Effect of Cortisone on Mumps Antibody Formation in Rabbits. (20136)

JOHN R. OVERMAN AND ROBERT HANAN. (Introduced by Karl Habel.)

From the National Institutes of Health, National Microbiological Institute, Laboratory of Infectious Diseases, Bethesda, Md.

It is well established from the work of Germuth(1) and Bjorneboe(2) that, in the rabbit, the administration of cortisone is capable of suppressing the production of antibody to crystalline protein and bacterial antigens. It is the purpose of this paper to extend these observations to the effect of cortisone on the development of antibody in the rabbit to a viral antigen. In order to relate such studies to previous experiments on the cortisone effect, rabbits were simultaneously immunized with mumps vaccine* and with crystallized bovine serum albumin (BSA).† Antibody to BSA was measured by the quantitative precipitin technic and antibody to mumps virus by 3 different serologic tests. The data form, then, a comparative study of the antibody response to 2 different types of immunological stimulus, as measured by several different means, in normal and cortisone treated rabbits.

Materials and methods. Immunization and cortisone schedules. Over a period of 2 weeks, 12 male albino rabbits weighing approximately 2 kg were given three 1 ml intramuscular injections of mumps vaccine, spaced 7 days apart. During this same period these rabbits were also given 7 intraperitoneal injections of alum-precipitated BSA for a total of 10 mg BSA. Beginning 3 days prior to the immunization period and continuing through and for 6 days afterwards, 6 of the 12 animals were daily given 4 mg of cortisone (Cortone, Merck) intramuscularly. The cortisone treated animals remained in apparent good health and showed no appreciable weight loss. All rabbits were bled from the ear prior to and at 7, 14, 21, 42, and 46 days after the immunization period. *Complement-fixation test.* Lederle mumps vaccine was used as antigen. In the test 2 units of antigen were used, usually necessitating a 1:8 dilution of the vaccine. The test was carried out in a final volume of 1 ml made up of 0.2 ml each of antigen, complement and serum and 0.4 ml of sensitized sheep cells. The end point was taken as the serum dilution producing 3+ or greater fixation of complement after incubation at 37°C for one hour and is expressed as the initial serum dilution. *Antihemagglutination test.* The procedure used was that reported by Robbins, Kilham, Levens, and Enders(3), except that incubation was for one hour at 37°C rather than overnight at 4°C. All sera used in the test were pretreated with cholera vibrio filtrate prepared as follows: a tryptose phosphate broth culture of the *Inaba* strain of *V. comma* was incubated at 37°C for 16 hours. The broth was then passed through a Seitz filter and, if sterile, stored at 4°C until used. In the test, serum and filtrate were mixed 1:5, incubated at 37°C overnight, then incubated at 56°C and used immediately. Dilutions are expressed as the final serum dilution. Treatment with the cholera filtrate efficiently removed the nonspecific mumps antihemagglutinin of rabbit serum without affecting the specific antibody titer. *Virus neutralization.* The Enders strain of mumps virus was cultivated in embryonated eggs and the allantoic fluid harvested, pooled and titered for egg infectivity. Neutralization tests were made with aliquots of this pooled fluid. The sera were inactivated at 56°C for 30 minutes and serial 2-fold dilutions of serum incubated with approximately 1000 egg infectious doses of virus for one hour at 37°C. Each dilution was then inoculated into 8 or more embryonated eggs by the allantoic route. Egg infection was then determined by the presence of hemagglutinin and the egg protective dose (EPD_{50}) was calculated by the method of Reed and Muench(4). *BSA antibody N.* A quantitative precipitin curve was obtained on each serum by adding increasing quantities of BSA to 1 ml portions of the antiserum, and collecting the precipitates after 48 hours in the cold. The precipitates were

* Lederle.

† Armour.

TABLE I. BSA and Mumps Antibody in Control Rabbits.

Rabbit No.			Pre-imm. bleeding	Bleeding–days after last dose of antigens 7	14	21	42
7	BSA Ab	*	—	165	248	155	—
	Mumps Ab	HI	<20	320	160	80	80
		CF	ac	16	16	< 4	<4
		Ne	< 5	20	18	<10	—
8	BSA Ab	*	—	91	121	76	—
	Mumps Ab	HI	<20	320	160	80	80
		CF	ac	16	16	< 4	<4
		Ne	< 5	12	13	<10	—
9	BSA Ab	*	—	202	218	147	—
	Mumps Ab	HI	<20	640	640	320	160
		CF	< 4	32	16	4	<4
		Ne	< 5	38	24	14	—
10	BSA Ab	*	—	111	276	153	—
	Mumps Ab	HI	<20	640	640	320	320
		CF	< 4	32	16	8	4
		Ne	< 5	40	37	11	—
11	BSA Ab	*	—	226	397	304	—
	Mumps Ab	HI	<20	640	320	160	160
		CF	< 4	32	16	4	<4
		Ne	< 5	32	21	<10	—
12	BSA Ab	*	—	204	265	193	—
	Mumps Ab	HI	<20	640	320	160	160
		CF	< 4	32	16	4	<4
		Ne	< 5	30	13	<10	—

* $\frac{\mu g \text{ Ab N}}{\text{ml antiserum}}$

HI = antihemagglutinin titer; CF = complement fixation titer; Ne = neutralization titer; ac = anticomplementary.

washed twice with cold saline and the N then determined by a micro-Kjeldahl procedure. The supernates were analyzed for antigen and antibody.

Results. The cortisone-treated animals had much lower levels of circulating anti-BSA than did the controls (Tables I and II). At the 7-day interval the cortisone rabbits had only about 25% of the antibody in the controls. In further agreement with the work of Germuth(1) were the results of an Arthus reaction done on all animals on the 7th post-immunization day, prior to bleeding. Injection of 1 mg of BSA intracutaneously resulted in much larger reactions in the control than in the cortisone treated group.

The mumps antibody shows a somewhat different picture. The control series showed that all the animals responded to the mumps vaccine in a fairly uniform manner, as measured by any one of the 3 serologic tests used. Moreover, there was a good general correlation of results obtained by the 3 methods so that rabbits showing the higher level of mumps antihemagglutinin also showed higher levels of complement-fixing and virus neutralizing antibody. The mumps antibody in the cortisone-treated animals showed levels which, on the average, were lower than those in the control group, but relatively not as low as the BSA antibody. Thus, the averages of mumps antibody measured by hemagglutination-inhibition ranged from 50 to 60% of the averages of the antibody levels in the controls. In considering individual animals, rabbits 3 and 5 had antibody levels very similar to those in the control group, yet the BSA antibody in these same rabbits was markedly suppressed. At 42 days after cessation of cortisone a booster dose of mumps vaccine (0.5 ml) was given and antihemagglutinin tests performed on the sera from all the animals 4 days later. All rabbits showed a rise of antibody to 320 with the exception of rabbit 4, whose response

TABLE II. BSA and Mumps Antibody in Cortisone Treated Rabbits.

Rabbit No.			Pre-imm. bleeding	Bleeding–days after last dose of antigens 7	14	21	42
1	BSA Ab	*	—	14	26	10	—
	Mumps Ab	HI	<20	80	80	40	40
		CF	< 4	4	<4	<4	<4
		Ne	< 5	<5	<5	<5	—
2	BSA Ab	*	—	51	52	32	—
	Mumps Ab	HI	<20	80	80	80	80
		CF	< 4	16	16	8	4
		Ne	< 5	<5	<5	<5	—
3	BSA Ab	*	—	57	77	64	—
	Mumps Ab	HI	<20	640	640	320	80
		CF	< 4	32	32	16	4
		Ne	< 5	17	12	10	—
4	BSA Ab	*	—	68	37	12	—
	Mumps Ab	HI	<20	80	40	40	20
		CF	< 4	4	4	4	4
		Ne	< 5	<5	<5	<5	—
5	BSA Ab	*	—	31	46	22	—
	Mumps Ab	HI	<20	640	320	160	160
		CF	< 4	32	16	8	4
		Ne	< 5	19	17	12	—
6	BSA Ab	*	—	6	9	0	—
	Mumps Ab	HI	<20	160	160	80	80
		CF	< 4	ac	8	4	4
		Ne	< 5	<5	<5	<5	—

* $\frac{\mu g \text{ Ab N}}{\text{ml antiserum}}$.

HI = antihemagglutinin titer; CF = complement fixation titer; Ne = neutralization titer; ac = anticomplementary.

was 80. This indicates that all the animals, when not under the influence of cortisone, were capable of responding to mumps vaccine to about the same degree with the exception noted. Rabbit 4, then, was probably a "poor antibody producer." This animal was included in the averages, but had it been omitted the mumps antibody averages would have been still closer to the control figures.

Discussion. The data obtained in the present experiments confirm the observation that cortisone administered to rabbits suppresses the level of antibody produced to purified protein antigens (BSA) and extends this finding to include antibody to mumps virus vaccine as measured by serologic methods. Under the experimental conditions herein employed, however, it is apparent that the suppression of mumps antibody was not as great as that of BSA antibody. The explanation for this is not clear from the present data. It may be that mumps virus is more antigenic than BSA, but this does not seem likely. Another possible explanation may be the difference in sensitivity of the methods used for detecting antibody. Thus, the serologic methods can only measure variations of 2-fold or greater so that a 40 to 50% lower level of antibody might not be detected serologically but would be readily measured by the precipitin technic. However, we feel that the relative insensitivity of the serologic measurements do not account for the fact that the BSA antibody levels in all the cortisone-treated animals were suppressed to the same marked degree, yet 2 of these animals by 3 different (and repeated) serologic tests showed the same levels of mumps antibody as did the controls. Further studies with antigen-antibody systems to which both quantitative and serologic methods can be applied may clarify this point and such experiments are now in progress.

Summary. 1. Serial antibody studies were performed on normal and cortisone treated rabbits immunized with mumps virus and bovine serum albumin. 2. In rabbits, corti-

sone suppresses development of antibodies to mumps virus as measured serologically, although the suppression is to a relatively lesser extent than that for BSA antibody measured quantitatively.

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3. Robbins, F. J., Kilham, L., Levens, J. H., and Enders, J. F., *J. Immunol.*, 1949, v61, 235.

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Renal Functions During Positive Pressure Respiration. (20137)

P. K. KNOEFEL, C. A. HANDLEY, AND R. A. HUGGINS.

From the Departments of Pharmacology, Baylor College of Medicine, Houston, Texas, and University of Louisville School of Medicine, Louisville.

Although the circulatory changes during respiration at pressures above atmospheric have been extensively studied, the effect of such a procedure on renal functions has not been investigated, except by Drury, Henry, and Goodman(1), who showed that marked reduction in urine volume and urea clearance resulted.

Methods. Eight experiments have been done on dogs in barbiturate anesthesia, in which respiration at pressures from 9 to 31 mm Hg above atmospheric was brought about by connecting the trachea with a large chamber through which air was passed at a rapid rate. Measurement of intratracheal pressure with a Hamilton manometer showed it to be close to the chamber pressure and essentially constant throughout the respiratory cycle. In 7 of the experiments, 1 kidney was denervated by stripping all visible nerves from artery, vein, and ureter. In 3 experiments, the vagus nerves were cut, to avoid respiratory inhibitory reflexes which limited the height to which the pressure could be raised. This did not appear to alter the type of response. In 5 of the experiments, pressure pulses in the aorta were recorded and cardiac outputs calculated from them(2). In all experiments, priming doses and continuous infusions of creatinine, p-aminohippurate, and glucose were given. Blood plasma and urine were analyzed for these substances, and clearances and maximum tubular transfer calculated. In most experiments, plasma and urine samples were analyzed for sodium and potassium by flame photometry, in some, urine chloride only was determined. Venous pressure was measured in some experiments.

Results. The experiments may be divided into 2 groups, one in which the TMg fell, one in which it rose. For the first group, the values, in the innervated kidneys, at maximal change were, in % of control (average, with range) C_{cr} *62* (33-85), C_{pah} *73* (43-100), TMg *70* (50-94). In the second group, these were C_{cr} *88* (63-115), C_{pah} *91* (76-103), TMg *187* (111-193). As an example of group 1, it can be seen in Fig. 1 that on the application of the pressure, the fall in cardiac output is associated with a fall in arterial pressure, but this returns to normal with an increase in total peripheral resistance. While the kidney appears to take part in this reflex vasoconstriction, with parallel decreases in clearances and TM, its response does not develop maximally until the third ten-minute period, by which time cardiac output and total peripheral resistance have returned essentially to normal. When respiration is returned to atmospheric pressure, there is a rise in cardiac output and a fall in total peripheral resistance, and renal functions return to normal, but as can be seen in the values for RF, that fraction which renal blood flow is of total blood flow, the kidney again only slowly takes part in the total change. This lag in response of the kidney was seen in the experiments cited above(1), and may resemble that seen in hemorrhage(3).