THE CHEMOSUPPRESSION OF CHEMOTAXIS

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PLATES 15 AND 16

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When immunologic agents are employed to induce acute injury to vascular structures of the skin and kidneys, serum complement (C′) has been shown to be required for the full expression of tissue damage in certain experimental models (1, 2). Fixation of the C′, detected by fluorescence microscopy as third component of C′ (β1C-globulin, C′3), has been shown to be associated with the influx of polymorphonuclear leukocytes (PMN's) (3). This finding, together with evidence obtained in vitro, has suggested that C′ functions as a chemotactic factor for the attraction of polymorphonuclear leukocytes in vivo (3, 4). Recently a C′-related chemotactic factor appearing after incubation of rabbit serum with antigen-antibody complexes has been identified and characterized as a protein-protein complex consisting of the fifth and sixth components of C′ (3), although the possibility exists of yet a third component of C′ in this biologically active complex (4). A similar chemotactic factor has also been found to exist transiently in the sera of rabbits after intravenous injection of C′-fixing agents (4).

It has been known for some time that the end result of immunologic injury, tissue damage, can be circumvented by the removal of circulating PMN's (1, 5–8).These cells presumably contain the proteolytic enzymes that are the effectors of tissue damage. Tissue damage can also be prevented by the reduction in levels of serum C′, the result of which is the lack of accumulation of PMN's at sites containing deposits of antigen-antibody complexes (1). In yet another approach to blocking injury mediated by PMN's, it has been shown that pretreatment of animals with corticosteroids will result in reduction of, or complete absence of, the inflammatory reaction that in some cases has been immunologically induced (9–13). In man, suppression of the inflammatory response by cortisone is well established (14). The mechanism or mechanisms by which corticosteroids inhibit the inflammatory response is obscure, although it has recently been demonstrated that hydrocortisone will inhibit serum C′ activity in vitro (15). In another study, reduction in ameboid activity of leukocytes has suggested a direct effect of hydrocortisone on cells (16).

In the present report it will be shown that hydrocortisone and chloroquine

inhibit chemotaxis in vitro by a direct effect on PMN's. It has been found that leukocyte migration to deposits of immunologic reactants in vivo is inhibited by treatment of animals with these drugs. These findings suggest that the antiphlogistic properties of corticosteroids, as well as chloroquine, can be ascribed to a direct effect of the drugs on PMN's, rendering the cells incapable of responding to chemotactic stimuli.

Materials and Methods[1]

Animals.—Adult male guinea pigs, 250 to 350 g, were used in studies of the effect of various drugs on the development of immunologic vasculitis. Housing and care of animals were provided by the Animal Care Branch of the Armed Forces Institute of Pathology.

Immunologic Vasculitis.—Reversed passive Arthus reactions were induced in guinea pigs by the intradermal injection of 50 μg N rabbit antibody to bovine serum albumin (BSA) followed by the intravenous injection of 1000 μg N BSA. The antibody (anti-BSA) employed was IgG, obtained by elution of rabbit γ-globulin from DEAE-cellulose column with low molarity, neutral phosphate buffer. 2½ hr after injection of immune reactants, guinea pigs were exsanguinated by cardiac puncture and sera obtained for $C'H_{50}$ assays. Skin sites were examined and the degree of edema and erythema noted. Leukocyte migration was assessed by light microscopy on formalin-fixed tissues. Cryostat sections of fresh frozen biopsy material were also obtained, and the localization of antigen (BSA, injected intravenously) and the third component of C′ (C′3, β1C-globulin) determined by fluorescence microscopy (1).

Chemotactic Experiments.—As has been reported recently, chemotactic experiments were carried out in simply constructed chambers containing a micropore filter (pore size, 650 mμ) separating each chamber into an upper and lower compartment (3). Chemotactic material was added to the bottom compartment, and various inhibitors were either added directly to this material or mixed with the cell suspension, which was then placed in the upper compartment. Quantitation of chemotaxis was accomplished by counting the number of cells migrating through each filter (3). In this procedure filters were fixed and stained after incubation of chambers at 37°C for 2 hr, and counts of migrating PMN's were determined by light microscopy. The source of the PMN's used in the chemotactic experiments was the exudate obtained after intraperitoneal installation of 0.1% glycogen (in saline) into adult New Zealand male rabbits.

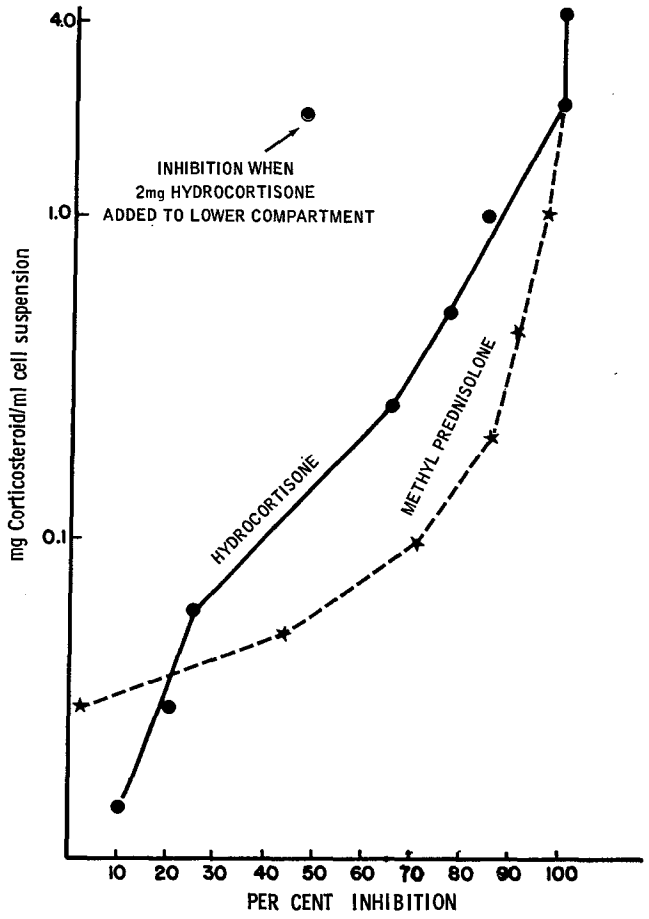
Experiments in Phagocytosis.—The degree of phagocytosis of zymosan particles by rabbit PMN's was carried out as previously described (1). Briefly, 5×10^5 PMN's in 1.4 ml of medium 199 and 10% fresh autologous serum were mixed with varying amounts of boiled, washed zymosan. After incubation for 2 hr at 37°C in sterile siliconized tubes in a constantly rotating rack, cell smears were made, stained with Wright's solution, and at least 200 cells counted. The number of cells with and without zymosan particles was recorded, as well as the number of particles of zymosan present in each cell.

$C'H_{50}$ Analysis in Serum.—The conventional analysis for guinea pig C′, according to the method of Osler, Strauss, and Mayer (17), was carried out in sera from animals after treatment in vivo with various agents. To determine the effect in vitro of corticosteroids and chloroquine on guinea pig C′, a more sensitive $C'H_{50}$ assay system was used, with a reaction volume of 2.0 ml and 5×10^7 sensitized erythrocytes, similar to that recently reported for rabbit C′ (18). By this method fresh guinea pig serum contained approximately 1000 $C'H_{50}$ units/ml.

[1] The "Principles of Laboratory Animal Care" as promulgated by the National Society for Medical Research were observed during this study.

In experiments to determine the effect of drugs on $C'H_{50}$ levels, the dose of drug was expressed as mg/ml-reaction mixture.

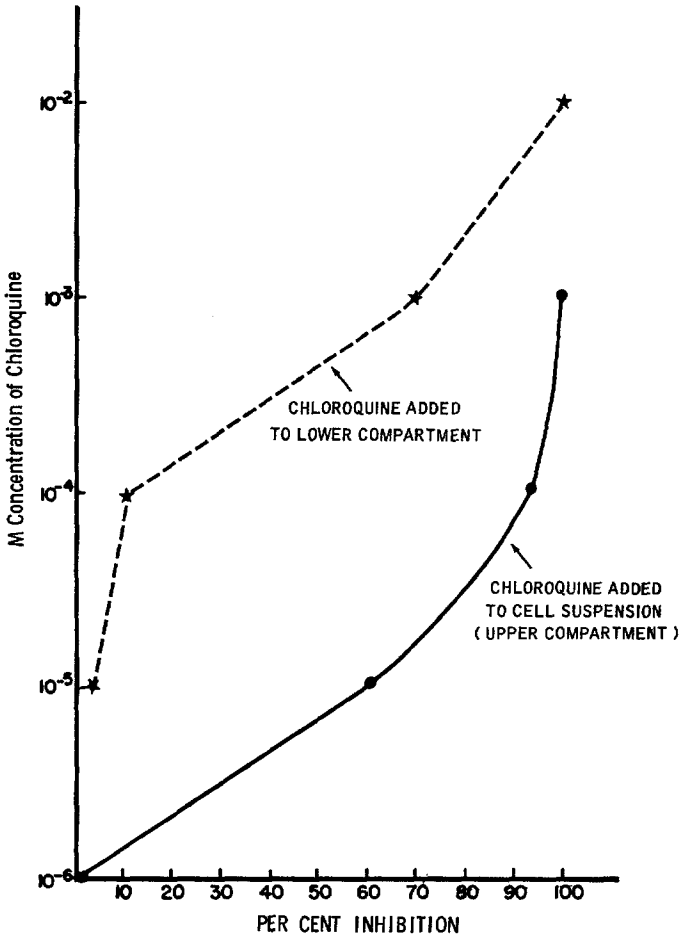
Drugs.—Soluble forms of hydrocortisone and methyl prednisolone (hydrocortisone succinate, Solu-Cortef; and methyl prednisolone succinate, Solu-Medrol, The UpJohn Co., Kalamazoo, Michigan) were used. Sodium succinate at equivalent molar concentrations was used as a control in the chemotactic experiments. Chloroquine hydrochloride (Winthrop Laboratories, New York) was also used. Estrone (Nutritional Biochemicals Corp., Cleveland) was dissolved in absolute alcohol and diluted appropriately in medium 199 to a final ethanol concentration of 0.5%, which, per se, was not inhibitory for chemotaxis of PMN's in vitro. 2,4-Dinitrophenol (DNP) was supplied in powder form (Eastman Kodak Co., Rochester, New York) and was



TEXT-FIG. 1. Inhibition of chemotaxis after addition of hydrocortisone and methyl prednisolone to PMN suspension.

readily soluble in medium 199. Endotoxin in the form of lipopolysaccharide B from *Escherichia coli* 0111:B4 (Difco Laboratories, Detroit) was used.

Two crystalline vitamin A preparations were used: vitamin-A aldehyde (all transretinal, stock number 953074) and vitamin-A acetate (all transretinyl acetate, stock number 942744).



TEXT-FIG. 2. Inhibition of chemotaxis by chloroquine. The drug was added either to cell suspension in the upper compartment or to the chemotactic factor in the lower compartment.

These products were obtained in vacuum-sealed ampules from Distillation Products Industries (Rochester, New York). The preparations were dissolved in absolute ethanol, diluted in medium 199, and used immediately in the chemotactic experiments.

RESULTS

Influence of Corticosteroids on Chemotaxis in Vitro.—Varying amounts of soluble hydrocortisone or methyl prednisolone preparations were added directly to the

cell suspensions in the upper compartments of chambers. The resulting inhibition in migration of PMN's toward chemotactically active material in the lower compartments of chambers (10% fresh rabbit serum with 100 μg N anti-BSA + antigen at equivalence) is indicated in Text-fig. 1. Both corticosteroids were effective in causing inhibition of chemotaxis, methyl prednisolone being more inhibitory in terms of dose. The drug concentrations giving 50% inhibition of chemotaxis were 0.14 mg/ml for hydrocortisone (approximately 2.9×10^{-4} M) and 0.06 mg/ml (1.2×10^{-4} M) for methyl prednisolone. That the suppressive effect of hydrocortisone was *not* through action on the chemotactic factor was

TABLE I

Effect of DNP (Added to Upper Compartment) on Chemotactic Activity of PMN's

Upper compartment (cells plus)	Lower compartment (10% rabbit serum plus)	Chemotactic counts
M		
DNP 10^{-4}	Ag-Ab*	178
DNP 10^{-5}	Ag-Ab	1050
DNP 10^{-6}	Ag-Ab	261
DNP 10^{-7}	Ag-Ab	267
DNP 10^{-4}	—‡	123
DNP 10^{-5}	—	323
DNP 10^{-6}	—	359
DNP 10^{-7}	—	159
—§	— (blank control)	29
—	Ag-Ab (positive control)	289

* 100 μg N anti-BSA plus 18 mg N BSA.

‡ Ag-Ab not added.

§ No DNP added.

demonstrated by the addition of 2 mg corticosteroid to the bottom compartment. Only 40% inhibition of chemotaxis occurred, in contrast to 99% inhibition when the same amount was added directly to the cell suspension in the upper compartment (Text-fig. 1.). Furthermore, the addition of 1.25 mg hydrocortisone to the lower compartment resulted in no inhibition of chemotaxis, although a comparable amount added directly to the cell suspension in the upper compartment would result in 92% inhibition of chemotaxis (Text-fig. 1.). It is likely that inhibition of chemotaxis after addition of steroid to the lower compartment was due to diffusion into the cell suspension of the upper compartment. In addition, it was found that the inhibitory effects of hydrocortisone were, to a large extent, irreversible, since washing the PMN's failed to result in significant restoration of the chemotactic capacity of these cells.

The restriction of the inhibitory effect to certain corticosteroids was demonstrated by the finding that another steroid, estrone, at a concentration in the cell

TABLE II

Effect of DNP (Added to Lower Compartment) on Chemotactic Activity of PMN's

Contents of lower compartment (in 10% rabbit serum)		Chemotactic counts
DNP	Chemotactic material	
M		
10^{-3}	—	234
10^{-4}	—	73
10^{-5}	—	67
10^{-3}	Ag-Ab*	481
10^{-4}	Ag-Ab	200
10^{-5}	Ag-Ab	227
—‡	— (blank control)	47
—	Ag-Ab (positive control)	293

* Antigen-antibody complexes, see Table I.

‡ Not added.

TABLE III

Effect of Vitamin A Preparations on the Chemotaxis of PMN's

Preparation*	Concentration	Chemotactic counts
	M	
All transretinyl acetate (vitamin A acetate)	0.012	167
	0.024	264
	0.048	415
	0.12	209
All transretinal (vitamin A aldehyde)	0.018	199
	0.035	242
	0.071	89
	0.18	43
—	Ag-Ab (positive control)	183
—	— (blank control)	36‡

* Added to cell suspension.

‡ Except for the blank control with 10% rabbit serum, all lower compartments contained 100 μg N anti-BSA plus BSA at equivalence in 10% rabbit serum.

suspension of 10^{-3} M, failed to cause inhibition of chemotaxis. Also, sodium succinate at the same concentration failed to exert any inhibitory effect on the ability of PMN's to respond to chemotactic stimuli, indicating that the succinate portion of the hydrocortisone and methyl prednisolone molecules (in ester

linkage) was not responsible for the inhibitory action exerted by these steroid preparations.

Effect of Chloroquine on Chemotaxis in Vitro.—The capacity of chloroquine to effect directly the ability of PMN's to respond to the chemotactic factor is demonstrated in Text-fig. 2. By extrapolation, the dose of chloroquine in the cell suspension required to cause 50% inhibition of chemotaxis was 8.4×10^{-6} M. That the drug effect was directly on cells was demonstrated by the finding that

TABLE IV

Effect of Hydrocortisone on Immunologic Vasculitis in Guinea Pigs

Guinea pig No.	Hydrocortisone dose§	$C'H_{50}$*	Skin sites							
			Anti-BSA				Anti-BSA + 10 mg hydrocortisone			
			Gross‡	PMN's	BSA	C′	Gross	PMN's	BSA	C′
	mg									
1	250‖	193	1+	2+	2+	1+	3+	1+	2+	1+
2	250	228	1+	2+	2+	2+	3+	±	2+	1+
3	100	142	±	2+	3+	2+	2+	±	2+	2+
4	100	136	±	2+	3+	2+	2+	±	1+	1+
5	50	232	2+	1+	4+	4+	3+	±	2+	ND
6	50	243	±	1+	2+	2+	1+	±	1+	1+
7	None	139	4+	4+	3+	3+	3+	0	3+	3+
8	None	226	4+	4+	3+	3+	4+	±	2+	2+

ND, Not done.

* Assays on sera obtained at time of sacrifice.

‡ Edema, graded ± to 4+.

§ Intravenous dose, given at time of injection of antigen. In addition, animals 1 to 6 also received 50 mg intravenously 1 hr later.

‖ Absolute PMN counts on guinea pig blood before and after treatment with hydrocortisone: guinea pig 1, 3870 PMN's/mm^3 (before)¶ and 9100/mm^3 (after); guinea pig 2, 3200 PMN's/mm^3 before and 15,900 PMN's/mm^3 after treatment.

upon addition of chloroquine directly to the lower compartment containing the chemotactic factor, approximately 100 times more drug was required to achieve the same degree of inhibition of chemotaxis (Text-fig. 2.).

Effect of DNP on Chemotaxis in Vitro.—DNP, when added in varying concentrations directly to the cell suspension, inhibited chemotaxis of PMN's only partially (approximately 38% at the highest concentration, 10^{-4} M, Table I). At all lower concentrations no inhibitory effect was noted. In fact, chemotactic activity was enhanced almost fourfold when DNP at 10^{-5} M was added to the cell suspension. It can also be seen that even in the absence of the chemotactic factor in the lower compartment, increased cell migration occurred, in proportion to the concentration of DNP (Table I).

If DNP was added, instead, to the lower compartments of the chambers, the highest concentration (10^{-3} M) caused increased nonspecific cell migration (count of 234) as well as augmentation in the response to the chemotactic factor, probably by diffusion of the DNP into the upper compartment (count of 481, Table II).

Influence of Vitamin A on Chemotaxis in Vitro.—The acetate and aldehyde preparations of vitamin A were tested. Only in relatively high concentrations,

TABLE V

Effect of Chloroquine and DNP on Immunologic Vasculitis in Guinea Pigs

Animal No.	Treatment	$C'H_{50}$*	PMN counts‡	Gross (edema/erythema)§	Skin sites		
					PMN's	BSA	C'
1		278	16,830	±/2	0	0	0
2	Chloroquine, 20 mg intramus-	256	7,590	2+/3	±	1+	±
3	cularly time zero and at 1 hr	204	ND	1+/2	1+	1+	1+
4		204	10,890	±/0	±	0	±
5	Chloroquine, 20 mg 30 min be-	ND	4,250	±/0	±	2+	2+
6	fore and 30 mg intramus-	ND	5,840	±/trace	2+	3+	3+
7	cularly at time zero	ND	8,100	1+/0	±	2+	2+
8		212	11,880	4+/8	4+	3+	3+
9	DNP, 5 mg intramuscularly at	430	12,200	3+/4	3+	4+	3+
10	time zero and at 1 hr	276	6,600	4+/4	4+	4+	3+
11	None	216	11,220	4+/7	3+	4+	3+
12	None	ND	9,200	4+/9	3+	3+	3+

ND, Not done.

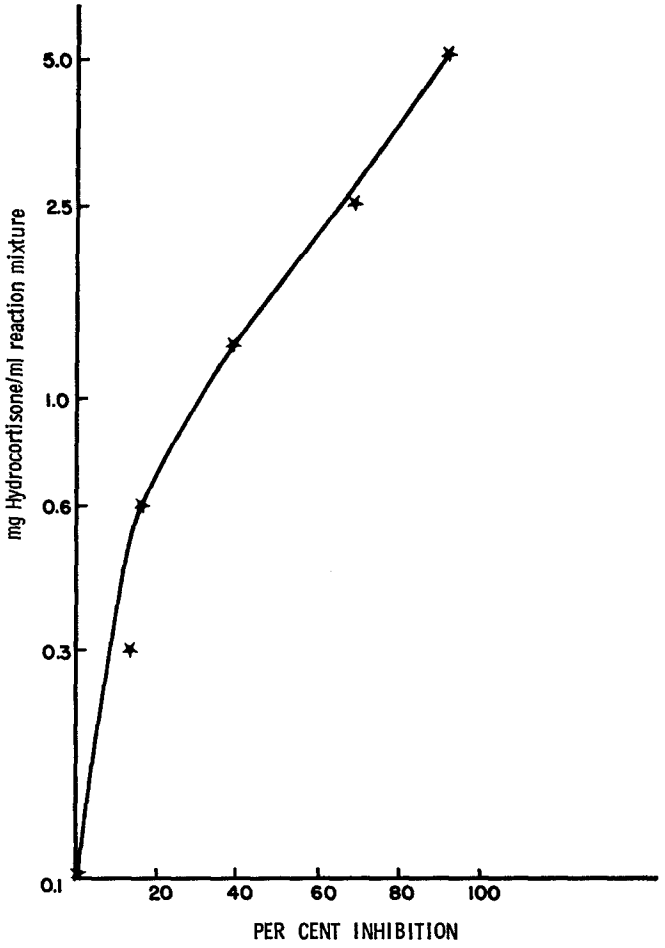
* Determined on sera obtained at the time of sacrifice.

‡ Absolute counts in blood, per cubic mm.

§ Edema graded from ± to 4+; erythema measured as mm diameter.

0.12 M with vitamin-A acetate and 0.07 M with vitamin-A aldehyde, was inhibition of chemotactic activity noted (Table III). In the former case the amount of ethanol required (3%) to maintain solubility of the acetate preparation could account for the drop in counts (to 209), since in previous experiments it was found that such a concentration of ethanol reduced the cell response by a value of 20%. However, at high concentrations (0.07 M and greater) the aldehyde preparation caused diminution of chemotaxis which could not be attributed to the presence of ethanol in the preparation. The vitamin-A acetate preparation at 0.048 M concentration caused a twofold enhancement of chemotaxis. At lower concentrations, no effect by either vitamin-A preparation on the chemotactic capacity of PMN's was noted.

Inhibitory Effect of Hydrocortisone on PMN Migration in Vivo.—Using the immunologic vasculitis model to determine whether hydrocortisone influences the ability of PMN's to migrate to deposits of antigen and antibody in vivo,



TEXT-FIG. 3. Inhibition of serum C′ activity by hydrocortisone.

reversed passive Arthus reactions were induced in guinea pigs. Varying amounts of hydrocortisone were given intravenously. In addition, in each animal two skin sites were prepared: one with anti-BSA alone, the other with the antibody mixed with 10 mg hydrocortisone. In animals treated systemically with hydrocortisone, inhibition of all skin sites receiving anti-BSA alone was noted (Table IV). Edema was minimal and, although circulating antigen (BSA), as well as

C'3, was localized in vascular structures, the degree of PMN infiltration was significantly reduced, compared with control animals not receiving hydrocortisone intravenously (Figs. 1 and 2). At the skin sites receiving anti-BSA mixed with hydrocortisone, reactions were inhibited, regardless of whether or not systemic treatment with hydrocortisone had been employed. Although, paradoxically, more edema was noted as compared with the sites receiving hydrocortisone-free anti-BSA, microspically few PMN's were present. The failure in migration of leukocytes was not due to the lack of fixation of circulating BSA and C', since both were readily demonstrable by fluorescence microscopy in vessel walls (Table IV). Correlating with this was the observation that serum C' levels were not significantly altered. The possibility that the inhibition could be attributed to insufficient levels of circulating PMN's was ruled out by the finding that the largest dose of hydrocortisone failed to reduce numbers of circulating PMN's. In fact, there was a substantial rise in the absolute PMN counts (Table IV).

Immunologic Vasculitis in Animals Treated with Chloroquine and DNP.—Treatment of guinea pigs with chloroquine resulted in significant inhibition of Arthus reactions. In 5 of 7 animals immunological reactants (circulating BSA and C') were found fixed in vascular structures of the skin (Table V). In spite of this, edema and erythema were minimal, and PMN infiltrates were barely detectable or completely absent in these animals (Fig. 3.). Levels of serum C' were not reduced, and there appeared to be adequate numbers of available circulating PMN's (Table V). In contrast with those results, treatment of guinea pigs with DNP did not suppress the Arthus reactions. The skin sites were characterized by severe edema and erythema and by abundant infiltrates of PMN's associated with the local fixation of immune reactants (Fig. 4). In all respects, the DNP-treated animals resembled the control animals (Table V). It is apparent that treatment with chloroquine or hydrocortisone, but not DNP, can lead to suppression of PMN migration into sites containing deposits of antigen, antibody, and C'. As a result, tissue injury is averted.

Inhibition of C' Activity in Vitro with High Levels of Hydrocortisone and Chloroquine.—When either hydrocortisone or chloroquine was present in the reaction mixtures of diluted guinea pig serum, reduction of C' activity could be demonstrated (Text-figs. 3 and 4). The amounts of hydrocortisone and chloroquine required to cause 50% inhibition of C' lysis of sheep red cells were 1.6 mg/ml and 0.95 mg/ml, respectively. If compared with the suppressive action of these same drugs on chemotaxis in vitro, it should be noted that substantially greater amounts of each drug were required for 50% inhibition of C' activity. For hydrocortisone, about 10 times greater drug concentration was required, while approximately 250 times more chloroquine was needed.

In separate experiments it was found that if erythrocytes were treated with large concentrations of hydrocortisone (20 mg/ml) before or during sensitization

with amboceptor, and then washed, they were equally active in the hemolytic system after exposure to C′. This indicates that inhibition in lysis of erythocytes by hydrocortisone is not due to an irreversible effect on the red cell membrane.

Inhibition of Phagocytosis by Corticosteroids.—Using the criteria of the percentage of cells with phagocytized zymosan particles as well as average counts of particles in positive cells, it was demonstrated that relatively high doses of

TABLE VI

Effect of Corticosteroids on Phagocytosis by PMN's

Amount of corticosteroid added*	Phagocytosis-positive cells (particles/cell)					
	Amount of zymosan added, *mg*					
	1		0.25		0.06	
	Per cent‡	Average§	Per cent	Average	Per cent	Average
mg						
Hydrocortisone						
20	0		0		0	
10	36	1.3	16	1.6	0	
5	81	2.4	34	2.3	15	1.7
1	96	4.1	25	1.7	21	2.0
None	98	6.1	64	4.0	20	1.6
None	97	5.8	75	3.0	20	1.6
Methyl prednisolone						
4.0	62	1.8	17	1.9	30	2.0
1.0	88	3.0	65	3.1	24	1.5
0.5	92	4.8	76	4.2	38	1.7
0.25	100	6.7	87	4.3	30	2.6
None	100	6.5	66	3.3	30	3.5
None	100	6.6	71	3.9	36	3.6

* Reaction volume of 1.4 ml.

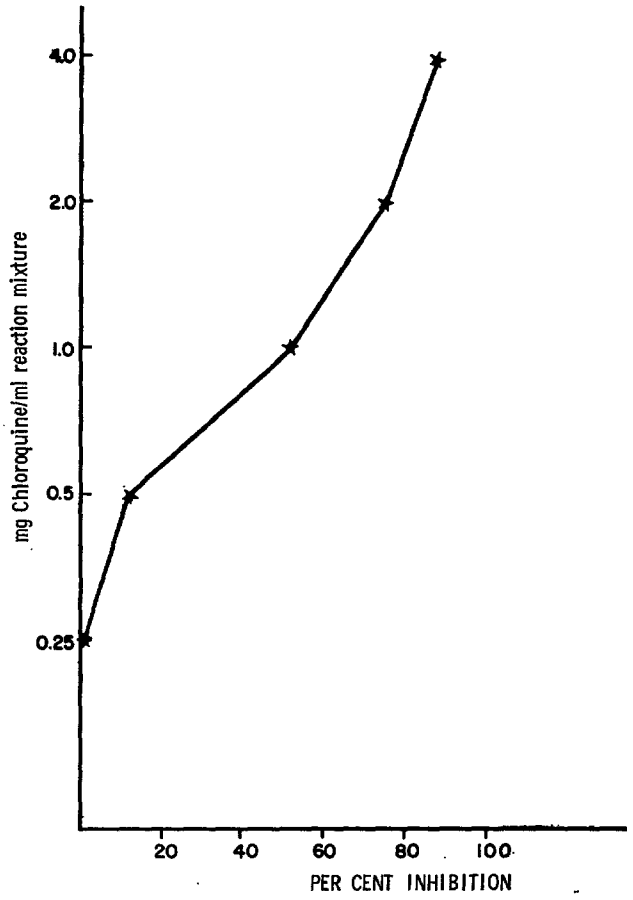
‡ Percentage of cells containing zymosan particles/200 PMN's.

§ Average number intracellular zymosan particles per positive cell.

either hydrocortisone or methyl prednisolone led to diminished phagocytosis (Table VI).

The results were also dependent upon the amount of zymosan added to the PMN suspension in that the inhibition of phagocytosis by methyl prednisolone was not consistent at the lowest dose of zymosan. In order to suppress phagocytosis, substantially larger amounts of hydrocortisone or methyl prednisolone were required, compared with inhibitory doses of steroids in chemotaxis. In addition, a clear difference in the efficacy of hydrocortisone and of methyl prednisolone for suppression of phagocytosis was not apparent in this system.

Phagocytosis in the Presence of Chloroquine, DNP, and Endotoxin.—Chloroquine was an effective suppressor for phagocytosis of zymosan particles by PMN's (Table VII). About 50% inhibition of phagocytosis (by cell numbers as well as particles taken up per cell) could be induced by concentrations of chloroquine between 10^{-3} and 10^{-4} M, again requiring substantially greater inhibitory concentrations than in the chemotaxis system. On the other hand, neither DNP nor endotoxin inhibited phagocytosis of zymosan particles.



TEXT-FIG. 4. Inhibition of serum C′ activity by chloroquine.

DISCUSSION

Employing the quantitative assay technique for chemotaxis, the data presented in this paper clearly suggest that hydrocortisone and chloroquine inhibit

the inflammatory response by a *direct* action on PMN's, rendering the cells incapable of responding to the chemotactic factor. There is also strong suggestive evidence that allergic inflammation (i.e. experimental immunologic vasculitis) is suppressed by a similar effect of these drugs on PMN's, since hydrocortisone and chloroquine did not interfere with the fixation of circulating antigen and C′ to antibody that had been locally injected (Table IV). It is likely that the

TABLE VII

Effect of Chloroquine, DNP, and Endotoxin on Phagocytosis by PMN's

Substance added*	Phagocytosis-positive cells (particles/cell)					
	Amount of zymosan added, *mg*					
	1		0.25		0.06	
	Per cent‡	Average§	Per cent	Average	Per cent	Average
None	100	7.6	100	5.4	93	4.3
None	100	7.0	100	6.4	94	3.3
Chloroquine 10^{-3} M	43	1.3	18	1.3	20	1.7
10^{-4} "	89	2.2	66	1.7	29	1.8
10^{-5} "	97	5.3	93	2.1	80	3.1
10^{-6} "	100	7.8	92	4.2	86	4.0
DNP 10^{-3} M	100	7.7	96	5.8	91	5.3
10^{-4} "	100	9.4	100	5.4	93	4.6
10^{-5} "	100	8.0	100	6.7	87	4.1
10^{-6} "	98	5.9	100	5.1	95	4.6
Endotoxin 100 μg	100	7.4	100	7.5	89	5.2
10 "	100	8.1	100	6.6	92	5.0

* Reaction volume of 1.4 ml.

‡ Percentage of cells containing zymosan particles/200 PMN's.

§ Average number intracellular zymosan particles per positive cell.

well documented suppressive effects of adrenal corticosteroids on "nonspecific" inflammatory reactions are the same as those under study in this report. Although inhibition of C′ activity by hydrocortisone has been emphasized (15) and is confirmed here, the relatively large concentration of steroid required, as well as the evidence that C′ levels in our experimental guinea pigs (Table IV) were unaffected (both by activity of serum C′ and fixation of C′ to antigen-antibody deposits in tissues), is strong evidence against the view that hydrocortisone and chloroquine suppressed the inflammatory response by blocking C′.

Some investigators have shown that corticosteroids reduce the adherence of PMN's to damaged endothelium, thus interfering with the subsequent migra-

tion of PMN's to extravascular sites (11, 13, 19). The findings presented in this paper suggest that hydrocortisone, by a direct action, renders PMN's incapable of responding to chemotactic stimuli. The data of Weissmann and Thomas have suggested that hydrocortisone can act directly upon PMN's by stabilizing lysosomes, thus rendering sequestered proteolytic enzymes enclosed within membranes unavailable for release and resultant production of tissue damage (20, 21). It is possible that such enzymes are also necessary for PMN's to respond to chemotactic stimuli, and, when the availability of these enzymes is curtailed by drugs, the leukocytes are unable to respond in the usual manner. It is rather interesting that the same drugs that stabilize lysosomes, i.e., hydrocortisone and chloroquine, inhibit chemotaxis, and at rather similar concentrations. In the chemotactic system, concentrations of 2.9×10^{-4} M hydrocortisone and 8.4×10^{-6} M chloroquine caused 50% inhibition, while Weissmann reports effective concentrations of 5×10^{-4} to 1×10^{-6} M for cortiscosteroids (depending upon the solvent employed) and 1×10^{-4} to 1×10^{-5} M for chloroquine (22, 23). Additional evidence to suggest that availability of lysosomal contents within PMN's is necessary for the response of these cells to chemotactic stimuli comes from the findings herein reported that vitamin A, DNP, and endotoxin, all labilizing agents of lysosomes (22), do not inhibit chemotaxis. In fact, these agents may enhance chemotaxis. The evidence to incriminate lysosomes with an essential role in chemotactic responses of PMN's is, admittedly, indirect. It can be argued that the effect on lysosomal membranes by the various drugs implies a similar direct effect on other cell membranes that may be involved in the cell response to the chemotactic factor. Such a possibility cannot be excluded at this time.

The inhibitory effects of hydrocortisone, methyl prednisolone, and chloroquine on phagocytic capacities of PMN's is yet another demonstration of a derangement within the PMN's caused by these drugs. While inhibition of phagocytosis was not nearly as impressive, as judged by effective drug concentrations, the correlation between inhibition, or lack of inhibition, by drugs in the two systems (chemotaxis and phagocytosis) is rather striking. It could be argued that the interference with phagocytosis by hydrocortisone and chloroquine is due to the C′-inhibiting properties of these drugs. In the case of hydrocortisone this cannot be ruled out. With chloroquine, however, a concentration of drug that is clearly inhibitory for phagocytosis (10^{-4} M, or 0.05 mg/ml) has no inhibitory activity for the serum C′ system. In the case of chloroquine, then, it seems likely that inhibition of phagocytosis is due to a direct effect of the drug on the PMN, rather than interference with some phagocytosis-promoting factor present in serum such as C′.

Hydrocortisone and chloroquine inhibit chemotaxis in vitro and in vivo, as demonstrated in this paper. The direct action of these drugs on PMN's may have significant clinical implications. In view of the various events in allergic

inflammation culminating in tissue damage (24), several approaches to treatment of immunological diseases can be considered. Following the combination of antigen with host antibody, complement is fixed, resulting in the generation of chemotactic factor consisting of a protein-protein complex of altered components of C′ (3, 4). As a result, circulating PMN's are attracted to the antigen-antibody-C′ deposits (1–3) and subsequently release tissue-damaging enzymes. Starting with the first step in this sequence, the synthesis of antibody can be turned off with cytotoxic and lympholytic drugs (25), agents interfering with protein synthesis, or by the administration of specific antibody itself (26). Next, systemic treatment that renders C′ unavailable will block the chain of events by preventing the release of chemotactically active material in tissues and thus the influx of PMN's to antigen-antibody deposits (1). By a direct attack on leukocytes, levels of circulating PMN's can be reduced with cytotoxic drugs or antibody to leukocytes, and tissues damage will be averted (1, 2, 5–8). As demonstrated in this paper, the therapeutic approach can also be directed at the PMN's per se, not diminishing their available numbers but altering them so that their response to chemotactic stimuli and their migration to areas containing immunologic deposits are blocked. Effective blockade at *any one* of these steps results in abrogation of the end-stage tissue damage that is due to release of lytic enzymes from accumulated PMN's. A better understanding of why and how PMN's respond to the chemotactic factor and how drugs interfere in this sequence will probably result in more effective and specific methods for the treatment of conditions that are mediated by the pathways described above.

SUMMARY

The effects of various drugs on chemotaxis of polymorphonuclear leukocytes (PMN's) in vitro and in vivo have been studied. Response of rabbit PMN's in vitro to the chemotactic factor of rabbit serum, consisting of an activated protein-protein complex of the fifth and sixth (and probably seventh) components of complement (C′), is suppressed by hydrocortisone, methyl prednisolone, and chloroquine. Drug concentrations causing 50% inhibition of chemotaxis in vitro were found to be: hydrocortisone, 2.9×10^{-4} M; methly prednisolone, 1.2×10^{-4} M; and chloroquine 8.5×10^{-6} M. The hydrocortisone effect on PMN's appeared to be irreversible, since washing of the cells did not restore their chemotactic response. 2, 4-Dinitrophenol (DNP), vitamin A, and endotoxin did not inhibit chemotaxis. Hydrocortisone and chloroquine did block serum C′ activity in vitro, but only at substantially higher concentrations.

Using the reversed passive Arthus reaction in guinea pigs as a model for chemotaxis in vivo, systemic treatment of animals with hydrocortisone or chloroquine inhibited development of the vasculitis. Circulating antigen and C′ were fixed in vascular structures, and serum C′ was not perceptibly altered. Nevertheless, PMN infiltrates failed to occur. Local administration of hydrocortisone

also prevented influx of PMN's in the Arthus reaction, in spite of the fact that immune reactants were found fixed in the vascular walls. Systemic treatment of guinea pigs with DNP did not diminish the intensity of the Arthus reactions.

Phagocytosis of zymosan particles by rabbit PMN's was inhibited by hydrocortisone, methyl prednisolone, and chloroquine, but not by DNP or endotoxin. The concentrations of drugs inhibitory in phagocytosis were substantially higher than those required for inhibition of chemotaxis in vitro.

These findings suggest that hydrocortisone and chloroquine inhibit the inflammatory process by preventing the response of leukocytes to chemotactic stimuli.

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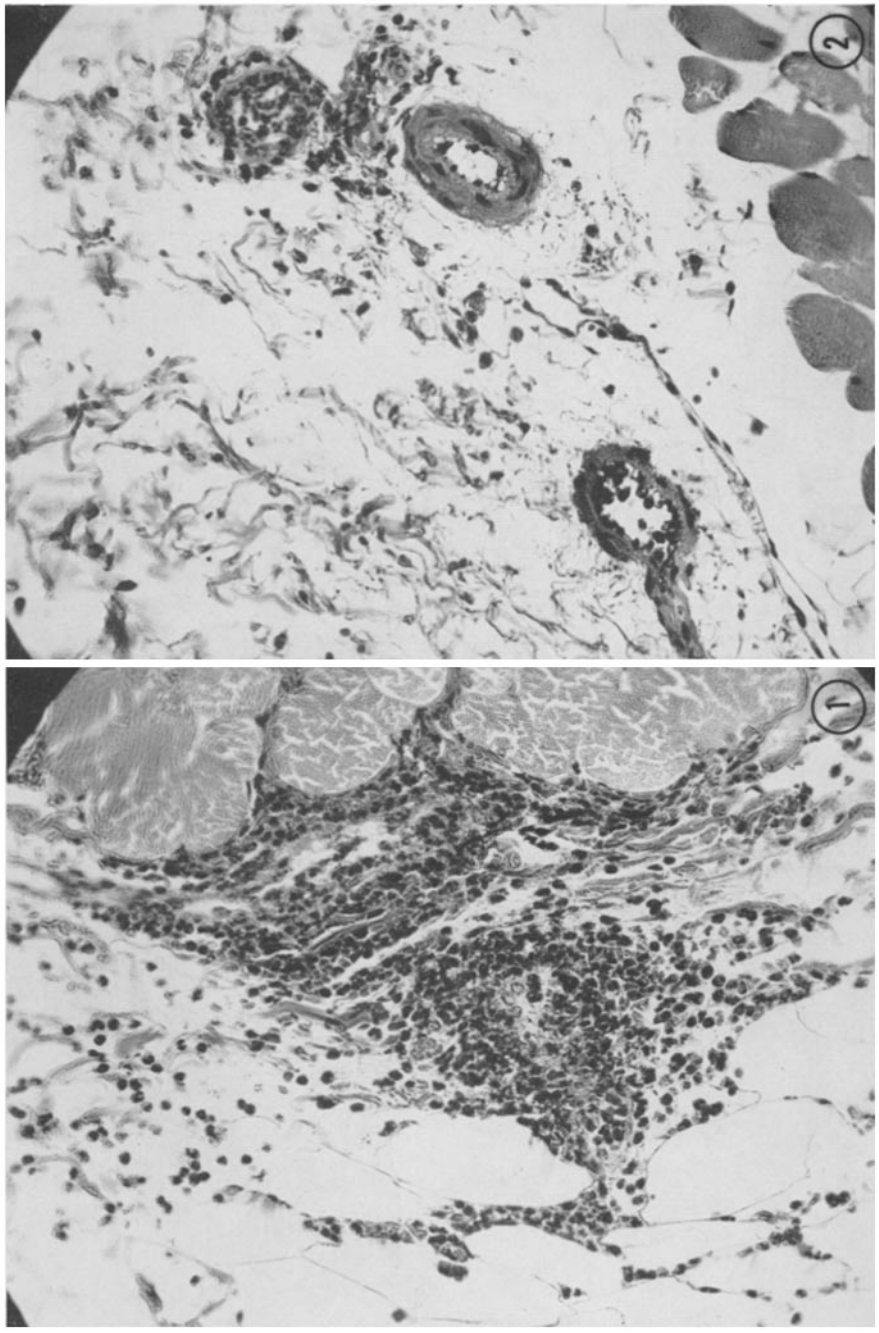
EXPLANATION OF PLATES

PLATE 15

FIG. 1. Arthus reaction from a normal control guinea pig. Walls of vessels adjacent to skeletal muscle contain abundant infiltrates of PMN's, which also extend into adjacent connective tissue. In other areas moderate hemorrhage is present. By fluorescence microscopy immune reactants (antigen, antibody, and complement) were found in walls of vessels containing the PMN infiltrates. Hematoxylin and eosin, × 265.

FIG. 2. Arthus reaction in a guinea pig given hydrocortisone intravenously (animal 5, Table IV). There is minimal edema of subcutaneous connective tissue, and minimal infiltrates of PMN's are seen in the wall of one vessel. Hemorrhage is absent. By fluorescence microscopy, immune reactants were found in nearly all vessel walls. In spite of this, few PMN's appeared. Hematoxylin and eosin, × 265.

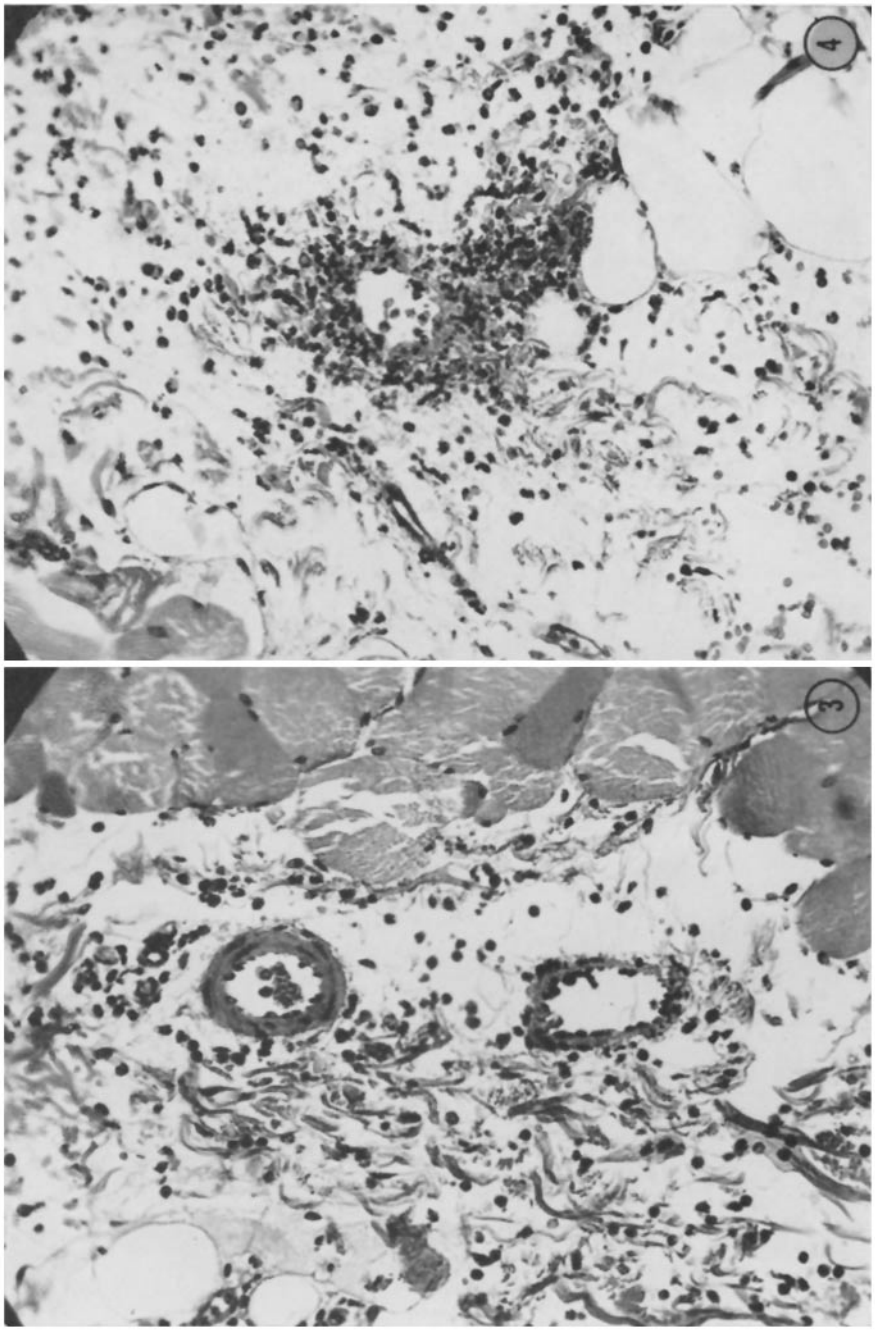


(Ward: Chemosuppression of chemotaxis)

PLATE 16

FIG. 3. Arthus reaction in a chloroquine-treated guinea pig (animal 5, Table V). Although a few PMN's are scattered in the connective tissue, few PMN's are found in the walls of the vessels. Hematoxylin and eosin, × 265.

FIG. 4. Skin reaction in a guinea pig treated with DNP. The acute, hemorrhagic vasculitis is similar in all respects to that found in the normal control animals (see Fig. 1). Hematoxylin and eosin, × 265.



(Ward: Chemosuppression of chemotaxis)