Leukokinetic Studies

XIII. A NON-STEADY-STATE KINETIC EVALUATION OF THE MECHANISM OF CORTISONE-INDUCED GRANULOCYTOSIS

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ABSTRACT The mechanism by which adrenocortical steroids induce granulocytosis in man has been investigated using granulocytes labeled with radioactive diisopropylfluorophosphate.

After an intravenous injection of 200 mg of cortisol was given to five normal subjects, the mean value for the total blood granulocyte pool increased from 79 to 138 $\times$ 10^7 cells per kg of body weight and reflected an increase in the size of both the circulating granulocyte pool and the marginal granulocyte pool.

When granulocytes in the circulation were labeled with diisopropylfluorophosphate and granulocytosis was induced later by the intravenous administration of cortisol, the rate of decline of granulocyte specific activity was increased, indicating that the blood pool was being diluted at an accelerated rate by unlabeled cells entering from the bone marrow.

The rate of egress of granulocytes from the blood pool to an inflammatory exudate was studied by the "skin window" technique. After the administration of cortisol, there was a mean reduction in the cellularity of induced inflammatory exudates of 75%. However, this reduction in cellularity varied considerably from subject to subject (45–98%).

From these studies we can infer that steroids induce an absolute granulocytosis by decreasing the rate of egress of cells from the total blood granulocyte pool as well as by increasing the influx of cells from the bone marrow.

By model simulation studies of the non–steady state induced by cortisol injection, it has been possible to quantitate these rate changes. In the present studies cortisol injection resulted in a mean decrease in blood granulocyte egress of 74% (1–99%) and a mean increase in cell inflow of 450% (300–750%).

INTRODUCTION

The measurement of certain parameters of granulocyte kinetics is made possible by labeling autologous blood granulocytes in vitro with radioactive diisopropylfluorophosphate ($DF^{32}P$) and by infusing the labeled cells back into the donor (1). Normal values for total blood granulocyte pool size (TBGP), circulating granulocyte pool size (CGP), marginal granulocyte pool size (MGP), and granulocyte turnover rate (GTR) have been reported (1, 2).

In previous studies it was noted that after 8 days of oral prednisone administration to normal subjects, there was an appreciable increase in the TBGP which was due to an increase in both the CGP and the MGP. The rate of turnover of granulocytes (GTR) at the new equilibrium level was the same after as before the prednisone was administered (2). The increase in the TBGP which follows the administration of adrenocortical steroids could be the result of a temporary accel-

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eration of cell inflow from the bone marrow, a temporary decrease in the rate of cell egress from the TBGP, or any combination of changes in inflow and egress resulting in a net increase in TBGP. The purpose of the present study is to determine which of these mechanisms is responsible for the granulocytosis after steroid administration [1] and to quantify the changes.

To assess the possibility that steroids produce granulocytosis by accelerating the flow of cells from the bone marrow into the TBGP, autologous granulocytes labeled with $DF^{32}P$ were infused into normal subjects. After the slope of the granulocyte disappearance curve was established, granulocytosis was induced by the rapid intravenous injection of cortisol. In such a study the bone marrow reservoir of granulocytes is not labeled (1, 2). Therefore, a sudden change in the blood granulocyte specific activity curve (increased rate of fall) should accompany a rapid influx of unlabeled cells from the marrow, as occurs, for example, when granulocytosis is produced by the administration of endotoxin (2). However, if the granulocytosis is due to a decrease in the rate of egress of the cells from the circulation, the slope of the specific activity curve should be influenced little if at all, because the specific activity of the TBGP is being diluted only by the normal rate of influx of unlabeled cells from the marrow.

To assess directly, the effect of steroids on the flow of granulocytes from the TBGP to the tissues, inflammatory lesions were produced on the skin. The accumulation of granulocytes in the inflammation was estimated before and at various time intervals after the administration of steroids.

Preliminary reports of these studies have been presented (3–5).

METHODS

Healthy, hematologically normal, male volunteers from the Utah State Prison, 23–40 yr of age, were used in these studies. Seven subjects ate breakfast at 6:00 a.m., and the infusion of autologous $DF^{32}P$-labeled blood was begun between 8:00 and 10:00 a.m. In two subjects the study was begun at 8:30 p.m. after a 6:00 p.m. supper.

The methods for labeling blood in vitro with $DF^{32}P$, for the isolation of leukocytes from blood samples, for the determination of granulocyte radioactivity, for the measurement of the TBGP, CGP, MGP, and $t_{\frac{1}{2}}$, and for the calculation of the GTR have been described (1, 2).

[1] Steroid, as used in this paper, refers to prednisone or cortisol.

Total leukocyte counts were made with a Coulter counter (6). Absolute counts were calculated from the total leukocyte count and 200 cell differential counts made on cover glass smears stained with Wright's stain.

Inflammation was induced by the "skin window" method of Rebuck et al. (7). Skin of the forearm or thigh was shaved and cleaned, and an area approximately 3×5 mm was scraped with a No. 10 Bard Parker blade until a slight serosanguinous ooze was evident. The trauma of denuding the skin served as the inflammatory stimulus. A round cover glass 12 mm in diameter was pressed against the lesion by a covering piece of cardboard taped firmly to the skin. Cover glasses were removed and replaced over the lesion 1, 3, 6, and 12 hr after the lesion was produced. The cover glass placed on the lesion at 12 hr after it was produced was removed 12 hr later.

A small drop of inflammatory exudate adhered to the surface of the cover glass when it was removed. The preparations were air dried and stained with Wright's stain. Comparison of the number of cells on the cover glass with the number removed by scraping the lesions indicated that approximately one-half of the free inflammatory cells were removed with the cover glass. This proportion remained unchanged after administration of steroids.

The total number of cells on the cover glass was counted. The value obtained represents an estimate, since the cells were not always evenly distributed and at times were too numerous to obtain accurate counts. Furthermore, a fivefold variation in the number of cells in the inflammatory exudate was observed when lesions were produced simultaneously in neighboring areas in the same subject. This marked variation in the number of cells in inflammatory exudates necessitated the use of large control and experimental groups for meaningful comparisons. A total of 152 inflammatory lesions was produced in six normal subjects. Although the values given are only comparative estimates, the differences before and after steroid administration were sufficiently great to be evident on gross scanning of the cover glasses.

Differential leukocyte counts were performed on 200 cells on each cover glass. The proportion of granulocytes and macrophages in simultaneously induced, adjacent lesions varied considerably. As a result, there was a large standard deviation in the mean percentage of granulocytes in 6, 12, and 24-hr-old inflammations. For example, the mean percentage of granulocytes in 12-hr samples of exudate from 50 control inflammations was 50, with a standard deviation of 25. 98% of the cells which appeared in exudates in the first 3 hr were granulocytes and, therefore, 3-hr-old exudate samples were used to determine the effect of steroids upon the number of granulocyts in an inflammation.

RESULTS

Influence of intravenous cortisol on the total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP) marginal granulocyte

TABLE I

Influence of Cortisol on the Total Blood Granulocyte Pool (TBGP), Circulating Granulocyte Pool (CGP), Marginal Granulocyte Pool (MGP), and Granulocyte (G) Count

Subject	Control			After Cortisol			Post steroid G/control G
	TBGP	CGP	MGP	TBGP	CGP	MGP	
	$\times 10^7 G/kg$	$\times 10^7 G/kg$	$\times 10^7 G/kg$	$\times 10^7 G/kg$	$\times 10^7 G/kg$	$\times 10^7 G/kg$	
V-70, 84	98	47	51	135	93	42	1.98
V-72, 86	95	39	56	180	93	87	2.39
V-98, 114	45	22	23	90	51	39	2.35
V-100, 116	94	29	65	148	66	82	2.25
V-102, 112	62	29	33	136	83	33	2.86
Mean	79	33	46	138	77	61	2.37

pool (MGP), and absolute granulocyte count. The size of the blood granulocyte pool was measured after the intravenous injection of cortisol in order to make certain that the granulocytosis was due to an absolute increase in the size of the TBGP, as was the case after 8 days of oral prednisone administration (2). The pools were measured in five subjects 3–5 hr after the rapid intravenous injection of 200 mg of cortisol. Each subject served as his own control. The results are presented in Table I.

The absolute granulocyte count increased progressively over the first 5 hr and reached a mean maximum value of 2.4 times the base line value. The mean value for the TBGP increased from 79 to 138×10^7 cells per kg of body weight and reflected an increase in the size of both the CGP and the MGP. There was a slightly greater increase in the size of the CGP than in the MGP in all five subjects. The mean CGP/TBGP ratio was 0.42 in control studies and 0.56, 5 hr after the intravenous cortisol injection.

Influence of steroids on the concentration of granulocytes in inflammatory exudates. As a

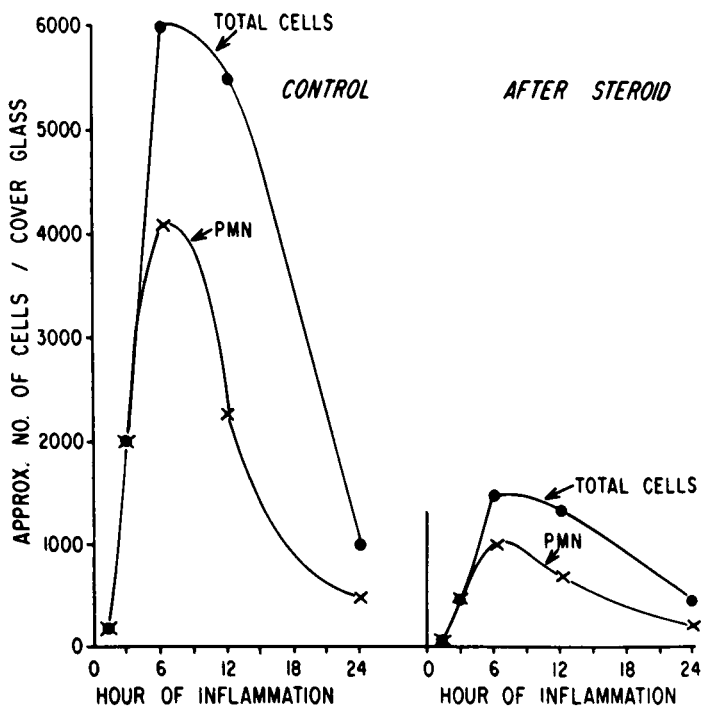

FIGURE 1 The cellularity of inflammatory exudates in normal subjects before and after the administration of steroid. The control curves represent the median values obtained in a total of 30 inflammatory lesions produced in six normal subjects. The subjects were then given in single intravenous injection of 200 mg cortisol. The poststeroid curves represent the median values obtained in a total of 38 inflammatory lesions produced 2–11 hr after the injection. Each of the lesions was sampled at the time intervals shown in the figure.

means of assessing the effect of steroids on granulocyte egress from the blood, the cellular composition of inflammatory exudates was studied in normal subjects before and after steroid administration.

The cellularity of inflammatory exudates is illustrated in Fig. 1. In normal subjects not given steroids, cells appeared within 1 hr after the inflammation was produced and reached maximum values after 6 hr. Thereafter, the cellularity of the exudates declined. 3 hr after the inflammation was produced, 98% of the cells present were granulocytes. Macrophages accounted for an increasing proportion of the cells in the exudates produced after 3 hr. In subjects given steroid (Fig. 1), the median cellularity of the exudates was reduced by approximately 75% as compared with the cellularity of exudates from control subjects. However, considerable variation was noted from subject to subject (45–98%). The time at which the cells first appeared in the lesions remained unchanged, as did the ratio of macrophages to granulocytes at each point on the curve.

To determine the rapidity with which the steroid effect became manifest, the cellularity of exudates was estimated in six normal subjects 24 hr before the administration of a single intravenous injection of 200 mg of cortisol. Inflammation was then produced 2 hr before the injection, at the time of the injection, and 2, 4, 6, and 11 hr after the injection. The cellularity of 3-hr-old exudates was estimated in each case (Fig. 2).

The cellularity of exudates arising from inflammations produced 2 hr before, or at the time of administration of cortisol (− 2 and 0 hr in Fig. 2) did not differ from the control values. The cellularity of exudates, produced by inflammations initiated 2, 4, 6, and 11 hr after the injection of cortisol, was greatly reduced.

To determine whether the effect of steroids on the cellularity of exudates persists with prolonged steroid administration, two subjects were given a single intravenous injection of cortisol followed 1 day later by the daily oral administration of 40 mg of prednisone for 11 days. The cellularity of 3-hr-old exudates was determined the day before steroid administration, on the 1st, 2nd, and 12th day of steroid administration, and 9 and 28 days after steroids were discontinued (Fig. 3).

The cellularity of the exudates remained greatly reduced throughout the period of steroid administration. In one of the subjects (V-82), the cellular response was still impaired 9 days after the last administration of steroids. 28 days after the last prednisone administration, the cellular response to an inflammatory stimulus had returned to normal in both subjects.

Influence of cortisol-induced granulocytosis on the specific activity of the granulocytes in the TBGP. Nine subjects were infused with autologous DF^{32}P-labeled leukocytes. The TBGP, CGP, and MGP were determined, and the blood granulocyte radioactivity curve was followed for 48 hr. During the 5–7 hr before cortisol was administered, the average $t_{\frac{1}{2}}$ was 6.0 hr, with a range of 4–9.5 hr. 5 or 7 hr after the infusion of labeled granulocytes, 200 mg of cortisol phosphate was injected intravenously over a 5 min period of time.

In all but one of the subjects, the determined

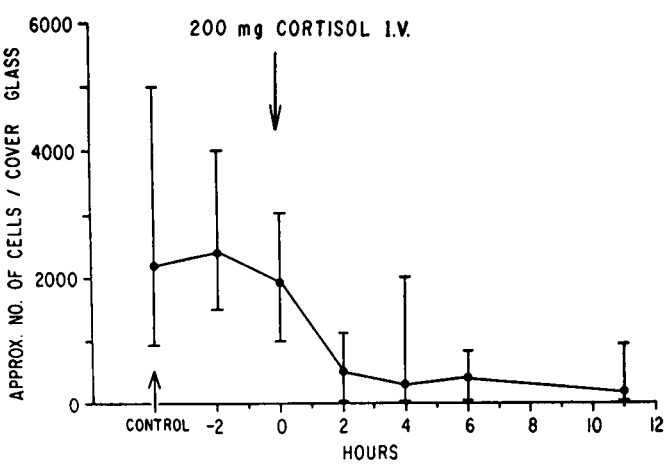

FIGURE 2 The cellularity of 3-hr old inflammatory exudates produced at various time intervals before and after the intravenous administration of cortisol. The values represent the median cellularity at each time interval of lesions in six subjects. The vertical bars delineate the range. A total of 88 inflammatory lesions are included in this figure.

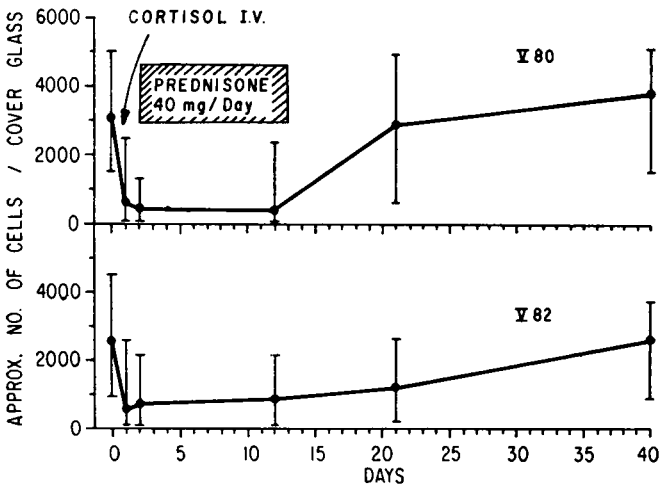

FIGURE 3 The cellularity of 3-hr old inflammatory exudates produced before, during, and after steroid administration. Inflammation was produced in each of two normal subjects the day before steroids were administered. Each subject was then given 200 mg of cortisol intravenously, followed by the daily administration of 40 mg of prednisone by mouth. The oral prednisone was discontinued on the 12th day. Each point on the curve represents median cellularity of eight, 3-hr-old inflammatory lesions. The vertical bars indicate the range.

specific activity of the granulocytes in the circulation at the time of maximum granulocytosis was significantly less than the value predicted by extrapolation of the slope of the line prior to steroid administration (Table II). A representative study is shown in Fig. 4. During the period of rapid increase in granulocyte count, granulocyte specific activity fell sharply. During the period of declining granulocyte values, the slope of the granulocyte specific activity curve was decreased so that little change in specific activity occurred over 4 hr.

In view of the known diurnal variation in plasma cortisol levels, it is interesting that in two subjects the mean GTR between 8:00 p.m. and 1:00 a.m. (before cortisol was given) was 242×10^7 cells/kg per 24 hr. In two subjects studied 12 hr earlier, the GTR was 233×10^7 cells/kg per 24 hr. After the administration of cortisol, the system was constantly changing, precluding calculation of the GTR.

TABLE II

Comparison of the Determined Specific Activity at the Time of Maximal Granulocytosis with the Specific Activity Extrapolated from the Initial Slope of the Disappearance Curve

Subject	Poststeroid G* / Control G	Determined specific activity	Extrapolated specific activity
IV-118	2.2	30	79
IV-120	2.3	133	190
IV-122	2.7	380	580
IV-158	2.3	122	86
IV-160	1.7	717	760
VIII-149	2.5	637	760
VIII-153	2.6	880	1450
VIII-157	2.2	1360	1525
VIII-161	2.6	851	1225
Mean		568	739

* G refers to granulocytes/mm³.

Simulation studies. Since the granulocyte system was continually changing and no longer in a steady state after the rapid infusion of cortisol (Fig. 4), a mathematical model was employed to aid in interpreting these data (Fig. 5). Specifically, we sought to quantify the changes in blood granulocyte inflow and egress rates produced by cortisol injection.

Equations describing the model are as follows:

$$SA_{(t)} = [A_{(t-1)} - (K_{out(t)} \times SA_{(t-1)}) \times DELT]/TBGP_{(t)} \quad (1)$$

$$TBGP_{(t)} = TBGP_{(t-1)} + [K_{in(t)} - K_{out(t)}] \times DELT \quad (2)$$

where A is the amount of radioactivity in the TBGP, TBGP is the size of the total blood granulocyte pool, and K_{in} and K_{out} are the number of cells entering and leaving the TBGP per hour. The two equations define values for SA and TBGP over a short time interval, DELT, which extends from time t to time $t + 1$. $SA_{(t)}$ is the specific activity value at any point in time t.

It is assumed in these simulation studies that changes in the granulocyte concentration reflect changes in TBGP size. This seems a reasonable approximation in view of the evidence presented in Table I.

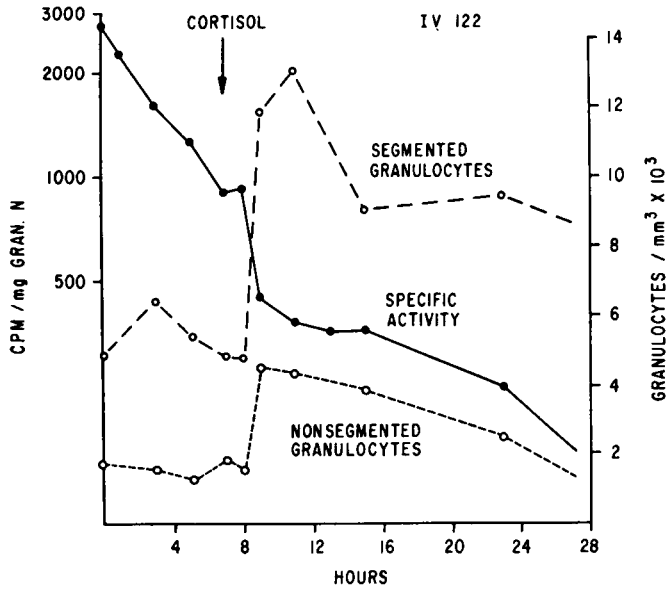

FIGURE 4 The effect of cortisol-induced granulocytosis on the granulocyte radioactivity disappearance curve. 200 mg of cortisol phosphate was injected intravenously 7 hr after the labeled granulocytes were returned to the donor. Granulocyte specific activity, depicted by the solid line, is plotted on the logarithmic scale on the left. The absolute granulocyte count is plotted on the arithmetic scale on the right.

To obtain rapid solution of the model, the equations were written into a computer program which made it possible to plot on an oscilloscope the granulocyte specific activity and granulocyte concentration, as well as K_{in} and K_{out} as a function of time (Fig. 6).

Fig. 6 A shows the steady state as predicted by the model when K_{out}, K_{in}, the granulocyte concentration, and the TBGP are constant. Fig. 6 B shows the effect of increasing K_{in} by 300%. The granulocyte count increased and the SA curve dropped below the extrapolation of the first part of the SA curve. In Fig. 6 C the effect of reducing K_{out} to zero at the 8th hr while K_{in} remains unchanged is shown. The rise in the granulocyte count is less rapid in Fig. 6 C than in Fig. 6 B, and any change in the SA curve is not clearly detectable for at least 6–8 hr after the change in K_{out}. Model studies in which the parameters

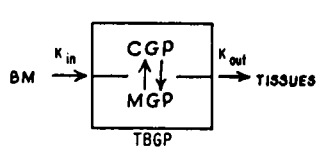

FIGURE 5 Model of blood granulocyte turnover in normal man. K_{in} is the rate of inflow of unlabeled cells from the marrow to the total blood granulocyte pool (TBGP). K_{out} is the rate of random egress of cells (labeled and unlabeled) from the TBGP. The TBGP is made up of the circulating granulocyte pool (CGP) and marginal granulocyte pool (MGP) which are in rapid equilibrium.

were varied in several combinations demonstrated clearly that the rate of rise in granulocyte count that occurs when K_{out} is decreased is determined by the GTR during the preceding steady state. In other words, changes produced by decreasing K_{out} are dependent on TBGP size and the slope of the preceding SA curve. The latter reflects K_{in} and K_{out}. On the other hand, K_{in} is a more independent variable, and increases in K_{in} can be 200, 300, or 400%, depending on the rate of cell release from marrow granulocyte reserves.

The computer was also programmed to plot and display simultaneously both model and data curves (Fig. 7). In the model it was impossible to obtain an increase in TBGP approaching that seen in the data from the experimental subjects by only decreasing K_{out} to zero. It was possible to approximate the rise in the granulocyte count seen in the experimental subjects by increasing K_{in} while keeping K_{out} constant. However, the drop in the model SA curve which resulted was greater than the drop that occurred in the experimental subjects (Table III). Thus, it appeared that a combination of changes in K_{in} (increase) and K_{out} (decrease) was necessary to match model and experimental curves in each of the nine subjects studied. The values for K_{out}, K_{in}, and TBGP which gave the best match between model and data curves are shown in Table IV. It is evident that there was considerable variation from study to

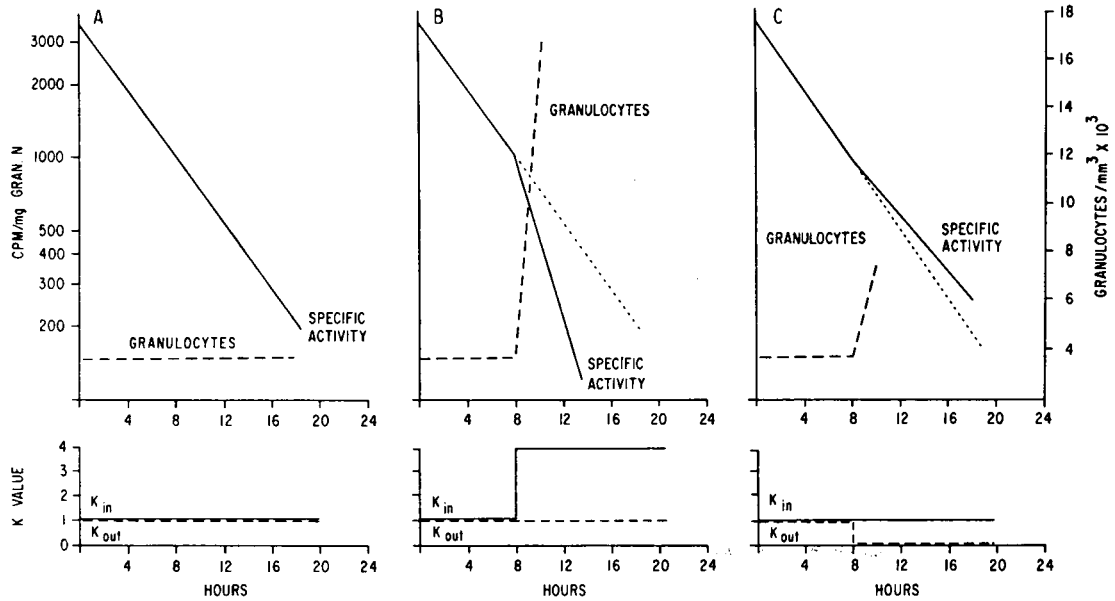

FIGURE 6 Model simulation of steady-state kinetics (A), an increase in K_{in} (B), and a decrease in K_{out} (C). Specific activity curves (solid lines) are plotted using the logarithmic scale on the left. Granulocyte counts are plotted using the arithmetic scale on the right. K_{in} and K_{out} are plotted using an arbitrary arithmetic scale with steady-state values set at unity.

TABLE III

*The Inability of Increasing Model K_{in} Alone to Match Both the Granulocyte and Specific Activity Curves from Experimental Subjects**

Subject	Determined specific activity at G Max‡	Model specific activity at G Max‡	% Difference§
IV-118	30	28	6.6
IV-120	133	120	9.8
IV-122	380	307	19.2
IV-158	122	113	8.0
IV-160	717	700	2.4
VIII-149	637	478	25.0
VIII-153	880	709	19.4
VIII-157	1360	978	28.1
VIII-161	851	646	24.1

* To obtain the data in this table, Model K_{in} was increased sufficiently to match the granulocyte curve. The SA curve then fell more sharply than the experimental curve.

‡ G Max is the time of maximum increase in granulocyte count.

§ Per cent difference is

$$\frac{\text{Determined specific activity} - \text{model specific activity}}{\text{Determined specific activity}} \times 100.$$

study in K_{in} values used to match the experimental data. However, there was a mean decrease in K_{out} of 74% (range 1–99%) and an increase in K_{in} of four- to fivefold (range 300–675%).

By similar analysis, it is apparent that the return of the granulocyte count toward a normal rate soon after the peak granulocytosis is due to

TABLE IV

Model Parameter Values for TBGP, K_{in} (Rate of Inflow) and K_{out} (Rate of Outflow) which Gave the Best Match of Model and Data Curves

Subject	TBGP Increased to*	K_{in} Increased to*	K_{out} Decreased to*
IV-118	265	695	4
IV-120	240	326	1
IV-122	330	660	17
IV-158	236	300	68
IV-160	181	589	99
VIII-149	241	603	4
VIII-153	263	750	17
VIII-157	235	600	17
VIII-161	259	494	9
Mean	250	558	26

* All values are expressed as per cent of initial value.

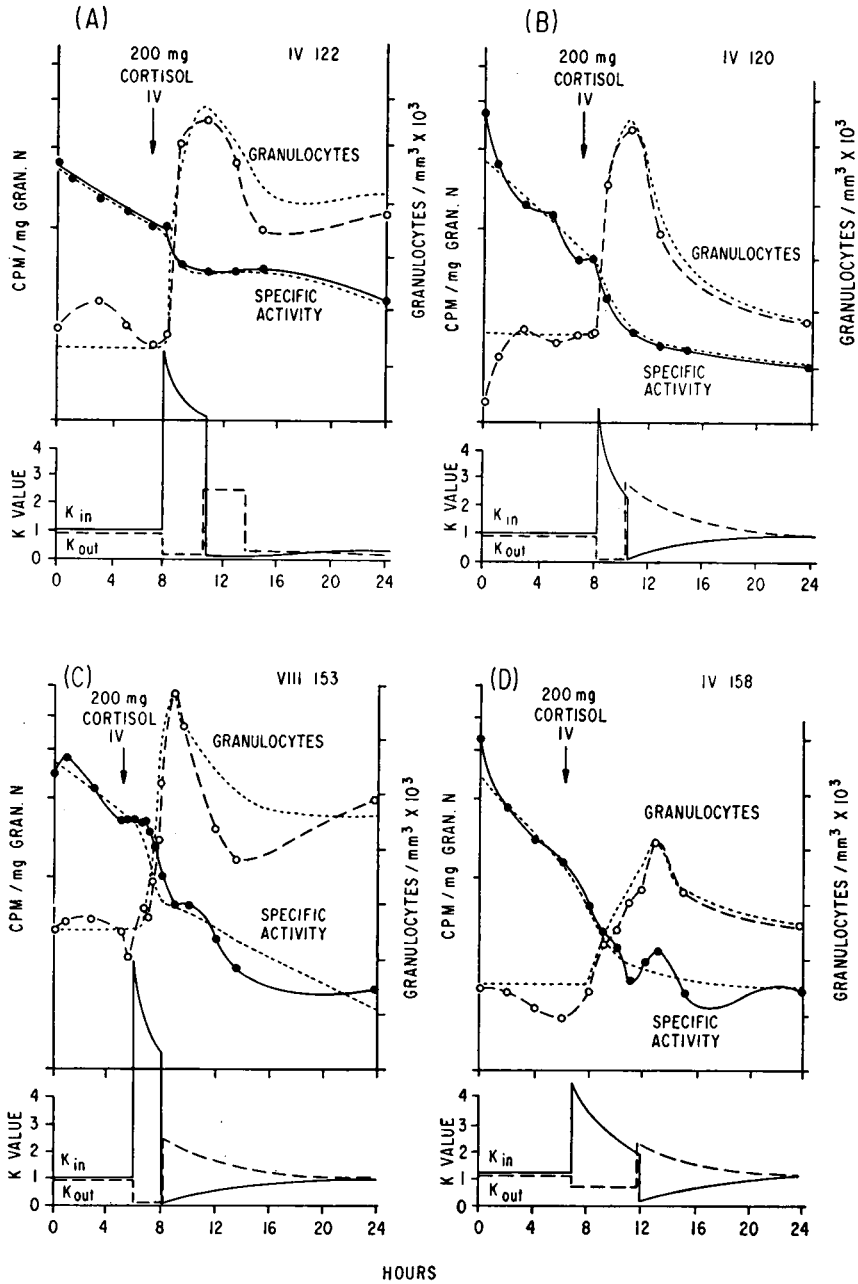

FIGURE 7 A comparison of the time course of model simulation and experimental data curves in four subjects given cortisol intravenously. Experimental specific activity values (solid circles) and granulocyte counts (open circles) are compared with model curves (dashed lines). Changes in influx (K_{in}) and efflux (K_{out}) are shown beneath each figure.

both an increase in K_{out} and a decrease in K_{in} (see Fig. 7).

In all instances the model curves did not match the experimental curves perfectly throughout, but representative curves from four such subjects can be seen in Fig. 7, and a reasonable fit is apparent.

Influence of cortisol on the differential count. In an attempt to obtain further evidence of mobili-

TABLE V

Absolute Granulocyte (G) Concentrations before and after Cortisol Injection

Subject	Mean Seg G/mm³		Mean Non-Seg G/mm³		Total G/mm³		Non-Seg/Seg*	
	Before	After	Before	After	Before	After	Before	After
IV-118	3709	7860	1381	2746	5090	10606	0.37	0.35
IV-120	3600	6318	1809	2888	5409	9206	0.50	0.46
IV-122	3756	6396	1563	3245	5319	9641	0.42	0.51
IV-158	2312	4022	1546	3477	3858	7499	0.67	0.86
IV-160	4307	4367	3237	4347	7544	8714	0.75	1.00
VIII-149	3051	5222	456	983	3507	6204	0.15	0.19
VIII-153	5012	7565	583	633	5595	8198	0.11	0.08
VIII-157	3866	4769	2985	7434	6851	12203	0.77	1.56
VIII-161	4563	8859	1113	2945	5676	11804	0.24	0.33

*Segmented neutrophil is defined as a neutrophilic granulocyte containing at least two nuclear lobes connected by a filament of nuclear material.

zation of marrow cells, the leukocyte differential counts observed before and after cortisol injection were evaluated. In eight of the nine cases, there was an increase in the absolute nonsegmented granulocyte concentration (WBC × % Non-Seg) after the cortisol injection. In six of the eight subjects, the nonsegmented to segmented neutrophil ratio increased by at least 10% (Table V). In each of the remaining three subjects this ratio was essentially unchanged after cortisol was injected.

DISCUSSION

It is clearly established that the administration of certain adrenal steroids results in blood granulocytosis (2, 8). It has also been demonstrated that this granulocytosis reflects an increase in TBGP size (4, 5). However, the mechanism by which the absolute granulocytosis is produced has been uncertain. Several investigators have thought that the granulocytosis is due to increased inflow of cells from the marrow (9, 10), while others have concluded that the granulocytosis results from a decrease outflow of cells from the blood (4, 11).

The present study provides evidence for increased inflow of marrow granulocytes into the blood, namely: (*a*) during the period when blood granulocyte concentration was increasing rapidly after cortisol injection, a drop in blood granulocyte radiocativity was detected in all subjects (Fig. 7 and Table II); (*b*) there was an increase in the absolute numbers of both segmented and nonsegmented neutrophils in the blood of eight of the nine subjects during this time (Table V); and (*c*) in six of the nine subjects a clear-cut shift to the left in differential count was evident.

Evidence also has been presented which suggests that there is decreased outflow of granulocytes from the blood; cortisol administration was associated with a 75% decrease in the number of granulocytes in induced exudates (Fig. 1–3). Although one cannot equate decreased cell migration into inflammatory exudates with events induced by cortisol in nonpathologic states, this finding raised the possibility that cortisol may interfere with blood granulocyte egress in more physiologic situations.

From these data it seemed reasonable to suggest that cortisol injection produces an absolute increase in TBGP size by both accelerating marrow granulocyte inflow into the blood and by decreasing granulocyte egress out of the blood. To quantify changes in inflow and egress and to thus delineate the relative importance of these two mechanisms, we compared the curves generated by a mathematical model with those obtained by actual experiment. These studies have demonstrated that simultaneous changes in blood granulocyte inflow and egress rates K_{in} and K_{out}) must occur in order to match the model curves to the data (Fig. 7 and Tables III and IV). For example, during the period when the granulocyte concentration was increasing rapidly, a drop in granulocyte radioactivity was noted. This drop reflects an inflow of unlabeled marrow granulocytes into the blood. However, manipulation of

the model parameters demonstrated that if the granulocytosis were only due to an increased inflow of unlabeled cells, a more rapid decline in granulocyte radioactivity would occur than was actually the case (Table III). In this manner, the mathematical simulation studies provide additional evidence for the dual mechanism by which cortisol produces granulocytosis. In addition, it became apparent that small changes in model parameters effected obvious changes in the shape of the curves generated. As a result, by matching model curves to the data curves, it has been possible to approximate the quantitative changes which occurred in blood granulocyte inflow and egress rates (K_{in} and K_{out}) (Table IV). It is concluded that cortisol injection produces granulocytosis by decreasing blood granulocyte egress (mean of 75%; range 1–99%), and simultaneously increasing granulocyte inflow from the marrow (mean of 450%; range 300–750%) (Table IV).

It is probably fortuitous that both studies (cell migration into exudates and model simulation) resulted in a mean reduction in cell egress of about 75% if for no other reason than because the studies were carried out on different subjects at different times. Nevertheless, the modification of inflammation cellularity by cortisol provides evidence, in addition to the kinetic studies, that cortisol changes granulocyte egress rates.

The usefulness of these model simulation studies is evident in quantitating granulocyte inflow and egress rates during the non–steady state produced by cortisol injection. A similar approach should have utility in a wide variety of biologic systems. However, the validity of any conclusions drawn from such studies depends on the validity of the model used. In the present instance the model of blood granulocyte turnover is a simple one (Fig. 5). It consists of two simultaneous equations describing the time course of blood granulocyte specific activity and granulocyte concentration. Since the number of independent variables in the model is limited to two (K_{in} and K_{out}), it is apparent that there is only one model solution which can match the data curves. The assumptions made in this model are: (*a*) that the blood granulocyte concentration reflects the size of the TBGP; (*b*) that cells in marginal (MGP) and circulating sites (CGP) are in rapid equilibrium; (*c*) that granulocyte turnover through the blood in the steady state is best described by a single exponential function; and (*d*) that there is no significant return of granulocytes to the blood during the course of these studies.

In regard to the first assumption, previous evidence has demonstrated a good correlation between blood granulocyte concentration and TBGP size in all clinical situations, except perhaps in patients with myelofibrosis or polycythemia who have high granulocyte counts (12, 13). Admittedly, the latter data were collected in steady-state situations, while the present studies were done during a non–steady state. For this reason the studies presented in Table I were carried out. From these studies it seems clear that cortisol injection resulted in a somewhat greater increase in the CGP than in the MGP. The effect of this deviation from the assumption of an equal increase in both CGP and MGP is an overestimate of K_{in}. As a result, the values for the K_{in} presented in Table IV represent maximum values.

The assumption that cells in the CGP and MGP are in rapid equilibrium has been demonstrated in normal subjects (1). It thus seems likely that in the present studies labeled cells were uniformly distributed between marginal and circulating sites before the injection of cortisol. It then follows that any decrease in blood granulocyte specific activity after cortisol injection must result from the influx of unlabeled cells from the bone marrow rather than from marginal sites. Further documentation of this assumption is provided by the already mentioned observation that in six of the nine subjects a clear-cut shift to the left in differential cell counts occurred after cortisol injection (Table V). The lack of significant shift to the left in the remaining three subjects is presumably due to the fact that they mobilized segmented and nonsegmented forms from their marrow granulocyte reserve in the same proportion as the blood concentration of these cells.

Evidence that blood granulocyte turnover is best described by a single exponential function has been presented previously (1, 14). The possibility of significant return of granulocytes to the blood from the tissues has also been considered previously (15). In the normal steady-state situation, the maximum possible rate of return of granulocytes from the tissues to the blood was about 0.3 of K_{out}. Even this degree of granulocyte recycling required the existence of a large tissue

pool of granulocytes, a hypothesis which has not been substantiated. Regarding this hypothesis, a recent study demonstrates that the major, if not the only source of granulocytes entering inflammations is the blood rather than a hypothetical tissue pool (16).

For the above reasons the model used seems a reasonable one. Nevertheless, several of the assumptions used in it are only approximately quantified, and thus the quantitative values derived for K_{in} and K_{out} (Table IV) must themselves be considered approximate values.

Since all portions of the model curves did not always match, the data curves would appear to be somewhat disturbing. This error is due to our current inability to vary K_{in} and K_{out} with complete freedom. The approximations were quite good, however, when the K_{in} and K_{out} changes were restricted to square wave or exponential curve configurations. We reasoned that intravenous injection of a drug might produce sudden step changes in K_{in} and K_{out} which then returned exponentially to the original values (Fig. 7). Further refinement of the model to permit more freedom in K_{in} and K_{out} variation should improve this matching process.

The means by which cortisol influences blood granulocyte inflow rates are unknown. Several agents which induce a granulocytosis, apparently by releasing marrow granulocytes, have been described (17–20). It is possible that cortisol acts by affecting one of these systems, that cortisol release is stimulated by these agents, or that there is no relationship betwen cortisol effects and these agents. In any case, leukopoiesis is quickly affected by cortisol. Such perturbations of steady-state situations appear to be a fruitful area for further study of control mechanisms.

Similarly, the means by which cortisol interferes with granulocyte egress from the blood into inflammatory exudates are unkown. The present demonstration of a decreased cellular content of inflammatory exudates confirms studies in man (7, 10, 21) and in other species (12–14, 22, 23), but sheds no light on whether this effect is on the granulocytes directly (24–26), on the vascular endothelium (24, 27), or results from an effect on the inflammatory stimulus (28–30).

No matter what the mechanism may be by which the number of phagocytic cells in inflammatory exudates is decreased, the observation that they are reduced in number may have implications for the use of steroids as therapy in certain conditions. An increase in the severity and possibly the frequency of bacterial infections attend steroid therapy (31). Although a number of mechanisms which explain this increased hazard of infection can be postulated (32), the magnitude of the demonstrated decrease in phagocytic cells in an inflammatory exudate may provide at least a partial explanation.

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