The *in Vitro* Effect of Steroids on Polymorphonuclear Leukocyte Metabolism[1] (36916)

M. Robert Cooper, Lawrence R. DeChatelet, and Charles E. McCall

The Departments of Medicine and Biochemistry, The Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103

The ingestion of a bacterium by a leukocyte is accompanied by a number of biochemical changes including increases in oxygen consumption, hydrogen peroxide formation, and hexose monophosphate shunt activity (HMS) (1). Although the precise enzymatic basis for these changes is controversial (2–4), the increased activities are generally conceded to be necessary for normal bactericidal activity. In particular, the production of H_2O_2 may be bactericidal in conjunction with iodide ion and myeloperoxidase which results in the fixation of iodine to the bacteria (5). The most compelling evidence for the importance of these reactions comes from the study of cells from patients with chronic granulomatous disease (6) in which the cells ingest particular matter normally, but do not show the usual increase in metabolism upon phagocytosis. This is accompanied by a defect in intracellular killing (6), and patients with this disease suffer from severe recurrent infections.

Animals receiving adrenocorticosteroids show an increased susceptibility to bacterial infection (7). Mandell, Rubin and Hook (8) have recently shown that hydrocortisone *in vitro* can inhibit the increased oxygen consumption and hydrogen peroxide production which normally accompany phagocytosis. This is accompanied by a decrease in the ability of the cells to kill bacteria. Chretien and Garagusi (9) have demonstrated that several steroids can affect leukocyte metabolism *in vivo* as measured by the ability of the cells to reduce nitroblue tetrazolium dye.

The present communication extends these observations by demonstrating that the *in vitro* addition of a number of steroids inhibits both HMS activity and iodination of bacteria. These effects are compatible with an inhibition of bacterial killing.

Materials and Methods. Reagents. Dexamethasone sodium phosphate (Decadron) and prednisolone sodium phosphate (Hydeltrasol) were obtained as injectable solutions from Merck, Sharpe and Dohme, West Point, PA. Hydrocortisone sodium succinate (Solu-Cortef) and methylprednisolone sodium succinate (Solu-Medrol) were obtained as injectable solutions from the Upjohn Company, Kalamazoo, MI. All steroids were diluted with phosphate buffered saline (PBS) (10) to give working stock solutions of 4.0 mg/ml. Glucose-1-^{14}C (sp act 52.2 mCi/mmole) was obtained from the New England Nuclear Corp., Boston, MA. The isotope was dissolved in distilled water to give a concentration of 0.5 μCi/ml and stored frozen. Na^{125}I (carrier free) was likewise obtained from the New England Nuclear Corp., Boston, MA. The isotope was dissolved in distilled water to give a concentration of 2.5 μCi/ml and was stored frozen. At the time of an experiment, an aliquot was counted in a gamma well scintillation counter and appropriate corrections were made for radioactive decay. Plasma gel, used in sedimenting the red blood cells was obtained from the HTI Corp., Buffalo, NY. Zymosan particles were obtained from Nutritional Biochemicals Corp., Cleveland, OH. They were suspended in PBS to a concentration of approximately 6×10^9 particles/ml. Latex particles (0.81 μm diam) were obtained from Difco Laboratories, Detroit, MI. The latex particles were washed three times in cold distilled water by repeated suspension and centrifugation (6040*g*

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for 15 min). The washed pellet was resuspended in isotonic saline such that a 1:100 dilution would have an absorbance of 0.420 at 525 nm. The final concentration of latex was approximately 3×10^9 particles/ml. All chemicals used were of reagent grade.

Isolation of leukocytes. Leukocytes were isolated by sedimentation with plasma gel as previously described (11). After washing with 0.9% saline, the cells were subjected to hypotonic lysis to remove the few contaminating red cells. The resulting white cells were suspended in PBS, counted in a white cell counting chamber, and brought to the desired concentration by adding PBS to the cell suspension. Viability was determined by the ability of the cells to exclude 1% trypan blue dye. Differential counts were performed on smears of the isolated white cell suspension. Appropriate corrections were made for the differential counts so that each study was based on an equal number of phagocytes. Approximately 90–95% of the cells thus isolated from normal venous blood were polymorphonuclear leukocytes.

Hexose monophosphate shunt activity. Glucose utilization via the hexose monophosphate shunt was estimated by a modification of the method of Sbarra and Karnovsky (12), employing glucose-1-^{14}C. Each flask contained 1.0 ml of Eagles medium 199, 0.20 ml of heat-inactivated pooled AB serum, and 0.10 ml of glucose-1-^{14}C. Phagocytosis was initiated to appropriate flasks by the addition of 0.15 ml of latex particles at a ratio of about 100 particles/phagocyte; control flasks received an equal volume of 0.9% saline. Where present, steroids were added in a volume of 0.25 ml; control flasks received an equal volume of PBS. Reaction was initiated by the addition of 1.0 ml of cell suspension (containing 5×10^6 cells in PBS) and allowed to proceed for 1 hr at 37°. $^{14}CO_2$ was collected in 0.5 ml of hydroxide of Hyamine and counted in a liquid scintillation spectrometer.

Respiration. Oxygen consumption was measured using a Clark oxygen electrode according to a previously reported method (13).

Phagocytic index. Phagocytes (1×10^7) were incubated with latex particles (0.81 μm diam) at a ratio of 100 particles/phagocyte for 15 min. Five hundred particles were then counted by phase microscopy and the number of particles in each cell was estimated. The index was calculated on the basis of 0–5 for each individual phagocyte. Thus, the phagocytic score could range from 0 to 2500.

TABLE I. Effect of Steroids on Hexose Monophosphate Shunt Activity of Human Neutrophil.

Experimental group	$^{14}CO_2$ (cpm) from glucose-1-^{14}C	
	Resting	Phagocytizing
Control	1025[a]	6953
Dexamethasone sodium phosphate[b]	415	1556
Hydrocortisone sodium succinate	685	4338
Methylprednisolone sodium succinate	538	3167
Prednisolone sodium phosphate	498	2055

[a] Each point represents the mean of 6 determinations.

[b] Steroids were added, where indicated, to give a final concentration of 0.7 m*M*.

Iodination. Iodination was quantitated by a modification of the method of Pincus and Klebanoff (14). Leukocyte suspensions were adjusted to a concentration of 6×10^7 phagocyte/ml. Each incubation tube contained in a total volume of 0.60 ml: 0.10 ml leukocyte suspension, 0.10 ml fresh AB serum, 0.10 ml glucose (1.50 mg), 0.10 ml Na^{125}I (0.25 μCi), 6×10^8 zymosan particles, and 0.10 ml of PBS. When employed, steroids were added in 0.10 ml of PBS. Controls were routinely run in which the zymosan particles were omitted.

Following gentle mixing, the test tubes were incubated for 60 min at 37°. The reaction was stopped by the addition of 2 ml of cold 5% trichloroacetic acid (TCA). The insoluble precipitate was collected by centrifugation, washed 4 × with 5% TCA, and suspended in 5.0 ml distilled water. The tubes were counted in a gamma well scintillation counter for 1 min. The values for the duplicate tubes were averaged and the control average (minus zymosan) was subtracted from its corresponding experimental value to

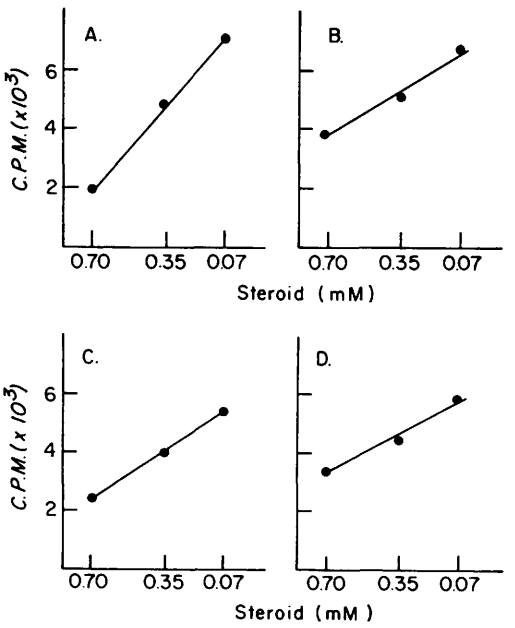

FIG. 1. Effect of various concentrations of steroids on oxidation of glucose-1-^{14}C to $^{14}CO_2$. (A) Dexamethasone sodium phosphate, (B) hydrocortisone sodium succinate, (C) methylprednisolone sodium succinate, (D) prednisolone sodium phosphate.

yield values attributable only to phagocytosis.

Results. Table I illustrates the effects of four steroids on glucose-1-^{14}C oxidation in normal human neutrophils. At a concentration of 0.7 m*M*, all four steroids inhibit the HMS activity in both resting and phagocytizing cells, but the degree of inhibition varies with the steroid used. The greatest degree of inhibition is observed with dexamethasone sodium phosphate and prednisolone sodium phosphate with relatively lesser effects shown by methylprednisolone sodium succinate and hydrocortisone sodium succinate. The inhibition given by all steroids is dose-dependent as illustrated in Fig. 1. The pattern of inhibition observed is very similar for all steroids used except dexamethasone sodium phosphate. This latter steroid causes the greatest inhibiton of HMS activity at a concentration of 0.7 m*M* (in accord with the data in Table I) but exerts the least inhibition when the concentration is reduced 10-fold to 0.07 m*M*. Thus, the inhibition by this compound is much more dependent upon concentration than that observed with the other steroids.

Since it has been shown that ascorbic acid will stimulate the HMS in human leukocytes (15), it was of interest to determine whether the various steroids could inhibit the ascorbate-stimulated HMS. The results in Table II confirm the previously noted stimulation of HMS activity by ascorbate and demonstrate that the steroids will inhibit HMS activity regardless of whether it is stimulated by phagocytosis of latex or by ascorbic acid. This implies that the steroids are not acting solely upon particle ingestion but rather are affecting some enzymatic reaction.

Results of oxygen consumption studies paralleled those of the HMS. Dexamethasone sodium phosphate (0.7 m*M*) caused the greatest inhibition of O_2 uptake by phagocytizing cells with significant, but lesser inhibition observed with the other steroids. The resting oxygen consumption is so low that it was difficult to establish inhibition (data not shown).

The effect of 0.70 m*M* steroids on the ingestion of latex particles is shown in Table III. Both dexamethasone sodium phosphate and prednisolone sodium phosphate cause a marked inhibition of particle uptake. Considerably less inhibition is observed with methylprednisolone sodium succinate, while little or no effect is observed with hydrocor-

TABLE II. Effect of Steroids on the Ascorbate-Induced HMS Stimulation of Human PMNL.

Experimental group	$^{14}CO_2$ (cpm) from glucose-1-^{14}C	
	Resting	+ .01 *M* ascorbate
Control	1025[a]	7025
Dexamethasone sodium phosphate[b]	435	3440
Hydrocortisone sodium succinate	796	4877
Methylprednisolone sodium succinate	692	3601
Prednisolone sodium phosphate	586	4062

[a] Each point represents the mean of 4 determinations.

[b] Steroids were added, where indicated, to give a final concentration of 0.7 m*M*.

TABLE III. Effect of Steroids on Phagocytosis of Polystyrene Particles by Human PMNL.

Experimental group	Phagocytic[a] index
Control	1777
Dexamethasone sodium phosphate[b]	345
Hydrocortisone sodium succinate	1645
Methylprednisolone sodium succinate	1365
Prednisolone sodium phosphate	670

[a] 500 cells were examined microscopically and scored arbitrarily from 0–5. Thus the potential range of the phagocytic index is from 0–2500.

[b] Steroids were added, where indicated, to a final concentration of 0.7 m*M*.

tisone sodium succinate. When normal ingestion occurs, the cells are observed to clump and form large aggregates. This "clumping" phenomenon was also observed to be diminished in cells incubated with either dexamethasone sodium phosphate or prednisolone sodium phosphate (data not shown).

The fixation of inorganic iodide to bacteria has been postulated as a major mechanism whereby the neutrophil kills bacteria (5). Accordingly, we studied the effects of steroids on the ability of intact neutrophils to iodinate zymosan particles. The results (Table IV) indicate that both dexamethasone sodium phosphate and hydrocortisone sodium succinate cause a profound inhibition of the iodination reaction, while methylprednisolone sodium succinate and prednisolone sodium phosphate have considerably less effect.

Discussion. The effects of steroids on neutrophil metabolism are complex and cannot be explained by a single common mechanism. All steroids tested caused an inhibition of hexose monophosphate shunt activity, but the magnitude of inhibition varied considerably, with dexamethasone sodium phosphate giving the greatest and hydrocortisone sodium succinate the least degree of inhibition at a concentration of 0.7 m*M*. Since the HMS inhibition was observed in resting as well as phagocytizing cells, one cannot explain the phenomena simply in terms of an inhibition of a particle uptake, although the steroids which caused the greatest effect on HMS activity did significantly reduce the ability of the cell to ingest particles. Further, the fact that the steroids could inhibit the ascorbic acid-induced HMS is additional evidence that the mechanism of action cannot be solely at the level of ingestion. One might speculate that the steroids might be inhibitors of glucose-6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase as has been observed in rat mammary tissue (16), but *in vitro* experiments employing sonic extracts of leukocytes showed no such effect with any of the compounds used here (unpublished data). Yielding and Tomkins (17) have demonstrated that many steroids inhibit NADH oxidase and Mandell, Rubin and Hook (8), have extensively investigated the effects of hydrocortisone sodium succinate on neutrophil metabolism and physiology. Mandell, Rubin and Hook reported that concentrations of hydrocortisone similar to those used here inhibited oxygen consumption, hydrogen peroxide production, and bactericidal activity without affecting ingestion or degranulation, and ascribed these effects to an inhibition of leukocyte NADH oxidase.

Our present results confirm some of the observations of Mandell, Rubin and Hook and extend them to demonstrate that the steroid likewise inhibits both HMS activity and the iodination of zymosan particles. However, it complicates somewhat the postulated mechanism since the steroids inhibit the ascorbate-induced HMS stimulation, this stimulation by ascorbate is presumably independent of the enzyme NADH oxidase (15).

TABLE IV. Effect of Steroids on the Iodination of Zymosan Particles by Human PMNL.

Experimental group	Net cpm[a]
Control	61,647[b]
Dexamethasone sodium phosphate[c]	4381
Hydrocortisone sodium succinate	4719
Methylprednisolone sodium succinate	44,206
Prednisolone sodium phosphate	37,190

[a] Net cpm represents the amount of ^{125}I fixed protein in the presence of zymosan minus that fixed to protein in the absence of zymosan particles.

[b] Each value represents the mean of 3 determinations.

[c] Steroids were added, where indicated, to give a final concentration of 0.7 m*M*.

Thus, it appears that the steroids can inhibit the HMS at least partially by a mechanism which is independent of effects on NADH oxidase.

The effects of different steroids likewise support the idea that a multiplicity of actions may be involved. Although hydrocortisone has only a minor effect on HMS activity, it exerts a profound inhibition on the iodination reaction. If its effect were solely on the NADH oxidase reaction, it would be expected to inhibit both HMS activity and iodination to a similar degree since both these reactions are dependent upon the NADH oxidase reaction in the sequence proposed by Baehner and Nathan (18). The inhibition of iodination is certainly compatible with an inhibition of bacterial killing as reported by Mandell, Rubin and Hook (8), but the present results make it uncertain that this can be explained simply by an inhibition of NADH oxidase.

The fact that a number of steroids can effect leukocyte metabolism was recently emphasized in a report by Chretien and Garagusi (9) who demonstrated the neutrophils isolated from patients receiving steroid therapy had an impaired ability to reduce nitroblue tetrazolium dye compared to normal cells. Steroids employed in this study included dexamethasone, hydrocortisone sodium succinate, and prednisone.

Thus steroids have a profound effect on neutrophil metabolism. Although the basic site of action is uncertain, the defects observed might well lead to decreased intracellular bactericidal activity which in turn could explain why corticosteroid therapy in patients occasionally leads to increased susceptibility to bacterial infections (9). Moreover, different steroid compounds might alter host defense parameters to varying degrees, as recently suggested by Peters *et al.* (19).

Summary. Steroids added *in vitro* to human neutrophils have numerous effects on cellular metabolism, including inhibition of hexose monophosphate shunt activity, particle uptake, and iodination of zymosan particles. The magnitude of these effects varies considerably with the steroid employed.

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