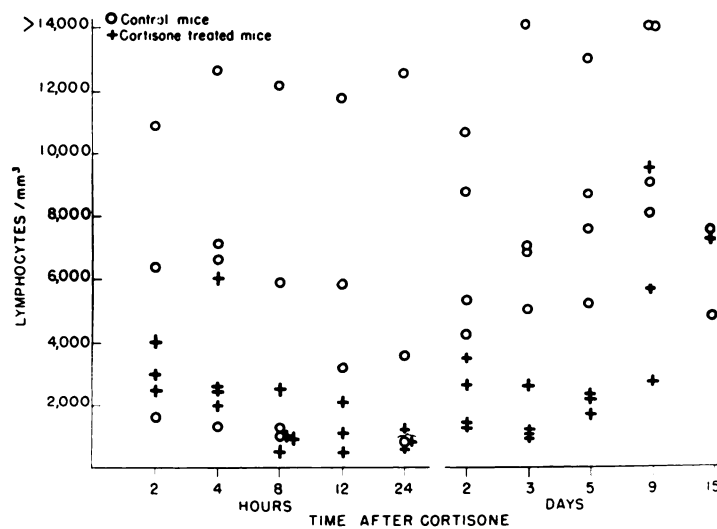


FIG. 1.—Total lymphocyte count.

the control animals had abnormally low total lymphocyte and eosinophile counts. However, since no data were available by this method concerning the leukocyte levels of the animals at the start of the experiment, it was impossible to evaluate the low count in the treated animals. To eliminate this factor the tail method was used. This permitted selection of a group of animals with a fairly uniform hemogram before treatment.

The mean data obtained by the tail method in 16 treated and 16 control mice followed for nine days after treatment is presented in table 1. Figure 3 is a scatter graph of the percentage change of the individual determinations of eosinophile count on treated and untreated animals. Figure 4 is a similar presentation of the total lymphocyte count. A mean average curve is interpolated on each graph. Figure 5 presents the total granulocyte counts in those mice on whom bone marrow studies were done.

It is apparent that subcutaneous administration of a single massive dose of cortisone is followed by a decided fall of the total lymphocytes and eosinophils

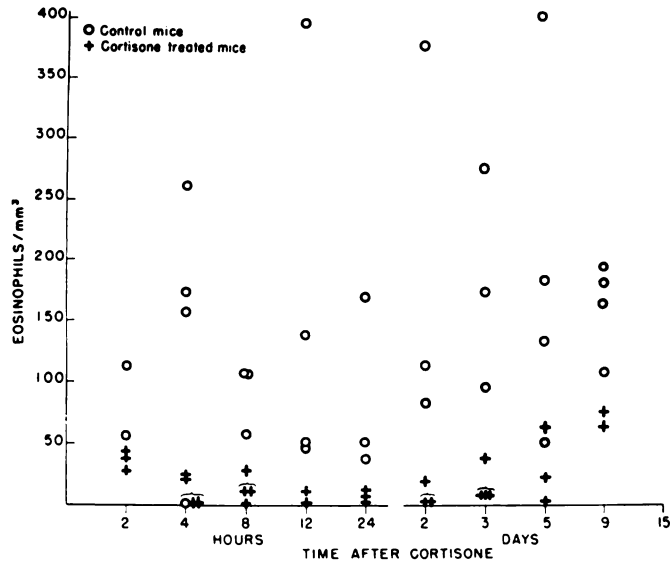


FIG. 2.—Total eosinophile count.

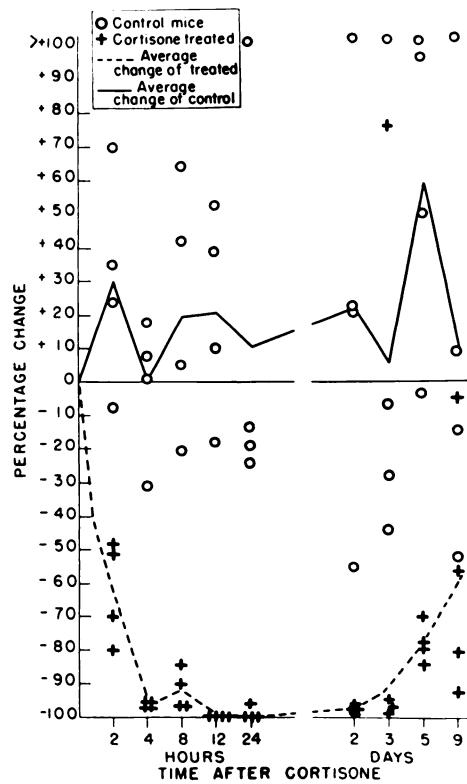


FIG. 3.—Percentage change in total eosinophile count.

which persists for several days. Additional observations made more than nine days after treatment demonstrated that the fall in count often lasted over two weeks. The granulocytes were diminished at four hours, remained at low levels through twenty-four hours, and abruptly returned to normal or higher at two days. Studies of red blood counts and hemoglobin showed no significant differences between control and treated mice. Seventy hemoglobin determinations made on untreated male mice gave a range of 12.5 to 17.0 Gm. with a mean value of 15.0 Gm. Fifty-three hemoglobin determinations on cortisone treated male mice gave a range of 13 to 17.5 Gm. with a mean value of 15.1 Gm.

Examination of the subcutaneous tissues of sacrificed mice revealed that small deposits of cortisone usually remained at the site of injection until five days after injection, and occasionally even nine days after injection.

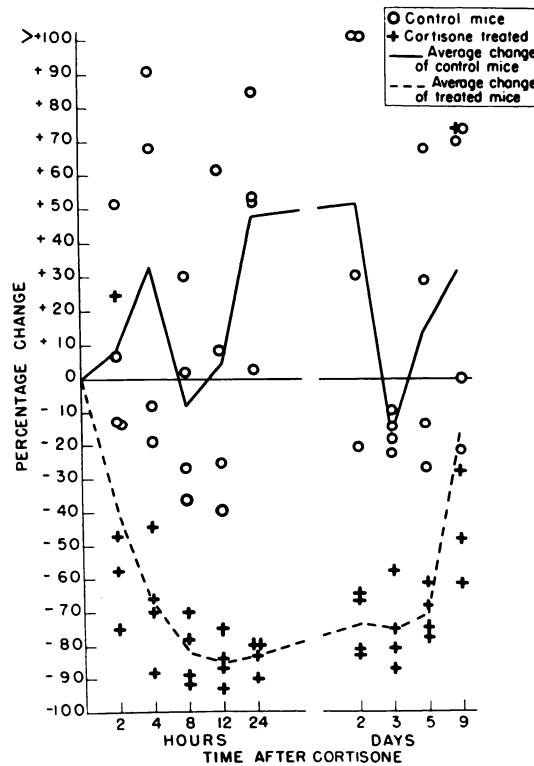


FIG. 4.—Percentage change in total lymphocyte count.

#### *Bone Marrow Studies*

The differential bone marrow findings of the 22 treated adult male mice are presented in table 2; the mean values and range for the 15 normal mature male mice are given at the bottom of the table. Myeloid-erythroid ratios (hereinafter referred to as ME ratios) were determined from the data on each animal.

Figure 6 shows the changes in the ME ratio in the marrow after cortisone treatment. The ME ratio increased, starting at twelve hours and reaching a maximum two days after injection. It fell considerably at three days and again rose, moderately, at five days. At nine days the ratios returned to the normal range in 2 out of 4 animals.

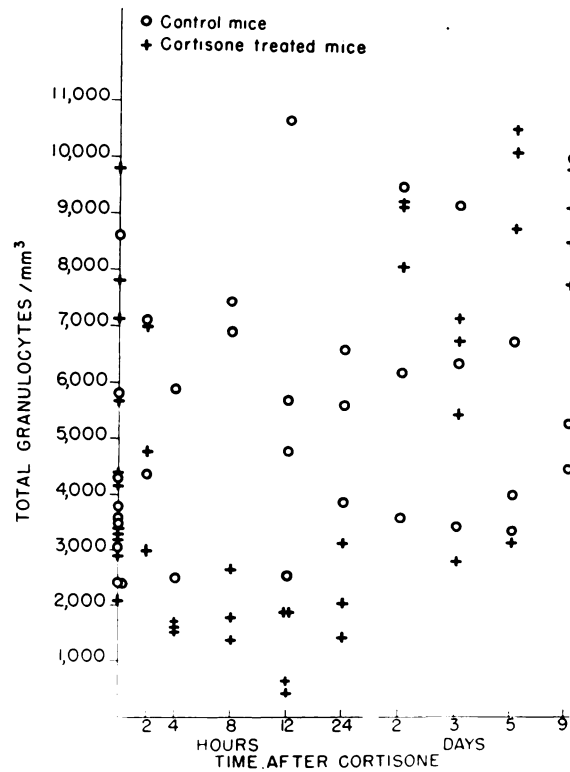


Fig. 5.—Peripheral total granulocyte counts of cortisone treated and control mice.

TABLE 1.—Mean Serial Blood Counts of Cortisone Treated and Control Mice

Treated Mice					Control Mice				
Number of Animals Used	Time After Cortisone	WBC	Total Lymphocytes	Total Eosinophils	Number of Animals Used	Time After Control Handling	WBC	Total Lymphocytes	Total Eosinophils
16	0	23,250	17,150	365	16	0	21,650	15,050	313
4	2 hr.	15,500	9,390	109	4	2 hr.	28,650	19,250	403
4	4 hr.	8,010	4,960	11	4	4 hr.	23,250	17,250	392
4	8 hr.	5,500	2,780	19	4	8 hr.	23,050	15,500	319
4	12 hr.	3,080	1,760	0	4	12 hr.	20,650	14,550	380
4	24 hr.	4,910	2,420	2	4	24 hr.	26,250	19,250	393
4	2 d.	14,900	5,330	11	4	2 d.	27,700	20,750	364
4	3 d.	10,650	4,430	92	4	3 d.	22,100	14,200	291
4	5 d.	16,750	5,890	96	4	5 d.	17,700	12,700	399
4	9 d.	21,150	11,250	154	4	9 d.	26,650	19,250	219

A slight increase in immature myeloid cells was seen in the first twelve hours after injection of cortisone (table 2). However, marked changes in distribution of cell type were first noted at twelve hours after injection. These consisted of a rise in myeloid and a fall in erythroid elements producing a rise in the ME ratio. From twelve hours through three days this marked increase in the ME ratio was mainly due to segmented cells (Animals 5, 7 to 13). At five and nine days the myeloid elevation resulted from a distinct increase in immature granulocytes (Animals 14 to 18, 20, 22). A less constant decrease in lymphocytes was seen in

TABLE 2.—The Differential Count in Bone Marrows of Cortisone Treated Mature Male Mice

Animal Number	Time after Cortisone	Myeloid Cells										Erythroid Cells			Miscellaneous Cells					Myeloid-Erythroid Ratios	
		Neutrophils					Eosinophils					Total	Eryth-roblasts	Normo-blasts	Total	Lym-pho-cytes	Mono-cytes	Plasma Cells	Reticu-endothelial Cells		Mega-karyo-cytes
		Myelo-blasts	Myelo-cytes	Ring Forms	Non-seg-mented Cells	Seg-mented Cells	Myelo-cytes	Ring Forms	Non-seg-mented Cells	Seg-mented Cells	Total										
1	2 hr.	0.8	6.8	8.2	5.6	29.0	0.4	1.0	1.0	1.0	53.8	4.6	29.0	33.6	12.2	0.2	0.0	0.0	0.0	0.2	1.6:1
2	2 hr.	0.6	8.0	10.1	9.4	16.6	0.4	0.4	0.0	45.9	5.2	33.5	38.7	14.8	0.4	0.0	0.0	0.0	0.0	0.2	1.2:1
3	8 hr.	1.6	10.4	13.0	7.4	13.0	0.0	1.8	0.4	48.6	5.4	32.4	37.8	12.6	1.0	0.0	0.0	0.0	0.0	0.0	1.3:1
4	8 hr.	1.4	5.0	16.4	6.6	25.6	0.8	0.8	0.4	58.0	4.6	31.0	35.6	5.8	0.6	0.0	0.0	0.0	0.0	0.0	1.6:1
5	12 hr.	2.4	7.4	14.8	5.8	34.8	0.8	4.2	2.6	75.0	2.8	12.0	14.8	9.8	0.2	0.0	0.0	0.0	0.2	0.2	5.1:1
6	12 hr.	1.0	5.6	22.2	4.2	27.2	0.0	1.2	1.0	63.2	4.8	20.4	25.2	10.8	0.6	0.0	0.0	0.0	0.0	0.2	2.5:1
7	24 hr.	2.2	5.0	9.2	4.0	40.0	0.2	7.0	2.6	73.2	2.0	13.8	15.8	10.6	0.4	0.0	0.0	0.0	0.0	0.0	4.6:1
8	24 hr.	1.4	7.6	9.0	5.2	41.8	0.2	0.6	0.8	70.0	1.8	23.0	24.8	5.2	0.0	0.0	0.0	0.0	0.0	0.0	2.8:1
9	24 hr.	1.2	6.0	6.8	4.8	51.0	0.4	0.8	0.8	74.2	2.4	20.4	22.8	2.8	0.0	0.0	0.0	0.0	0.2	0.2	3.3:1
10	2 d.	1.4	3.8	7.2	4.6	55.6	0.2	2.0	0.6	77.4	1.0	11.4	12.4	9.0	0.8	0.0	0.0	0.0	0.4	0.4	6.2:1
11	2 d.	0.8	8.2	19.6	10.0	36.8	0.0	0.2	0.2	76.2	4.4	14.0	18.4	4.6	0.2	0.4	0.0	0.0	0.2	0.2	4.1:1
12	3 d.	2.0	3.0	11.2	3.8	34.2	0.2	2.2	0.8	64.6	4.4	20.2	24.6	9.0	0.8	0.8	0.0	0.0	0.2	0.2	2.6:1
13	3 d.	1.6	4.4	8.8	5.2	41.6	0.0	1.0	1.0	65.2	3.8	19.6	23.4	11.4	0.0	0.0	0.0	0.0	0.0	0.0	2.8:1
14	5 d.	7.6	11.8	15.4	9.8	25.4	0.2	2.0	1.0	77.2	2.6	17.2	19.8	2.6	0.0	0.2	0.0	0.0	0.2	0.2	3.9:1
15	5 d.	4.0	7.6	18.0	11.0	31.8	0.2	0.0	0.0	72.8	4.4	19.6	24.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	3.0:1
16	5 d.	2.4	6.6	22.8	9.0	29.2	0.2	1.0	1.4	75.0	1.8	17.4	19.2	5.0	0.6	0.2	0.0	0.0	0.0	0.0	3.9:1
17	8 d.	2.0	12.0	14.4	8.4	30.6	0.4	1.2	0.4	69.6	4.4	22.0	26.4	3.4	0.0	0.2	0.2	0.2	0.2	0.2	2.6:1
18	9 d.	4.2	9.6	13.6	4.6	36.2	0.0	0.0	0.0	68.2	4.4	21.0	25.4	5.8	0.2	0.2	0.0	0.0	0.2	0.2	2.7:1
19	9 d.	2.4	7.2	12.8	4.6	23.0	0.6	1.2	2.8	55.8	6.2	29.8	36.0	6.4	0.6	0.4	0.0	0.0	0.2	0.2	1.6:1
20	9 d.	4.0	5.6	17.2	8.2	33.8	0.0	0.4	0.2	69.6	2.4	19.0	21.4	8.6	0.4	0.0	0.0	0.0	0.0	0.0	3.2:1
21	9 d.	0.8	4.8	13.2	7.4	34.2	0.2	0.4	0.0	61.4	2.4	27.8	30.2	8.0	0.2	0.0	0.0	0.0	0.2	0.0	2.0:1
22	15 d.	1.8	9.6	18.8	10.4	20.8	0.2	1.4	0.6	63.8	3.4	23.2	26.6	9.2	0.2	0.2	0.0	0.0	0.0	0.0	2.4:1
<i>Control Mice</i>																					
Mean*		1.4	6.0	10.6	4.9	24.2	0.2	1.3	0.6	50.4	4.6	31.3	35.7	12.9	0.4	0.1	0.4	0.2	0.2	0.2	1.49:1 (S.D. = 0.47)
Range* High		2.8	9.0	14.4	9.0	34.6	0.6	4.8	2.4	64.6	6.0	42.6	48.6	24.0	0.8	0.4	0.2	0.4	0.4	0.4	2.4:1
Low		0.2	1.2	7.6	2.8	15.2	0.0	0.0	0.0	38.2	2.4	21.2	26.8	7.8	0.0	0.0	0.0	0.0	0.0	0.0	0.8:1

\* Fifteen mature male mice (controls).

Animals 8, 9, 11, 14 to 20. In immature mice similar findings were present after cortisone, although the untreated controls had ME ratios below the mean found for adults.

Comparison of the paraffin sections of the bone marrow of the treated and control mice revealed an overall increase in marrow cellularity in the treated animals with morphologic changes corresponding to those seen in the marrow smears.

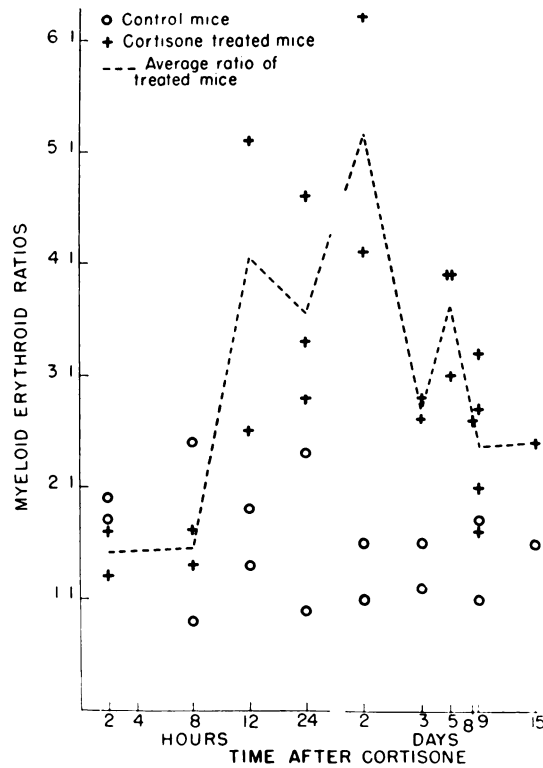


FIG. 6.—Myeloid-erythroid ratios of cortisone treated and control mice.

DISCUSSION

The administration of 2.5 mg. of cortisone to normal adult male mice produces a prompt and prolonged depression of the level of circulating lymphocytes and eosinophils. This dose represents roughly 50 times the dosage now employed in humans. The sustained depression of lymphocyte and eosinophile levels by this dose is of very long duration. However, it must be emphasized that cortisone is slowly absorbed from the site of injection; five or more days are necessary for the depot to disappear. The sustained low level of lymphocytes and eosinophils seen in our experiments may then be ascribed to the massive dose given, to a maintenance of the cortisone effect due to continued absorption from its depot, or to both. A similar response has also been observed after subcutaneous administration of 1.25 mg. of cortisone to immature albino mice (21 to 30 days old), 10

mg. to mature male albino rats, and 5 to 10 mg. to 9 week old hypophysectomized rats injected two weeks after hypophysectomy.

The total granulocyte levels in the peripheral blood follow a different course: there is a period of absolute granulocytopenia lasting more than twenty-four hours, followed by a period of granulocytosis.

A pronounced increase in the myeloid-erythroid ratio occurs after a massive dose of cortisone. Examination of marrow sections suggest that this change is due to an absolute increase of myeloid cells with a resultant increase in total marrow cellularity. This eliminates the possibility of an increase in ME ratio due solely to the disappearance of nucleated erythrocytes, since this would decrease marrow cellularity. No decrease in hemoglobin or erythrocyte levels of the peripheral blood are noted.

Since the rise in ME ratio and the increase in bone marrow cellularity indicates an absolute myeloid increase after cortisone treatment, the fall in marrow lymphocytes, as well as in erythroid cells, appears to be relative. The percentage of eosinophilic cells in the marrow does not change. Cortisone does not seem to inhibit cell proliferation in bone marrow. On the other hand, there is a striking absolute decrease in both eosinophils and lymphocytes in the peripheral blood concurrent with the involution of lymphoid tissues.<sup>1</sup> In this regard, it is of interest to note that the marrow of the majority of leukemic patients also does not respond to cortisone.<sup>7</sup>

The myeloid increase in the marrow may depend upon increased proliferation of myeloid cells, interference with myeloid release from the bone marrow into the peripheral blood, or a combination of both factors. The coexistence of a peripheral granulocytopenia, and bone marrow myelocytosis due to mature myeloid cells seen twelve and twenty-four hours after the administration of cortisone, is compatible with the concept of blocking of the bone marrow. Five days after the administration of cortisone, when the peripheral granulocyte count is normal, and when there is a secondary rise in ME ratio, a significant increase in immature myeloid forms is seen in the marrow which persists as long as nine days. This rise may indicate myeloid proliferation in response to the resumption of cellular discharge from the marrow.

#### SUMMARY

1. The subcutaneous administration of a single massive dose of cortisone to mature male albino mice produces: (1) A prompt and prolonged fall in the level of circulating lymphocytes and eosinophils which persists as long as nine days after the administration of the hormone. (2) A granulocytopenia which resolves at two days and is accompanied by a period of bone marrow blocking followed by resumption of delivery and myeloid hyperplasia.

2. No inhibitory effect of cortisone on cell proliferation is seen in the bone marrow.

3. There is no change in hemoglobin concentration following cortisone treatment.

4. Adult male albino mice are satisfactory animals for serial observations of the effect of cortisone on the circulating blood elements and the bone marrow.



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