THE EFFECT OF GLUCOCORTICOSTEROIDS ON THE KINETICS OF MONONUCLEAR PHAGOCYTES*

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Recently, several investigators have shown that both the tissue macrophages present in many organs under normal conditions and the mononuclear phagocytes occurring at sites of inflammation, originate from monocytes in the peripheral blood (1–10). The monocytes in the circulation derive from precursor cells in the bone marrow, called promonocytes (7, 10, 11).

Glucocorticosteroids are known to have an anti-inflammatory action, one of the expressions of which is a decrease in the number of mononuclear phagocytes at the sites of inflammation (12–15). This effect may be brought about by either a decrease in the number of circulating monocytes (16) or a decreased permeability of the vascular wall (17).

In contrast to the well documented effect of glucocorticosteroids on the number of circulating lymphocytes, their effect on the number of monocytes in the peripheral blood has rarely been mentioned (18–22). There are no reports on systematic studies concerning the effect of glucocorticosteroids on the number of circulating monocytes in relation to the decreased number of mononuclear phagocytes at sites of inflammation during treatment with these compounds.

The present study was undertaken to investigate the effect of glucocorticosteroids on the kinetics of mononuclear phagocytes during an inflammatory reaction.

Materials and Methods

Animals.—In all of the present studies, specific pathogen-free male Swiss mice weighing 25–30 g (Central Institute for the Breeding of Laboratory Animals, TNO, Bilthoven, Holland) were used.

Blood Leukocyte Counts.—Leukocyte counting was done in a Bürker hemocytometer; the blood was diluted in a leukocyte pipette 1:20 with Türk's solution, which contains 6% acetic acid. The total leukocyte counts of each animal were done in duplicate for each of two samples from tail vein blood, thus giving for each animal four leukocyte values, from which the mean was calculated.

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Differential counts were done on 200 leukocytes from at least two different blood smears fixed in absolute methanol and stained with Giemsa stain. From the total leukocyte number and values of the differential counts, the absolute number of cells for each category of leukocytes was calculated. For 20 normal mice, these values were: monocytes 348 (range 185–570) per mm^3, lymphocytes 8,270 (range 4,600–15,170) per mm^3, and polymorphonuclear leukocytes 1,400 (range 360–4,480) per mm^3.

Peritoneal Cell Counts.—Mice were killed rapidly with chloroform. The skin over the abdominal cavity was stripped off, and the peritoneal cells were washed out under strict standard conditions. 2 ml of phosphate-buffered saline, pH 7.2, (Difco Laboratories, Inc., Detroit, Mich.) containing 50 units/ml heparin, was injected into the peritoneal cavity; 1 min later, after gentle kneading, the leukocyte-rich fluid was removed with a capillary pipette and brought into a plastic tube.

The total cell counts were done in undiluted cell suspensions without staining; in the inflammation experiments the cells were sometimes diluted 1:10 in a 0.2% trypan blue solution in saline. These counts were done in duplicate with a Bürker hemocytometer.

Differential counts were done in preparations made with a sedimentation apparatus, the cells being sedimented on a microscope slide,[1] fixed in absolute methanol, and stained with Giemsa stain. For each animal, 500 consecutive cells were differentiated. From the total peritoneal cell number and the values of the differential counts, the total number of peritoneal macrophages and lymphocytes per milliliter was calculated, and in the inflammation experiments, this was also done for the polymorphonuclear cells.

For 20 normal mice, the peritoneal cavity was found to contain 160 (range 110–190) $\times 10^4$ macrophages per ml and 42 (range 20–95) $\times 10^4$ lymphocytes per ml.

Glucocorticosteroids.—The glucocorticosteroid preparations used in this study were dexamethasone sodium phosphate (Oradexon, Organon, Inc., Oss, Holland) and hydrocortisone acetate (Merck, Sharp & Dohme, Haarlem, Holland), kindly provided by these firms. Both preparations were used either in the commercially available form or diluted in phosphate-buffered saline, pH 7.2. The dilutions were made such that the volume to be injected was 0.5 ml. The glucocorticosteroids were injected subcutaneously in the nuchal region. The vehicle preparation (which contains 0.5% carboxymethyl cellulose, 0.9% benzyl alcohol, and 0.4% polysorbate 80 in saline) used as control in the hydrocortisone experiment, was the commercial preparation without hydrocortisone.

Inflammation.—An inflammatory reaction in the peritoneal cavity was provoked by one injection of 1 ml sterile newborn calf serum (NBCS) (Grand Island Biological Co., Grand Island, N. Y.) into the peritoneal cavity (7) with a small needle (Yale, 26G ⅜, Becton-Dickinson & Co., Ltd., Drogheda, Ireland). Mice showing a hemorrhagic exudate were discarded.

Labeling Studies.—Details of the technique have been described previously (7). The cells were labeled with methyl-^{3}H-thymidine (specific activity, 6.7 mCi/mmole; New England Nuclear Corp., Boston, Mass.); four intramuscular injections of ^{3}H-thymidine (1 μCi/g body weight) were given at 4-hr intervals. The in vitro labeling of peritoneal macrophages was done by culturing the cells for 24 hr on cover slips in a medium containing 0.1 μCi/ml ^{3}H-thymidine (7).

The labeling index of peritoneal macrophages on cover slips and of monocytes in blood smears was determined from stained radioautographs. At least 400 cells were counted.

Identification of the Cells.—The mononuclear phagocytes of the blood, i.e., the monocytes, are cells with a diameter of 12–16 μ, a pale blue cytoplasm, and an indented, horseshoe-

[1] van Furth, R., J. G. Hirsch, and M. E. Federko. 1970. Morphology and peroxidase cytochemistry of mouse promonocytes, monocytes, and macrophages. To be published.

shaped or reniform nucleus. The cell suspension obtained from the peritoneal cavity of normal mice consists of a population of predominantly two types of cells, namely lymphocytes and macrophages. According to the morphological differentiation of these cells as judged from the sedimentation preparations on slides, the lymphocytes are cells with a diameter of 8–10 μ, a small rim of cytoplasm, and a nucleus with a dense chromatin structure. These cells are not phagocytic and do not attach to the glass surface.[1] Peritoneal macrophages are cells with a diameter of 11–18 μ and a rim of a light basophilic cytoplasm; some of these cells have a round nucleus[1] with a characteristically loose chromatin structure. Since previous experiments have demonstrated that the cells with these morphologic characteristics adhere firmly to the glass surface, grow out into classical macrophages after incubation in vitro, and are capable of phagocytosis, they can be considered as mononuclear phagocytes.[1]

General Remarks.—Each animal was only used once for leukocyte and peritoneal cell sampling. At each time point, the results represent the mean value of at least four animals.

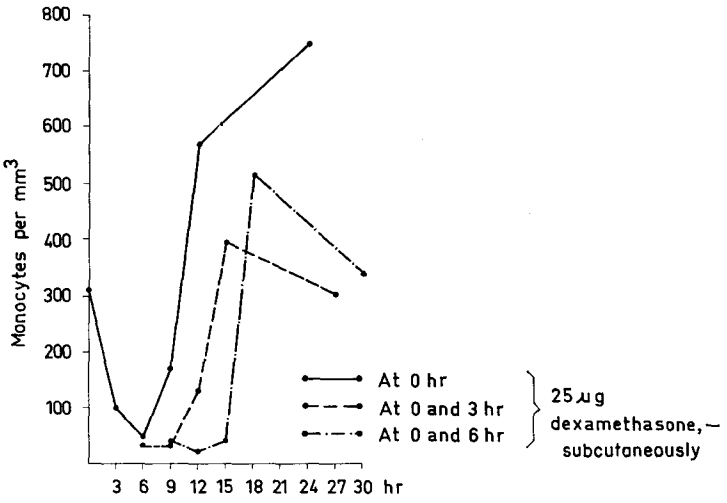

FIG. 1. The effect of one and two subcutaneous injections of 25 μg dexamethasone on the number of monocytes in the peripheral blood.

RESULTS

The Effect of Dexamethasone on the Number of Peripheral Blood Monocytes, Lymphocytes, and Polymorphonuclear Leukocytes in Normal Mice.—

A single injection of 25 μg dexamethasone, a water-soluble synthetic glucocorticosteroid, is followed by a rapid decrease in the number of blood monocytes (Fig. 1). This effect was observed to be maximal at 6 hr after the injection. 12 hr after injection the number of monocytes is normal, and after 24 hr it is even higher than the preinjection level.

The period during which the number of monocytes in the blood is reduced can be prolonged by repeated injection of dexamethasone. When dexamethasone

is given at time points 0 and 3 hr, the decrease of the monocytes lasts 9 hr; after injections at 0 and 6 hr, the effect persists for as long as 15 hr. In all experiments, 12 hr after the last injection, the number of monocytes had returned to a normal level or become even higher (Fig. 1).

A smaller dose of dexamethasone (12.5 μg or 2.5 μg per animal) was not observed to have any effect on the number of monocytes, and this number did not fall below normal after an injection of 0.5 ml buffered saline.

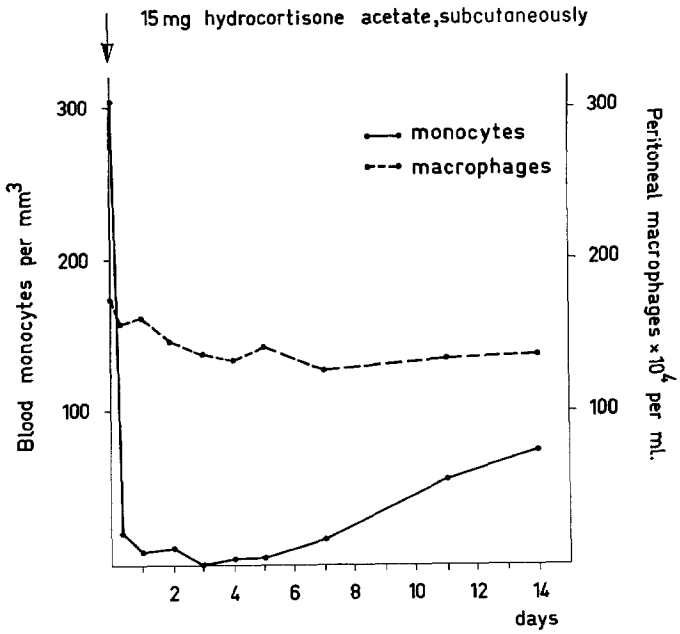

FIG. 2. The effect of one subcutaneous injection of 15 mg hydrocortisone on the number of peripheral blood monocytes and peritoneal macrophages.

The number of lymphocytes, too, decreases after an injection of dexamethasone. The magnitude and duration of the effect are similar to those found for the monocytes. Dexamethasone has little effect on the number of polymorphonuclear leukocytes; 12 hr after the last injection the value is usually twice normal.

The Effect of Hydrocortisone on the Number of Peripheral Blood Monocytes, Lymphocytes, and Polymorphonuclear Leukocytes in Normal Mice.—

A much more prolonged reduction of the number of monocytes can be obtained with a single injection of 15 mg hydrocortisone (Fig. 2), given as an emulsion of hydrocortisone acetate in a vehicle. This dose led to the formation

of a subcutaneous depot, remnants of which were found throughout the period of the experiment.

The administration of 15 mg hydrocortisone results in an almost complete disappearance of the monocytes from the peripheral blood. The effect is maximal 3 days after the injection; on the 7th day there is a gradual increase of the monocytes. On the 14th day, when the experiment was discontinued because too many animals were dying in a cachectic state, the monocyte count had not yet returned to a normal level (Fig. 2).

A reduction of the dose of hydrocortisone also gave an almost complete disappearance of the monocytes, but the duration of this effect was much shorter; 48 hr after an injection of 250 μg hydrocortisone, the number of monocytes had returned to normal and a dose of 100 μg hydrocortisone resulted in a normal level after 24 hr. A dose of 25 μg hydrocortisone no longer affected the number of blood monocytes. After an injection of the vehicle alone, the number of monocytes did not drop below the normal level.

Hydrocortisone also affects the number of lymphocytes in the peripheral blood. The magnitude and duration of this decrease are almost the same as was found for the monocytes (Fig. 3). The number of polymorphonuclear leukocytes remained constant during the first 3 days, but thereafter gradually increased to 5–10 times the normal number 12–14 days after the injection.

The Effect of Hydrocortisone on the Number of Macrophages and Lymphocytes in the Peritoneal Cavity of Normal Mice.—

After an injection of 15 mg hydrocortisone, the number of lymphocytes in the peritoneal cavity diminished rapidly during the first 48 hr, and remained low; simultaneously, the number of lymphocytes in the circulation was reduced (Fig. 3).

In contrast to the almost complete disappearance of the monocytes from the peripheral blood after this dose of hydrocortisone, only a slight decrease (about 30%) of the total number of peritoneal macrophages was found during the first week (Fig. 2).

Since these macrophages derive from monocytes in the circulation (7), it was of interest to know whether there would be a continued influx of new mononuclear phagocytes into the peritoneal cavity. This was studied by labeling the mononuclear phagocytes with ^{3}H-thymidine.

The Effect of Hydrocortisone on the Influx of Labeled Mononuclear Phagocytes into the Peritoneal Cavity of Normal Mice.—

When normal mice are injected with four doses of 25 μCi ^{3}H-thymidine 6.3% of the peritoneal macrophages were labeled 12 hr after the last injection; the highest percentage of labeled cells was seen after 60 hr (Table I).

When 15 mg hydrocortisone was injected 3 hr after the last ^{3}H-thymidine

injection, 5.0% of the peritoneal macrophages showed labeling 12 hr after the last thymidine injection, but no further increase in the percentage of labeled macrophages was found (Table I). The peripheral blood of these mice contained a small number of labeled monocytes, but the total number of mono-

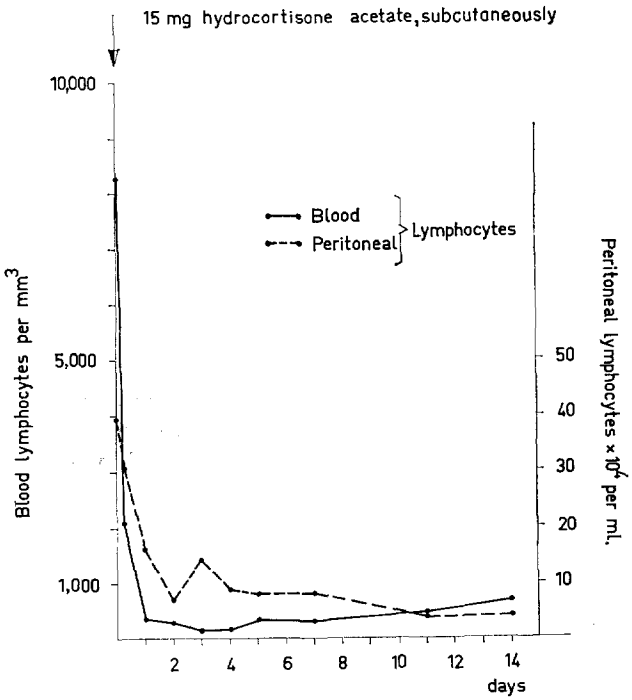

FIG. 3. The effect of one subcutaneous injection of 15 mg hydrocortisone on the number of lymphocytes in the peripheral blood and peritoneal cavity.

TABLE I

The Effect of Hydrocortisone on the Percentage of Labeled Peritoneal Macrophages

Time after labeling	^{3}H-thymidine*	^{3}H-thymidine-hydrocortisone‡	Hydrocortisone-thymidine§
hr	%	%	%
12	6.3	5.0	0.1
36	7.4	3.4	0.2
60	15.6	2.5	0.1
84	12.3	3.0	0.2
108	11.9	2.4	0.1

* 4 × 25 μCi ^{3}H-thymidine.

‡ 4 × 25 μCi ^{3}H-thymidine; after 3 hr, 15 mg hydrocortisone.

§ 15 mg hydrocortisone; after 24 hr, 4 × 25 μCi ^{3}H-thymidine.

cytes was too low to permit reliable determination of the percentage of labeled cells.

In the reverse experiment, when 15 mg hydrocortisone was injected first and ^{3}H-thymidine 24 hr later, only 0.1–0.2% of the peritoneal macrophages were labeled (Table I).

The results of these labeling experiments demonstrate that during the period in which hydrocortisone is effective and almost no monocytes are present in the peripheral blood, there is almost no influx of labeled mononuclear phagocytes into the peritoneal cavity.

In Vitro Labeling of Peritoneal Macrophages.—

Glucocorticosteroids do not directly affect the incorporation of ^{3}H-thymidine in vitro by peritoneal macrophages. The labeling index of these cells after being cultured for 24 hr in the presence of 1 or 10 μg/ml dexamethasone sodium phosphate was 4.3 and 5.4% respectively, whereas the labeling index of these cells from normal mice was 4.0%.

The in vitro labeling of peritoneal macrophages collected 48 hr after an injection of 15 mg hydrocortisone, was only 0.2%. When mice were treated with 15 mg hydrocortisone and received an intraperitoneal injection of 1 ml newborn calf serum 48 hr later, the labeling index of the peritoneal macrophages obtained 24 hr after the serum injection was 1.2%.

The Effect of Hydrocortisone on the Course of an Inflammatory Reaction in the Peritoneal Cavity.—

As a model of an inflammatory reaction, we used the increase in the number of peritoneal macrophages after an intraperitoneal injection of 1 ml sterile newborn calf serum (7).

In normal mice this stimulus evoked an initial decrease in the total number of peritoneal macrophages during the first 6 hr, followed by an increase that reached a maximum after 72 hr (Fig. 4). During the same period, the number of monocytes in the peripheral blood increased to about three times the normal value (Fig. 5).

When mice were treated with 15 mg hydrocortisone 48 hr before the inflammatory stimulus, an intraperitoneal injection of 1 ml newborn calf serum also led to an initial drop in the number of peritoneal macrophages 6 hr after the injection. During the next 24 hr the number of macrophages increased slightly, but remained low, at a level even lower than that found in mice treated with hydrocortisone only (Fig. 4). In the peripheral blood, the monocytes showed a small peak 6 hr after the inflammatory stimulus, but after 12 hr the number of monocytes lay in the same range as that seen in mice treated with hydrocortisone only (Fig. 5).

In the reverse experiment, newborn calf serum was injected first and 15 mg hydrocortisone 24 hr later. Here, the increase in the number of peritoneal

macrophages stopped after hydrocortisone was given, and the number of blood monocytes decreased to a low level (Fig. 6).

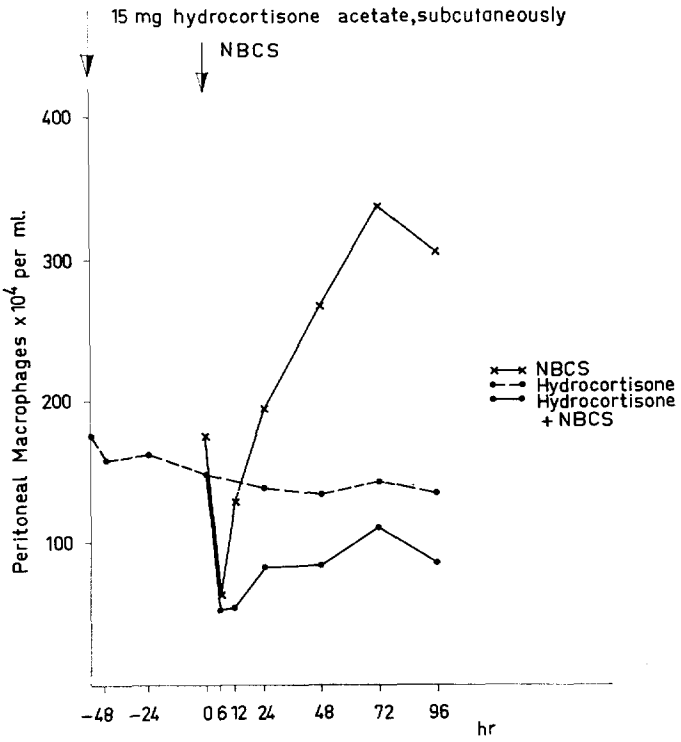

FIG. 4. The effect of an intraperitoneal injection of 1 ml newborn calf serum on the number of peritoneal macrophages of normal mice (×——×), and of mice, pretreated 48 hr earlier with 15 mg hydrocortisone (●——●). The third line (●. . . . .●) shows the effect of an injection of 15 mg hydrocortisone alone.

The Effect of Hydrocortisone on the Influx of Labeled Mononuclear Phagocytes into the Peritoneal Cavity during an Inflammatory Reaction.—

To determine the effect of 15 mg hydrocortisone on the influx of mononuclear phagocytes into the inflammatory exudate, mice were injected with four doses of 25 μCi ^{3}H-thymidine intramuscularly. 3 hr after the last thymidine injection, 15 mg hydrocortisone was given, and 36 hr later, 1 ml newborn calf serum was injected intraperitoneally.

During the first 24 hr after the inflammatory stimulus, there was an increase in the relative percentage and total number of labeled peritoneal macrophages; the highest number of labeled cells was reached at time point 60 hr, i.e. 24 hr

after the serum injection (Table II). In a control experiment done under similar conditions but in which no hydrocortisone was given, a much larger increase in the percentage and total number of labeled macrophages was obtained, a maximum occurring at time point 108 hr, i.e. 72 hr after the injec-

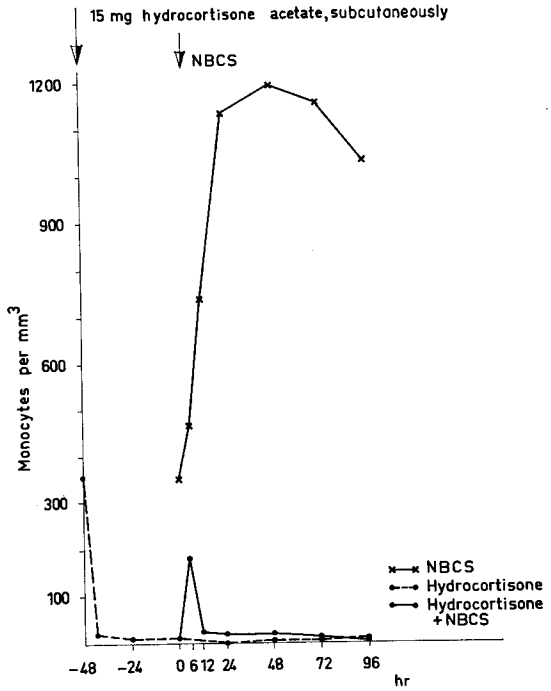

FIG. 5. The effect of an intraperitoneal injection of 1 ml newborn calf serum on the number of peripheral blood monocytes of normal mice (×——×), and of mice pretreated 48 hr earlier with 15 mg hydrocortisone (●——●). The third line (●. . . . .●) shows the effect of an injection of 15 mg hydrocortisone alone.

tion of newborn calf serum (Table II). These experiments thus demonstrate that hydrocortisone suppresses, albeit not entirely, the influx of labeled mononuclear phagocytes into the inflammatory exudate in the peritoneal cavity.

DISCUSSION

The present studies were performed to investigate two possible ways in which glucocorticosteroids could affect an inflammatory reaction, as expressed by a diminished exudation of mononuclear phagocytes. The two modes of action considered were a decreased vascular permeability for mononuclear phagocytes and a decrease in the number of monocytes in the circulation.

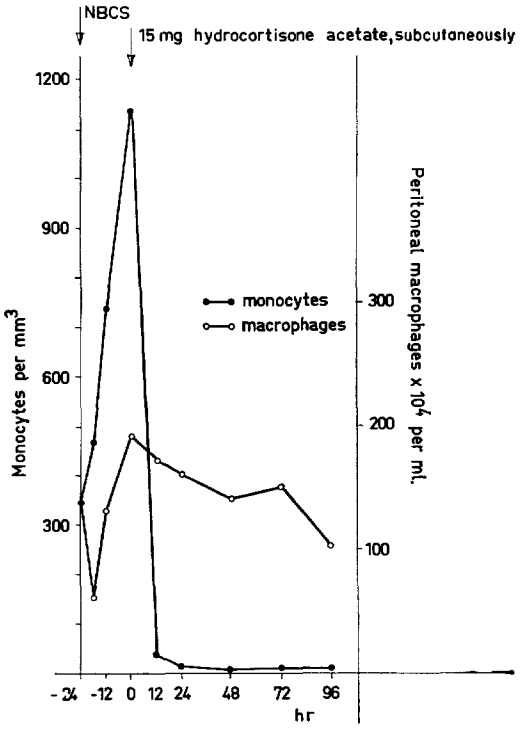

FIG. 6. The effect of one subcutaneous injection of 15 mg hydrocortisone in mice given 1 ml newborn calf serum intraperitoneally 24 hr earlier, on the number of monocytes and peritoneal macrophages.

TABLE II

The Effect of Hydrocortisone on the Labeled Peritoneal Macrophages after an Intraperitoneal Injection of Newborn Calf Serum

Time after ^{3}H-thymidine*	Labeled peritoneal macrophages			
	Serum‡	Hydrocortisone + serum§	Serum‡	Hydrocortisone + serum§
hr	%	%	× 10⁴/ml	× 10⁴/ml
36	7.4	3.4	12	5
60	46.1	26.5	89	22
84	61.3	25.5	164	21
108	69.4	11.2	234	12
132	63.2	9.9	193	8

* 4 × 1 μCi/g body weight intramuscularly.

‡ 1 ml newborn calf serum intraperitoneally 36 hr after ^{3}H-thymidine.

§ 15 mg hydrocortisone subcutaneously 3 hr after ^{3}H-thymidine and 1 ml newborn calf serum intraperitoneally 36 hr after hydrocortisone.

The results of the present studies provide clear evidence that in normal mice, subcutaneously administered glucocorticosteroids rapidly cause (within 3–6 hr) a decrease in the number of monocytes in the circulation. The duration of this effect on the monocytes is dependent on the nature of the glucocorticosteroid and the dose administered.

The number of lymphocytes in the peripheral blood is similarly affected by glucocorticosteroids. Hydrocortisone did not greatly influence the number of polymorphonuclear leukocytes initially, but after a few days the number of these cells increases considerably. The effects observed in the lymphocytes and polymorphonuclear cells are in agreement with previous reports (23–25).

In normal mice, in contrast to the great reduction in the number of the monocytes in the circulation, hydrocortisone has only a slight effect on the number of macrophages already present in the peritoneal cavity. The peritoneal macrophages, which derive from monocytes in the peripheral blood, have a turnover time of about 40 days (7). The reduction in macrophage population during the period in which there are almost no monocytes in the peripheral blood can be explained by a normal disappearance rate of macrophages from the peritoneal cavity in the absence of any influx of mononuclear phagocytes from the peripheral blood. The results of the experiments with labeled mononuclear phagocytes support this view.

The same reasoning holds for the response after an inflammatory stimulus. In hydrocortisone-treated animals, newborn calf serum induced only a small, transient increase in the number of monocytes in the peripheral blood. In these animals, the number of peritoneal macrophages decreases initially, as is also seen in normal animals, and thereafter remains at a constant low level. This effect of hydrocortisone pretreatment is in contrast with the results obtained in normal animals, in which newborn calf serum induces a doubling of the number of peritoneal macrophages deriving from the monocytes in the circulation (7).

The labeling studies demonstrated that hydrocortisone does not entirely suppress the migration of mononuclear phagocytes from the blood into the peritoneal cavity. Only during the first 24 hr after an inflammatory stimulus there is an influx of mononuclear phagocytes; no evidence for a delayed migration of these cells has been found. It therefore seems unlikely that glucocorticosteroids diminish the vascular permeability for the monocytes migrating to the site of inflammation. The diminished exudation of mononuclear phagocytes is most easily explained by the great reduction in the number of monocytes in the peripheral blood.

The effect of glucocorticosteroids on granuloma formation reported by Nicol et al. (15) can now be interpreted in a similar way. A decrease in granuloma weight was seen only at a hydrocortisone dose shown by this study to have an effect on the number of monocytes.

In contrast to the results of the present study, Boggs et al. (22) found no demonstrable relation between the number of monocytes and lymphocytes in the blood and the reduced number of macrophages in the inflammatory exudate on skin windows, under the influence of glucocorticosteroids.

Whether hydrocortisone renders the inflammatory stimulus ineffective or prevents this stimulus from activating the monocyte–macrophage system, is not clear. The slight increase in the number of monocytes and the small influx of labeled cells into the peritoneal cavity shortly after the inflammatory stimulus under the influence of hydrocortisone, shows that this stimulus can still mobilize a pool of newly formed cells, either in the bone marrow or somewhere else.

The moderate decrease in the number of macrophages during the action of glucocorticosteroids makes a direct lytic action of these drugs on the macrophages unlikely. Furthermore, the structure of mononuclear phagocytes from mice treated with hydrocortisone and the morphology of these phagocytes from normal mice incubated in the presence of dexamethasone, showed no distinct divergence from that of normal peritoneal macrophages, which is in accordance with other reports (26, 27). Their capacity to incorporate ^{3}H-thymidine in vitro also remained unchanged when dexamethasone was added to the culture medium. In considering these in vitro experiments, however, it must be taken into account that the steroid effect in vivo may be caused by a metabolite of administered glucocorticosteroids rather than by the preparation itself.

The mechanism underlying the rapid disappearance of the monocytes from the circulation is still obscure. An inhibited release of monocytes from the bone marrow cannot account for this phenomenon, because the disappearance of these cells from the blood during glucocorticosteroid treatment is much quicker than the average blood transit time, which amounts to 32 hr under normal steady-state conditions (7). Since a direct lytic action seems unlikely, it can be postulated that the monocytes are sequestrated in a pool, the localization of which, however, is unknown. A similar mechanism has been assumed to bring about the effect of hydrocortisone on the disappearance of eosinophilic granulocytes (28). The rapid return of monocytes to the circulation after the effect of dexamethasone has ceased, could be due to a recruitment of these cells from the sequestration pool or to a release of newly formed monocytes from the bone marrow. Furthermore, the continuous administration of glucocorticosteroids by means of a depot-forming preparation may also inhibit the production of monocytes in the bone marrow and/or delay their release into the circulation.

SUMMARY

The effect of glucocorticosteroids on the kinetics of mononuclear phagocytes, i.e., peripheral blood monocytes and peritoneal macrophages, was studied in

normal mice, as well as in mice in which an inflammatory reaction was evoked in the peritoneal cavity.

The administration of glucocorticosteroids resulted in a rapid decrease (within 3–6 hr) in the number of circulating monocytes, the duration being dependent on the nature and dose of the compound. The water-soluble dexamethasone sodium phosphate is only briefly active (less than 12 hr), but hydrocortisone acetate, which forms a subcutaneous depot, reduced the number of monocytes for more than 2 wk.

In normal mice, hydrocortisone did not affect the number of macrophages already present in the peritoneal cavity, but the transit of mononuclear phagocytes from the circulation into the peritoneal cavity was arrested.

During an inflammatory response in the peritoneal cavity, hydrocortisone suppresses both the increase in the number of monocytes in the peripheral blood and the increase in the number of peritoneal macrophages. This reduction of the inflammatory exudate appeared to be due to a diminished influx of mononuclear phagocytes from the peripheral blood. No lytic action of glucocorticosteroids on the mononuclear phagocytes could be demonstrated.

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