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Influence of Splenectomy in Rats on the Formation of 19S and 7S Antibodies

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Summary. Adult rats were immunized with human γ-globulin, in heat aggregated form intravenously, and with complete adjuvant intravenously or in the footpads. In all animals an intravenous booster injection was given on day 25. Splenectomy or sham splenectomy was performed 7 days prior to or 3 and 21 days after immunization, and 19S and 7S antibody activities were determined separately in the sera of these animals in the primary and the secondary response. Splenectomized young rats immunized with human γ-globulin in complete adjuvant intravenously or in the footpads were similarly studied.

The formation of 19S and 7S antibody was affected separately depending upon the route of antigen administration and time of splenectomy. Furthermore, the inhibitory effect of splenectomy on antibody formation was more pronounced in young than in adult rats.

INTRODUCTION

The spleen is known to be an important site of antibody production. Splenectomy results in a reduction of antibody formation in many species, including monkeys (Saslaw and Carlisle, 1964), rabbits (Taliaferro and Taliaferro, 1950), chickens (Rosenquist and Wolfe, 1962) and rats (Rowley, 1950). In rats, splenectomy also causes a reduction of normal γ-globulin synthesis (Andersen and Bierring, 1964). Preliminary studies on the formation of 19S and 7S antibodies in splenectomized rabbits after immunization with particulate (Davidsohn, Lee and Zandrew, 1964) or soluble antigen (Sahiar and Schwartz, 1965) have given divergent results.

In this paper, the effect of splenectomy on the formation of 19S and 7S antibodies in young and adult rats is reported. Different routes of antigen administration were studied and splenectomy was performed at different times before and during immunization. Human γ-globulin was used as the antigen since it has been shown regularly, in preliminary studies, to elicit the formation of 19S antibody followed by the appearance of 7S antibody.

MATERIAL AND METHODS

Animals. Adult CFE male rats (*Bartonella* free) weighing 200–250 g and young (2–3 weeks) CFE male rats weighing 80–100 g were used in these studies.

Antigen. Human γ-globulin (HGG) (Cohn Fraction II from Squibb Laboratories) was used either heat aggregated (20 minutes at 63°) or emulsified in complete Freund's adjuvant (Difco Laboratories).

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Immunization. The animals were immunized either intravenously or in the footpads with 500 μg HGG either as a heat aggregated preparation, or emulsified in 0·1 ml of complete Freund's adjuvant for intravenous injection, or 0·2 ml for footpad injection. Twenty-five days later, all animals received a booster injection of 100 μg HGG intravenously, using the same antigen preparation as the one used for immunization (heat aggregated HGG without adjuvant or non-heat aggregated HGG emulsified in 0·1 ml of Freund's adjuvant).

Splenectomy and sham splenectomy

The spleen was removed from anaesthetized rats through a left lower abdominal incision under aseptic conditions. Sham splenectomy consisted in the opening of the left abdominal cavity under similar aseptic conditions.

Experimental design (see Fig. 1)

(a) *Adult animals. Group I*: Five control animals and five experimental animals, splenectomized 7 days before antigen injection, were immunized by the intravenous injection of aggregated HGG.

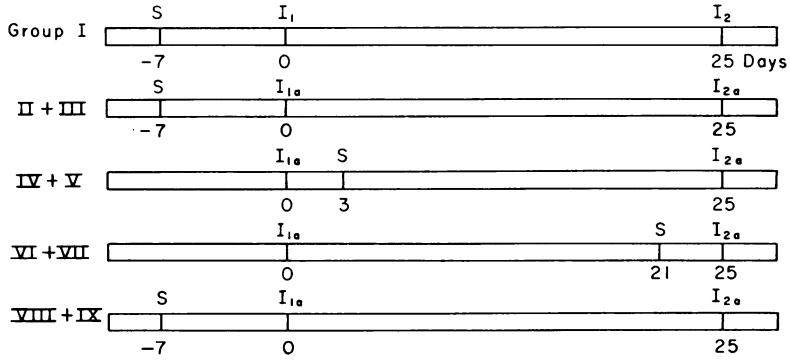

FIG. 1. S = Splenectomy or sham splenectomy; I_1 = injection of 500 μg of heat aggregated human γ-globulin (HGG); I_{1a} = injection of 500 μg of HGG in complete Freund's adjuvant; I_2 = booster injection of 100 μg of heat aggregated HGG; I_{2a} = booster injection of 100 μg of HGG in complete Freund's adjuvant.

Group II: Eight animals which had received sham operations and eight animals which had been splenectomized were treated as Group I but the antigen was given intravenously in complete Freund's adjuvant.

Group III: Eighteen animals, nine splenectomized and nine sham operated, were treated as Group II but the initial injections of antigen were given in complete Freund's adjuvant into the footpads.

Group IV: Twelve animals, six splenectomized and six sham operated, were immunized intravenously with antigen in complete adjuvant 3 days before operation.

Group V: Ten animals, five splenectomized and five sham operated, were immunized in the footpads with antigen in complete adjuvant. They were operated on 3 days after immunization.

Group VI: Twelve animals were immunized intravenously with antigen in complete Freund's adjuvant and operated on 21 days later, six by splenectomy and six by sham operation. A booster injection was given 4 days after the operation.

Group VII: Twelve animals were immunized in the footpads with antigen in complete adjuvant and treated as those in Group VI.

(b) *Young rats. Group VIII*: Eight animals were operated (four with sham operation and four with splenectomy) 7 days before an intravenous immunization with antigen in complete Freund's adjuvant.

Group IX: Sixteen animals were similarly treated as Group VIII but the antigen was injected into the footpads.

Serological tests

Animals were bled at 5-day intervals from the tail vein.

Treatment of the serum with 2-mercaptoethanol (ME)

Rat serum was diluted 1:5 in buffered saline and incubated with ME (in a final concentration of 0·1 M) for 30 minutes at 37°. After incubation, the serum was dialysed in the cold overnight against buffered saline containing 0·02 M iodoacetamide.

Antibody activity

Passive haemagglutination according to a modification of the micro method of Heller, Kolodny, Lepow, Jacobson, Rivera and Marks (1955), using formolized sheep red blood cells, was performed with both untreated and ME treated sera.

Chromatography on Sephadex G-200

Two millilitres of serum were applied to a (2·5 × 90 cm) column of Sephadex G-200 at 5° and eluted in the cold at the rate of 4–9 ml/hr with 0·2 M NaCl–0·1 M Tris (hydroxymethylaminoethane) buffer pH 8·0. Three millilitre fractions were collected. Protein concentration was measured at 280 mμ. Individual fractions or pooled fractions, corresponding to either the 19S or the 7S peak, were dialysed overnight against buffered saline and treated with ME. Passive haemagglutination of the untreated or ME treated (individual or pooled) fractions were performed.

Sucrose density gradient ultracentrifugation

The method used was that described by Edelman, Kunkel and Franklin (1958). Serum, 0·3 ml, was layered over 5 ml of a continuous gradient of sucrose from 40 to 10 per cent in 0·15 M NaCl prepared in a 5·5-ml centrifuge tube. Centrifugation was performed in a Spinco model L ultracentrifuge with a SW 39 swinging bucket rotor at 32,500 rev/min for 18 hours (115,700 ***g***). Twelve to fourteen fractions of six drops each were collected through a small perforation at the bottom of the centrifuge tube. After dialysis against 0·15 M saline, the protein concentration of the fractions was determined by the method of Lowry, Rosebrough, Farr and Randall (1951). In some experiments, fractions were pooled to obtain a rapidly sedimenting fraction rich in 19S globulin and essentially free of 7S globulin and a fraction containing the bulk of 7S globulin. In other experiments, individual fractions were examined serologically. Aliquots of each individual or pooled fraction were then diluted 1:2 with buffered saline and treated with ME. Haemagglutinin activity was determined in each untreated and ME treated sample. In some experiments human macroglobulin (19S) was used as a marker.

Passive cutaneous anaphylaxis (PCA)

PCA was done according to a modification of the method described by Binaghi and Benacerraf (1964). Freshly prepared serum was used, 0·1 ml of which was

injected intradermally into the dorsal region of recipient animals. After a latent period of 16–20 hours, 10 mg of HGG in 1 per cent Evans blue was injected intravenously and the results recorded after 60 minutes. Three animals were used for each experiment and the PCA was considered positive when present in at least two animals.

Complement fixation

Complement fixation was performed according to the method described by Kabat and Mayer (1961).

RESULTS

I. EXPERIMENTS IN ADULT RATS

(a) *Splenectomy 1 week prior to immunization* (Fig. 2)

All control animals of Group I given heat aggregated HGG intravenously formed ME sensitive antibody in the primary and the secondary responses. In one animal part of the

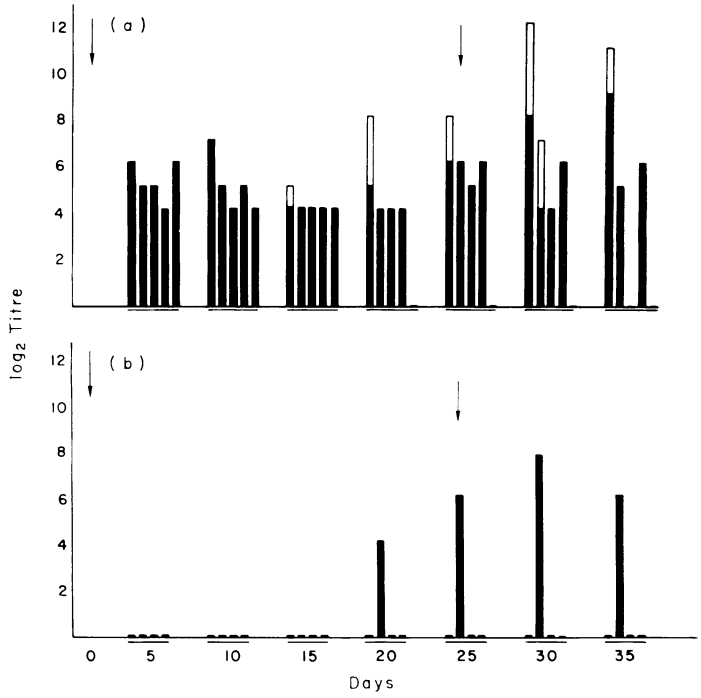

FIG. 2. Result of passive haemagglutination test of animals of Group I. (a) Control animals, (b) splenectomized animals. Each column represents one animal. Black columns, ME sensitive antibody activity; white columns, ME resistant antibody activity. The arrows indicate intravenous injection of heat aggregated human globulin (500 μg at day 0 and 100 μg at day 25).

haemagglutinin antibody activity of the primary response was ME resistant. Two animals had a small proportion of ME resistant antibody activity in the secondary response. Only one of four splenectomized animals formed antibody late in the primary, as well as in the secondary responses. This antibody was ME sensitive and as shown in Fig. 3, sedimented in the 19S fraction on density gradient centrifugation.

Intravenous injection of the antigen in complete adjuvant in eight sham operated

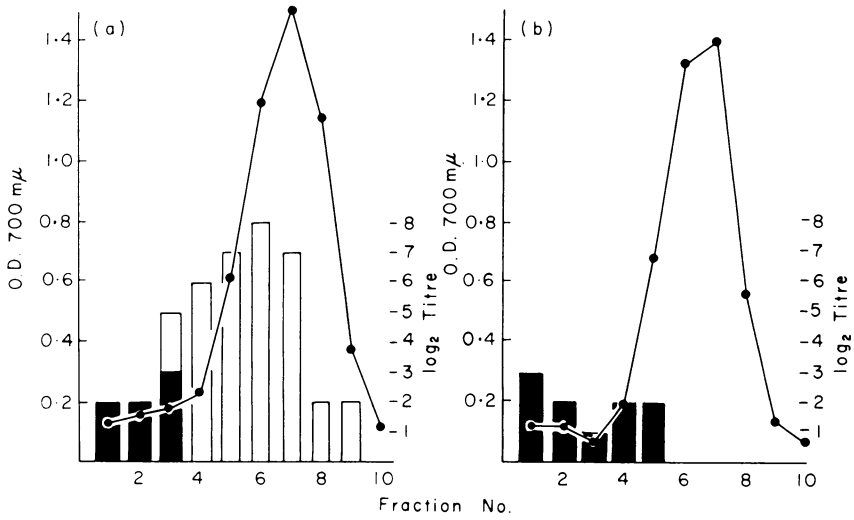

FIG. 3. Results of passive haemagglutination test of serum fractions obtained by sucrose gradient ultracentrifugation. (a) Serum from sham operated animal of Group I at day 30 (see Fig. 2). (b) Serum from splenectomized animal 2 of Group I at day 30 (see Fig. 2). The haemagglutination titre is indicated by the heights of the column. Black columns, ME sensitive antibody activity; white columns, ME resistant antibody activity.

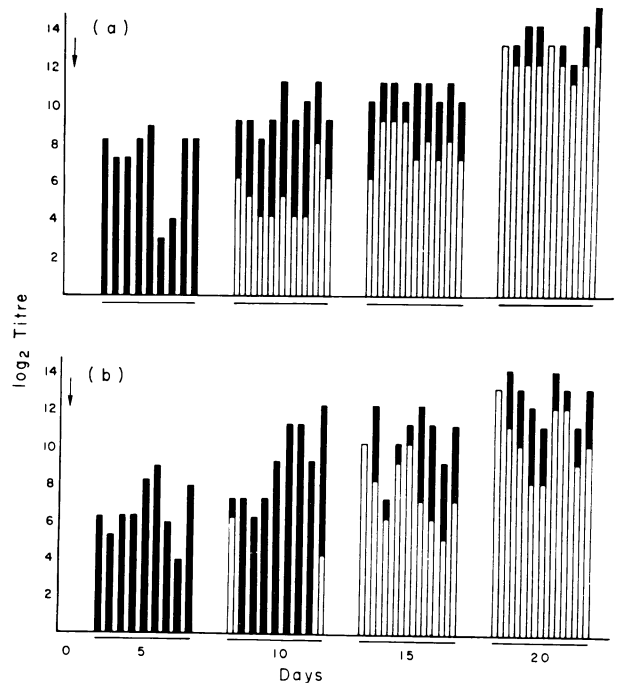

FIG. 4. Result of passive haemagglutination test of sera from animals of Group III. (a) Sham operated animals, (b) splenectomized animals. Each column represents one animal. Black columns, ME sensitive; white columns, ME resistant. The arrow indicates injection of 500 μg of human γ-globulin in complete Freund's adjuvant into the footpads.

animals and eight splenectomized animals (Group II) resulted in the appearance of a similar titre of antibody in the primary and the secondary response. The mean titre of 7S antibody 20 days after immunization was 1:256 in both groups.

The injection of the antigen in complete Freund's adjuvant into the footpads of control animals resulted in the earlier appearance of 7S antibody than in the intravenously injected control animals (Group III). In splenectomized animals the appearance of the ME resistant antibody was somewhat delayed (Fig. 4). The result of sucrose gradient centrifugation of the serum of a splenectomized animal 10 days after immunization is given in Fig. 5.

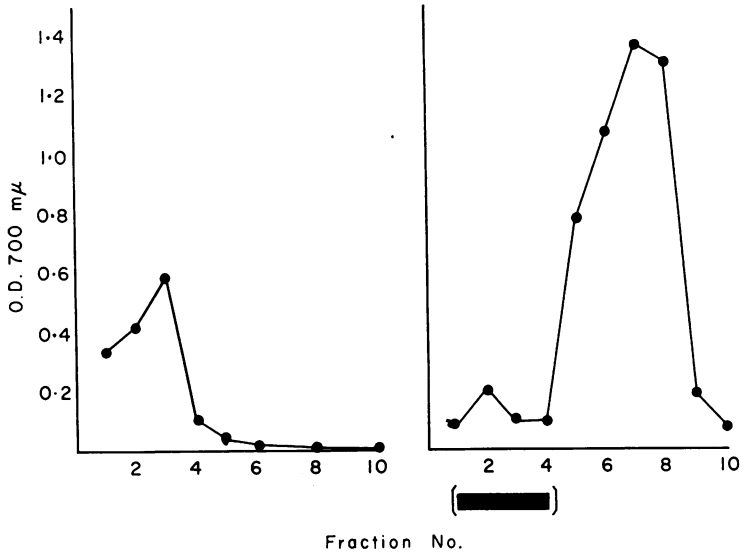

FIG. 5. The right-hand graph shows the result of ultracentrifugation in a sucrose gradient of serum 6 from a splenectomized animal of Group III bled 10 days after immunization (see Fig. 4). It contains only ME sensitive haemagglutinin activity. The serological activity (black bar) is confined to the fractions that correspond to the range of human 19S macroglobulin used as a marker and shown in the left-hand graph.

(b) *Splenectomy 3 days after immunization*

The haemagglutinin antibody activity of animals sham operated or splenectomized 3 days after immunization with antigen in complete adjuvant either intravenously or in the footpads is summarized in Fig. 6.

The intravenous administration of the antigen (Group IV) resulted in a delayed appearance of ME sensitive antibody activity and in a slight diminution of the titre of ME resistant antibody activity which was found not to be statistically significant ($P = 0{\cdot}4$) according to Student's *t*-test. This effect was not observed in the secondary response of these animals. Sera were analysed by chromatography on Sephadex G-200 in order to evaluate better the results obtained by ME treatment of whole sera. ME sensitive antibody was only found in the fractions corresponding to the 19S peak while ME resistant antibody activity was detectable in the second peak corresponding to 7S globulin (Fig. 7).

When the antigen was injected in the footpads (Group V), the effect of splenectomy was much less pronounced (Fig. 6). There was no delay in the appearance of antibody formation. The titres of ME resistant antibodies were slightly lower 20 days after

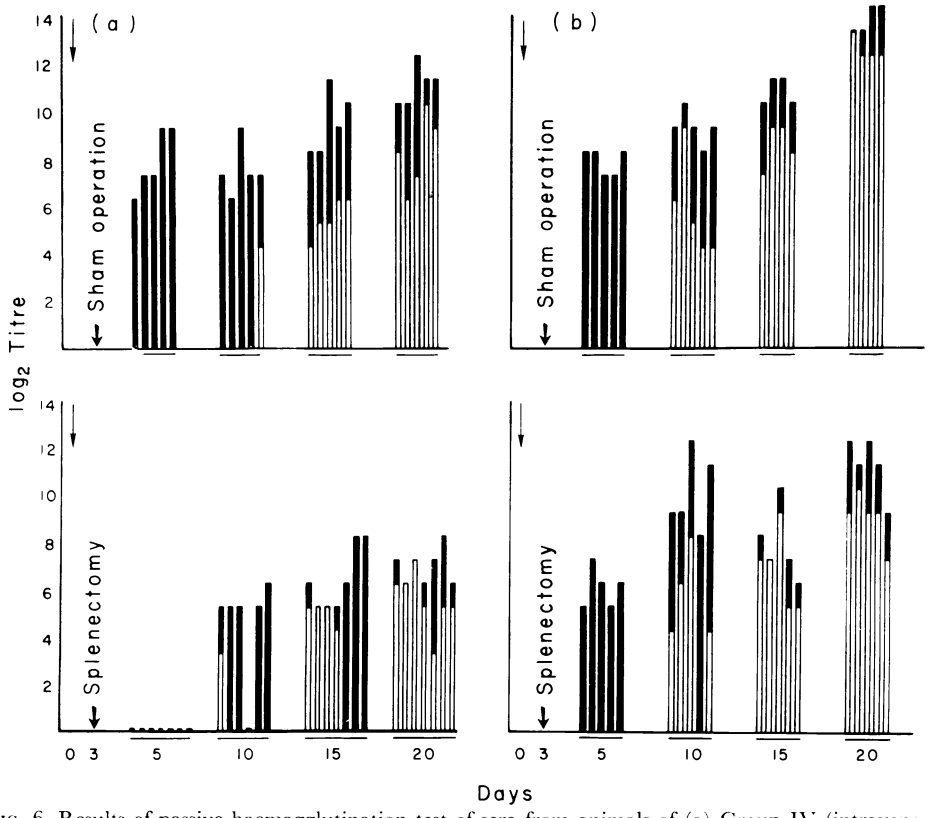

FIG. 6. Results of passive haemagglutination test of sera from animals of (a) Group IV (intravenous immunization), and (b) Group V (immunization in footpads). The arrow at day 0 refers to the injection of 500 μg of HGG in complete adjuvant. Each column represents one animal. Black columns, ME sensitive; white columns, ME resistant activity.

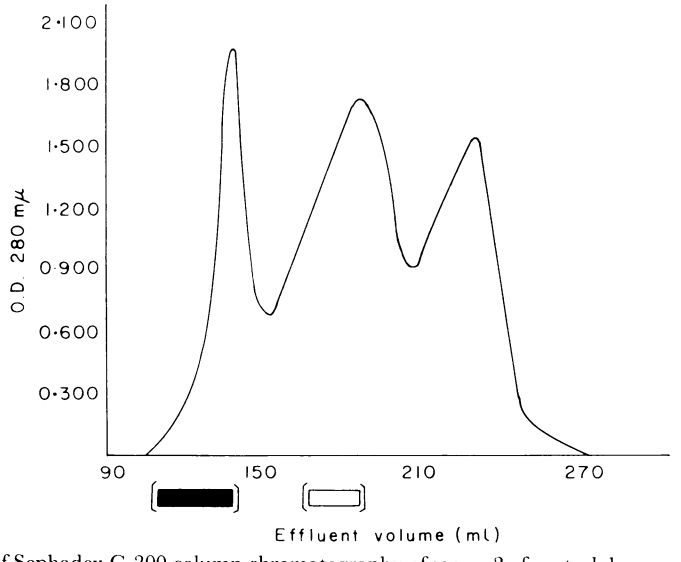

FIG. 7. Result of Sephadex G-200 column chromatography of serum 2 of control sham operated animals Group IV. The ME sensitive activity (black bar) is confined to the first peak, the ME resistant activity (white bar) to the second peak.

immunization. However, the difference in titres between control and experimental animals was found not to be statistically significant ($P = 0{\cdot}15$) according to Student's *t*-test.

(c) *Splenectomy 21 days after immunization*

In order to investigate the influence of splenectomy on the secondary immune response, the operation was performed 4 days prior to an intravenous booster injection of antigen in animals immunized 21 days previously. Splenectomized and sham operated animals of Group VI (immunized intravenously with antigen in complete adjuvant) and of Group VIII (immunized with antigen in complete adjuvant in the footpads) exhibited a similar anamnestic response with a maximum titre of ME resistant antibody of 1:8192 in Group VII.

II. EXPERIENCE IN YOUNG ANIMALS SPLENECTOMIZED A WEEK PRIOR TO IMMUNIZATION

(a) *Intravenous route (Group VIII)*

Splenectomy of 2–3-week-old rats resulted in a marked delay in antibody formation in the primary response (Fig. 8).

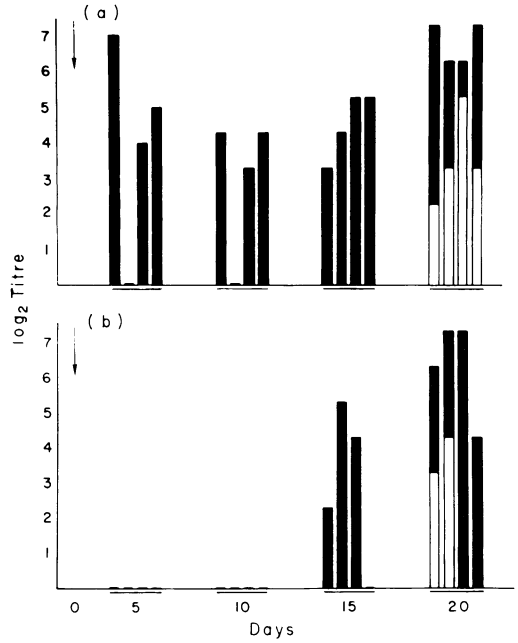

FIG. 8. Result of passive haemagglutinin test of sera of Group VIII (young animals) immunized intravenously. (a) Sham operated animals. (b) splenectomized animals. Each column represents one animal. Black columns, ME sensitive antibody activity; white columns, ME resistant antibody activity.

(b) *Footpad route (Group IX)*

The total antibody titres of rats splenectomized at 2–3 weeks of age and immunized 7 days later in the footpads in complete adjuvant was reduced in comparison to those of sham operated control animals. The difference at day 20 was found to be statistically significant ($P = 0{\cdot}05$) according to Student's *t*-test. The ME resistant antibody response was more markedly reduced than the ME sensitive response; the difference was found to be statistically highly significant at day 20 ($P = 0{\cdot}01$) and still significant at day 25 ($P = 0{\cdot}05$) according to Student's *t*-test.

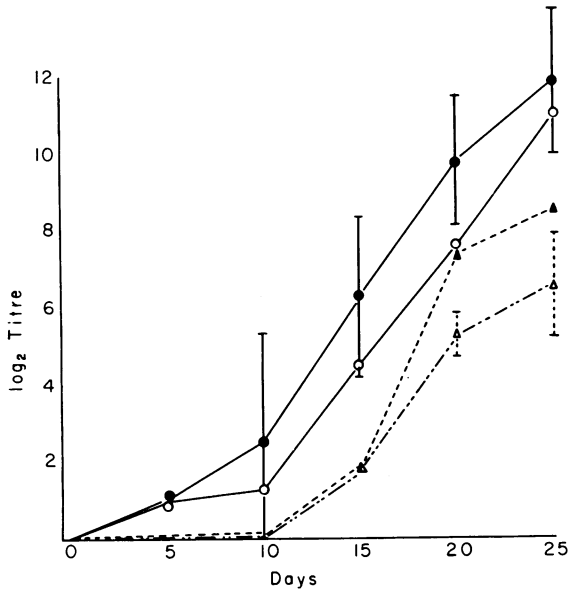

FIG. 9. Mean titre of haemagglutinin antibody activity of eight young splenectomized animals and eight young sham operated animals immunized in the footpads (Group IX). Total serum titre: ●, sham operated animals; ○, splenectomized animals; ME resistant serum titre: ▲, sham operated animals; △, splenectomized animals. The vertical bars demonstrate the confidence limits for a P value of 0·05. All points within this zone are not considered to be significantly different from the reference value. Statistical analysis according to Student's t-test.

INFLUENCE OF SPLENECTOMY ON THE FORMATION OF COMPLEMENT FIXING ANTIBODY AND ANAPHYLACTIC ANTIBODY

The sera of animals of Groups II, IV and VII were examined for complement fixing and anaphylactic (PCA) antibody activity.

Complement fixing antibody appeared in a few animals on day 20 and was present in all animals at day 35. There was no difference in the time of onset and the antibody titres were the same (1:50 to 1:200) in the various groups.

Anaphylactic antibody activity became detectable in some animals at day 30 and was found in ten of eleven sham operated animals of the various groups at day 35 or 45. Anaphylactic antibodies could not be detected in four animals splenectomized 3 days after intravenous immunization (Group IV) and in two animals splenectomized 21 days after immunization (Group VII) 20, 25, 30, 35 and 45 days after immunization. However, splenectomy prior to immunization did not seem to influence the appearance of this antibody. Two of the three splenectomized animals studied in Group II had PCA antibody on day 45.

DISCUSSION

It has been shown previously that splenectomy results in a diminution of antibody formation, particularly when a small dose of antigen is given intravenously. This effect of splenectomy in rats has been well documented for different doses and routes of antigen

administration by Rowley (1959) and more recently by Winebright and Fitch (1962) and Fitch and Winebright (1962).

The results of the present study confirm these data. In addition, they show that splenectomy can have different effects on 19S and 7S antibody formation, depending upon the route of antigen administration and the time of splenectomy. Furthermore, splenectomy was found to affect antibody formation to a greater extent in young animals than in adult animals.

In adult animals, the effect of splenectomy was most pronounced when no Freund's adjuvant was used for intravenous immunization (Group I): most of the splenectomized animals failed completely to form antibody. When the same amount of antigen was given intravenously in complete adjuvant, splenectomized and control animals did not differ in their immune response suggesting that the strength of immunization had overcome the effect of splenectomy. However, when splenectomy was performed 3 days after intravenous immunization with antigen in complete adjuvant, 19S antibody and in some animals 7S antibody formation was delayed; and PCA antibody activity was suppressed.

It is interesting to note that the appearance of 7S antibodies was detected 5 days earlier in control animals immunized in the footpads as compared to control animals immunized intravenously. This early appearance of 7S antibody was not observed in splenectomized animals immunized in the footpads. This result appears to indicate a role of the spleen in the early 7S immune response under these experimental conditions, i.e. when the primary 19S response takes place in the regional lymph nodes. In animals splenectomized 3 days after immunization into the footpads this delay in the appearance of 7S antibody did not occur, suggesting that the spleen has already initiated the formation of the primary 7S response at the time of splenectomy.

The immune function of the spleen with respect to the age of the animal has been investigated in chickens (Rosenquist and Wolfe, 1962). In this species the bursa of Fabricius plays an important role in the development of the spleen. In young chickens when the bursa is still functioning, splenectomy is of little consequence for the immune response while in adult animals when the bursa is undergoing involution, splenectomy results in a marked impairment of the immune response (Cooper, Paterson, South and Good, 1966). A reversed behaviour has been suggested to be operative with human species. An increased susceptibility to infection in small children has been reported after splenectomy (Horan and Colebatch, 1962). While no change in the resistance towards bacterial infections was observed in adults, our data in rats clearly indicate that the immune function of the spleen of the mammal is more important in young than in adult animals. Intravenous immunization of the antigen with Freund's adjuvant resulted in a marked delay in antibody production in splenectomized young rats in contrast to adult animals which had under those experimental conditions a normal immune response. Also, administration of the antigen in complete adjuvant in the footpads of splenectomized young rats resulted in a significant decrease of 7S antibody production which was not observed in adult splenectomized rats.

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