RESEARCH PAPERS

THE EFFECT OF SPLENECTOMY ON THE PRODUCTION OF ANAPHYLACTIC SHOCK IN THE GUINEA PIG AND THE RAT

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Splenectomy in the guinea pig affords protection against active anaphylactic shock, if the operation is performed a short time before the challenge with antigen. When the challenge is made under ether anaesthesia, the maximal protective action is present 24 hours after splenectomy but is lost during the next 48 hours. If the challenge is made under urethane anaesthesia, the protective action is present 72 hours after the operation. The mechanism of the protective action of splenectomy is not clear, although it may be due to a loss of tissue antibodies or of complement since passive anaphylaxis is not altered by the operation. In contrast, removal of the spleen in the rat fails to modify the symptoms of anaphylactic shock.

THE spleen is claimed to be a site of antibody formation[1–3], and its removal may modify the production of anaphylactic shock. In this paper, the role of the spleen in anaphylaxis has been studied in the guinea pig and the rat.

METHODS

Female guinea pigs weighing about 400 g. and albino rats weighing about 150 g. were used in this study. Adult rabbits provided the anti-serum for the passive sensitisation experiments. The rats were fed on cubes (No. 41, Associated London Flour Millers Ltd.), the guinea pigs and rabbits on diet No. 18B. Drinking water was allowed *ad lib.*

Sensitisation, challenge and assessment of shock. Guinea pigs were actively sensitised by an intraperitoneal injection of 0·5 ml. of horse serum and challenged under ether or urethane anaesthesia with an intravenous dose of 1 ml. of horse serum either three or ten weeks later. Other guinea pigs were passively sensitised by an intraperitoneal injection of 2 ml. of anti-serum obtained from rabbits bled 10 days after the last of a series of 6 daily intraperitoneal injections each of 1 ml. of horse serum. The recipient guinea pigs were then challenged 24 hours later with an intravenous dose of 1 ml. of horse serum. Shock was assessed as follows; (i) mild shock, consisting of prolongation of anaesthesia and occasional sneezing; (ii) moderate shock, consisting of retching, sneezing, coughing and hurried respiration; (iii) severe shock, consisting of dyspnoea, periodic cessation of respiration and occasional violent respiratory efforts together with opening of the mouth at each inspiration.

Rats were sensitised by an intraperitoneal injection of either horse serum or diluted eggwhite and shock assessed on challenge, as previously described[4].

Collection of guinea pig lung. Guinea pig lung was collected before and after challenge in each animal. On the day of challenge with antigen, the guinea pig was anaesthetised with urethane (1·5 g./kg. intraperitoneally) and blood pressure recordings were taken from the carotid artery. Artificial respiration was applied through a tracheal cannula and a piece of right lung was removed for the extraction of histamine. A portion of the right phrenic nerve was also removed before challenge for studying tissue mast cells in the attached pleura. Twenty to thirty minutes after the challenging dose of antigen had been given, similar pieces of left lung and phrenic nerve were removed from each animal. The lung tissues were weighed, extracted with trichloroacetic acid and assayed on the isolated guinea pig ileum[5]. The phrenic nerves were mounted on microscope slides, fixed in alcohol, stained with toluidine blue and mounted.

Splenectomy. The spleen was removed from guinea pigs under ether anaesthesia. Through a left lateral incision in the abdominal cavity, the spleen was mobilised by tearing the lienophrenic ligament with the finger and then removed after ligaturing the pedicle. The abdominal cavity was closed with atraumatic ophthalmic catgut, and the skin incision joined with suture clips. In other guinea pigs, the spleen was exposed but not removed, and these mock-splenectomised animals served as controls. Groups of rats were similarly splenectomised or mock-splenectomised, though the operation in this species is easier, there being no lieno-phrenic ligament.

Bleeding in all operated animals was minimal. After the operation, they were always placed in sterilised cages and allowed food and water. A few guinea pigs died in the ensuing weeks, but most recovered rapidly from the operation.

Results

Experiments with Guinea Pigs

Splenectomy before sensitisation. Splenectomy in the guinea pig failed to modify the process of sensitisation to foreign protein. When the operation was performed 24 hours before sensitisation and the animals were challenged under ether anaesthesia 3 weeks later, 4 out of 5 guinea pigs suffered fatal anaphylactic shock, the other exhibiting severe shock. A similar result was also obtained when 5 mock-splenectomised animals were similarly challenged.

Splenectomy before challenge. Splenectomy in the guinea pig a short time before challenge considerably reduced the severity of anaphylactic shock. When the challenge was made under ether anaesthesia, the maximal effect occurred 24 hours after splenectomy. At this time interval, only 1 out of 18 animals had severe shock, 1 moderate shock, 12 mild shock whilst 4 failed to exhibit any shock. In contrast, all 17 mock-splenectomised animals at this time interval had severe shock, 15 dying within 30 minutes of the challenge. These results are shown in Table I. When the interval between operation and challenge was extended to 72 hours, the severity of the shock was not reduced, mortality rates exceeding 75 per cent in both splenectomised and mock-splenectomised groups.

When the challenge was made under urethane anaesthesia, the protective effect of splenectomy on anaphylactic shock was demonstrable 72 hours after the operation. Whereas the mortality rate of mock-splenectomised guinea pigs (4 out of 10) was similar to that of unoperated sensitised animals (5 out of 12) given the challenging dose of antigen, there were no deaths and only mild shock in 10 splenectomised animals. Several of the

TABLE I

THE INFLUENCE OF SPLENECTOMY OR MOCK-SPLENECTOMY, PERFORMED AT VARYING TIME INTERVALS BEFORE INTRAVENOUS CHALLENGE BY HORSE SERUM, ON THE PRODUCTION OF ANAPHYLACTIC SHOCK IN SENSITISED GUINEA PIGS. CHALLENGE WAS GIVEN UNDER ETHER ANAESTHESIA

Time interval (hours)	Splenectomy				Mock-splenectomy			
	Degree of shock			Mortality rate (per cent)	Degree of shock			Mortality rate (per cent)
	Mild	Moderate	Severe		Mild	Moderate	Severe	
0	1	4	16	72	0	5	19	75
6	3	1	1	40	0	1	3	75
24	12	1	1	11*	0	0	17	94
48	0	2	7	66	0	0	8	75
72	0	3	5	75	0	1	7	87

* Four animals in this group failed to exhibit any degree of shock.

surviving guinea pigs in each group were killed 1 hour after the challenge and examined. In both the unoperated and mock-splenectomised animals, there were haemorrhagic patches and consolidation in the lungs, which microscopically showed collapse with oedematous inter-alveolar septa (Fig. 1A and B). On the other hand, the microscopic appearance of the lungs of the splenectomised animals after challenge was normal (Fig. 1C).

TABLE II

THE INFLUENCE OF SPLENECTOMY OR MOCK-SPLENECTOMY, PERFORMED THREE DAYS BEFORE INTRAVENOUS CHALLENGE BY HORSE SERUM, ON THE HISTAMINE CONTENT (μG./G.) OF LUNGS OF SENSITISED GUINEA PIGS. CHALLENGE WAS GIVEN UNDER URETHANE ANAESTHESIA

Treatment	Histamine content		Change (per cent)
	Pre-shock value	Post-shock value	
Splenectomy	20·0	20·0	0
	22·4	22·7	+1
	44·0	45·0	+2
	50·0	50·0	0
	80·0	75·0	−6
	85·0	80·0	−6
	180·0	186·0	+3
Mock-splenectomy	12·6	10·1	−19
	13·4	11·2	−16
	28·6	18·0	−37
	30·0	21·2	−29
	30·3	10·4	−66
	36·0	28·0	−22
	120·0	66·0	−45

Pleural mast cells of unoperated guinea pigs undergoing anaphylactic shock showed marked distortion, degranulation and disruption (Fig. 2A) and similar changes were found in mock-splenectomised animals given the challenge either under ether anaesthesia 24 hours after the operation or

under urethane anaesthesia 72 hours after the operation (Fig. 2B). On the other hand, pleural mast cells from animals similarly treated after splenectomy were normal in appearance (Fig. 2C).

The intravenous injection of horse serum into unoperated sensitised guinea pigs under urethane anaesthesia resulted in a rise of the arterial blood pressure which sometimes lasted for 30 minutes. A similar

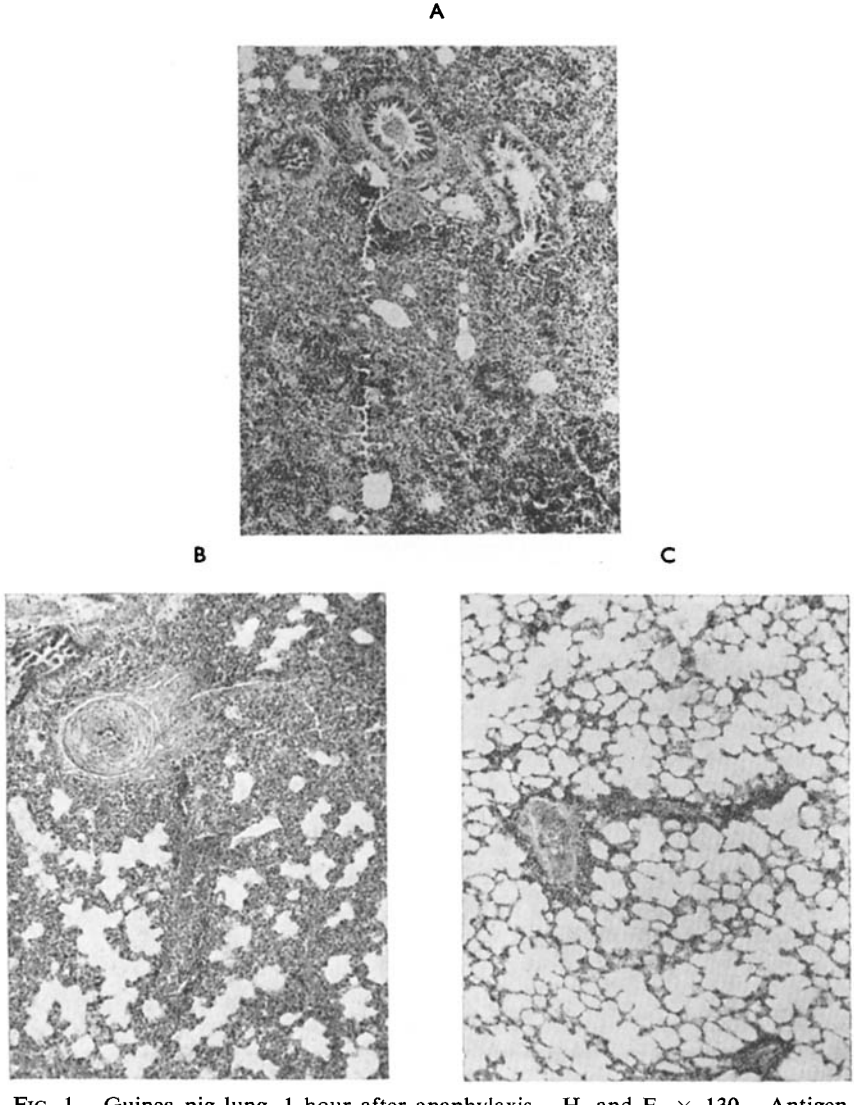

FIG. 1. Guinea pig lung, 1 hour after anaphylaxis. H. and E. × 130. Antigen is horse serum. Challenge performed under urethane anaesthesia. A, unoperated; B, mock-splenectomised 3 days before challenge; C, splenectomised 3 days before challenge. Note that splenectomy before challenge protects the lungs from damage by antigen.

pressor response was obtained when mock-splenectomised sensitised animals were given the antigen 72 hours after the operation. In sharp contrast, however, the injection of horse serum into splenectomized sensitised animals at this time interval failed to alter the blood pressure.

The histamine content of the lungs of mock-splenectomised guinea pigs was reduced by about 36 per cent (range 16–66) after anaphylaxis under urethane anaesthesia 72 hours after the operation. On the other hand,

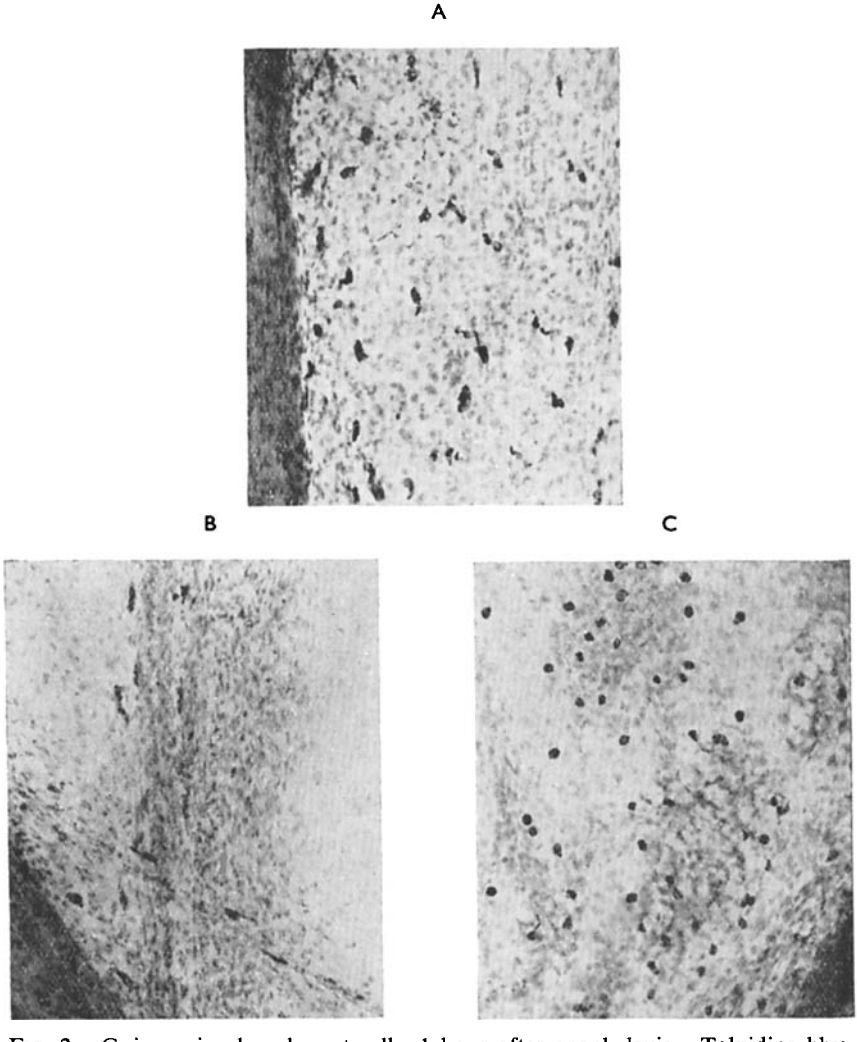

FIG. 2. Guinea pig pleural mast cells, 1 hour after anaphylaxis. Toluidine blue × 200. Antigen is horse serum. Challenge performed under urethane anaesthesia. A, unoperated; B, mock-splenectomised 3 days before challenge; C, splenectomised 3 days before challenge. Note that splenectomy before challenge protects the pleural mast cells from damage by antigen.

the histamine content of the lungs of splenectomised animals similarly challenged was not altered. These results are shown in Table II and also illustrate the wide range of histamine-content of guinea pig lung before anaphylaxis.

The protective action of splenectomy on anaphylactic shock was also noted after a period of sensitisation of 10 weeks. When the operation was performed under ether anaesthesia 24 hours before the challenging dose, 6 mock-splenectomised guinea pigs died within 30 minutes whereas none of the 6 splenectomised animals showed more than mild shock. When the challenge was delayed till 72 hours after the operation, there was no significant difference between the splenectomised and mock-splenectomised animals, 5 out of 6 in each group dying within 1 hour.

When the time course of the return of anaphylactic sensitivity was studied in splenectomised guinea pigs which had survived anaphylactic shock, it was found that a period of 10 days was necessary for the full recovery. Mock-splenectomised animals similarly treated also needed about 10 days for recovery of anaphylactic sensitivity.

When the uterus or intestine of a sensitised guinea pig which had been mock-splenectomised 72 hours previously was suspended in Tyrode's solution in an organ bath, the addition of the specific antigen resulted in a contraction and the tissue exhibited desensitisation. On the other hand, uteri or intestine of splenectomised sensitised animals generally showed no response on addition of antigen.

Splenectomy and passive anaphylaxis. Splenectomy did not protect the guinea pig against passive anaphylaxis. When rabbit anti-serum having a precipitin titre of 1/400 was injected into groups of 6–8 guinea pigs which had been splenectomised or mock-splenectomised 1 or 48 hours previously, and the animals were challenged with the antigen 24 hours after sensitisation, the shock value of both groups was similar, about half in each group dying within 1 hour.

In another experiment, two groups of 6 guinea pigs 1 hour after splenectomy were injected with rabbit anti-serum, the complement of which had been destroyed by heat at 56° for 30 minutes. On the next day, one group was given 2 ml. of fresh guinea pig serum containing complement whilst the other group was injected with 2 ml. of normal saline. All animals were challenged 3 hours later. There were no fatal reactions in either group but the shock was more severe in those animals which had received guinea pig complement.

Splenectomy and amine sensitivity. The influence of splenectomy on the sensitivity of guinea pigs to histamine and 5-hydroxytryptamine (5-HT) was studied by determining their preconvulsion times when exposed to aerosols of these amines[6]. In general, splenectomy did not alter the preconvulsion time to histamine but considerably increased that to 5-HT. For example, the preconvulsion time to aerosols of histamine (0·4 per cent w/v) of groups of 8 splenectomised, mock-splenectomised or unoperated animals was the same (about 50 sec.). Likewise, intravenous doses of 0·8 mg./kg. histamine killed groups of 4 splenectomised, mock-splenectomised or unoperated guinea pigs in 2–3 minutes. But

the average preconvulsion time to aerosols of 5-HT (0·5 per cent w/v) of groups of 8 mock-splenectomised or unoperated animals was 67 seconds whereas that of splenectomised animals was 174 seconds.

Experiments with Rats

Splenectomy performed either before sensitization or before challenge failed to modify the production of anaphylactic shock in the rat. Shock values were similar in splenectomised rats to those in mock-splenectomised animals.

Discussion

The present results show that if splenectomy in guinea pigs is performed a short time before the challenge with the antigen there is a considerable reduction in the intensity of the anaphylactic shock. This is manifest by (i) a reduced mortality rate, (ii) no macroscopic or microscopic changes in the lung, (iii) no alteration in the appearance of pleural mast cells, (iv) no change in the arterial blood pressure, and (v) no reduction in the lung histamine. These effects cannot be attributed to the stress of the operation, since the symptoms noted in mock-splenectomised animals undergoing anaphylactic shock resemble those of unoperated animals receiving similar treatment. The maximal protective effect occurs 24 hours after splenectomy when the challenge is made under ether anaesthesia but is lost by 72 hours. If the challenge is made under urethane anaesthesia, the effect is still demonstrable on the 3rd post-operative day. This difference in duration of effect may be accounted for by the fact that urethane itself possesses a partial anti-anaphylactic property[7,8].

The removal of the spleen protects the guinea pig from active, but not passive, anaphylaxis. To explain this action, the following possibilities exist: (i) the tissues for a short period of time become resistant to the substances released during anaphylaxis, (ii) the union of antigen and antibody is prevented, or (iii) subsequent steps of the antigen-antibody union are inhibited.

It has been shown that splenectomised guinea pigs are more resistant to the action of 5-HT than are mock-splenectomised animals. But histamine is the major toxic substance released during anaphylactic shock in this species and its toxicity is not reduced, and so it is unlikely that the protective action of splenectomy can be solely explained on the basis of increased resistance to 5-HT. As the Dale–Schultz reaction is prevented and pleural mast cells do not show any major change, the union of antigen and antibody and its subsequent steps are more likely to be involved. If the spleen is the major source of antibody formation, then its removal may temporarily prevent their replenishment to the tissues, which in consequence will lose their sensitivity to antigen. This sensitivity will return when non-splenic sources take up the function of the spleen. Since the maximal protective effect is obtained within 24 hours of the operation, a very rapid turn-over of antibodies in the tissues is indicated. Such a possibility however is unlikely since anaphylactic shock can be induced up to at least 7 days after passive sensitisation[9].

It is possible that splenectomy removes temporarily a tissue constituent essential for anaphylaxis. Rice[10] has recently confirmed that there is a lowering of complement titre after anaphylaxis and showed that the return of the titre to pre-shock levels only occupies 24 hours. The transient protective effect of splenectomy may therefore be explained in terms of a diminution of the complement titre. However, the injection of fresh guinea pig serum (containing complement) only slightly aggravated the shock in splenectomised animals, passively sensitised with anti-serum heated to 56°. Direct estimations of complement titre have not been made in the present experiments and it is doubtful if a sufficient reduction occurs to modify the anaphylactic reaction.

Ungar[11] postulated in 1953 that a proteolytic enzyme, namely fibrinolysin, is activated during anaphylaxis. He suggested that fibrinolysin is neutralised by antifibrinolysin, the activity of which is stimulated by a constituent of the spleen termed "Splenin-A" and depressed by another constituent termed "Splenin-B". Normal spleen is said to form more of "Splenin-A" than of "Splenin-B" (Ungar and Damgaard[12]) so that splenectomy would be expected to aggravate, rather than alleviate, anaphylactic shock. This is contrary to the results reported in this paper.

If circulating antibodies protect against anaphylaxis[13], the absence of the symptoms when the challenge is given after splenectomy may be due to the antigen-antibody reaction occurring in the blood. Guinea pigs once sensitised maintain the sensitised state for many years[14] and the present results show that the protective effect of splenectomy can be demonstrated in animals which have reached a steady state of anaphylactic sensitisation (after 10 weeks of sensitisation). This state is possible only if antibodies are constantly being produced to replace those lost from the tissues. If a delicate balance exists between the antibodies in the tissues and those in the blood, and if the blood antibody titre is controlled by the spleen, then its removal will upset this balance and the tissues will rapidly give up their antibodies. On challenge, the reaction will occur mostly in the blood and the animal will be temporarily immune. As a result of the high blood antibody content, some of the antibodies will return to those tissues with the highest avidity. The lungs, for example, acquire partial anaphylactic sensitivity 3 days after splenectomy, whereas the uterus at that time is still in a comparatively sensitised state.

It is thus impossible to name a single factor to account for the protective effect of splenectomy on anaphylaxis in the guinea pig. The loss of antibodies and of complement from the tissues as well as the increased resistance of the animal to released 5-HT may all contribute to this action.

The rat, unlike the guinea pig, remains sensitised to foreign protein for only a short time after sensitisation[4]. When the spleen is removed in this species before sensitisation and the animal is challenged 12–14 days later, the production of anaphylactic shock is unaltered. Further, when the spleen is removed a short time before challenge, anaphylaxis is again unaltered, and it appears that the spleen in the rat is not such an important site of antibody formation as it is in the guinea pig.

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