ADRENAL STEROIDS AND INFECTION: THE EFFECT OF CORTISONE ADMINISTRATION ON POLYMORPHONUCLEAR LEUKOCYTIC FUNCTIONS AND ON SERUM OPSONINS AND BACTERICIDINS *

By JAMES G. HIRSCH and ALICE B. CHURCH

(From the Rockefeller Institute, New York, N. Y.)

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Abundant clinical and laboratory experience has established clearly the increased susceptibility to certain infections of animals receiving large doses of cortisone (1–3). There is general agreement that excessively high levels of glucocorticoid hormones diminish the efficiency of host resistance to microbial invasion, but considerable uncertainty remains as to the precise alterations and mechanisms involved (4, 5). Suppression of the inflammatory reaction, impaired function of phagocytic cells, and lowering of antibody levels have all been incriminated to explain the reduced ability to handle microbes of the animal receiving large doses of cortisone.

Host resistance to infections is based ultimately on the activity of many systems: phagocytic cells (polymorphonuclear leukocytes, fixed and wandering macrophages); serum factors (bactericidins, complement, antibody); and the microenvironment of tissue spaces (vascular and neural functions, chemical composition). This communication presents the results of experiments in which the effect of cortisone administration was studied on function of certain of these host resistance systems—namely, the polymorphonuclear leukocyte and serum bactericidins and opsonins.

METHODS

Several experiments were done with different dosages of cortisone. The results were in all cases the same; accordingly, only experiments involving the administration of large doses will be described here.

Young adult New Zealand red rabbits were used. At weekly intervals (× 3) these animals were given an intraperitoneal injection of 300 ml of 0.1 per cent glycogen (Amend Drug and Chemical Co.) in pyrogen-free physiological saline. These injections were intended to prepare the animal for collections of peritoneal exudates at a later date. Before administration of cortisone the rabbits were bled from the heart; after clotting and standing at room temperature for 1 hour, serum was separated by centrifugation and stored in the frozen state for later investigation.

The animals were then given cortisone (Cortone Acetate suspension, Merck Sharp and Dohme) by intramuscular injection on a dosage schedule of 25 mg per kg per day for a total of 11 consecutive days. On Day 11 of cortisone administration blood was again obtained by cardiac puncture and serum prepared and stored as described above.

On Days 10 and 11 of steroid injections, peritoneal exudates were produced and polymorphonuclear leukocytes harvested by a technique described in detail previously (6, 7). In one animal peritoneal exudate leukocytes were also obtained for study 14 days following discontinuation of cortisone injections.

Three different aspects of polymorphonuclear leukocytic function were investigated: *1*) ability to ingest and kill certain microorganisms, *2*) details of cellular morphology before and after ingestion of bacteria, and *3*) content of certain bactericidal agents.

The suspending medium for the tests of phagocytic function was Gey's balanced salt solution containing a final concentration of 0.1 per cent gelatin, hereafter referred to as *gel-Gey's solution.* A portion of the peritoneal exudate was centrifuged (International PR 2) at 1,000 rpm for 5 minutes. The cell button was then washed twice on the centrifuge in 40 ml of gel-Gey's. Exudate cells were in all instances over 95 per cent polymorphonuclear leukocytes. The washed white cells were finally suspended in gel-Gey's solution at a concentration of 20,000 per mm^3. Phagocytic tests were done in 10 × 75 mm Pyrex test tubes into which were placed 0.8 ml of the leukocyte suspension, 0.1 ml of whole or diluted serum, and 0.1 ml of the bacterial inoculum. After capping with sterile rubber stoppers, the tubes were rotated end over end at 38° C. Before incubation and at suitable intervals, samples were removed from each tube and the numbers of surviving bacteria determined by serial dilution in penassay broth and plating on penassay agar. Control tubes containing no white cells always showed a stable bacterial count, indicating that bactericidal effects observed in the test specimens were based on phagocytosis and intracellular killing. The microorganisms employed were *Staphyloccus albus* (Greaves) (nonpigmented, coagulase negative) and *Salmonella typhimurium* RIA, a nonvirulent variant. The mi-

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crobes were cultured overnight on penassay agar slants. The surface growth was suspended in gel-Gey's and washed once on the centrifuge in this medium. Appropriate dilutions were made in gel-Gey's so that the phagocytic test system would contain initially between 10^5 and 10^6 bacteria per ml.

Polymorphonuclear leukocytes from the fresh exudates were examined for morphology and for degranulation following ingestion of foreign material by a procedure described previously (8). In brief, this technique involved sedimentation of granulocytes onto glass slides followed by phagocytosis of bacteria *in situ*. Examination of Wright's stained preparations of these specimens permitted evaluation of various morphological features, and especially of the degranulation response to phagocytosis, in cells obtained from cortisone-treated rabbits.

The remaining exudate leukocytes were collected in the centrifuge, freed from contaminating red blood cells and washed according to the methods described in an earlier report (7). The washed white cell buttons were stored in the frozen state and examined for their content of antimicrobial substances at a later date. This examination was done in parallel with similarly collected and treated white cell buttons from control animals which had not received cortisone. The cell buttons were frozen and thawed 3 times in dry ice-alcohol mixtures and a 38° C bath. The frozen-thawed buttons were suspended in saline at a concentration of 5×10^7 leukocytes per ml, and allowed to stand at room temperature for 30 minutes. These suspensions were then spun at 12,000 G for 15 minutes. The supernatant, called *saline extract,* was stored in the cold. The pellet was washed twice with saline by high speed centrifugation, and the supernatant discarded. The deposit was next suspended in 0.01 M citric acid and, after standing at room temperature for 30 minutes, was centrifuged at 12,000 G. The supernatant of this citric acid extraction was saved for subsequent testing. After two washings with 0.01 M citric acid, the deposit was finally extracted with 0.2 N HCl. This procedure, taken after that described previously (9), permitted separation of three of the major intraleukocytic antimicrobial substances: lysozyme, present in the saline extract; phagocytin, found in the citric acid extract; and histones, located in the hydrochloric acid extract. Assay for lysozyme was performed by the method of Shugar (10) with slight modification. Phagocytin and histone titrations were performed by a technique described previously (9), using *Salmonella* RIA as the test organism.

Comparisons were made of the opsonic activity of serum from normal and cortisone-treated rabbits by employing the phagocytosis system described above. Addition of serial dilutions of serum enabled a titration of opsonic power; i.e., a determination of the lowest concentration of a given serum which manifested a distinct phagocytosis-promoting effect. These observations were made in systems with leukocytes from cortisone-treated animals, as well as in those containing normal rabbit granulocytes.

Measurement of serum bactericidins was done by the technique described in an earlier publication (11), using *Bacillus subtilis* for estimation of β-lysins, and *Escherichia coli* K-12 for titrations of the complement-serum antibody bactericidal system.

RESULTS

General effects of cortisone administration on the experimental rabbits. The rabbits given large doses of cortisone (25 mg per kg per day) for 11 consecutive days lost approximately 10 per cent of their body weight during this period. None showed gross signs of infection or other compli-

TABLE I

Performance in a phagocytic system of polymorphonuclear leukocytes and of serum from normal and cortisone-treated rabbits

Serum, final concentration %		Numbers of surviving bacteria per ml after 60 min at 38° C					
		Normal rabbit leukocytes		Leukocytes from rabbit given cortisone for 11 days		Leukocytes from rabbit 14 days after end of cort. admin.	
		Staph. albus (4×10^5)*	*Sal. RIA* (1×10^6)*	*Staph. albus* (5×10^5)*	*Sal. RIA* (6×10^5)*	*Staph. albus* (6×10^5)*	*Sal. RIA* (8×10^5)*
Normal†	10	9×10^3	3×10^3	2×10^3	8×10^3	3×10^3	7×10^3
Normal	3	6×10^3	2×10^4	2×10^3	2×10^4	6×10^3	2×10^4
Normal	1	6×10^3	6×10^5	1×10^3	3×10^5	5×10^3	6×10^5
Normal	0.3	5×10^4	1×10^6	8×10^3	7×10^5	6×10^4	1×10^6
Cortisone‡	10	8×10^3	4×10^3	1×10^3	6×10^3	1×10^3	1×10^4
Cortisone	3	5×10^3	3×10^4	1×10^3	2×10^4	4×10^3	3×10^5
Cortisone	1	2×10^3	1×10^6	2×10^3	5×10^5	5×10^4	1×10^6
Cortisone	0.3	3×10^4	1×10^6	1×10^4	8×10^5	1×10^5	1×10^6
None		2×10^5	1×10^6	3×10^5	8×10^5	3×10^5	8×10^5

* Bacterial counts at start of incubation.
† From experimental rabbit prior to cortisone administration.
‡ From experimental rabbit after 11 days of cortisone injections.

TABLE II

Performance in a phagocytic system of polymorphonuclear leukocytes and of serum from normal and cortisone-treated rabbits

Polymorphonuclear leukocytes from	Minimal concentration of serum required for >90% phagocytosis and killing in 1 hour at 38° C with			
	Normal rabbit serum		Serum from cortisone-treated rabbit	
	Staph. albus	*Sal. RIA*	*Staph. albus*	*Sal. RIA*
Normal rabbit	1	3	0.3	3
Cortisone-treated rabbit	0.3	3	0.3	3

cating illness before or during cortisone treatment. The sera separated from blood drawn after 10 or 11 days of hormone injections were opalescent, and showed an elevated sugar content (approximately 150 mg per 100 ml) with no detectable ketone bodies, in contrast to the clarity and normal sugar concentration of the pretreatment sera.

Peritoneal exudates, harvested from the rabbits given large doses of cortisone, contained numbers of cells (5×10^8 to 1.5×10^9) similar to those produced in normal animals. Over 95 per cent of these exudate cells in the treated rabbits were polymorphonuclear leukocytes; they differed grossly from normal rabbit granulocyte suspensions only in showing less clumping and less adherence to glass upon standing at room temperature.

Function in phagocytic systems of polymorphonuclear leukocytes and of serum from rabbits treated with cortisone. Comparisons were made *in vitro* of the ability of polymorphonuclear leukocytes from normal and cortisone-treated rabbits to engulf and kill a *Staph. albus* and an avirulent strain of *Sal. typhimurium*. By including in these phagocytic systems dilutions of serum obtained prior to and during cortisone administration, it was also possible to study the effect, if any, of steroid injections on serum opsonic power.

Results of a typical experiment are recorded in Table I, and are also presented in condensed form in Table II. Phagocytic and bactericidal capacity of leukocytes from cortisone-treated animals was the same as that of cells from normal rabbits. Granulocytes obtained 2 weeks after cessation of hormone injections demonstrated no detectable difference in their behavior.

The phagocytosis-promoting power of serum obtained prior to and 11 days after the start of cortisone therapy was not significantly different.

These studies thus demonstrated that large doses of cortisone had no detectable effect on polymorphonuclear leukocytic function or on serum opsonins as measured in this particular phagocytic system.

Studies on morphology and on the degranulation process in polymorphonuclear leukocytes obtained from rabbits receiving large doses of cortisone. Examination of preparations stained by Wright's

TABLE III

Content of antimicrobial agents in granulocytes from normal and cortisone-treated rabbits

Polymorphonuclear leukocyte extracts	Leukocytes from	Content of		
		Lysozyme	Phagocytin	"Histones"
		μg/ml	*U**	*U**
Saline	Normal rabbit	42	160	
	Cortisone-treated rabbit	45	160–320	
Citric acid	Normal rabbit	trace	1,280	
	Cortisone-treated rabbit	trace	1,280	
HCl	Normal rabbit	0		2,560
	Cortisone-treated rabbit	0		2,560

* Reciprocal of highest dilution of the extract which produced greater than a 90% kill of *Salmonella* RIA in 1 hour at 38° C.

TABLE IV

Serum bactericidins in normal and cortisone-treated rabbits

Serum from rabbit	Bactericidal activity* on *Bacillus subtilis* (β-lysins)	Bactericidal activity* on *Escherichia coli* K-12 (complement-antibody)
Prior to cortisone administration	2,000	8–16
After 11 days of cortisone injections	4,000	8

* Reciprocal of highest dilution which produced > 90% kill in 90 min at 38° C.

revealed no significant differences in the appearance of polymorphonuclear leukocytes from peritoneal exudates of normal and of cortisone-treated rabbits. The cells from animals receiving steroid hormone did show some hypersegmentation of nuclei, and perhaps a slight increase in numbers of cytoplasmic granules. The specific granules in these leukocytes showed size, structure and staining properties similar to those of normal rabbit granulocytes.

After ingestion of streptococci or zymozan particles, leukocytes from the cortisone-treated animals showed loss of cytoplasmic granules. This degranulation subsequent to phagocytosis was not quantitatively or qualitatively different from that previously reported for normal rabbit polymorphonuclear white cells (8).

Study of the effect of cortisone administration on the content of antimicrobial substances in polymorphonuclear leukocytes. Polymorphonuclear leukocytes from normal and from cortisone-treated rabbits were extracted in a fashion which permitted estimation of their content of three antimicrobial agents: lysozyme, phagocytin, and histones. Results of these studies, presented in Table III, show that cortisone therapy did not alter the quantity of these bactericidins present in granulocytes.

The lack of demonstrable effect of cortisone therapy on activity of serum bactericidins. Serum samples obtained from the same rabbit prior to and after 11 days of cortisone injections were assayed for their content of two types of bactericidins: β-lysins, and the complement-antibody system. As is seen in Table IV, the level of these serum bactericidal systems was not changed by steroid administration.

DISCUSSION

The increased susceptibility to infections of animals with abnormally high levels of certain adrenal steroids could be due to one of two gross mechanisms: *1*) a direct effect of steroids on the microbes, somehow altering their properties so as to increase their virulence; or *2*) an action on host defense systems resulting in lowered resistance to microbial invasion. Efforts to demonstrate an influence of corticoid hormones on virulence of microorganisms cultured *in vitro* have met with no success; there remains the remote possibility that microorganisms growing *in vivo* might be directly altered by adrenal steroids. At the moment, however, it seems best to assume that native or acquired host resistance mechanisms are disturbed in the presence of high levels of cortisone-like compounds.

The present studies serve to eliminate from consideration several of the host resistance factors which might conceivably be suppressed by elevated corticosteroid concentrations. The morphology, the phagocytic and bactericidal functions, and the content of antimicrobial agents of polymorphonuclear leukocytes were not detectably altered in rabbits injected repeatedly with large doses of cortisone. These results thus support and extend those of Mogabgab and Thomas (12) and of Germuth, Ottinger and Oyama (13). They serve furthermore to cast doubt upon previous work purporting to show diminished phagocytic function of granulocytes from cortisone-treated animals (14, 15).

The mobilization of granulocytes in the cortisone-treated rabbits of the present study was grossly normal; i.e., abundant numbers of these cells were obtained in exudates produced by intraperitoneal injection of glycogen. However, the migration of granulocytes into tissues of these animals in response to implantation of small numbers of certain microbes might well have been deficient or significantly delayed, in keeping with the findings of several previous studies on this point (reviewed in Reference 5).

Investigations into relationships between serum antibodies and cortisone administration (4, 5) have yielded somewhat conflicting results, but there seems now to be a measure of agreement that suppression or elimination of antibodies is not primarily responsible for the altered host resistance. The present observations support this view, and they also demonstrate that serum from cortisone-treated rabbits possesses a normal concentration of heat-labile opsonins. The lack of influence of cortisone therapy on the level of serum bactericidins for coliform microbes provides further evidence that high levels of glucocorticoids do not significantly suppress antibodies or complement, since these components are both essential for this bactericidal activity.

The present experiments thus indicate that the depression of host resistance to infection which occurs in association with high levels of corticosteroid hormones is not based upon alterations in polymorphonuclear leukocytic functions or in serum opsonins and bactericidins. Studies done by others (reviewed in References 4 and 5) indicate furthermore that changed behavior of mononuclear phagocytes is not primarily responsible for the cortisone effect on susceptibility to microbial disease. Thus, the evidence available at present suggests that adrenal glucocorticoids depress host resistance by suppressing the inflammatory response, and perhaps also by altering metabolism so as to render the local tissue chemical environment more favorable for proliferation of various microbes.

SUMMARY

Polymorphonuclear leukocytes collected from rabbits given large doses of cortisone exhibit normal capacity to engulf and kill certain staphylococci and enteric bacteria. The general morphology and degranulation response to phagocytosis of these cells are not detectably different from those of leukocytes from normal rabbits.

Granulocytes from normal and from cortisone-treated rabbits contain similar amounts of the antimicrobial agents lysozyme, phagocytin and histone.

Administration of large doses of cortisone to rabbits is not associated with significant changes in opsonic or bactericidal activities of the serum.

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