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# Stimulation of antibody synthesis induced by surgical trauma in rats

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## SUMMARY

The effect of a standard laparotomy on antibody synthesis was studied in Wistar R/A rats receiving an intravenous injection of $10^9$ sheep red blood cells (SRBC) during the surgical procedure. Anti-SRBC antibody titres were significantly higher in operated animals than in controls.

When SRBC were given 2 hr after the surgical procedure, stimulation of antibody synthesis still persisted, but when the antigen was administered 24 hr after laparotomy, no significant difference could be detected between the operated animals and controls. Surgery also enhances the secondary humoral response.

## INTRODUCTION

It is usually admitted that surgery depresses the immune response to foreign antigens. However, in a recent survey of the literature, Howard & Simmons (1974) mentioned the need for studies on the effect of surgical trauma on humoral immunity. Cooper, Irvine & Turnbull (1974) have shown a depression of the antibody response in guinea-pigs injected with sheep red blood cells (SRBC) 2–3 hr after a cholecystectomy. We have studied the humoral response when the antigen was administered to rats at the time of operation, i.e. during the period of highest risk of contamination by micro-organisms. The present data indicate that antibody synthesis is enhanced in this situation.

## MATERIALS AND METHODS

*Active immunization.* Inbred white Wistar R/A rats were used for all experiments. Male animals weighing 200± 20g were anaesthetized with ether, bled by cutting the tail vein to determine the anti-SRBC antibody titres prior to surgery and submitted to a midline laparotomy. The bowel loops were allowed to stay outside the abdominal cavity for 2 to 3 min. Surgical procedures were usually performed with a clean, but not sterile, technique. In one set of experiments, however, a rigid aseptic technique was used. Some animals of this group underwent a left nephrectomy instead of a simple laparotomy. The kidney was removed through a left subcostal incision. The rats were injected i.v. with $10^9$ SRBC just prior to the closure of the abdomen. Anaesthetized unoperated controls were injected on the same day as the operated animals with the same batch of SRBC. The body temperature was maintained around 38°C throughout the operative procedure. All the rats were observed for 25 days and bled on days 4, 7, 11, 18 and 25 for the determination of serum haemolysin and haemagglutinin levels. In two experiments, SRBC were given either 2 hr or 1 day after surgery and the time of injection of the antigen was taken as time 0. The secondary antibody response was studied in rats which had received $10^9$ SRBC i.v. 58 days prior to the booster injection. On day 0, the animals were anaesthetized and bled through the tail vein. During the operation, they received a second i.v. injection of $10^9$ SRBC and were then submitted to the same protocol as the animals used for the experiments on the primary antibody response. The effect of anaesthesia was studied by comparing normal controls with rats anaesthetized with ether or with 30 mg per 100 g body weight of chloral hydrate administered i.p. The antigen was injected during anaesthesia.

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*Passive immunization.* Anaesthetized unoperated animals and rats, submitted to a laparotomy, were injected i.v. with $10^9$ SRBC. 7 days later, they were bled to death. The pool of serum obtained from operated rats was diluted 1/4 with normal rat serum in order to reach a haemolysin titre of 1/512, similar to that of the control serum. 3 ml of the diluted serum of operated animals were injected i.v. on post-operative day 7 to rats which had undergone a laparotomy. A second group of rats received 3 ml of control serum 7 days after a simple anaesthesia. The animals were bled 2 hr after the serum injection and on days 1, 2, 3, 4 and 7. The anti-SRBC antibody titres of the samples were determined.

*Haemolysin and haemagglutinin titration.* The method for haemolysin titration was derived from the microtechnique of platelet complement fixation of Colombani *et al.* (1971). It is described in detail elsewhere (Kinnaert *et al.*, 1976). The highest dilution of rat serum causing complete haemolysis was defined to be the titre of the serum. Haemagglutinin levels were determined after 2 hr incubation at 37°C. The last dilution where haemagglutination was observed was taken as the titre of the serum. Titres are expressed according to the formula $1-\log_2$ of the titre and 0 corresponds to no detectable antibody.

*Mercaptoethanol (2ME) treatment.* Groups of five rats were submitted to the protocol of active immunization. Each time time they were bled, their sera were pooled. The total anti-SRBC activity and 2ME-resistant anti-SRBC antibody titre of these pools were determined. Inactivation of 19S antibodies was done with 2ME according to Uhr & Finkelstein (1963). The aliquots used for the determination of total anti-SRBC activity were treated under the same conditions, except that 2ME was omitted. All the samples of serum were dialysed overnight at 4°C against phosphate buffer before the anti-SRBC acitivity was measured. Five groups of operated animals were compared to three groups of controls. The results were expressed as the means of these experiments. Incubation with 2ME entails a 1 : 4 dilution of the sera prior to antibody titration. Therefore, when no haemolytic activity was detected, this was expressed as an antibody titre lower than 1/4 or less than 3, according to the formula $1-\log_2 1/4$.

*Detection of plaque-forming cells (PFC).* Control rats and operated animals were actively immunized and killed at various intervals after antigen injection. The number of direct PFC in the spleen was measured by Jerne's technique (Jerne *et al.*, 1974). Base layers were not used and triplicate plates were counted for each determination.

*Statistical analysis.* A two-way analysis of variance was performed for each set of experiments (Snedecor, 1956). This method takes into account all the time-points of the curves to be compared and yields $F$ values for effects of treatment, chronologic variation and interaction of these two factors. When interaction was present, it was calculated separately for each successive period of time of the experiment. Because all the curves of primary antibody response have the same origin, we could conclude that they started to differ as soon as the interaction was significant. The $P$ values given in the text correspond to $F$ values of interaction calculated for the corresponding period of time.

## RESULTS

A simple laparotomy performed with a clean non-sterile technique induces a significant increase in anti-SRBC haemolysin levels (Fig. 1; $P<0{\cdot}001$). At day 4, the antibody titres do not differ significantly in the controls and operated rats, but later on they are much higher in rats submitted to surgery ($P<0{\cdot}001$). Because these results could eventually be due to stimulation of the immune system by bacterial contamination, another series of animals was operated on with a strict aseptic technique. Furthermore, in order to test the possible influence of the intensity of surgical trauma, the rats were divided into three groups: (a) controls, (b) rats submitted to a standard laparotomy and (c) rats which underwent a left nephrectomy. Fig. 2 shows that haemolysin levels are similar in the nephrectomized rats and the laparotomy group, but between controls and operated animals (nephrectomy and laparotomy) there is a significant difference ($P<0{\cdot}001$), which is mainly due to an increase in the late antibody response. When SRBC are injected 2 hr after a laparotomy, the same phenomenon is again observed (Fig. 3; $P<0{\cdot}001$). However, when the antigen is given 24 hr after operation, there is no significant difference between the controls and operated rats. Surgery also enhances the secondary antibody response to SRBC when the booster injection is given intraoperatively (Fig. 4; $P<0{\cdot}001$).

Fig. 5 shows that anti-SRBC antibodies obtained from operated animals injected into rats submitted to a laparotomy disappear from the serum at the same rate as antibodies from unoperated rats injected into control animals. The slope is the same for both curves.

On day 4, no haemolytic activity could be detected in any of the sera after inactivation of IgM antibodies. The levels of 2ME-resistant antibodies are higher in operated rats than in controls (Fig. 6). This increase is present from day 7 on, and seems to be responsible for the difference between the last part of the curves representing total anti-SRBC activity, i.e. between days 18 and 25. However, our data do not preclude a possible increase of 19S antibody synthesis in the early humoral response after surgery. Indeed, our results are expressed according to a $\log^2$ scale and, for instance, on day 7 the difference

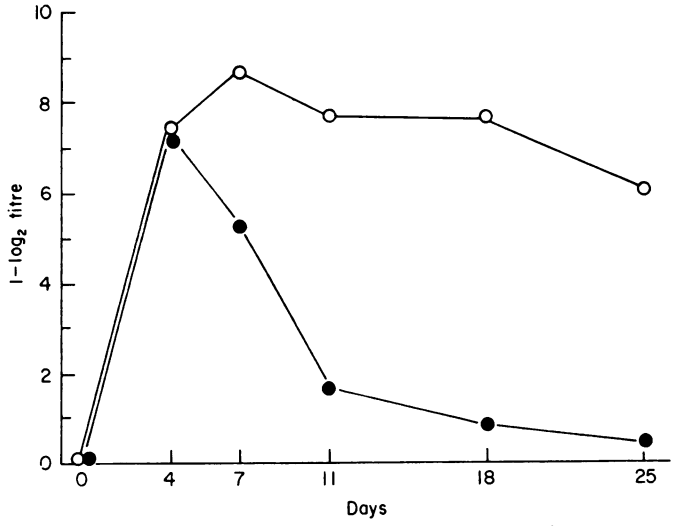


FIG. 1. Haemolysin titre primary immune response after an i.v. injection of $10^9$ SRBC into ten anaesthetized control animals (●) and into ten rats submitted to a laparotomy with a clean but not aseptic technique (○).

| Source of variation | Degrees of freedom | *F* | *P* |
|---|---|---|---|
| Surgery | 1 and 108 | 387·82 | < 0·001 |
| Time | 5 and 108 | 134·95 | < 0·001 |
| Interaction | 5 and 108 | 42·61 | < 0·001 |

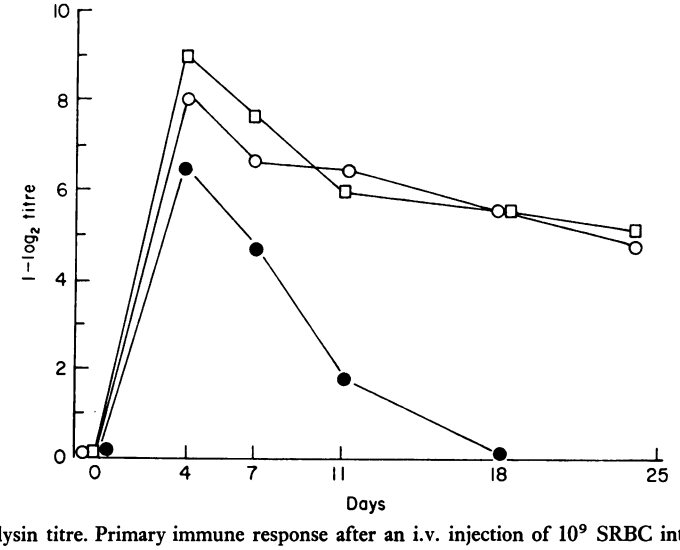


FIG. 2. Haemolysin titre. Primary immune response after an i.v. injection of $10^9$ SRBC into anaesthetized control animals (four rats) (●) and into rats operated on with a strict aseptic technique. Laparotomy (five rats) (○); nephrectomy (five rats) (□).

| Source of variation | Degrees of freedom | *F* | *P* |
|---|---|---|---|
| Laparotomy *vs* nephrectomy | 1 and 66 | 2·06 | n.s. |
| Controls *vs* surgery | 1 and 66 | 270·38 | < 0·001 |
| Time | 5 and 66 | 156·91 | < 0·001 |
| Interaction | 10 and 66 | 9·79 | < 0·001 |

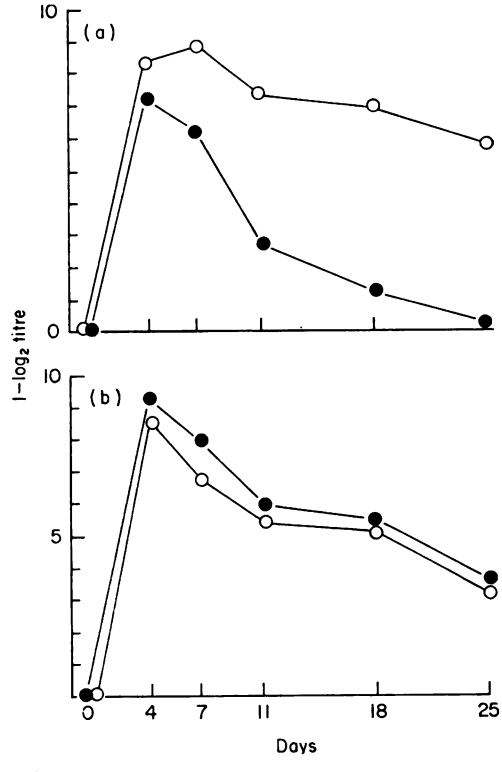


FIG. 3. Haeomolysin titre. Primary immune response after an i.v. injection of $10^9$ SRBC (a) 2 hr or (b) 24 hr after operation (○) or ether anaesthesia (controls) (●). For (a): six controls and six operated on; for (b) five controls and five operated on. When the antigen is injected 1 day after surgery, there is no significant difference. For an antigenic challenge 2 hr following operation, see below.

| Source of variation | Degrees of freedom | $F$ | $P$ |
|---|---|---|---|
| Surgery | 1 and 60 | 261·69 | < 0·001 |
| Time | 5 and 60 | 139·05 | < 0·001 |
| Interaction | 5 and 60 | 22·85 | < 0·001 |

between 2ME-resistant haemolysin levels of animals and controls (7 and 5) is smaller than the discrepancy between the total haemolytic activity of these animals (10·6 and 8·8). Direct PFC assays detect mainly IgM-synthesizing cells (Jerne *et al.*, 1974). Animals undergoing a laparotomy have higher numbers of direct PFC than non-operated rats (Fig. 7) after antigenic challenge with $10^9$ SRBC. Besides, the curves of response have a different shape in both groups. In controls, the peak of the immune reaction occurs on day 4 and is followed by a sharp decrease in the number of PFC on day 5, while in operated animals, there is no significant difference between the numbers of PFC on days 4 and 5 and the drop is observed between days 5 and 6. It may thus be concluded that IgM synthesis is also increased.

All these experiments show an enhanced antibody synthesis in operated animals. This increased response is relative to unoperated but anaesthetized controls. Because anaesthetic agents are able to impair immune mechanisms (Duncan & Cullen, 1976), we also investigated the effect of anaesthesia on circulating antibody levels and we obtained variable results. In a first experiment, ether anaesthesia was accompanied by a slight, but significant, reduction in haemolysin and haemagglutinin titres ($P < 000{\cdot}1$), while chloral hydrate decreased only the haemolysin levels (Fig. 8; $P < 0{\cdot}001$). The difference

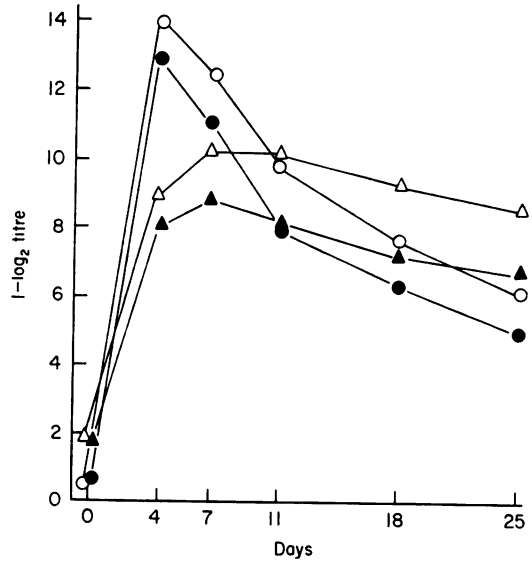


FIG. 4. Secondary humoral response after a booster injection of $10^9$ SRBC into rats which had received the same dose of antigen 58 days earlier. The diagram compares the curves obtained from nine controls (●) and (▲) and eight operated rats (○) and (△). Haemolysin titres are represented by circles and haemagglutinin titres by triangles.

| Source of variation | Degrees of freedom | *F* | *P* |
|---|---|---|---|
| Surgery | 1 and 90 | 13·92 | < 0·001 |
| Time | 5 and 90 | 183·32 | < 0·001 |
| Interaction | 5 and 90 | 3·62 | < 0·05 |

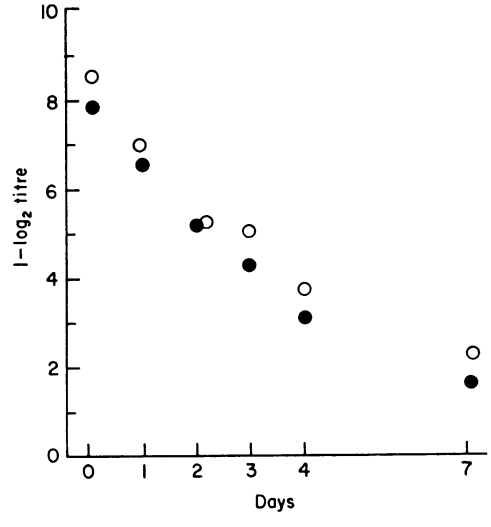


FIG. 5. Haemolysin titre. *In vivo* fall of anti-SRBC antibodies obtained from unoperated rats injected into five control rats (●) compared to the disappearance rate of anti-SRBC antibodies from operated rats administered to four animals submitted to a laparotomy (○).

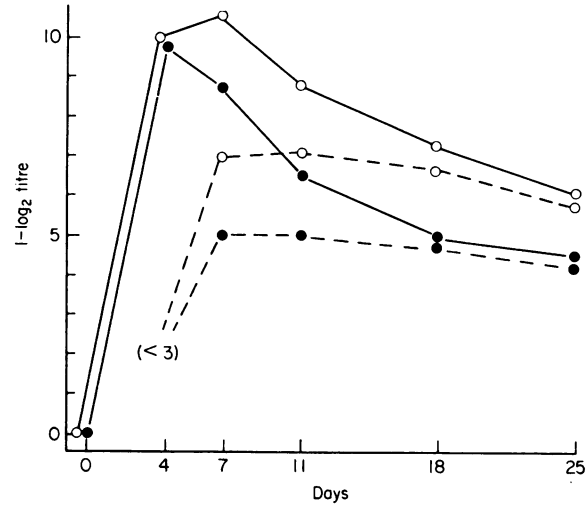


FIG. 6. Haemolysin titre. Effect of surgery on total the haemolytic activity of the serum (——) and on 2ME-resistant antibody levels (– – –). Inactivation of 19s antibodies with 2ME entails a 1/4 dilution, therefore when no antibody activity was detected after incubation with 2ME the results are expressed as < 3, i.e. ($1-\log_2 1/4$). (●) Controls; (○) operated rats.

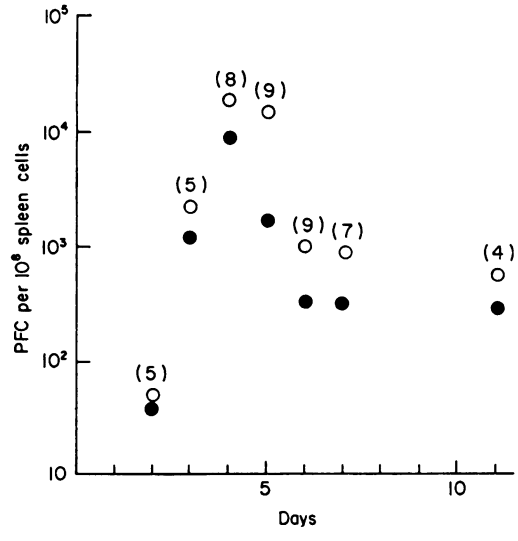


FIG. 7. Effect of surgery on the production of spleen PFC. Results are expressed as the geometric means of the data. (○) Operated rats; (●) controls. The figures in parentheses represent the numbers of rats tested in each group on the various days.

| Source of variation | Degrees of freedom | *F* | *P* |
|---|---|---|---|
| Surgery | 1 and 79 | 39·93 | < 0·001 |
| Time | 6 and 79 | 63·89 | < 0·001 |
| Interaction | 6 and 79 | 1·70 | n.s. |

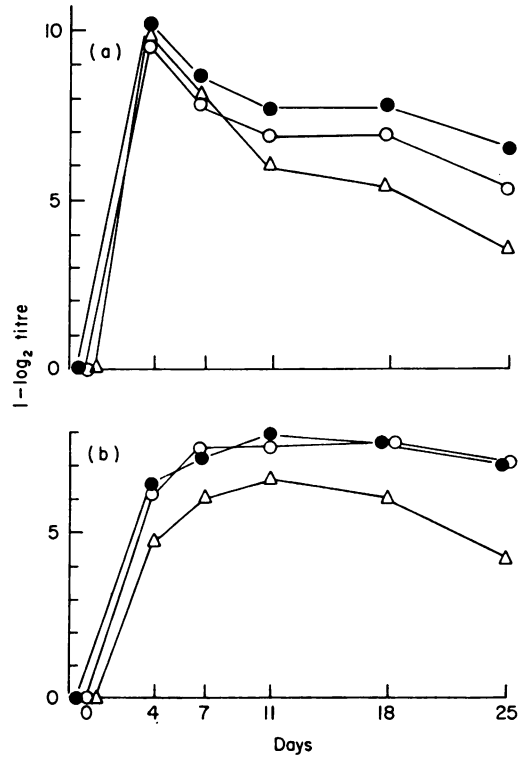


FIG. 8. Effect of anaesthesia on primary immune response of rats injected with $10^9$ SRBC. Five rats were tested in each group. Unanaesthetized controls (●); ether anaesthesia (△); chloral hydrate (○). (a) Haemolysin titre; (b) haemogglutinin titre.

| Source of variation | Degrees of freedom | *F* | *P* |
|---|---|---|---|
| Ether *vs* Chloral hydrate | 1 and 72 | 13·89 | < 0·001 |
| Controls *vs* Anaesthesia | 1 and 72 | 52·78 | < 0·001 |
| Time | 5 and 72 | 481·11 | < 0·001 |
| Interaction | 10 and 72 | 5·56 | < 0·001 |

between the three haemolysin curves was significant from day 11 on ($P<0·05$). The depression in haemolysin titres was less marked with chloral hydrate than with ether ($P<0·001$). In a second experiment, however, there was no difference between the controls and rats undergoing ether anaesthesia, but these two groups had lower anti-SRBC titres than operated animals (Fig. 9; $P<0·001$).

## DISCUSSION

Our results show that 4 days after antigenic challenge with SRBC, the levels of circulating antibodies are similar, or only slightly different, in operated rats and anaesthetized unoperated controls. However, from day 7 on, the titres are higher in the operated group. This stimulation of the immune response is observed when SRBC are given during operation or 2 hr later. However, if the antigen is injected 24 hr after a laparotomy no significant effect of surgery the can be detected. The higher anti-SRBC activity in the serum of operated rats could be due to an increase in the late antibody response or to a lower catabolic rate of circulating antibodies. Our studies of passive immunization show that the second hypothesis is not correct, and one may thus conclude that surgery enhances antibody synthesis. In the

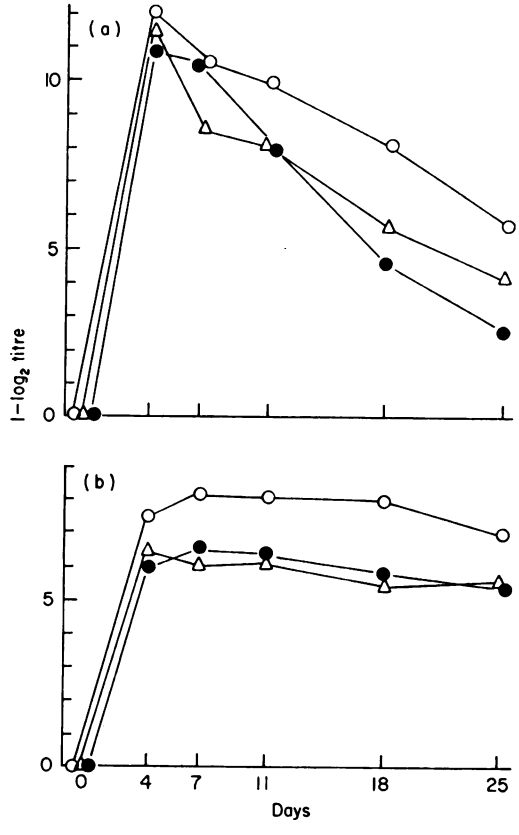


FIG. 9. Comparison of the humoral response in unanaesthetized controls (●); rats submitted to ether anaesthesia (△); and operated animals (○). Six rats were tested in each group. (a) Haemolysin titre; (b) Haemagglutinin titre.

| Source of variation | Degrees of freedom | *F* | *P* |
|---|---|---|---|
| Controls *vs* anaesthesia | 1 and 89 | 0·54 | n.s. |
| Surgery *vs* (controls plus anaesthesia) | 1 and 89 | 44·08 | < 0·001 |
| Time | 5 and 89 | 259·95 | < 0·001 |
| Interaction | 10 and 89 | 4·13 | < 0·001 |

present experimental set-up, the phenomenon was not influenced by the intensity of surgical trauma, as the rats had comparable anti-SRBC antibody levels after a simple laparotomy or a left nephrectomy. Bacterial contamination of the abdominal cavity with subsequent activation of macrophages does not seem to play an important role, because the increase in humoral response was observed as clearly with an aseptic technique as with a clean surgical technique.

Cooper *et al.* (1974) have shown a depression in the immune response of guinea-pigs injected with SRBC 2–3 hr after a cholecystectomy. A possible explanation of this discrepancy could be that the method used to test the immune response was different in both series. Cooper *et al.* (1974) measured *in vitro* the number of cells synthesizing anti-SRBC antibodies in the spleen (Jerne plaque technique), while we measured the circulating antibody levels. A lack of correlation has been demonstrated between these two techniques (Ferreira, Moreno & Hoecker, 1973; Petranyi, Benczur & Alföldy, 1971). Mice treated with cortisone had a lower number of PFC in the spleen than control animals, but there was no

difference in the haemolytic titres of the sera. Ferreira *et al.* (1973) have shown that the reduction in the number of spleen PFC was at least partly compensated by an increase in bone marrow cells synthesizing anti-SRBC haemolysins. Therefore we also performed spleen PFC assays and again we found an increased response in operated animals. The memory function for SRBC in spleen cells from operated guinea-pigs was severely impaired following passive transfer (Cooper *et al.*, 1974), but in our experiments the secondary humoral response was slightly, but significantly, enhanced. Consequently the discrepancy between Cooper's results and ours should be explained by species differences. Finally, our data on direct PFC and on 2ME-resistant antibodies indicate that surgery enhances IgM and IgG synthesis.

The effects of various types of trauma on humoral immunity have been reported in the literature. However, the comparison of the results of these studies is difficult, because the methods used to test antibody synthesis and the timing of administration of the antigen vary widely. In man, battle injuries do not impair the humoral response to diptheria toxoid (Havens, Bock & Siegel, 1954) or the anamnestic response to tetanus toxoid (Balch, 1955). Severely burned patients are unable to produce antibodies against erythrocytes from cayman sclerops injected 2 to 27 days following injury, but have higher circulating antitoxin levels than normal individuals after a booster injection of tetanus toxoid (Alexander & Montcrief, 1966). Burn injury has also been studied in laboratory animals. The primary antibody production is normal in burned mice injected with SRBC 30 min to 1 hr after trauma (Markley, Smallman & Evans, 1967) and in rabbits receiving bovine gamma globulins immediately prior to injury (Malt & Cope, 1956). The humoral response to human red blood cells (HRBC) is also normal in rats receiving a 20% third-degree burn. A 30% third-degree burn does not alter the antibody synthesis when HRBC are administered 24 hr prior to trauma, but when HRBC are given 1 to 24 hr following burn injury, a depression of the immune reaction is observed. On the other hand, the antibody response is not decreased after injection of Pseudomonas or typhoid antigens (Alexander & Montcrief, 1967). Amputation performed 6 days following antigenic challenge has no effect on circulating antibody levels in rats (Humphrey Wingard & Lang, 1969) but a fracture of the tibia inflicted to rabbits immediately after an injection of BCG enhances antibody synthesis (Malt & Cope, 1956). In conclusion, it seems that usually the various types of trauma studied up to now are either without effect on the humoral immunity or stimulate the primary and secondary antibody response, which is in keeping with our results. Only severe burns depress the primary antibody production, mainly when the antigen is given after trauma. Severe thermal injury is always associated with hypovolaemia and infection, moreover a burn toxin has been recently described (Allgöwer, Städtler & Schoenenberger, 1974). These three factors, which could be responsible for alterations in the immune response, are not present in our model of surgical trauma.

Reports on the influence of anaesthesia on the humoral immune response are scarce. Cooper *et al.* (1974) observed no effect of i.p. pentobarbitone sodium in guinea-pigs when the antigen (SRBC) was given 2 hr after drug administration. Halothane does not modify the circulating antibody levels in chicken or rats anaesthetized 4 to 7 days after antigenic challenge (Viljanen *et al.*, 1973; Humphrey *et al.*, 1969). However, in rats there is a drop in the number of spleen cells producing antibodies for 48 hr after exposure to halothane. Our experiements do not yield reproducible results. Once ether anaesthesia induced a slight depression of antibody production and on another occasion it did not alter the humoral response. We have no explanation for this discrepancy but for this reason we think that the influence of surgical trauma on immune mechanisms is best studied by comparing operated rats with animals anaesthetized on the same day and immunized with the same batch of SRBC.

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