amounts of amide groups that can alter the net charge of the protein molecule.

SPERO et al.[7] have shown that partial deamidation was responsible for the multiple forms of enterotoxin B. If this explanation is applicable to enterotoxin A, the pI 8.60 component would correspond to the most fully amidated molecule; this would be converted to the more acid fractions of the toxin by deamidation.

The apparent discrepancy in the pI value found by CHU et al.[1] for enterotoxin A (pI-6.8) and in the results obtained in this laboratory (pI-8.1 major peak) can be explained by assuming that the more basic components were not eluted off the CM-cellulose column used for the purification of enterotoxin A by CHU et al.[1].

While this work was in progress SCHANTZ et al.[8] reported heterogeneity of enterotoxin A from *S. aureus* strain 13N-2909. By isoelectric focusing they separated 4 components with pI values of 6.64, 7.26, 7.68, and 8.10 (at 4 °C). The component present in greatest concentration focused at pH 7.26.

Résumé. L'entérotoxine A a été divisée en six composants par focalisation isoélectrique. Les points isoélectriques (pI) variaient entre 6.78 et 8.60 à 25C; le composant prédominant se focalisait à un pH de 8.10. Les caractéristiques immunologiques et le poids moléculaire des 6 composants furent identiques.

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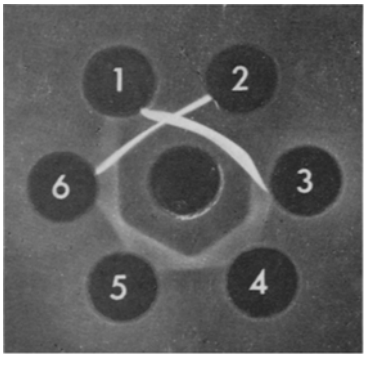
Effects of Splenectomy on Amphibian Antibody Responses

In mammals, splenectomy has repeatedly been shown to impair antibody responses to various antigens, particularly those introduced into the circulation[1–4]. Similar effects have been observed in birds[5,6] and in reptiles[7]. Conversely, splenectomized teleosts (*Lutjanus griseus*) and sharks (*Ginglymostoma cirratum* and *Negaprion brevirostris*) have produced antibodies in titres comparable with intact animals, irrespective of the route of antigen administration[8], whilst good antibody production to both soluble and particulate circulating antigens has been demonstrated in splenectomized amphibians (*Xenopus laevis*)[9]. There is thus some indication that extra-splenic sites play a more significant immunological role in the lower vertebrates than in the amniotes. Information on the poikilothermic species is relatively sparse at present, however, and restricted to a few antigens.



Double diffusion in agar gel, demonstrating similar antigen persistence in the sera of intact and splenectomized toads at 4 weeks after injection of whole human serum (WHS). Central well, 1:5 anti-WHS produced in goat; well 1, HGG (1 mg/ml); well 2, HSA (1 mg/ml); wells 3 and 4, experimental sera from splenectomized toads; wells 5 and 6, experimental sera from sham-operated toads. All 4 experimental sera have a single line of persisting antigen which shows identity with HSA (but no identity with HGG).

The present study is designed to investigate further the immune capabilities of splenectomized *Xenopus*, and to establish whether their ability to elicit a response depends on the antigen presented. A complex antigen, whole human serum (WHS), has been used as a convenient means of testing the animals' ability to respond to a variety of protein antigens.

Materials and methods. *Xenopus* used in these experiments were all mature adult males weighing between 33 and 65 g. They were maintained in tap water at 20 °C and fed weekly on minced meat. Splenectomies were performed as described previously[9]. Sham-splenectomized animals were subjected to the same operative trauma as the splenectomized group, but leaving the spleen intact. All animals were immunized 6 days after the operation.

Antigen consisted of 10 mg/ml freeze-dried WHS (Hyland, California) in saline, emulsified with an equal volume of Freund's complete adjuvant (Difco, Detroit). This emulsion was injected into the dorsal lymph sac at a rate of 5 µl/g body weight.

Toads were divided into 3 groups, each consisting of 3 splenectomized and 3 sham-operated animals. Those of groups I and II were given a single injection of antigen and killed at week 4 and week 8 respectively; group III was given a second injection at week 4 and killed at week 8. Blood was collected from the heart, allowed to clot, and

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Anti-HGG titres ($-\log_2$) of sham-operated versus splenectomized *Xenopus* immunized with whole human serum

	Group I (1°; week 4)		Group II (1°; week 8)		Group III (2°; week 8)	
	Sham	Splenectomized	Sham	Splenectomized	Sham	Splenectomized
Total antibody	10, 14, 10	8, 7, 10	8, 11, 10	12, 13, 13	12, 13, 10	7, 12, 8
MER antibody	–, 4, –	–, –, –	–, 6, 6	7, 8, 6	9, 7, 5	–, 8, –

the serum decomplemented at 56 °C for 30 min. Splenectomized toads were examined at the time of sacrifice for signs of splenic regeneration. Only in one case did regeneration occur, and the resultant splenule was small (4.6 mg).

Using the technique of double diffusion in agar gel, fresh serum samples were tested against undiluted and 1:5 WHS or anti-WHS (Hyland, California) produced in goats, to demonstrate precipitating antibodies and persistence of antigen respectively. Precipitin responses were further examined by immunoelectrophoresis as previously described[10]. Anti-HGG antibody titres were estimated using a passive haemagglutination technique: two-fold serial dilutions of serum were tested against a suspension of tanned sheep erythrocytes (SRBC) coated with 0.1% HGG[11]. Sera treated with 2-mercaptoethanol (0.1 *M*) were incubated for 1 h at 37 °C before titrating.

Results. Antigen persistence. In all 6 *Xenopus* (splenectomized and sham-operated) examined at 4 weeks after immunization (group I), a single line of persisting antigen could be detected, which showed identity with human albumin (HSA, Figure). No other components of human serum remained in the circulation in this way. At 8 weeks after immunization (group II), the albumin component also had been cleared, all 6 animals of this group being negative for persisting antigen. In the group which had received a second injection of antigen (group III), results were again similar between splenectomized and sham-operated toads: 2 out of 3 splenectomized animals versus 1 out of 3 controls showed persisting albumin.

Antibody production. In toads examined 4 weeks after primary injection of antigen, neither splenectomized nor sham-operated animals showed precipitating antibodies. By week 8, however, both were showing precipitins to a number of human serum components. In the primary series (group II), immunoelectrophoretic analysis showed a similar intensity and spectrum of response in splenectomized and control toads, the most readily detectable antibodies being those directed against human serum components with slow electrophoretic mobility, just on the anode side of the origin (see MANNING and TURNER[10]). Splenectomized animals given a secondary injection (group III) showed precipitins of similar range and intensity to those in group II animals, but a wider spectrum of response was seen in 2 of the 3 control toads of this secondary series.

BEDNARIK and CAJTHAMLOVA[3] have shown that in rabbits immunized with heterologous serum proteins, the antibody response most impaired in the splenectomized animal is that directed against the immunoglobulin fraction. In the present experiments, splenectomized *Xenopus* immunized with whole human serum showed slightly poorer anti-human immunoglobulin (HGG) titres in 2 of the 3 groups examined, but in the remaining group (group II), titres were somewhat higher than sham-operated controls (Table). Comparison of total anti-HGG titres with titres of mercaptoethanol-resistant (MER) antibody showed a significant proportion of antibody in all groups (and particularly in those examined at 4 weeks) to be of the mercaptoethanol-sensitive (MES) type. This MES antibody in *Xenopus* appears to belong to the same immunoglobulin class (IgM) as the 19S antibodies of mammals[12]. Its presence in splenectomized *Xenopus* contrasts with the findings of PIERCE[13] and BOREK et al.[14], where spleenless mammals failed to elicit antibody of the 19S type.

Discussion. The present work has shown removal of the spleen in the amphibian to have had comparatively little effect on its ability to respond to circulating antigens, either in terms of antigen clearance or antibody production. Since *Xenopus* fail to respond consistently to protein antigens administered in saline[10], all injections were made using adjuvant, and the latter may have stimulated the involvement of primitive lymphoid tissues present in organs such as the kidney and liver. Previous studies on this species using particulate antigens (SRBC) – which do elicit a response in the absence of adjuvant – have also shown comparable titres in intact and splenectomized animals[9], but only to high doses of antigen, and recent work from this laboratory has demonstrated an inferior response to low doses of SRBC following splenectomy[15]. Similarly with threshold doses of protein antigen (in adjuvant), splenectomized toads are unable to respond as well as intact controls[15]. Extra-splenic sites in the amphibian are thus competent to respond to both soluble and particulate antigens in the circulation, but lack the efficiency of the spleen in dealing with small amounts.

Résumé. La liquidation des antigènes et la production des précipitines et des macroglobulines à la suite de l'injection de sérum humain complet (mélangé à de l'adjuvant de Freund) ont été semblables chez les *Xenopus laevis* intacts et ceux qui avaient subi une splénectomie. Les résultats suggèrent qu'au stade amphibie, les zones extra-spléniques jouent un rôle important dans les réactions aux antigènes en circulation.

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