Immunology, 1965, **9**, 553.

The Influence of Sex upon the Antibody Response to an Incompatible Tumour

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(*Received* 15*th April* 1965)

Summary. (BALB/c × C57Bl) hybrids and strain 129 hosts of both sexes were immunized with heavily irradiated cells of the C3H ascites sarcoma, B.P.8. It was found that both the primary and secondary haemagglutinin responses were higher in females than males of the same strain. After passive immunization, antibody titres declined at equal rates in the two sexes. It is concluded that antibody synthesis is sustained at a higher rate in females than males. Further experiments were performed to investigate the basis of this difference. The effects of oöphorectomy and oestrogen replacement treatment indicate that the superior reactivity of females is not due to the presence of ovarian tissues. It is concluded from the effects of orchidectomy that the immunological inferiority of males is due partly to the presence of the testes. However, treatment with testosterone propionate failed to influence the haemagglutinin response to irradiated B.P.8 in two experiments.

An acute illness resembling severe 'runt disease' was observed in oestrogen treated mice after immunization.

INTRODUCTION

Immunological enhancement of the C3H ascites sarcoma, B.P.8, occurs more readily in male than female hosts (Gorer and Kaliss, 1959). It has also been noted by Kaliss (1957) that C57Bl females produce higher antibody titres than males in response to immunization with an A strain tumour. These observations suggest that there may be a difference in immunological potency between the sexes. The experiments described here were performed to test this possibility, and to make an initial attempt to analyse the basis of any difference.

Although the experiments cited above suggest that there may be a sex difference in immunological potency, another possibility has to be considered. The antigenic material in these experiments consisted of dividing tumour cells. Many tumours are hormone sensitive (Gardner, 1953; Gardner, Pfeiffer and Trentin, 1958) and thus growth rates of any one tumour may not be identical in males and females of the same strain. A valid comparison of immune responses can only be made if both sexes receive the same total amount of antigen. In some preliminary experiments, the effect of hormonal environment upon the intraperitoneal growth rate of B.P.8 in (BALB/c × C57Bl) hosts was tested. It was found that during the first 4 days after injection, B.P.8 grew twice as rapidly in females as in males. The growth rate was further increased in oöphorectomized females treated with large doses of oestradiol monobenzoate; oöphorectomy without concomitant oestrogen treatment caused a reduction in growth rate. In males, removal of the testes favoured a more rapid growth rate than was found in sham operated controls. It was

evident that the hormonal status of the host significantly affected the growth of B.P.8. Therefore in the following experiments, in which the immune responses of the two sexes are compared, non-replicating antigen, in the form of heavily irradiated B.P.8 cells has been employed (Klein, 1959).

MATERIALS AND METHODS

Mice

(BALB/c × C57Bl)F_1 hybrids, and strain 129 hosts of both sexes were used.

Immunization

Mice were immunized intraperitoneally with heavily irradiated suspensions of the C3H ascites sarcoma, B.P.8. Doses for each experiment are given with the results. Before inoculation all tumour suspensions were exposed to 15,000 R γ-irradiation from a cobalt 60 source. The dose rate was approximately 1000 R/15 sec. A preliminary experiment confirmed that this treatment prevented B.P.8 from multiplying after transplantation into (BALB/c × C57Bl) hosts.

Haemagglutination tests

Sera from immunized mice were titrated individually by the method of Gorer and Mikulska (1954). All sera were titrated against strain A red cells. These cells contain K region antigens of the H-2 locus in common with B.P.8 which are absent from (BALB/c × C57Bl) and strain 129 mice. Strain A red cells were chosen in preference to C3H cells since the former are particularly reactive in haemagglutination tests and therefore provide a very sensitive indicator system for the presence of antibody.

Mononuclear cell counts

Blood samples were diluted in Turck's fluid and counted individually with the use of a microscope cell counting chamber. Cells were classified either as mononuclear or polymorphonuclear.

Statistical methods

The significance of differences between groups of mean haemagglutinin titres or mononuclear cell counts were tested by 'Student's' *t*-test.

Operative procedures

Bilateral oöphorectomy was performed through an incision in each flank. The ovary and terminal part of each fallopian tube was resected. In sham operated controls, the ovary was delivered through the incision and then returned into the peritoneal cavity. The vaginal washings of oöphorectomized mice were examined twice weekly after operation for evidence of oestrous cycles. The occasional failures in which oestrous cycles were still observed after operation were excluded from the experiments. Bilateral orchidectomy was performed through a single midline incision in the scrotum. Sham operated controls were similarly treated but the testes were not removed.

Hormone treatment

Oestradiol monobenzoate implants (Organon Laboratories Ltd), containing either 1 or 2 mg of hormone, were administered subcutaneously. Recipients were given a total dose of 4 mg. Testosterone propionate (Neo-Hombreol, Organon Laboratories Ltd) dissolved in ethyl oleate with 1 per cent of benzyl alcohol was injected into recipients

intramuscularly twice weekly. Dose schedules for each experiment are given in the section on results.

RESULTS

The primary and secondary haemagglutinin responses of (BALB × C57Bl) hybrids and strain 129 hosts of both sexes were compared after immunization with heavily irradiated B.P.8. Three experiments upon the primary response were made, using a total of fifty-four mice. The results are given in Table 1.

TABLE 1

COMPARISON BETWEEN SEXES OF PRIMARY HAEMAGGLUTININ RESPONSE

Experiment	Antigen dose	Strain of host	No. of mice	Sex	Mean haemagglutinin titre (reciprocal of $\log_2$) on Day					
					6	10	13	17	20	27
1	47 million B.P.8	(BALB/c × C57Bl)	10	F	8·6	8·2	7·4	6·1	6·5	2·7
			10	M	8·4	7·1	5·8	5·1	5·1	4·0
		Difference between sexes			0·2	1·1[a]	1·6[a]	1·0[a]	1·4[b]	0·3
					6	10	13	17	21	
2	50 million B.P.8	129	10	F	1·6	5·5	6·1	3·5	6·0	
			10	M	0·3	3·4	1·7	1·1	3·0	
		Difference between sexes			1·3	2·1[b]	4·4[c]	2·4[b]	3·0[b]	
					4	7	11	18	24	81
3	53 million B.P.8	(BALB/c × C57Bl)	7	F	6·9	9·6	9·7	5·3	5·1	3·9
			7	M	6·1	9·0	7·7	0·4	0	0
		Difference between sexes			0·8	0·6	2·0[b]	4·9[c]	5·1[c]	3·9[c]

Significant differences between groups are indicated as: a = $0{\cdot}05 > P > 0{\cdot}01$, b = $0{\cdot}01 > P > 0{\cdot}001$, c = $P < 0{\cdot}001$.

All three experiments showed the same result. Except for a single occasion (Day 27, Experiment 1) the haemagglutinin titres of females were consistently higher than those of the males. Differences in titre did not become statistically significant until the 10th to 11th days after immunization. Maximum differences occurred during the 3rd week after immunization. The length of time for which the differences continued was not studied in detail, but there is a suggestion that they may have persisted for a considerable period. In Experiment 3, six out of seven females had detectable titres on the 81st day after immunization, whereas no haemagglutinins were detected in any of the male sera.

In two experiments, numbers 1 and 3, the mice were re-immunized for observation of the secondary responses. The results of this are given in Table 2.

It can be seen from Table 2 that the secondary haemagglutinin titres of females were also higher than those of males of the same strain. In Experiment 1, the differences are small and only of statistical significance at Day 19; but in this experiment, two out of the ten females showed atypically low titres throughout. If they are excluded, a highly significant difference exists, similar to that seen in Experiment 3.

A further difference in the response of the two sexes was also seen. Haemagglutinins produced by females consistently caused more effective red cell clumping than the haemagglutinins present in male antisera. Agglutinates formed by incubation of red cells in male

TABLE 2

COMPARISON BETWEEN SEXES OF SECONDARY HAEMAGGLUTININ RESPONSE

Experiment	Challenge dose of antigen	Sex of hosts	Mean haemagglutinin titre (reciprocal of $\log_2$) on Day		
			6	12	19
1	55 million B.P.8	F	11·0	10·8	11·6
		M	9·2	8·9	9·4
	Difference between sexes		1·8	1·9	2·2[a]
			6	10	15
2	30 million B.P.8	F	10·9	12·1	10·1
		M	7·7	8·7	8·3
	Difference between sexes		3·2[b]	3·4[c]	1·8

See Table 1 for details of primary immunization. In Experiment 1, antigenic challenge was performed 27 days after the primary stimulus, and in Experiment 2, 81 days afterwards. Significant differences between groups are indicated as follows: a = $0{\cdot}05 > P > 0{\cdot}01$, b = $0{\cdot}01 > P > 0{\cdot}001$, c = $P < 0{\cdot}001$.

sera were smaller and more easily broken up. Grading of agglutination is of course subjective, but may be quite accurate when readings are made by the same observer. This qualitative difference between male and female antisera was regularly seen both after primary and secondary immunization.

At the same time that blood was collected for antibody titration in the previous experiments, mononuclear cell counts were performed on part of each sample. In most strains immunization with allogeneic tissues provokes changes in peripheral blood lymphocyte levels (Murphy and Morton, 1915; Blumenthal, 1941). In the first 2–4 days after primary immunization the peripheral blood mononuclear cell count may fall slightly, and then it rises sharply to a peak which is usually reached at approximately the 6th and 8th days. Its subsequent return to normal levels may be interrupted by secondary or tertiary peaks. It is reasonable to suppose that this cellular response consists of the descendants of antigenically stimulated lymphoid stem cells. The response may be inhibited by administering specific antiserum (Chantler, 1965). Furthermore, injections of syngeneic tissue do not cause comparable changes.

Whatever is the mechanism for the development of antigen-induced mononuclear cell responses, the response itself may be used as an index of immunization. For this reason the cellular responses of the two sexes were compared. No attempt was made to subclassify mononuclear cells into different histological types, but most were members of the lymphocyte series.

The results in all three experiments were qualitatively similar. Mononuclear cell counts rose to higher peaks in males than females. Statistically significant differences between the peak counts of the two sexes were observed in two out of three experiments. The results of these two experiments (Experiments 1 and 2) are illustrated in Figs. 1 and 2. The most violent responses were observed in strain 129 hosts (Fig. 2). Since higher cell counts were observed in males, the difference in haemagglutinin responses of the sexes cannot be attributed to a numerically superior cellular response on the part of the females. Nor was the cell response more prolonged in females.

Since it appeared that antibody titres of actively immunized hosts of the two sexes declined at different rates, two possible explanations were considered. Either antibody synthesis might continue in females for longer than in males, or alternatively antibody

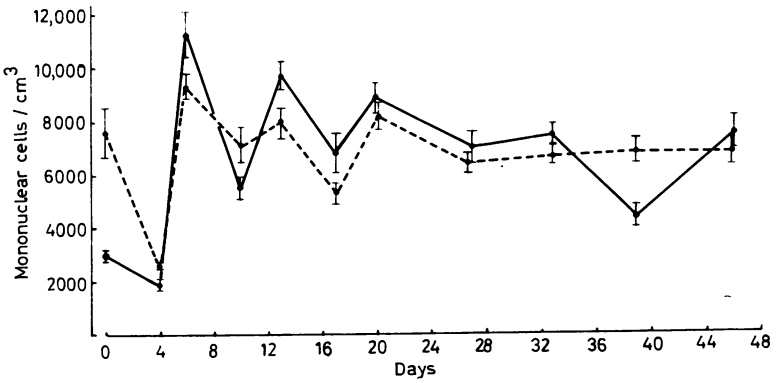

FIG. 1. The mononuclear cell response of (BALB/c × C57Bl) hybrids immunized with irradiated B.P.8. Immunization schedule given in Table 1, Experiment 1. – – – –, Females; ————, males.

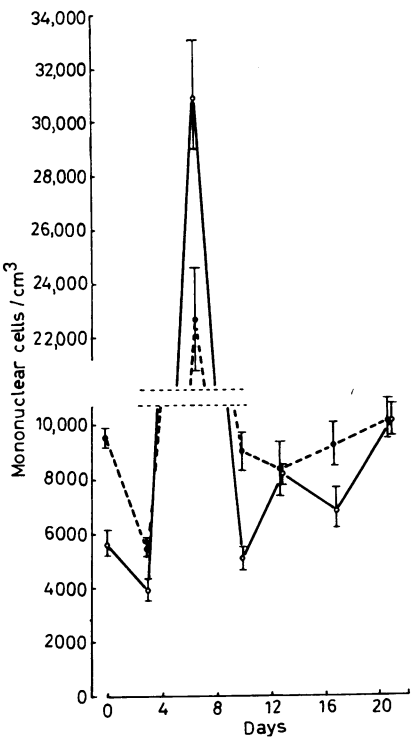

FIG. 2. The mononuclear cell response of strain 129 hosts immunized with irradiated B.P.8. Immunization schedule given in Table 1, Experiment 2. – – – –, Females; ————, males.

catabolism could take place at different rates in the two sexes. The second possibility was tested by following the rate at which haemagglutinin titres declined in passively immunized hosts of both sexes. A pool of (BALB/c × C57Bl) anti B.P.8 serum with a titre of 1 : 16,000 against strain A red cells was used. Twelve (BALB/c × C57Bl) hybrids of each sex received a weight adjusted dose of antiserum intraperitoneally. Males were given 0·22 ml and

females, 0·20 ml. The mice of each sex were divided into two groups, which were bled alternately so as to avoid too frequent bleeding of any of the subjects. The results are shown in Table 3. No significant difference between the sexes was observed. It appeared likely therefore that catabolism of haemagglutinins occurred at similar rates in the two sexes, and that the differences in titre after active immunization must be due to more prolonged synthesis of antibody by females.

TABLE 3

DECLINE OF HAEMAGGLUTININ TITRES AFTER PASSIVE IMMUNIZATION OF MALES AND FEMALES

Sex	Group	Mean haemagglutinin titre (reciprocal of $\log_2$) on Day							
		4 hours	2	4	7	11	14	21	25
M	1	11·0	–	7·3	–	5·8	–	5·7	–
	2	–	9·0	–	9·0	–	4·2	–	0·3
F	1	10·7	–	8·2	–	6·7	–	5·3	–
	2	–	8·8	–	9·0	–	4·7	–	2·3

Hosts = (BALB/c × C57Bl) hybrids. Six animals per group.
Serum = (BALB/c × C57Bl) anti B.P.8.
Serum dose = 0·22 ml for males, 0·20 ml for females.

Experiments were then performed to examine the effect of gonadectomy and hormone treatment. The influence of oöphorectomy and oestrogen treatment was tested in three experiments. Adult (BALB/c × C57Bl) or 129 females were subjected to bilateral oöphorectomy or to an equivalently severe sham operation. Subcutaneous implants containing 4 mg of oestradiol monobenzoate were given to one group of oöphorectomized females 7–10 days later. Further groups of oöphorectomized and sham operated hosts received no

TABLE 4

THE EFFECT OF OÖPHORECTOMY AND OESTROGEN TREATMENT ON THE PRIMARY HAEMAGGLUTININ RESPONSE OF MICE IMMUNIZED WITH HEAVILY IRRADIATED B.P.8

Experiment	No. of mice	Treatment	Mean titre (reciprocal of $\log_2$) on Day							
			8	11	15	18	22	26	32	40
5	6	Oöphorectomy	6·2	7·5	4·7	4·0	5·3	6·3	4·7	5·7
	6	Oöphorectomy+4 mg oestradiol	3·5[b]	4·0[b]	1·0[b]	0[b]	2·4[b]	3·4[b]	1·6[b]	2·6[b]
	7	Sham oöphorectomy	6·1	5·1[b]	2·9[a]	0·9[b]	3·4[b]	5·0[b]	2·0[b]	5·4
			7		11		14		18	21
6	7	Oöphorectomy	6·0		9·6		8·1		7·0	6·6
	7	Oöphorectomy+4 mg oestradiol	5·3[a]		7·0[b]		6·8[b]		6·3	5·8
	(2 died on Day 4)									
	7	Sham oöphorectomy	6·3		6·3[b]		7·9		6·9	4·4[a]
			6		14		18		21	
7	7	Oöphorectomy	5·1		3·7		0		1·9	
	7	Oöphorectomy+4 mg oestradiol	4·1		2·3		0		0[a]	
	(3 died on Day 9)									
	7	Sham oöphorectomy	5·9		4·9		0		3·6	

Recipients: (BALB/c × C57Bl) ♀ in Experiments 5 and 7, strain 129 ♀ in Experiment 6.
Antigen: 48–78 million irradiated B.P.8 cells.
Significant differences from group treated by oöphorectomy only are indicated as follows: a = $0{\cdot}05 > P > 0{\cdot}01$, b = $P < 0{\cdot}001$.

oestrogens. Approximately $2\frac{1}{2}$ weeks after operation, all mice were immunized intraperitoneally with γ-irradiated B.P.8. Both the haemagglutinin and mononuclear cell responses elicited by this treatment were measured. Later the mice were rechallenged, and the secondary responses observed.

The primary haemagglutinin titres are recorded in Table 4. Titres of oöphorectomized mice were significantly higher than those of the sham operated groups in two out of three experiments. In the third experiment they were slightly lower, but the difference is not statistically significant. It is concluded that the effect of oöphorectomy alone is weak, and may not always be detectable. Treatment of oöphorectomized hosts with oestrogen caused a marked depression of the antibody response in all experiments. The secondary haemagglutinin responses of Experiments 5 and 6 were followed (Table 5) but no significant differences were observed.

TABLE 5

THE EFFECT OF OÖPHORECTOMY AND OESTROGEN TREATMENT ON THE SECONDARY HAEMAGGLUTININ RESPONSE OF MICE IMMUNIZED WITH HEAVILY IRRADIATED B.P.8

Experiment	No. of mice	Treatment	Mean titre (reciprocal of $\log_2$) on Day	
			4	9
5	6	Oöphorectomy	8·5	10·0
	2 (4 died on Day 3)	Oöphorectomy+4 mg oestradiol	7·5	11·5
	7	Sham oöphorectomy	8·0	10·1
			4	7
6	7	Oöphorectomy	12·1	18·0
	5	Oöphorectomy+4 mg oestradiol	13·2	17·3
	7	Sham oöphorectomy	11·3	17·7

Recipients: (BALB/c × C57Bl) ♀ in Experiment 5; strain 129 ♀ in Experiment 6.
Antigen: 48–52 million irradiated B.P.8 cells.

The mononuclear cell responses following primary immunization correlated directly with the antibody responses of the different groups. Highest cell counts were observed in oöphorectomized mice. Peak cell counts of sham operated animals were significantly lower than those of the oöphorectomized mice (Experiments 5 and 6, Table 4). Oöphorectomized mice treated with oestrogen implants showed strikingly depressed mononuclear cell responses in all three experiments. A typical result (Experiment 6) is illustrated in Fig. 3.

It is unlikely that the more sustained antibody production characteristic of females was due to stimulation of the immune response by ovarian tissue. If this had been the case, one would expect oöphorectomy to have an inhibitory effect. Furthermore oöphorectomy had no effect on the secondary haemagglutinin response. The results of treatment with oestradiol show that at the dose levels used, primary antibody synthesis was depressed. Although these doses are greatly in excess of physiological levels, the results are in keeping with the opposite effect of oöphorectomy.

The next possibility considered was that the immunological response of males was suppressed by the testis. The influence of orchidectomy was therefore investigated. Male hosts were submitted to orchidectomy; subsequently their primary and secondary

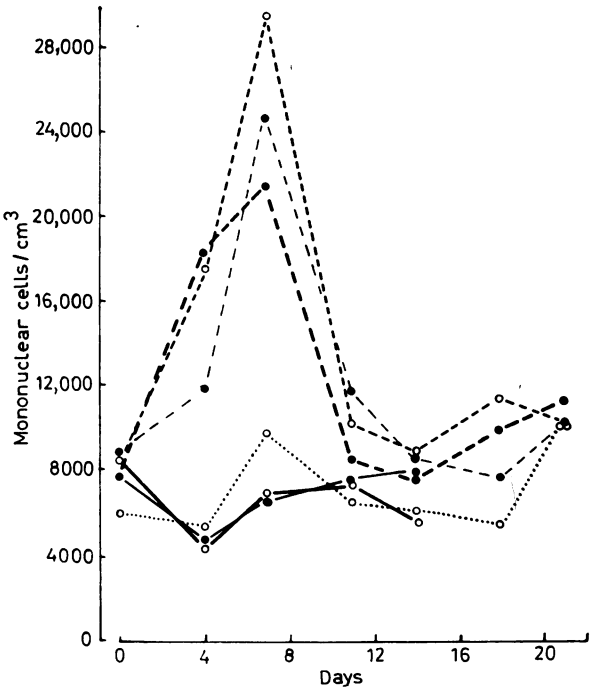

FIG. 3. The effect of oöphorectomy and oestradiol treatment on the mononuclear cell response of strain 129 hosts to primary immunization with 48 million irradiated B.P.8 cells. ● – – – ●, Oöphorectomy only; ○ · · · · · · · ○, oöphorectomy plus 4 mg oestradiol implant; ● – – – – ●, sham oöphorectomy (females). Control groups: ○ – – – ○, 129 males immunized with 48 million irradiated B.P.8 cells; ●————●, 129 females injected with 50 million 129 spleen cells; ○————○, 129 males injected with 50 million 129 spleen cells.

haemagglutinin responses were compared with those of sham operated controls. Two experiments were performed. In the first, adult (BALB/c × C57Bl) males were submitted to operation, and in the second, orchidectomy was performed just before the onset of puberty when the mice were 16–19 days old. Primary immunization was performed 4–6 weeks after orchidectomy in both experiments. The results are given in Table 6.

TABLE 6

THE EFFECT OF ORCHIDECTOMY UPON THE HAEMAGGLUTININ RESPONSE OF (BALB/c × C57Bl) ♂ IMMUNIZED WITH IRRADIATED B.P.8

Experiment	Group	No. in group	Mean titre (reciprocal of $\log_2$) on Day						
			Primary response				Secondary response		
			7	10	14	18	0	6	9
8	Orchidectomy	7	9·9	5·7	4·0[a]	4·0[b]	1·6	11·4[a]	14·9[c]
	Sham-operated	7	9·0	5·0	2·4	1·0	1·3	9·1	11·7
			4	12	19	27	5	13	20
9	Orchidectomy	6	4·0	5·7	1·8	1·3	9·0	12·0	12·2[a]
	Sham-operated	5	3·8	5·4	1·6	0·6	8·4	10·4	8·8

Antigen dose = 30–60 million irradiated B.P.8 cells.
Significant differences between groups are indicated as follows: a = $0{\cdot}05 > P > 0{\cdot}01$, b = $0{\cdot}01 > P > 0{\cdot}001$, c = $P < 0{\cdot}001$.

Table 6 shows that orchidectomy produced a weak but statistically significant stimulation of the primary haemagglutinin response in the first experiment, but no effect on the primary response of the second experiment. In both experiments a significant stimulation of the secondary response was observed. Furthermore, the red cell agglutinates, produced by antisera from orchidectomized males resembled those formed by the action of female antisera. Clumps were both larger and more cohesive than those formed in the presence of sera from intact males. It appeared likely therefore that at least one factor which explained the immunological inferiority of males was that the presence of the testes exerted an inhibitory effect.

Two further experiments were then performed to investigate the influence of testosterone upon haemagglutinin responses. In the first, orchidectomized (BALB/c × C57Bl) hosts were injected with 0·5 mg of testosterone twice weekly starting 2 weeks before primary immunization and continuing throughout the experiment. Control groups of orchidectomized and sham operated males were given a similar schedule of treatment with the hormone solvent only. In the second experiment, intact males were injected with 1·25 mg of testosterone twice weekly. As previously, treatment started 2 weeks prior to immunization and continued throughout the experiment. Controls were given hormone solvent only. Immunization was performed intraperitoneally with 36–39 million irradiated B.P.8 cells.

TABLE 7

THE EFFECT OF TESTOSTERONE TREATMENT UPON THE HAEMAGGLUTININ RESPONSE OF (BALB/c × C57Bl) ♂ IMMUNIZED WITH IRRADIATED B.P.8

Experiment	Treatment	No. of mice	Mean titre (reciprocal of $\log_2$) on Day						
			Primary response					Secondary response	
			7	10	14	16	22		
10	Orchidectomy + testosterone	6	6·3	4·0	3·8	4·4	1·4	Not tested	
	Orchidectomy + hormone solvent	5	6·6	4·2	3·6	3·6	1·4		
	Sham operation + hormone solvent	5	6·2	3·2	2·4	2·8	0·6		
			7			11		4	9
11	Testosterone	5	8·0			4·8		4·6	8·0
	Hormone solvent	5	8·8			5·0		5·0	8·6

Antigen dose = 30–39 million irradiated B.P.8 cells.

The results are given in Table 7. It can be seen that in neither experiment did treatment with testosterone produce a significant effect on the primary antibody response. In Experiment 10, orchidectomy produced a weak stimulatory effect on the primary response as in earlier experiments. The effect of testosterone treatment upon the secondary response was examined in Experiment 11. Here also no significant difference was observed between the experimental and control groups.

During the course of these experiments it was noted that mice given 4 mg implants of oestradiol became acutely ill shortly after immunization. They remained apparently in good health until 2 days after injection of irradiated B.P.8. At that time they began to develop a syndrome characterized by listlessness, hunched posture, severe dehydration, and many of the mice died. Of a total of twenty mice which were given oestrogen implants, nine animals died between the 3rd and 16th day after antigenic stimulation. No deaths occurred in the other experimental groups.

Microscopic examination of the lymphoid tissue of other oestrogenized (BALB/c × C57Bl) and 129 mice showed that hormone treatment resulted in a marked loss of small lymphocytes from lymph nodes and spleen. There was also severe involution of the thymus within 10 days of the insertion of hormone pellets. The residual thymic tissue consisted of small areas of medullary epithelial cells and scattered Hassall's corpuscles, the usual cortex of thymic lymphocytes having disappeared entirely.

DISCUSSION

In the experiments described here it was found that both primary and secondary haemagglutinin responses of (BALB/c × C57Bl) and strain 129 hosts immunized with irradiated B.P.8 were sustained for longer in females than males. Passively transfused antibody disappeared from the circulation at the same rate in the two sexes. It follows therefore that the higher titres observed in the females after active immunization must be attributed to differences between the sexes in antibody synthesis. It seems unlikely that the difference is only a quantitative one. Red cell agglutinates formed by incubation in female antisera were larger and more cohesive than those formed in the presence of male sera and this suggests that there may be qualitative differences in the populations of antibody molecules synthesized by the two sexes.

Changes in the level of circulating mononuclear cells occur following immunization. In females, peak mononuclear cell counts, usually reached at approximately the 6th day, were lower than those observed in males, and the cellular responses subsided at the same rate. There was no evidence that females exhibited a more prolonged mononuclear cell response than males.

The sex difference in antibody synthesis could not be attributed to the influence of ovarian function. Primary haemagglutinin formation was found to be either increased or unchanged as a result of bilateral oöphorectomy, and the secondary antibody response was unaffected. Treatment of females with 4 mg implants of oestradiol depressed the primary response, but had no effect on the secondary one. The results of orchidectomy, however, suggested that the testes exert a weak inhibitory effect upon the antibody response of males, and that this may be one of the factors which explains the immunological inferiority of males. Removal of the testes led to a slightly increased antibody response, more marked after secondary immunization. Antisera from orchidectomized males produced large cohesive red cell agglutinates similar to those observed after incubation in female antisera. Two experiments were performed to test the influence of testosterone but the results were negative in both. The reason for this discrepancy between the negative results of testosterone treatment and the effect of orchidectomy is unknown. One possibility is that the dosage schedules were unsuitable. The effect of orchidectomy is weak, and one might expect correspondingly weak inhibitory effects in hormone replacement experiments.

During the course of these experiments it was noted that most of the mice treated with 4 mg implants of oestradiol became severely ill shortly after being immunized with irradiated B.P.8. The syndrome resembled an acute form of 'runt disease' in its clinical features and severe lymphopaenia. Histological examination of similarly oestrogenized mice showed that hormone treatment caused marked thymic involution and the loss of small lymphocytes from spleen and lymph nodes. Procedures which induce runting or allied states are known to cause the loss of functioning thymic tissue and disappearance of

small lymphocytes (Billingham and Brent, 1959; Miller, 1962; Simonsen, 1962; Barnes, Loutit and Micklem, 1962; Schlesinger and Marks, 1964). Mice given 4 mg of oestradiol implants develop thymic involution and lymphopaenia within 2 weeks, but they normally survive for several months. It seems likely that artificial immunization increases lymphocyte turnover rates, and thus accelerates the appearance of florid runt disease.

There is some evidence to suggest that immunological superiority of females over males may not be limited only to the system used in the present experiments. Data on the induction of tolerance to bovine γ-globulin in adult CBA hosts (Dresser, 1962) suggests that non-reactivity is more easily obtained in males than in females. Females tended to develop immunity to doses of BGG which induced tolerance in males of the same age. Spontaneous autoimmune haemolytic anaemia of the NZB/BL strain described by Bielschowsky, Helyer and Howie (1959) develops in a higher incidence in virgin females than in males of the same age. Helyer and Howie (1961) have crossed NZB/Bl with an unaffected strain, and observed lupus erythematosus cells in 41·2 per cent of the female offspring but only 13·6 per cent of the male progeny. Wheater and Hurst (1961) have reported that females of an outbred colony of mice are less susceptible to several bacterial pathogens than males. However, the same arguments that have been levelled against the use of viable incompatible cells for immunization apply equally to live bacteria and viruses. It is possible that live organisms may proliferate at different rates in the two sexes, and therefore the immune responses of the sexes would differ for this reason. In other species, Pearson (1959) has observed that the incidence of Freund's adjuvant induced arthritis in rats is greater in females than in males. In human diseases, where autoimmune reactions have been implicated, females are more commonly affected than males. It is stated by Cecil and Loeb (1959) that systemic lupus erythematosus, rheumatoid arthritis, Hashimoto's thyroiditis, myasthenia gravis, and acquired autoimmune haemolytic anaemia, affect females more commonly than males. This type of evidence is of course only circumstantial, but it should be a simple matter to compare the immune responses of the two sexes in human subjects.

ACKNOWLEDGMENT

This investigation has been supported by a grant from the British Empire Cancer Campaign.

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