Immunology, 1962, 5, 211.

Effect of Splenectomy at Different Ages on Precipitin Production in Chickens

GRACE L. ROSENQUIST* AND H. R. WOLFE

Department of Zoology, University of Wisconsin, Madison, Wisconsin

(*Received 14th July*, 1961)

Summary. Immunologically immature and mature Arbor Acre White Rock fowls were splenectomized and precipitin production after a single inoculation of bovine serum albumin was assayed. Fowls splenectomized at 4, 9 or 17 weeks of age were intravenously injected at 6, 12, 20 weeks; a subcutaneous or intravenous inoculation was given to 12-week-old birds splenectomized at 8 weeks. The splenectomized animals showed a delayed antibody appearance and a later day of peak titre. Only the animals which were splenectomized at 17 weeks showed a depression. These results suggest that a greater number of immunologically competent cells are present in the spleen and that non-splenic tissue of young animals only can compensate for the removal of this organ.

INTRODUCTION

Indirect evidence, such as the presence of antigen or antibody in the spleen or the effect of splenectomy on antibody production, suggests the importance of the spleen in the immune response. Direct evidence for antibody production by the spleen may be obtained from *in vivo* or *in vitro* tissue culture experiments. This paper is concerned with the effect of splenectomy on precipitin production.

Motohashi (1922) reported a slower and depressed haemolysin production in splenectomized rabbits after an intravenous injection of sheep red blood cells. Similar results were secured by Rowley (1950 a, b) with splenectomized rats. Splenectomized rabbits injected intravenously with stromata of heated sheep red blood cells gave lower peak haemolysin titres, longer induction period, and a later peak day (Taliaferro and Taliaferro, 1950, 1951; Draper and Süssdorf, 1957).

The effect of splenectomy on precipitin production by fowls against a soluble antigen was first reported by Wolfe, Norton, Springer, Goodman and Herrick (1950). The fowls were bled only on the seventh day after inoculation of bovine serum albumin (BSA). A large reduction in antibody nitrogen values was noted.

The route of injection was found to affect haemolysin production of splenectomized rabbits (Draper and Süssdorf, 1957). Splenectomized animals injected subcutaneously responded like the controls but those injected intravenously gave a lower and delayed production of antibodies.

The age at which the animal is splenectomized may have an effect on the results. Workers experimenting with mammals have used only adults. Wolfe *et al.* (1950) splenec-

* Present address: Division of Biology, California Institute of Technology, Pasadena, California.

tomized young and mature birds but did not attempt to distinguish whether the age at which the operation occurred affected the production of antibody.

In the present study chickens were splenectomized at various ages and the antibody response to a single injection of a soluble antigen was followed. Chickens in a second experiment were injected subcutaneously to determine whether splenectomy changed the antibody response in animals which do not normally have discrete lymph nodes. The chickens were given a single injection of antigen and daily bleedings were made from the fourth to the tenth day. Additional samples were drawn until the fifteenth or nineteenth day.

MATERIALS AND METHODS

ANIMALS. Arbor Acre White Rock chickens, secured from a local hatchery at 1 day of age, were used. Ninety per cent of the birds were males. They were housed in air-contioned animal quarters with 12 hours' light per day during the duration of the experiments. The animals were fed Master Mix pullet grower and water *ad libitum*.

SPLENECTOMY. Splenectomies were performed at 4, 8, 9 or 17 weeks of age. The animals were anaesthetized with an intravenous injection of nembutal (26 mg. per kg. body weight) and supplementary ether inhalation was given if necessary. The incision was made just ventral and parallel to the left pubic bone. After the spleen was removed, powdered sulphathiazole was placed in the peritoneal cavity and between the muscle and skin layers. Post-operative mortality was 2–3 per cent.

TYPES OF EXPERIMENTS AND PROCEDURES. The chickens were divided into two groups. Birds of Group I were splenectomized at different ages and their primary response was determined after a single intravenous injection. Operated and control animals of Group II were given either an intravenous or subcutaneous injection and their responses were assayed. Each chicken received an injection of 40 mg. crystalline BSA per kg. The antigen was obtained in crystalline form from Armour & Co. and was injected as a 4 per cent solution.

All animals were bled on each of several days after antigen injection. Samples of blood were obtained by venepuncture of the brachial vein or by cardiac puncture. Sera were analysed for antibody nitrogen by the quantitative precipitin method of Heidelberger, Kendall and Soo Hoo (1933). The 1 ml. reaction mixtures contained 0.25 ml. each of antiserum and BSA at 1 μg. intervals. A final NaCl concentration of 8 per cent was used, since this was shown to be optimum for the chicken system (Goodman, Wolfe and Norton, 1951). Duplicate tubes were incubated at $37° \pm 1°$ for 3 hours, then centrifuged in the cold at 1500 *g* for 30 minutes. In some samples incubation was continued at $4° \pm 1°$ for 18 hours before centrifugation. The supernatants were tested to obtain the region of slight antigen excess and antibody determinations were made at this point. The antigen-antibody precipitates were washed once with cold, phosphate-buffered 8 per cent NaCl solution (pH 7.0). The digestion and colorimetric determinations were carried out according to the method of Johnson (1941) except the digestion mixture contained 4.5 N instead of 2 N H_2SO_4 to shorten the time in the oven to 3 hours. High titred antisera were diluted with phosphate buffered 1 per cent NaCl (pH 7.0) and the antibody values adjusted according to the formulation of Gengozian and Wolfe (1956).

Some of the experiments of Group I were assayed for equivalence points only. Determinations were made to the nearest μg. of BSA nitrogen. Reaction mixtures identical to

those used for the quantitative precipitin technique above were incubated for 3 hours at $37° \pm 1°$ and at $4° \pm 1°$ for 18 hours. After the incubation period, the tubes were centrifuged at 680 *g* for 5 minutes, and the supernatants were tested for excess antigen or antibody. The first tube of antigen excess was the value reported as that of equivalence.

All the data were tested for significance by either the Student-Fisher *t*-test or the analysis of variance.

RESULTS

The splenectomized and control groups were compared in regard to length of time from the injection of antigen to the first day of antibody appearance (induction period), period from the first appearance of antibody to the peak titre (rise to the peak) and day after injection on which the peak titre occurred (peak day). Mean peak titres and maximum mean titres were also determined. The maximum mean titre was found by determining the mean of all titres for one particular day and was the maximum antibody titre as plotted on the antibody production curves. The mean peak titre was the mean of the maximum titres of birds in one experimental group, regardless of the day on which the maximum fell. The statistical analyses are shown in Table 2 (Student-Fisher *t*-test) and in Table 5 (analysis).

PRIMARY RESPONSE AFTER SPLENECTOMY AT 4, 9 OR 17 WEEKS OF AGE

Chickens splenectomized at 4 weeks were injected at either 6 or 12 weeks. The mean equivalence values for the different bleedings are plotted in Fig. 1. Chickens injected at 6 weeks showed a significant difference ($P<0.001$; Table 2) in the time antibody appeared in the circulation as compared to the controls. The induction period (Table 1) in the

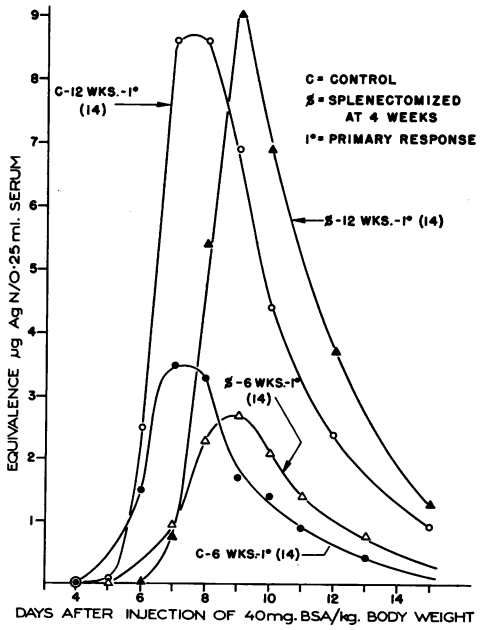

FIG. 1. Precipitin production of chickens splenectomized at 4 weeks and intravenously injected at 6 or 12 weeks. Numbers in parentheses are numbers of animals used in experiment.

fourteen splenectomized birds ranged from 7–9 days with a mean of 7.8 ±0.3 days, while the controls first showed antibody at 5–7 days with a mean of 6.3 ±0.03 days. The peak day occurred at 9.1 ±0.2 days in the splenectomized animals; for the controls this was reached at 7.5 ±0.04 days. This difference was statistically significant ($P<0.001$). A

TABLE 1

EFFECT OF SPLENECTOMY AT VARIOUS AGES ON PRECIPITIN RESPONSE

*Age at operation**	*Age at injection*	*Induction period in days ±S.E.*	*Peak day ±S.E.*	*Rise to peak in days ±S.E.*	*Mean peak titre† ±S.E.*
		Intravenous injection			
4 wks. $	6 wks.	7.8 ±0.3	9.1 ±0.2	1.2 ±0.3	3.3 ±0.7‡
None	6 wks.	6.3 ±0.03	7.5 ±0.04	1.2 ±0.1	3.5 ±0.7‡
4 wks. $	12 wks.	7.6 ±0.2	9.1 ±0.1	1.5 ±0.1	9.3 ±1.3‡
None	12 wks.	6.0 ±0.1	7.5 ±0.2	1.5 ±0.1	9.6 ±1.8‡
9 wks. $	12 wks.	7.6 ±0.1	8.6 ±0.1	1.04 ±0.1	352 ±54
None	12 wks.	6.4 ±0.3	7.4 ±0.2	1.0 ±0.3	348 ±57
17 wks. $	20 wks.	8.1 ±0.2	9.6 ±0.2	1.5 ±0.2	129 ±45
None	20 wks.	6.2 ±0.2	9.0 ±0.1	2.8 ±0.2	239 ±52
17 wks. Sham	20 wks.	5.9 ±0.2	7.6 ±0.2	1.7 ±0.7	396 ±82
		Intravenous (IV) or Subcutaneous (SubC) injection			
8 wks. $	12 wks. IV	7.7 ±0.2	9.4 ±0.2	1.6 ±0.2	109 ±28
None	12 wks. IV	6.3 ±0.1	7.8 ±0.2	1.5 ±0.2	139 ±23
8 wks. $	12 wks. SubC	8.5 ±0.3	9.7 ±0.3	1.2 ±0.3	121 ±44
None	12 wks. SubC	6.7 ±0.3	8.1 ±0.1	1.4 ±0.2	133 ±30

* $ — splenectomy.
† μg. Ab N/ml. ±S.E. unless otherwise stated.
‡ Equivalence in μg. antigen N/0.25 ml.

TABLE 2

SUMMARY OF THE RESULTS OF STATISTICAL ANALYSIS PRIMARY RESPONSE FOLLOWING IV INJECTION

Groups compared	*Age $ or Sh*	*Age injected*	*Days in induction*	*Antibody rise to peak*	*Day of peak titre*	*Mean peak titre*
$* *vs.* C	4	6	$P<0.001$ $-Longer	NS†	$P<0.001$ $-Later	NS
$ *vs.* C	4	12	$P<0.001$ $-Longer	NS	$P<0.001$ $-Later	NS
$ *vs.* C	9	12	$P<0.001$ $-Longer	NS	$P<0.001$ $-Later	NS
$ *vs.* C	17	20	$P<0.001$ $-Longer	$P<0.001$ $-Shorter	$0.01<P<0.02$ $-Later	NS
Sh *vs.* C	17	20	NS	$0.001<P<0.01$ Sh-Shorter	$P<0.001$ Sh-Earlier	NS
$ *vs.* Sh	17	20	$P<0.001$ $-Longer	NS	$P<0.001$ $-Later	$0.001<P<0.01$ $-Lower

* $ — splenectomized, Sh — sham operation. All animals operated on were compared to controls of same age except in last group in which splenectomized animals were compared to sham operated.
† NS — not significant, $P>0.05$.

comparison of the mean equivalences of splenectomized animals and controls revealed a significantly lower ($P<0.001$) equivalence in the controls only on days 6 and 7. Since there was no difference in the mean peak titre or the maximum mean titre as measured by equivalence (μg. BSA N combining with 0.25 ml. anti-serum), the longer induction period and later peak day were interpreted as a delayed and not as a depressed response.

The group of birds injected at 12 weeks of age gave results similar to those animals receiving an injection at 6 weeks of age, except that the antibody titres were higher in the older animals (Fig. 1). Again, the splenectomized animals showed a statistically significant delay ($P<0.001$) in antibody appearance and a later peak day ($P<0.001$), but no significant difference in mean peak titre or maximum mean titre. A comparison of the mean equivalences for each day showed that the titres were significantly higher in the control animals on days 6 and 7 ($0.001<P<0.01$, $P<0.001$, respectively).

TABLE 3

PRECIPITIN PRODUCTION OF 12-WEEK-OLD CHICKENS SPLENECTOMIZED AT 9 WEEKS

	Splenectomized			*Controls*		
		μg. *Ab N/ml.*			μg. *Ab N/ml.*	
*Day**	*Response†*	*Range*	*Mean‡ ±S.E.*	*Response*	*Range*	*Mean ±S.E.*
5	Ag+(14)			Ag+(3) Ag−Ab−(3) Ab+(3)		
7	Ab−(9) Ab+(5)	0–318	44±24	Ab+(10)	64–549	337±56
8	Ab+(14)	32–844	280±59	Ab+(10)	64–531	295±51
9	Ab+(14)	152–803	322±49	Ab+(10)	52–376	160±33
10	Ab+(14)	96–710	275±57	Ab+(10)	15–256	96±24

* Day after antigen injection.

† 'Ag+' — antigen in the circulation; 'Ag−' — no antigen in circulation; 'Ab+' — antibody in the circulation; 'Ab−' — no antibody in circulation. The numbers in parentheses refer to the numbers responding in that manner.

‡ Non-producers are averaged as 'O' Ab N.

Animals splenectomized at 9 weeks and challenged at 12 weeks with BSA responded in essentially the same manner as those splenectomized at 4 weeks. Although splenectomized animals responded with a longer induction period ($P<0.001$) and later peak day ($P<0.001$), the mean peak titre and maximum mean titres were not different from the controls. Table 3 records the mean antibody nitrogen values and the range of titres for the operated and control groups. When the daily mean titres were analysed, the splenectomized animals showed a lower seventh day titre, as had been previously reported for the birds splenectomized at 4 weeks. However, as the circulating antibody titre decreased in the controls, it continued to rise in the splenectomized animals. These splenectomized animals showed a significantly higher titre than the controls on day 9 ($0.01<P<0.02$) and day 10 ($0.001<P<0.01$). This heightened response in the splenectomized animals after the peak day of the controls was evident in other experiments, but the difference in mean titres was never statistically significant ($P>0.05$).

Birds that were close to serological maturity (Wolfe, Mueller, Neess and Tempelis, 1957) were splenectomized at 17 weeks of age. In this experiment a surgical control group was added to determine if any of the effects seen were due to the surgical trauma. The mean antibody titres are presented in Table 4. The experimental group showed an induction period of 8.1 ±0.2 days, which was longer ($P<0.001$) than the unoperated (6.2 ±0.2 days) or the surgical controls (5.9 ±0.2 days). The peak day in the splenectomized group (9.6 ±0.2 days) was significantly later than in the unoperated (9.0 ±0.2 days; $0.01<P<0.02$) or the surgical controls (7.6 ±0.2 days; $P<0.001$).

TABLE 4

PRECIPITIN PRODUCTION OF 20-WEEK-OLD CHICKENS SPLENECTOMIZED AT 17 WEEKS

	Splenectomized			*Controls*			*Sham-operated*		
	Mean body weight in grams 3012 ± 118			3200 ± 107			3332 ± 81		
		μg. *Ab N/ml.*			μg. *Ab N/ml.*			μg. *Ab N/ml.*†	
*Day**	*Response*†	*Range*	*Mean*‡ ± *S.E.*	*Response*	*Range*	*Mean* ± *S.E.*	*Response*	*Range*	*Mean* ± *S.E.*
4	Ag + (13)			Ag + (12)			Ag + (11)		
5	Ag + (13)			Ag + (8)			Ag + (4)		
				Ag − Ab − (3)			Ag − Ab − (5)		
				Ab + (1)	0–15	1.2 ± 1.2	Ab + (2)	0–15	2.7 ± 1.8
6	Ag + (13)			Ag + (1)			Ag + (1)		
				Ag − Ab − (3)			Ag − Ab − (1)		
				Ab + (8)	0–452	57 ± 36	Ab + (10)	0–260	129 ± 25
7	Ag + (9)						Ag + (1)		
	Ag − Ab − (3)						Ab + (10)	0–742	335 ± 79
	Ab + (1)	0–15	1.2 ± 1.2	Ab + (11)	0–607	135 ± 45			
				Ag + (1)					
8	Ag + (3)						Ag + (1)		
	Ag − Ab − (2)						Ab + (10)	0–582	319 ± 70
	Ab + (8)	0–146	30 ± 13	Ab + (11)	0–679	201 ± 51			
9	Ag + (2)						Ag + (1)		
				Ag − Ab − (1)			Ab + (10)	0–535	252 ± 56
	Ab + (11)	0–465	92 ± 36	Ab ± (11)	0–418	232 ± 52			
10	Ag + (2)						Ag + (1)		
	Ag − Ab − (2)			Ag − Ab − (1)			Ab + (10)	0–326	185 ± 37
	Ab + (8)	0–479	100 ± 41	Ab + (11)	0–418	163 ± 34			
11	Ag + (2)						Ag + (1)		
	Ag − Ab − (2)			Ag − Ab − (1)			Ag + (10)	0–299	148 ± 34
	Ab + (9)	0–460	84 ± 36	Ab + (11)	0–270	105 ± 22			
12	Ag + (2)								
	Ag − Ab − (2)			Ag − Ab − (1)			Ag − Ab − (2)		
	Ab + (9)	0–320	65 ± 25	Ab + (11)	0–220	73 ± 18	Ab + (9)	0–202	103 ± 24
15	Ag + (1)								
	Ag − Ab − (5)			Ag − Ab − (2)			Ag − Ab − (2)		
	Ab + (7)	0–48	19 ± 8	Ab + (10)	0–104	29 ± 10	Ab + (9)	0–155	43 ± 14

* Day after antigen injection.

† 'Ag −' — antigen in circulation; 'Ag − Ab −' — neither antigen nor antibody in the circulation; 'Ab +' — antibody in circulation. The number in parentheses refers to the number responding in that manner.

‡ Non-producers are averaged as 'O' Ab N.

Splenectomy at 17 weeks appeared to depress the antibody response as measured by mean peak or maximum mean titres. Compared with the surgical controls, the splenectomized group showed a lower mean peak ($0.001 < P < 0.01$) and lower maximum mean titre ($0.001 < P < 0.01$). However, only the maximum mean titre was lower ($0.02 < P < 0.05$) when the splenectomized group was compared to the intact controls. Further evidence for the depression in the antibody response was found by comparing the daily mean Ab N titres in the splenectomized and control animals. The response was much lower ($P < 0.001$) in the splenectomized than in the surgical controls on days 6, 7, 8, and significantly lower ($0.02 < P < 0.05$) on day 9. If the daily mean titres of the splenectomized animals were compared to the intact control animals, the response of the splenectomized animals was significantly lower on days 7, 8 and 9 ($0.001 < P < 0.01$, $0.001 < P < 0.01$, and $0.02 < P < 0.05$, respectively).

The induction period was similar in the surgical and intact controls (6.2 ± 0.2 days and 5.9 ± 0.2 days, respectively). The rise to the peak titre for the intact controls was 2.8 ± 0.2 days (Table 1), which was generally much longer than for the other control or operated groups. This longer rise no doubt resulted in a later ($P < 0.001$) day of peak titre for the intact controls.

It can be concluded from these experiments that chickens splenectomized at 4, 9 or 17 weeks of age and given a single, intravenous injection of 40 mg. BSA/kg. body weight showed a longer induction period and a later peak day. Only the animals splenectomized at 17 weeks gave a marked depression of antibody titre; this was not evident in the animals splenectomized at an earlier age.

SPLENECTOMY AT 8 WEEKS FOLLOWED BY INJECTION OF BSA EITHER BY INTRAVENOUS OR SUBCUTANEOUS ROUTE

Two groups of chickens were splenectomized at 8 weeks of age and injected either intravenously or subcutaneously at 12 weeks of age. Results of this experiment are found in Tables 1 and 5 and Fig. 2.

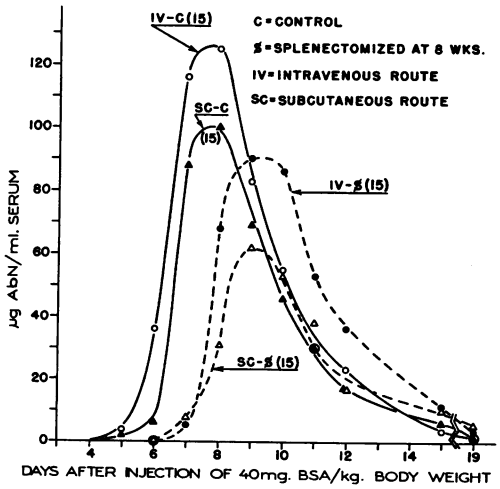

FIG. 2. Precipitin production of chickens splenectomized at 8 weeks and injected either intravenously or subcutaneously at 12 weeks. Numbers in parentheses are numbers of animals used in experiment.

No differences were found between the two control groups in any of the parameters tested (Table 5). A response similar to that described in the first experiment was shown by the splenectomized animals injected by either intravenous or subcutaneous route. There was one difference between birds injected by the two different routes after splenectomy. Those injected intravenously had a shorter ($0.01 < P < 0.025$) induction period than those injected subcutaneously.

TABLE 5

ANALYSIS OF VARIANCE OF DATA OF 12-WEEK-OLD CHICKENS INJECTED INTRAVENOUSLY OR SUBCUTANEOUSLY AFTER SPLENECTOMY AT 8 WEEKS

*Groups analysed**		*Days in induction*	*Antibody rise to peak*	*Day of peak titre*	*Mean peak titre*
IV-$†	*vs.* IV-C	Longer $P<0.005$	NS‡	Later $P<0.005$	NS
SubC-$†	*vs.* SubC-C	Longer $P<0.005$	NS	Later $P<0.005$	NS
IV-$†	*vs.* SubC-$	Shorter $P>0.01$ $P<0.025$	NS	NS	NS
IV-C	*vs.* SubC-C	NS	NS	NS	NS

* IV — intravenous; $ — splenectomized; C — control; SubC — subcutaneous.
† Refers to group showing significant difference.
‡ NS — not significant, $P>0.05$.

Splenectomized animals which were injected intravenously or subcutaneously showed differences in length of induction and peak day when compared to control animals injected by the same routes (Tables 1 and 5). The splenectomized chickens which were injected intravenously showed a longer ($P<0.005$) induction period (7.7 ± 0.2 days) than the control group (6.3 ± 0.1 days) injected in the same manner. A similar difference was noted between the splenectomized and non-splenectomized groups which were injected subcutaneously ($P<0.005$). The peak day for the intravenously injected splenectomized group was later (9.4 ± 0.2 days) than that of the control group injected intravenously. This difference was significant ($P<0.005$). Comparison of the splenectomized and control groups injected subcutaneously also revealed a later peak day in the splenectomized animals ($P<0.005$). No significant differences were noted in rise to the peak titre, mean peak titre or maximum mean titre, when a comparison was made between the splenectomized animals injected intravenously and the control animals similarly injected. The same parameters revealed no differences between the subcutaneously injected splenectomized and control groups.

Splenectomized animals injected intravenously had significantly lower antibody titres than the intravenous controls on days 7 and 8 ($P<0.001$ and $0.02<P<0.05$, respectively). The splenectomized animals injected subcutaneously showed lower antibody titres than controls only on day 7 ($0.001<P<0.01$).

In summary, splenectomy followed by either a subcutaneous or intravenous injection of BSA caused a delay in the appearance of antibody and time of maximum Ab N in the circulation. No differences were found between the subcutaneous and intravenous controls. The induction period was longer in the splenectomized chickens after subcutaneous injection than after an intravenous injection.

DISCUSSION

The spleen may be considered as containing part of a large population of immunologically competent cells which may respond differently, depending on their location. Differences in the potentialities of this cell population, including the ability to compensate for the loss of a large number of cells, might vary with the age of the organism. The present data suggests that splenectomy at different ages affects antibody production of chickens injected with BSA, a soluble antigen.

Animals were splenectomized at various ages and the amount of circulating precipitin was determined. In another experiment the effect of the route of injection on antibody production of splenectomized animals was studied. The use of a soluble antigen in antibody production studies makes possible the application of a quantitative nitrogen technique as an assay method. Previously, most workers have utilized either haemolysin or agglutinin titres to assay the antibody response in the splenectomized animal.

Antibody production was determined from daily bleedings and statistical analyses have been applied to evaluate the data. This is necessary because of the great variation in antibody response. Previously few workers have used statistics to interpret their findings (Taliaferro and Taliaferro, 1950, 1951; Draper and Süssdorf, 1957).

Splenectomy experiments have most often been done with adult animals. With particulate antigens a depression in the response as well as a lag in antibody appearance was reported (Luckhardt and Becht, 1911; Hektoen, 1915; Motohashi, 1922; Rowley, 1950 and 1950a; Taliaferro and Taliaferro, 1950, 1951; Draper and Süssdorf, 1957). These results were similar to the data presented for the birds splenectomized at 17 weeks, an age comparable to that used by previous workers. A possible explanation for the depression found only in the older animals is the inability of the adult animals to compensate as well as the younger animal for the loss of a large lymphoid organ. The capacity of chickens splenectomized at the early age of 4, 8 or 9 weeks to respond with the same peak titres as unoperated controls may be attributed to the hyperplasia or increased activity of cells capable of antibody production.

The lag in antibody production in splenectomized animals injected either intravenously or subcutaneously suggests that the spleen synthesizes antibody earlier than other antibody-forming tissue. This may be ascribed to the presence of a greater number of immunologically competent cells in the spleen or to a more effective uptake of antigen by this tissue. Supporting evidence for the early antibody synthesis by the spleen has been shown by many workers. The later antibody appearance and later peak day in splenectomized animals challenged with an antigen as reported by Taliaferro *et al.* (1950, 1951), Draper and Süssdorf (1957) and by us is consistent with this hypothesis. Secondly, if animals are splenectomized at the peak of the antibody-production curve, there is no difference between the descending portion of the curve in the operated or control groups (Taliaferro and Taliaferro, 1950). More recently, Thorbecke, Asofsky, Hochwald and Siskind (1961) have reported direct evidence for the early role of the spleen. After an intravenous injection of bovine γ globulin into rabbits only the spleen produced antibody in tissue culture as early as the fifth day after immunization.

Several studies supply pertinent information on a differential role of the spleen after subcutaneous as compared to intravenous injection. Pfeiffer and Marx (1898) suggested that the spleen was the major site of antibody production after subcutaneous injection of cholera vibrios. Further support of this has come more recently from the work of Stavitsky

(1954) who reported similar titres in rabbits receiving spleen transfers from animals which had been injected either intravenously or into the foot pad. On the other hand, popliteal lymph nodes transferred antibody-producing capacity only following a subcutaneous injection of alum-precipitated diphtheria toxoid. Thorbecke and Keuning (1953), also using tissue-culture techniques, concluded that the spleen is the main source of antibody after subcutaneous injection. In their experiments young rabbits injected intravenously or subcutaneously with paratyphoid B vaccine showed four to five times more antibody in the red pulp of the spleen than in the lymph nodes.

The experiments of Draper and Süssdorf (1957) support the lesser role of the spleen in antibody production after subcutaneous injection. There was no difference between the responses of splenectomized and control rabbits to antigen injected intravenously or subcutaneously. Extracts of rabbit spleen showed just a trace of antibody following a subcutaneous injection of *Shigella paradysenteriae* (Harris, Rhodes and Stokes, 1948). Stavitsky (1957) found relatively low agglutinin titres in rabbits which received spleen homotransplants from animals injected with 16 LF diphtheria toxoid in the hind foot pad. These results differed greatly from those in which more than twice this dose (40 LF) was injected (Stavitsky, 1954). Harris and Harris (1950) found that titres of spleen extracts were similar to those obtained from the regional lymph nodes when a 5 mg. dose of *Shigella* was administered to rabbits. When a smaller amount (1 mg.) was injected, the spleen produced much less antibody than did the lymph nodes.

The splenectomized chicken showed similar results in antibody production regardless of the route of injection. The only variation between the two groups of splenectomized animals was a longer induction period in the subcutaneously injected animals. This difference may indicate a small difference in the time it takes for a given amount of antigen to reach and stimulate the non-splenic lymphoid tissue.

Discrepancies between our experiments and those described earlier, regarding the function of the spleen, might be explained by differences in type and dosage of antigen, animal used and bleeding schedule.

ACKNOWLEDGMENTS

This project was supported in part by the National Science Foundation and in part by the Research Committee of the University of Wisconsin Graduate School, from funds supplied by the Wisconsin Alumni Research Foundation. The authors wish to express their appreciation to J. Peter Wall for technical assistance.

REFERENCES

DRAPER, L. R. and SÜSSDORF, D. H. (1957). 'The serum hemolysin response in intact and splenectomized rabbits following immunization by various routes.' *J. infect. Dis.*, **100**, 147-61.

GENGOZIAN, N. and WOLFE, H. R. (1956). 'Precipitin production in chickens. XIV. Effect of dilution of chicken antisera on the amount of precipitin.' *J. Immunol.*, **77**, 172-80.

GOODMAN, M., WOLFE, H. R. and NORTON, S. (1951). 'Precipitin production in chickens. VI. The effect of varying concentrations of NaCl on precipitate formation.' *J. Immunol.*, **66**, 225-36.

HARRIS, T. N. and HARRIS, S. (1950). 'Biological and technical factors in the demonstration of antibody production by lymphatic tissue.' *J. Immunol.*, **64**, 45-56.

HARRIS, T. N., RHOADS, J. and STOKES, J. (1948). 'A study of thymus and spleen in the formation of antibodies in the rabbits.' *J. Immunol.*, **58**, 27-32.

HEIDELBERGER, M., KENDALL, F. E. and SOO HOO, C. M. (1933). 'Quantitative studies on the precipitin reaction. Antibody production in rabbits injected with an azoprotein.' *J. exp. Med.*, **58**, 137-52.

HEKTOEN, L. (1915). 'The influence of X-rays on the production of antibodies.' *J. infect. Dis.*, **17**, 415-22.

LUCKHARDT, A. B. and BECHT, F. C. (1911). 'The relation of the spleen to the fixation of antigens and the production of immune bodies.' *Amer. J. Physiol.*, **28**, 257-74.

MOTOHASHI, S. (1922). 'The effect of splenectomy upon the production of antibody.' *J. med. Res.*, **43**, 473-85.

PFEIFFER, R. and MARX, D. (1898). 'Die Bildungstätte der Choleraschutzstoffe.' *Z. Hyg. InfektKr.*, **27**, 272-97.

ROWLEY, D. A. (1950a). 'The effect of splenectomy on the formation of circulating antibody in the adult male albino rat.' *J. Immunol.*, **64**, 289-95.

ROWLEY, D. A. (1950b). 'The formation of circulating antibody in the splenectomized human being following intravenous injection of heterologous erythrocytes.' *J. Immunol.*, **65**, 515-21.

STAVITSKY, A. B. (1954). 'Participation of the popliteal lymph node and spleen in the production of diphtheria antitoxin in the rabbit.' *J. infect. Dis.*, **94**, 306-18.

STAVITSKY, A. B. (1957). 'Antibody synthesis by homotransplanted cells and tissues. I. Study of factors which influence the process in the rabbit.' *J. Immunol.*, **79**, 187-99.

TALIAFERRO, W. H. and TALIAFERRO, L. G. (1950). 'The dynamics of hemolysin formation in intact and splenectomized rabbits.' *J. infect. Dis.*, **87**, 37-62.

TALIAFERRO, W. H. and TALIAFERRO, L. G. (1951). 'The role of the spleen in hemolysin production in rabbits receiving multiple antigen injections.' *J. infect. Dis.*, **89**, 143-68.

THORBECKE, G. J. and KEUNING, F. J. (1953). 'Antibody formation *in vitro* by haemopoietic organs after subcutaneous and intravenous immunization.' *J. Immunol.*, **70**, 129-34.

THORBECKE, G. J., ASOFSKY, R., HOCHWALD, G. M. and SISKIND, G. W. (1961). 'Antibody production *in vitro* by the spleen and bone marrow at various days after injection.' *Fed. Proc.*, **20**, 25.

WOLFE, H. R., MUELLER, A., NEESS, J. and TEMPELIS, C. (1957). 'Precipitin production in chickens. XVI. The relationship of age to antibody production.' *J. Immunol.*, **79**, 142.

WOLFE, H. R., NORTON, S., SPRINGER, E., GOODMAN, M. and HERRICK, C. (1950). 'Precipitin production in chickens. V. The effect of splenectomy on antibody formation.' *J. Immunol.*, **64**, 179-84.