IMMUNOLOGY

Synthesis of Antibody *in vitro* by Lymph Node Fragments using Absorbable Gelatin Sponges

It has been conclusively demonstrated that fragments of lymph nodes obtained from previously immunized rabbits can be secondarily stimulated *in vitro* with the specific antigen[1,2]. Richards *et al.*[3] and Richardson[4] have shown that the antibody released by fragments *in vitro* is actually synthesized *de novo*. In the main, glass wool pads have been used to support the fragments in the culture. Unfortunately, the use of glass wool pads impairs histological investigation of the fragments during antibody synthesis. The glass wool cannot be sectioned on a microtome so that any fragment studied must be removed from the pad, resulting in loss of the periphery of the fragment which clings tightly to the glass wool. The cells which migrate from the tissue into the interstices of the pad are also not available for histological study. An ideal supporting medium for combined histological and antibody studies should be capable of being sectioned so that the cells can be observed in their relationships to each other and to the supporting medium. In order to pursue this type of investigation, we decided to investigate the possible use of absorbable gelatin sponges, as a replacement for the glass wool.

Young, adult New Zealand rabbits were immunized with rat serum albumin (RSA) (prepared by ammonium sulphate fractionation of whole rat serum, pure by the criterion of immunoelectrophoresis) and bovine serum albumin (BSA) (obtained from Pentex Incorporated, Kankakee, Illinois). The rabbits were given three intravenous injections of antigen (20 mg per injection) and three foot pad injections, bilaterally (10 mg per foot pad per injection) over a period of 10 days. All injections were made in saline. After a period of 3–4 months, during which time the serum antibody titre decreased to a low level, the rabbits were killed by intravenous administration of pentothal (50 mg per kg body weight) and the popliteal lymph nodes were excised within a period of 5 min. The nodes were trimmed of excess fat and cut into fragments of about 1–2 mm^3 in sterile Hanks's solution (obtained from Difco Laboratories, Detroit, Michigan). Six to eight fragments were placed into each Leighton tube and either a glass wool pad or absorbable gelatin sponge ('Gelfoam', Upjohn Co. of Canada, Montreal) was then overlaid. The fragments were then incubated, at room temperature for 2 h, with 0·5 mg of the antigen in 1 ml. Eagle's medium (HeLa, ten times, obtained from Difco Laboratories) containing fresh normal rabbit serum (20 per cent), glutamine (0·04 per cent), sodium bicarbonate (0·135 per cent), streptomycin (50 μg per ml.) and penicillin (100 u./ml.)[1,2]. The fragments in the control tubes were incubated with the modified Eagle's medium only. The fragments were then washed three times with aliquots of the medium and incubated with 1 ml. of medium at 37° C. The medium was changed every 3 days and tested for the presence of antibody by the tanned cell haemagglutination technique[5].

Table 2. Synthesis of Antibody to Bovine Serum Albumin *in vitro* using Glass Wool Pads and Absorbable Gelatin Sponges

Table No.	BSA incubated with fragments	Type of support used	Antibody titres* of supernatants in days of culture 6	9	12	15	18
1		Glass	320	640	320	320	40
2	–	wool	20	0	0	0	0
3			160	320	160	160	40
4		Glass	40	640	640	640	160
5	+	wool	40	320	1,280	640	80
6			40	320	640	320	160
7		Gelatin	0	0	0	0	0
8	–	sponge	0	0	0	0	0
9			0	0	0	0	0
10		Gelatin	0	0	0	20	0
11	+	sponge	0	80	320	320	40
12			0	80	160	80	80

* Titres less than 10 are considered to be negative.

As can be seen from Tables 1 and 2, the gelatin sponges functioned at least as well as glass wool pads in the facilitation of the immune response *in vitro*. In point of fact, the sponges appeared to act in a more discriminate fashion in the presence of BSA (Table 2) since the control tubes with gelatin sponges contained no detectable antibody whereas the control tubes with glass wool pads invariably contained antibody, but in much lower titre than the experimental tubes.

In summary, it has been demonstrated that absorbable gelatin sponges can replace glass wool pads in the maintenance of antibody-forming lymph node fragments *in vitro* without any adverse effect in so far as the peak titre of antibody formation is concerned or with respect to the temporal aspects of the *in vitro* antibody formation curve. The utilization of gelatin sponges should facilitate histological examination of the fragments since they can easily be fixed and sectioned, properties which are not possessed by glass wool.

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M. Richter*
A. Juhasz
B. Drechsler
J. Myers

Division of Immunochemistry and Allergy,
McGill University Clinic,
Royal Victoria Hospital,
Montreal.

* Medical Research Associate, Medical Research Council, Canada.

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Cytotoxic Effects of Antisera against Dye–Protein Complexes

It has been shown in rabbits that antibodies are formed following immunization with dye–protein complexes. These antibodies react with stained cells and reveal a cytotoxic activity in the presence of active complement[1,2]. Sulphorhodamine *B* was coupled to bovine albumin according to Uehleke[3]. Rabbits were immunized with this dye–protein complex and an antiserum (DPC-antiserum)

Table 1. Synthesis of Antibody to Rat Serum Albumin *in vitro* using Glass Wool Pads and Absorbable Gelatin Sponges

Tube No.	RSA incubated with fragments	Support used	Antibody titres* of supernatants in days of culture 6	9	12	15	18	21	24	27	30	33
1		Glass wool	1,280	1,280	1,280	640	160	160	0			
2	–		1,280	1,280	640	320	40	20	0			
3			640	640	160	80	20	0	0			
4		Glass wool	5,000	10,000	5,000	5,000	1,280	640	320	160	40	0
5	+		5,000	10,000	5,000	2,560	640	320	160	160	20	0
6			2,500	5,000	2,560	1,280	320	160	20	0	0	0
7		Gelatin sponge	80	80	80	40	40	40	0			
8	–		80	80	80	40	40	20	0			
9			40	80	80	40	20	0	0			
10		Gelatin sponge	320	5,000	10,000	5,000	5,000	5,000	5,000	640		0
11	+		320	5,000	10,000	5,000	5,000	5,000	2,560	640		0
12			0	5,000	5,000	1,280	1,280			Contaminated		

* Antibody titres less than 10 are considered to be negative.