EXPERIMENTALLY-INDUCED AGAMMAGLOBULINEMIA IN JAPANESE QUAILS

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SUMMARY: Induction of agammaglobulinemia in Japanese quails was attempted by various procedures of bursectomy (BX) including hormonal BX (H-BX) with testosterone, surgical BX (S-BX), chemical BX (C-BX) with cyclophosphamide and irradiation with γ -ray. Immunosuppressive effect of BX was evaluated by antibody production in response to immunization with bovine serum albumin and sheep red blood cells, by the level of natural antibody, and by the amount of immunoglobulins in sera. As a result, two procedures, i.e. combination of H-BX, S-BX, and C-BX and combination of H-BX, S-BX and irradiation were found to induce most frequently complete agammaglobulinemia in which neither immunoglobulin nor antibody production was detected. Mechanisms of immunosuppression of these BX procedures were discussed from the morphological aspect of development of the bursa.

INTRODUCTION

In avian species the bursa of Fabricius and thymus serve as the central immune organs; the former controls humoral immunity and the latter cell-mediated immunity. Removal of the bursa or thymus selectively suppresses either antibody responses or cell-mediated immunity in chickens. Thus, the bursectomized (BX) chickens provide a unique model of experimental agammaglobulinemia for the study on the role of humoral immunity in microbial infections and in oncogenesis as well as for the study of ontogenesis of the immune system. For these purposes, various techniques of BX have been developed in chickens (Cooper et al., 1966; Warner et al., 1969; Lerman et al., 1970). However, only a few reports have been made in regard to experimental agammaglobulinemia in avian species other than the chicken (Glick et al., 1963; Sugimura et al., 1975).

Although Japanese quails have been extensively utilized for the study of tumor immunity in this laboratory (Yamanouchi and Hayami, 1970, 1971), only surgical BX was employed and no detailed study for production of complete

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agammaglobulinemia has been attempted. In the present study, various procedures of BX were applied to quails, and their suppressive effects on humoral immunity and bursal development were investigated.

MATERIALS AND METHODS

Quails: White eggs of Japanese quails (JQ-NIBS: *Conturnix conturnix japonica*) and quail feeds were obtained from the Nippon Institute for Biological Science, Tokyo, Japan. In each experiment 200–300 eggs were used. A group of 10–20 hatched quails were kept in a brooder-type cage for chicks and fed with quail-feed powder and water *ad libitum*. At 2 weeks of age, they were transferred into special quail cages equipped with an automatic watering device in a group of five birds each and fed with quail-feed pellets.

Hormonal bursectomy (H-BX): Testosterone propionate (TP: Iwaki Seiyaku, Co. Ltd., Tokyo, Japan) was used. In Exp. 3, eggs were dipped in 2% TP solution in ethyl alcohol for a few seconds. In the other experiments, TP was emulsified in fresh yolk of quail eggs added with saline to inhibit coagulation of yolk and inoculated into the white cavity of quail embryos. Unless otherwise specified, 0.05 ml of yolk emulsion containing 0.4 mg of TP was injected.

Surgical bursectomy (S-BX): Within 24 hr after hatching, the bursa was removed surgically with ophthalmological forceps. At the same time, sham operation was done in the control group.

Chemical bursectomy (C-BX): Quails were injected twice intraperitoneally with 0.6 mg of cyclophosphamide (CY: Endoxan, Shionogi Seiyaku, Co. Ltd., Osaka, Japan) dissolved in 0.1 ml of phosphate buffered saline (PBS) at 1–3 and 3–5 days after hatching, except in Exp. 2 in which 0.3 to 1.2 mg CY were employed.

Irradiation: Quails were irradiated with ^{60}Co at a dose of 1,000 rads (r) at 1 week of age. In Exp. 1, doses of 500 to 2,000 r were used.

Immunization: Bovine serum albumin (BSA) and sheep red blood cells (SRBC) were used as antigens to estimate the ability of antibody production. Immunization was conducted by the following schedule except in Exp. 8. A BSA solution of 80 mg/ml was mixed with an equal volume of Freund's complete adjuvant and 0.05 ml of the mixture was injected intramuscularly into each quail at 4 weeks of age. At the same time, 0.1 ml of 10% SRBC suspension was injected intraperitoneally. Two weeks later, quails received the second immunization with a mixture of BSA and SRBC. One week after the booster injection, all the quails were bled and their sera were tested. In Exp. 8, quails were immunized by a single intravenous injection at 4 weeks of age and bled 1 week later.

Titration of anti-BSA antibody: Anti-BSA antibody was measured by the ordinary passive hemagglutination (HA) method with slight modification. Since quails were immunized with SRBC at the same time, guinea-pig red blood cells (GPRBC), that had been confirmed not to contain cross-reacting antigens with

SRBC or with normal quail serum, were used. Coating of GPRBC with BSA was made as follows; four volumes of 10% GPRBC suspension was mixed with one volume of 10% phosphate buffered formalin and left at 37 C for 20 hr. The formalin-fixed GPRBC were quickly frozen in a dry ice-acetone bath and stored at -80 C until used. After thawed, the fixed GPRBC were washed with PBS (pH 7.0) and treated with 1:40,000 tannic acid solution at 37 C for 20 min. One volume of 2% tanned RBC suspension, three volumes of PBS (pH 5.6) and one volume of 2% BSA solution (in PBS at pH 5.6) were mixed and the mixture was left at 37 C for 20 min. The BSA-coated RBC were stabilized by adding normal quail serum and gelatin at final concentrations of 2% and 0.01%, respectively. Titration of the antibody was conducted on microtiter plates. Twenty-five microliters of serial twofold dilutions of the sera were tested by adding an equal volume of 1.5% BSA-coated RBC suspension and titers were expressed as reciprocals of the highest serum dilution giving a definite agglutination pattern after incubation at 37 C for 1 hr.

Titration of anti-SRBC antibody: HA antibody to SRBC was titrated by the same methods as described in passive HA titration except for use of 0.5% SRBC suspension instead of BSA-coated GPRBC suspension.

Titration of natural antibodies: In preliminary experiments, quail sera were examined for natural antibodies against RBC of various mammalian species. HA and hemolytic (HL) antibodies to rabbit RBC (RRBC) were detected at relatively high levels and those to rat and mouse RBC at low levels. Natural antibodies to hamster, guinea pig, sheep, African green monkey, cynomolgus monkey and human RBC were not detected. Natural HA antibody to RRBC was considered to be acquired after hatching, as incidence of this antibody in the sera was 0% at hatching to 3 to 4-week old, 30% at 5-week old, and 100% at 8 to 10-week old, respectively. Natural HL antibody to RRBC was detected at hatching, decreasing until 3 to 4-week old and increasing again at 8 to 10-week old. Therefore, this antibody detected at hatching was considered to be transferred from the mother to the embryos.

Immunoelectrophoresis: Agar (Special agar Noble, Difco Lab., Detroit, MI, U.S.A.) was dissolved at 1.1% (w/v) in commercial barbital buffer (pH 8.6, ionic strength 0.05; Iatron Lab., Inc., Tokyo, Japan) and an agar layer with 1 mm of thickness was prepared in plates. Electrophoresis was conducted under electric current of 3 mA/cm of the agar plate by using rabbit antiserum to quail whole serum. In a preliminary study, identification of quail Ig was attempted in double agar gel diffusion on quail globulin fractions obtained by Sephadex G-200 filtration or precipitation with sodium sulfate by using rabbit antisera to chicken IgG and to chicken IgM. Thus, the pattern of immunoelectrophoresis of quail serum was analyzed as illustrated in Fig. 1. The amount of Ig was roughly estimated by observing the precipitating arcs according to the following criteria; clear and thick arc as ++, weak and thin one as +, trace as ±, and none as -. Typical patterns of the sera with various levels of Ig deficiency are shown in Fig. 2.

Fig. 1. Proteins of normal quail serum analyzed in immunoelectrophoresis by rabbit anti-serum to quail whole serum.

Fig. 2. Typical patterns of the sera with various types of Ig deficiency.

A: agammaglobulinemia. 1 and 2. IgG (-), IgM (-).

H: hypogammaglobulinemia. 1. IgG ($\pm$), IgM (+); 2. IgG ($\pm$), IgM (-); 3. IgG (+), IgM (-).

N: Normal. 1. IgG (++) , IgM (++) ; 2. IgG (++) , IgM (+).

Morphological examinations of the bursa: At autopsy, the bursa was measured and fixed in neutral buffered formalin for histological examinations. The size of the bursa was given a score according to its length (L) and width (W) as follows: score 5, $L > 8.25$ mm and $W \geq 4.25$ mm; score 4, $L \geq 8.25$ mm, $W < 4.25$ mm, or $L < 8.25$ mm, $W \geq 4.25$ mm; score 3, $L = 6.25-8.25$ mm, $W < 4.25$ mm, or $L < 8.25$ mm, $W = 3.25-4.25$ mm; score 2, $L = 4.25-6.25$ mm, $W < 3.25$ mm, or $L < 6.25$ mm, $W = 2.25-3.25$ mm; score, 1, $L < 4.25$ mm; $W < 2.25$ mm.

Degree of development of the lymphoid elements in the bursa was examined morphologically on the tissue sections, and classified as follows; 3, normal; 2, depletion of the lymphoid cells in either the medulla or cortex with a few

TABLE I
Experimental design

Exp.	Group	Procedure
1	A	S-BX+500 r
	B	S-BX+1,000 r
	C	S-BX+2,000 r
	Ct-1	Sham-operated control
	Ct-2	Untreated-unimmunized control
2	A	S-BX+C-BX (0.3 mg)×2
	B	S-BX+C-BX (0.6 mg)×2
	C	S-BX+C-BX (1.2 mg)×2
	Ct-1	Sham-operated control
	Ct-2	Untreated-unimmunized control
3	A	H-BX (dipping) in 2% TP at 3 day
	B	H-BX (dipping) in 2% TP at 6 day
	C	H-BX (dipping) in 2% TP at 10 day
	Ct-1	Untreated control
	Ct-2	Untreated-unimmunized control
4	A	H-BX (injection) 0.2 mg at 5 day
	B	H-BX (injection) 0.4 mg at 5 day
	C	H-BX (injection) 0.3 mg at 10 day
	D	H-BX (injection) 0.6 mg at 10 day
	Ct-1	Untreated control
	Ct-2	Untreated-unimmunized control
5	A	H-BX (injection) 0.4 mg at 5 day
	B	H-BX (injection) 0.4 mg at 8 day
	C	H-BX (injection) 0.4 mg at 11 day
	Ct-1	Untreated control
	Ct-2	Untreated-unimmunized control
6	A	H-BX (injection) 0.4 mg at 4 day+C-BX (0.6 mg)×2
	B	H-BX (injection) 0.4 mg at 7 day+C-BX (0.6 mg)×2
	C	H-BX (injection) 0.4 mg at 11 day+C-BX (0.6 mg)×2
	Ct-1	Untreated control
	Ct-2	Untreated-unimmunized control
7	A	H-BX (injection) 0.4 mg at 4 day+1,000 r
	B	H-BX (injection) 0.4 mg at 7 day+1,000 r
	C	H-BX (injection) 0.4 mg at 11 day+1,000 r
	Ct-1	Untreated control
	Ct-2	Untreated-unimmunized control
8	A	H-BX (injection) 0.4 mg at 3 day+S-BX+C-BX (0.6 mg)×2
	B	H-BX (injection) 0.4 mg at 3 day+S-BX+1,000 r
	Ct-1	Untreated control
	Ct-2	Untreated-unimmunized control

TABLE II
Effect of BX on the rates of hatching and survival

Exp.	Group	Hatching rate*	Survival rate**
1	A		8/33
	B		11/33
	C		4/33
	Ct-1		18/35
2	A		9/35
	B		8/35
	C		2/35
	Ct-1		26/35
3	A	42/50	11/42
	B	40/50	13/40
	C	40/50	14/40
	Ct-1	37/46	7/37
4	A	15/34	8/15
	B	18/37	6/18
	C	18/35	11/18
	D	8/35	3/8
	Ct-1	24/36	17/24
5	A	30/53	17/30
	B	36/49	32/36
	C	35/49	30/35
	Ct-1	41/49	34/41
6	A	30/51	13/30
	B	23/51	11/23
	C	29/50	8/29
	Ct-1	37/50	30/37
7	A	37/64	19/37
	B	26/53	17/26
	C	32/53	26/32
	Ct-1	42/53	36/42
8	A	24/60	7/24
	B	30/60	9/30
	Ct-1	34/40	29/34

* Number of hatched/Total Number of eggs incubated.

** Exp. 1-7: Number of survived at 7 weeks/Number of hatched quails.

Exp. 8: Number of survived at 5 weeks/Number of hatched quails.

number of immature follicles; 1, depletion of the lymphoid cells in both the medulla and cortex with many immature follicles; 0, complete depletion of the lymphoid cells without formation of follicles.

Experimental design: As summarized in Table I, eight experiments each consisting of 4-6 groups treated by various protocols of BX were conducted. Quails were immunized with the mixture of BSA and SRBC twice at 4 and 6

weeks of age or once at 4 weeks of age for evaluation of the suppressive effect of BX on production of antibodies to soluble protein and to particulate antigens. At 7 weeks of age, all the quails were autopsied, their sera were examined for the presence of natural antibody, and the lymphoid tissues histologically.

RESULTS

Effects of BX on the Rates of Hatching and Survival

To obtain basic information on general effects of various BX procedures before practical application, rates of hatching and survival after BX were investigated. As shown in Table II, hatching rates of the groups receiving H-BX by dipping in 3, 6, and 10 days of incubation (Exp. 3) were almost on a normal level, while those in the groups receiving H-BX by injection (Exp. 4-8) were found to become lower than those of the control group.

Survival rates of the groups receiving BX were generally lower than those of the control groups. Especially low survival rates were observed in Exp. 2, 6 and 8 when compared with those of control groups.

Production of Immunoglobulin (Ig) in BX-quails

Effects of various BX procedures on production of Ig are summarized in Table III. Sera with IgM deficiency but with normal levels of IgG were frequently observed in the groups receiving BX, suggesting that immunoelectrophoresis may be relatively insensitive for detection of IgM. Therefore, IgG appeared to be a more suitable parameter than IgM to evaluate the effect of BX on Ig production.

Most of the quails in the untreated and unimmunized control groups exhibited production of both IgG and IgM at normal levels. Normal levels of IgG production were also observed in the three groups receiving H-BX either by dipping or injection with or without irradiation (Exp. 3, 4, 5 and 7). Relatively severe IgG deficiency was demonstrated in Exp. 1-B, 2-B, 6-A, 6-B, 6-C, 8-A, and 8-B. Especially in Exp. 8-A in which H-BX by injection combined with S-BX and C-BX were made, six out of seven quails lacked detectable amounts of IgG.

Production of Antibodies in BX-quails

The effect of BX on antibody production was evaluated by immunization with SRBC as a particulate antigen and BSA as a soluble antigen, and also by demonstration of natural antibody to RRBC. As summarized in Table IV, anti-SRBC antibody was noticed in 8 of 77 (10%) sera in the unimmunized control groups (Ct-2 in Exp. 2, 6, 7 and 8). Immunization of untreated quails (Ct-1 in Exp. 1-8) with SRBC induced production of anti-SRBC antibody in

TABLE III
Effect of BX on Ig production

Exp.	Group	IgG			IgM			Total Number of quails tested
		Normal	HGG*	AGG**	Normal	HGG	AGG	
1	A	6***	1	1	2	2	4	8
	B	1	4	6	2	1	8	11
	C	2	1	1	1	1	2	4
	Ct-1	5	0	0	3	2	0	5
	Ct-2	4	0	0	4	0	0	4
2	A	7	2	0	1	2	6	9
	B	1	5	2	0	1	7	8
	C	1	0	1	0	1	1	2
	Ct-1	8	0	0	7	1	0	8
	Ct-2	5	0	0	4	1	0	5
3	A	8	3	0	9	2	0	11
	B	13	0	0	11	2	0	13
	C	14	0	0	13	1	0	14
	Ct-1	4	1	0	4	1	0	5
	Ct-2	5	0	0	5	0	0	5
4	A	8	0	0	5	3	0	8
	B	5	1	0	3	3	0	6
	C	11	0	0	9	2	0	11
	D	3	0	0	0	3	0	3
	Ct-1	6	0	0	5	1	0	6
	Ct-2	6	0	0	4	2	0	6
5	A	14	3	0	9	7	1	17
	B	31	1	0	25	7	0	32
	C	28	2	0	24	5	1	30
	Ct-1	11	0	0	8	3	0	11
	Ct-2	6	0	0	5	1	0	6
6	A	4	8	1	0	4	9	13
	B	6	4	1	2	6	3	11
	C	1	5	2	0	4	4	8
	Ct-1	10	0	0	7	3	0	10
	Ct-2	6	0	0	6	0	0	6
7	A	19	0	0	12	7	0	19
	B	17	0	0	13	4	0	17
	C	22	4	0	22	4	0	26
	Ct-1	9	1	0	9	1	0	10
	Ct-2	8	0	0	7	1	0	8
8	A	0	1	6	0	0	7	7
	B	4	3	2	1	2	6	9
	Ct-1	9	0	0	6	3	0	9
	Ct-2	7	0	0	7	0	0	7

* HGG: hypogammaglobulinemia

** AGG: agammaglobulinemia

*** Number of quails

TABLE IV
Effect of BX on antibody responses

Exp.	Group	anti-SRBC antibody		anti-BSA antibody		anti-RRBC antibody	
		Incidence*	Titer**	Incidence	Titer	Incidence	Titer
1	A	2/8	1.0	3/8	2.6	4/8	3.3
	B	0/11	—	1/11	2.0	2/11	4.0
	C	0/4	—	1/4	3.0	1/4	5.0
	Ct-1	6/18	3.7	5/18	3.4	5/18	3.2
	Ct-2	0/7	—	1/7	—	1/7	2.0
2	A	4/9	1.5	3/9	1.3	2/9	2.0
	B	6/8	1.3	0/8	—	1/8	2.0
	C	1/2	1.0	0/2	—	0/2	—
	Ct-1	15/26	2.0	4/26	2.0	7/26	2.6
	Ct-2	3/10	1.0	0/10	—	8/10	3.0
3	A	8/11	1.4	4/11	1.5	6/11	3.0
	B	10/13	2.7	2/13	2.0	7/13	2.6
	C	8/14	2.8	5/14	1.8	8/14	2.6
	Ct-1	2/7	2.5	1/7	2.0	2/7	1.5
	Ct-2	0/10	—	0/10	—	4/10	2.0
4	A	4/8	2.0	5/8	3.2	6/8	2.5
	B	6/6	3.8	2/6	2.5	6/6	2.7
	C	4/11	3.0	4/11	2.3	6/11	2.8
	D	0/3	—	2/3	3.0	2/3	3.0
	Ct-1	4/17	2.2	3/17	2.0	7/17	3.3
	Ct-2	0/10	—	3/10	1.3	3/10	2.3
5	A	6/17	3.2	11/17	2.9	7/17	2.3
	B	27/32	2.7	20/32	3.0	21/32	2.5
	C	18/30	2.4	17/30	3.4	19/30	2.7
	Ct-1	23/34	2.3	17/34	2.8	24/34	2.2
	Ct-2	0/10	—	2/10	1.5	2/10	2.5
6	A	3/13	1.0	3/13	3.7	5/13	3.4
	B	6/11	2.2	6/11	2.8	4/11	2.5
	C	2/8	2.0	1/8	2.0	4/8	2.5
	Ct-1	22/30	3.3	18/30	3.0	28/30	2.9
	Ct-2	2/10	1.5	1/10	2.0	10/10	2.7
7	A	13/19	4.3	14/19	2.9	10/19	2.4
	B	11/17	2.9	9/17	2.9	13/17	2.0
	C	18/26	3.5	13/26	1.9	22/26	2.7
	Ct-1	24/36	3.3	24/36	2.7	30/36	2.7
	Ct-2	1/10	1.0	1/10	2.0	8/10	2.8
8	A	0/7	—	0/7	—	0/7	—
	B	1/9	4.0	0/9	—	1/9	1.0
	Ct-1	16/29	5.8	19/29	2.0	25/29	2.0
	Ct-2	2/10	1.5	1/10	2.0	9/10	2.3

* Number of antibody-positive/Number of quails tested.

** Mean antibody titer of positive quails ($\log_2$)

112 out of 197 quails (56%). A marked suppressive effect of BX on production of anti-SRBC antibody was observed in the quails receiving S-BX and irradiation with 1,000 r (Exp. 1-B), in those receiving H-BX in 4 days of incubation and C-BX (Exp. 6-A), in those receiving combined H-BX, S-BX and C-BX (Exp. 8-A), and in those receiving combined H-BX, S-BX and irradiation (Exp. 8-B).

Similar results were obtained in production of anti-BSA antibody. Nine of 77 sera (12%) in the unimmunized control groups possessed anti-BSA antibody. Immunization with BSA resulted in production of the antibody in 91 out of 197 untreated quails (47%). The results in Exp. 1-B and 2-B could not be taken to indicate effectiveness of BX, as rates of responders in the untreated control groups in Exp. 1 and 2 were lower than 30%. Significant suppression of anti-BSA antibody production was observed in Exp. 6-A, 6-C, 8-A and 8-B. Thus, suppression of antibody production to BSA was obtained by combination of H-BX and C-BX (Exp. 6-A, 6-C), combination of H-BX, S-BX and C-BX (Exp. 8-A) and combination of H-BX, S-BX and irradiation (Exp. 8-B).

Natural antibody to RRBC was detected in the control groups immunized with BSA and SRBC, and in the unimmunized control groups at rates of about 65% (128 out of 197 quails) and 60% (45 out of 77 quails), respectively. The results of Exp. 1-B and 2-B could not be considered to be valid because of such low incidence of the natural antibody in the control groups. Suppression of production of the natural antibody was observed in Exp. 8-A and 8-B.

Thus, it may be concluded that combined H-BX, S-BX, and C-BX (Exp. 8-A) and combined H-BX, S-BX and irradiation (Exp. 8-B) were the most effective procedures as indicated by suppression of the three types of antibody production.

Correlation between Ig Production and Antibody Responses

In order to investigate correlation between the level of Ig deficiency and

TABLE V

Correlation between Ig production and antibody response

Ig production	Antibody to	Number of quails tested	Antibody production	
			Number of quails	%
Normal	SRBC	198	129	65.1
	BSA		96	48.5
	RRBC		126	62.9
Hypogammaglobulinemia	SRBC	79	29	36.7
	BSA		30	37.9
	RRBC		31	39.2
Agammaglobulinemia	SRBC	20	0	0
	BSA		0	0
	RRBC		0	0

TABLE VI
Effect of BX on morphological development of the bursa

Exp.	Group	Development of lymphoid elements				Size of bursa					No. of quails examined
		3	2	1	0	5	4	3	2	1	
3	A	11*	0	0	0	2	5	4	0	0	11
	B	11	2	0	0	6	6	1	0	0	13
	C	14	0	0	0	8	4	2	0	0	14
	Ct-1	7	0	0	0	3	4	0	0	0	7
	Ct-2	10	0	0	0	3	6	1	0	0	11
4	A	6	1	1	0	0	1	1	3	3	8
	B	6	0	0	0	0	0	3	2	1	6
	C	10	1	0	0	5	5	0	1	0	11
	D	3	0	0	0	2	0	1	0	0	3
	Ct-1	16	1	0	0	7	9	1	0	0	17
	Ct-2	10	0	0	0	4	4	2	0	0	10
5	A**	10	6	0	0	0	1	6	8	2	17
	B	28	4	0	0	2	6	16	8	0	32
	C	29	1	0	0	19	7	4	0	0	30
	Ct-1	33	1	0	0	24	6	4	0	0	34
	Ct-2	10	0	0	0	8	1	1	0	0	10
6	A	0	4	5	4	0	0	0	3	10	13
	B**	4	2	2	2	0	0	2	5	4	11
	C	2	3	3	0	0	1	5	2	0	8
	Ct-1	29	1	0	0	19	9	2	0	0	30
	Ct-2	10	0	0	0	3	7	0	0	0	10
7	A	17	2	0	0	0	1	11	6	1	19
	B	17	0	0	0	1	3	7	4	2	17
	C	24	2	0	0	9	12	5	0	0	26
	Ct-1	34	2	0	0	26	8	2	0	0	36
	Ct-2	10	0	0	0	6	4	0	0	0	10

* Number of quails.

** One sample was not examined histologically because of failure of sampling.

capacity of antibody production, all the quails bursectomized by different procedures in the eight experiments were classified into three groups, normal, hypogammaglobulinemia and agammaglobulinemia, based on the immunoelectrophoretic patterns of their sera. Close correlation between the level of Ig production and antibody responses to the three antigens, i.e. SRBC, BSA and RRBC, was noticed as shown in Table V. Especially, all the quails with agammaglobulinemia completely lacked antibody responses to any of the three antigens. The results indicated usefulness of Ig determination by immunoelectrophoresis as a parameter to evaluate the suppressive effect of BX on humoral immunity.

Morphological Examination of the Bursa of BX-quails

The effect of BX on the morphological development of the bursa was examined on all the experimental groups except Exp. 1, 2, and 8 in which S-BX was conducted. The results are shown in Table VI. H-BX by dipping (Exp. 3) did not exhibit any significant effect on the size. In contrast, H-BX by injection induced reduction in the size of the bursa; the suppressive effect seemed to be more marked by administration of TP at an early embryonic stage (Exp. 4-A, 4-B, 5-A and 5-B) than at later stages. Combined H-BX and C-BX showed a more marked suppressive effect on the size of the bursa, thus decrease in size was noticed in all the groups including those receiving TP injection at later stages (Exp. 6-C).

Histological examinations revealed various types of morphological abnormality in the bursa as shown in Figs. 3-6. Such markedly suppressive effects on development were consistently observed only in Exp. 6, in which combined H-BX and C-BX was conducted. In Exp. 6-A, many bursae lacked follicles, while some of them had immature ones with various sizes. In Exp. 6-B, the bursae with different types including those without follicles and those consisting of a small number of mature follicles were observed. On the other hand, all the bursae examined in Exp. 6-C had relatively large follicles without the lymphoid cells, thus showing a form of large epithelial buds. In Exp. 4, 5, and 7 in which H-BX with or without irradiation was made, the follicles in the bursa were generally reduced in size and number. However, most of them were found to have almost intact structure except the presence of a few immature follicles.

DISCUSSION

In the present study, various procedures of BX developed in the chicken system were applied to quails. Although several reports indicated successful suppression of bursa-dependent humoral immunity in chickens by H-BX by dipping in a TP solution (Lerner *et al.*, 1971; Fezer *et al.*, 1973; Sato *et al.*, 1975), the same dipping procedure was shown not to be effective in quails. H-BX of quails by injection of TP was found to suppress more effectively development of the bursa when given at an early embryonic stage than by dipping. Thus, decrease in size of the bursa as well as reduction in size and number of follicles were noticed after this procedure. However, the follicle structures were almost intact and such immunological functions as antibody formation and Ig production were not so severely suppressed as in chickens (Warner *et al.*, 1969; Bryant *et al.*, 1973). These results suggested that TP was not so effectively absorbed into quail embryos as in chicken embryos and that the suppressive effect of this hormone on both the development of the bursa and immune functions was transient.

Combined H-BX and C-BX induced marked morphological abnormality of

Fig. 3. The bursa lacking both the follicles and lymphoid elements. Classified in 0. $\times 40$.

Fig. 4. The follicles showing a form of epithelial buds. A few number of immature follicles are observed. Classified in 1. $\times 40$.

Fig. 5. The bursa containing many immature follicles of various sizes. Classified in 2. $\times 40$.

Fig. 6. Normal bursa consisting of mature follicles. Classified in 3. $\times 40$.

the bursa as well as relatively severe immunosuppression. Administration of TP at different stages of embryos followed by C-BX after hatching exerted different effects on morphology of the bursa; the complete absence of the lymphoid follicles in the bursa was observed in several quails that received TP injection at an early embryonic stage, while development of large follicles without lymphoid cells was demonstrated in the group that received TP at a late stage. In the preliminary experiments, it was confirmed that the quail embryonal bursa started to invaginate around the 7th day of incubation, small epithelial buds appeared on the 11th to 12th days, large lymphoid cells accumulated in the buds by the 14th day, and both the medulla and cortex consisting of one to two layers of lymphoid cells were noticed around hatching days (16th to 17 days). Therefore, it may be concluded that the TP treatment at an early embryonic stage exerted a transient inhibitory effect on the development of the epithelial buds, and that the TP treatment at a late stage influenced the accumulation of the lymphoid cells. C-BX with CY was considered to exert the inhibitory effect by destruction of the remaining lymphoid elements which were not affected by H-BX.

Combined H-BX and irradiation decreased the size of the bursa but failed to inhibit the maturation of the remaining lymphoid follicles. Thus, inhibition of antibody response and Ig synthesis was not achieved by this procedure. On the contrary, combined S-BX and irradiation markedly suppressed the immune functions. The most marked immunosuppression was achieved by combination of H-BX, S-BX and C-BX (Exp. 8-A) and by that of H-BX, S-BX and irradiation (Exp. 8-B). These results may indicate that the remaining lymphoid elements having escaped the effect of H-BX are responsible for antibody response in H-BX quails, and that they can be destroyed or eliminated either by C-BX or S-BX, but are resistant to irradiation at 1 week of age.

Hatching and survival rates corresponded to the effectiveness of BX procedures. Thus, hatching rates of quails receiving H-BX by injection were lower than those of quails receiving H-BX by dipping. Low survival rates were also noticed in Exp. 2, 6, and 8 in which relatively severe immunosuppression was obtained.

The immunosuppressive effects of various BX procedures were estimated by measuring the amount of Ig, antibody titers to SRBC, BSA, and RRBC. There was a good correlation between the degree of antibody responses to the three antigens and the amount of Ig (Table V). However, antibody responses could not be regarded as a suitable parameter for estimating the immunosuppressive effect, since the degree of antibody response in the untreated quails was not high enough, sometimes showing the presence of nonresponders up to half of the animals. As relatively consistent results were obtained by immunoelectrophoresis, the amount of Ig was considered to be a practical parameter for evaluation of immunosuppressive effects. Natural antibody to RRBC should also be considered to be a useful parameter for rapid and simple determination of immune function in quails older than 4 weeks of age, as immunization with

foreign antigens which may modify the immune status can be avoided.

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