Influence of Cortisone on Experimental Viral Infection. II. Effects on Antibody Formation and Acquired Immunity.* (22136)

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It has been demonstrated that infection of a number of mammalian species with a variety of microorganisms is altered to the detriment of the host by cortisone(see reviews(1,2)). It has also been established with less unanimity that the formation of humoral antibody to non-replicating antigens is suppressed by the administration of cortisone(3,4). However, few studies(5,6) have been published of the influence of cortisone on the development of circulating antibody during actual infection. Perhaps the rapidly lethal outcome of most experimental infections studied precludes the formation of detectable antibody unless antimicrobial therapy is used to arrest infection (5,6).

In the present study, 2 avirulent influenza A virus strains capable of multiplication to high titer without the concomitants of pneumonia or death have been used to permit study of antibody formation resulting from unrestricted multiplication of a viable antigen. In a companion experiment, study has been made of the effectiveness of the immunity so acquired in resisting challenge with a virulent strain of one of the viruses used in immunization.

Materials and methods. Mice. CFW mice which averaged 20 g in weight were employed in groups of 6-21 as indicated in the protocols. Mice were inoculated intranasally with viral suspensions while under light ether anesthesia. Blood for antibody determinations was removed under chloroform anesthesia by cardiac puncture exsanguination. *Viruses.* The 1947 FM-1 influenza A strain was employed in two forms: 1) as unadapted first mouse lung passage virus (10% mouse lung suspension), and 2) as a 10% mouse lung suspension of 29th passage mouse-adapted virus. A 1950 non-mouse-adapted influenza A virus (Rockefeller Institute JB strain)(7) was also employed as undiluted allantoic fluid. *Cortisone.* Cortisone was used in the form of cortisone acetate (Cortone® Merck); the aqueous vehicle used in its suspension was employed for the subcutaneous injection of control animals.‡ *RDE.* The filtrate of a 48-hour neopeptone broth culture which had been inoculated with the Inaba strain of *Vibrio comma* and incubated at 37°C was used as receptor destroying enzyme to remove serum inhibitor. *Viral titrations.* Assay of pulmonary virus was accomplished by inoculation of four 10-day-old White Leghorn embryos with the indicated dilution of mouse lung. Allantoic fluids were harvested after 40-48 hour incubation at 35°C and titered for hemagglutinin at 1:4 dilution with 1% human "O" RBC. MID_{50} (mouse infective dose) was determined as previously described(7). *Scoring of pulmonary lesions.* When it became apparent that cortisone did not kill control mice under the conditions employed, the conventional maximum score method(8) (which considers mortality) was used in grading pulmonary lesions, but with a percentile expression of results to facilitate comparison among mouse groups of varying size. *Antibody titrations.* Sera were heated at 56°C for 30 minutes prior to their use. Neutralizing antibody was measured by the mouse-egg technic which can determine neutralization in mice of non-mouse-adapted virus (7). Hemagglutination-inhibiting antibody was measured by a method described earlier (9) after prior incubation of sera with equal volumes of RDE for 18 hours. Human "O" RBC in initial concentrations of 1% were used.

Results. Effect of cortisone on formation of antibody in mice infected with influenza A

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TABLE I. Effect of Varying Doses of Cortisone on Formation of Neutralizing Antibody in Mice Infected with Influenza A Virus (JB Strain).

Serum from mice inj. with:		Neutralizing antibody titer v.s.	
Cortisone (mg)	Virus	50 MID_{50}*	5000 MID_{50}
0	+	>32†	12
5	+	>32	12
1	+	>32	6
2.5 q 3 days × 6	+	6	<4
5	0	<4	<4

* Mouse infectious doses.
† Reciprocal of serum dilution 50% neutralization.

viruses. Formation of neutralizing or hemagglutination-inhibiting antibody was suppressed during infection of cortisone-injected mice with either of 2 strains of influenza A virus. In one experiment groups of 18-21 mice were killed with chloroform and bled from the heart 19 days following the intranasal inoculation of the JB strain of influenza A virus and the administration of cortisone or control material. The results of titrations of serum neutralizing antibody are presented in Table I. Definite suppression of neutralizing antibody formation followed prolonged cortisone dosage initiated on the day of infection, but was not evident after single injections of 1 and 5 mg of cortisone administered at the time of viral inoculation.

In a second experiment, mice were inoculated with unadapted FM-1 (1947) virus, then subjected to either of two cortisone regimens, as shown in Table II. One schedule consisted of administration of 5 mg doses on the day preceding, the day of, and the day after virus inoculation; the second schedule involved the protracted administration of smaller doses as used in the first experiment. Pooled sera (15 mice/group) were obtained on the 18th day and titrated for hemagglutination-inhibiting antibody. Results are shown in Table II. As in the first experiment, only prolonged administration of cortisone inhibited development of antibody.

Effect of cortisone on acquired active immunity against FM-1 virus. Six groups of 6 mice each were inoculated intranasally with avirulent non-adapted FM-1 virus or saline (Table III). Fourteen days later mice were challenged with mouse-virulent FM-1. Mice received concomitant injections of cortisone as indicated. No fatalities occurred in mice previously infected with FM-1, indicating that such mice possessed at least partial immunity when compared with mice not previously infected with virus. However, pulmonary lesions involving 33% of total lung volume were found in mice given large amounts (15 mg) of cortisone at the time of challenge. It is also of interest that two groups of mice given cortisone (5 or 15 mg) had demonstrable pulmonary virus 7 days after challenge, in contrast to mice injected with saline, who were found to have none. Thus, cortisone in large doses may lower acquired host resistance to allow viral multiplication, and result in the appearance of manifest disease, as well.

Discussion. To the author's knowledge the present study is the only published investigation which describes inhibition by cortisone of antibody formation during infection. A study by Kass *et al.*(10) with a virulent influenza A strain failed to demonstrate humoral antibody in either control or cortisone-treated mice 6 days after inoculation—leaving the question of antibody inhibition unanswered. Studies of streptococcal infection in rabbits(5) and man(6) have shown no suppression of antistreptolysin O formation with cortisone. Germuth *et al.*(11) found no interference by cortisone with the development of an immune response to pneumococcal infection of the rabbit, but made no study of circulating antibody.

The demonstration in the present study

TABLE II. Effect of Varying Doses of Cortisone on Formation of Hemagglutination-Inhibiting Antibodies in Mice Infected with Influenza A (FM-1) Virus.

Serum from mice inj. with: Cortisone (mg)	Virus*	Antibody titers†
0	+	64
5 × 3	+	32
0	0	<8
5 × 3	0	<8
2.5 q 3d × 6	+	<8

* 10^6 EID_{50}.
† Reciprocal of highest dilution of serum which inhibited hemagglutination with 8 hemagglutinating units of virus.

TABLE III. Effect of Cortisone on Aquired Active Immunity against FM-1 Virus; Challenge of Immunized Mice with Virulent Virus.

Day 1	Day 14		Day 21		
Initial inoculation	Challenge inoculum*	Cortisone (mg)	D/T†	Lesions, maximum score (%)	Virus‡ in lungs
FM-1 (1st mouse pass.)	FM-1 (29th pass.)	5	0/6	10	+
"	"	0	"	0	0
"	"	5 × 3§	"	33	+
Saline	FM-1 (29th pass.)	0	4/6	72	
"	"	5	4/5	80	
FM-1 (1st mouse pass.)	Saline	0	0/6	3	0

* 10^5 MID_{50}. † No. of dead mice/total in group. ‡ +, 10^{-2} lung suspension infects eggs; 0, 10^{-2} lung suspension does not infect eggs. § On days 13, 14, 15.

that antibody suppression is dependent on cortisone dosage suggests an explanation for the inhibition of antibody formation noted here but not observed by others. Species difference would not appear to be an important factor in view of evidence of profound inhibition by cortisone in the rabbit of antibody formation to non-multiplying antigens(3,4).

The present study has demonstrated that cortisone may suppress the formation of antibody during the course of a viral infection, and further, that the immunity conferred by infection may be partially surmounted by administration of cortisone with a challenge inoculum. However, reduction in pre-existing acquired immunity is effected by cortisone dosages inadequate to suppress antibody formation. Thus, the persistence of pulmonary virus and the induction of pneumonia in immune mice given cortisone cannot be ascribed to effects on circulating antibody. These findings are in accord with previous observations that 1) lethal infections may be rapidly induced with cortisone in naturally resistant hosts(5,12) and 2) that increased viral multiplication and mortality may be effected by cortisone in a host (the chick embryo) incapable of demonstrable antibody formation(13).

It is conceivable that the demonstrated multiplication or persistence of virus in an immune host may be explicable on the basis of cortisone-induced reactivation of pre-existing pulmonary virus, but thus far the phenomenon of viral reactivation with cortisone has been studied only in the chick embryo(14).

Summary. 1) The formation of neutralizing and hemagglutination-inhibiting antibodies was suppressed by cortisone administered during infection of mice with influenza A viruses. 2) Suppression of antibody was dependent on prolonged administration of cortisone and was not effected by large doses given at the onset of infection. 3) Acquired active immunity induced by infection of mice with avirulent virus was partially surmounted by administration of cortisone with a challenge inoculum of virulent virus. 4) Cortisone in doses insufficient to suppress antibody formation induced lesions and persistence of pulmonary virus following challenge of mice with previously acquired immunity.

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