seems likely that antibodies of this type may play a part in the defence against invasion of micro-organisms.

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Polyaminostyrene Purified Chicken Antibodies

SEVERAL workers have used polyaminostyrene (PAS)-antigen complexes for the purification of mammalian sera[1]. We have attempted to use this method to purify chicken antibodies to either beef serum albumen (BSA) or Bryan strain Rous sarcoma virus (BS-RSV) for use in the reversed bisdiazobenzidine technique[2], a method of passive haemagglutination which detects antigen.

The general technique of Webb and Lapresle[3] was used for the preparation and use of the PAS-antigen complexes.

It was found that PAS–BSA adsorbed antibody specifically from hyperimmune chicken anti-BSA serum. The immunoadsorbant could be washed free of contaminating serum proteins and the attached antibodies eluted by reducing the *p*H with 0·5 molar acetate buffer (*p*H 3) followed by 0·1 molar hydrochloric acid containing 1 per cent sodium chloride. The elution pattern from an immunoadsorbant column is shown in Fig. 1. The proteinaceous material was eluted in two major fractions corresponding to the two buffers. The shoulder seen in the first peak sometimes formed another peak of activity. Precipitating activity against BSA, shown by gel diffusion[4], could only be found in material eluted by reducing the *p*H to 3. Two areas of anti-BSA activity were shown by a passive haemagglutination technique[5,6] both of which closely followed the protein curves eluted by the two buffers. Rabbit-antichicken plasma showed that the material eluted by *p*H 3 buffer, even after concentration, had only three components which migrated in the globulin region on electrophoresis (Fig. 2). The material eluted by the second buffer had one component only which also

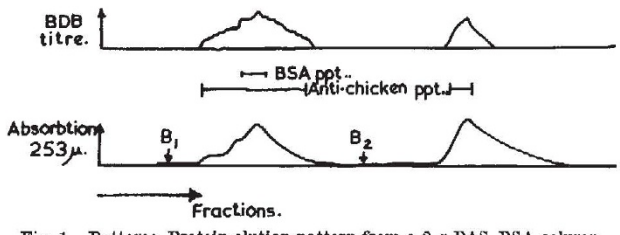

Fig. 1. Bottom: Protein elution pattern from a 2 g PAS–BSA column after non-adsorbed serum proteins had been washed off. B_1 is the point of application of the 0·5 molar acetate buffer, *p*H 3, B_2 the application point of 0·1 molar hydrochloric acid, 1 per cent sodium chloride buffer. Top: The agglutination titre of each fraction for bisdiazobenzidine (BDB)–BSA sensitized red cells. The fractions in which BSA precipitating antibody (BSA ppt) and chicken proteins (anti-chicken ppt) were found without further concentration are also shown.

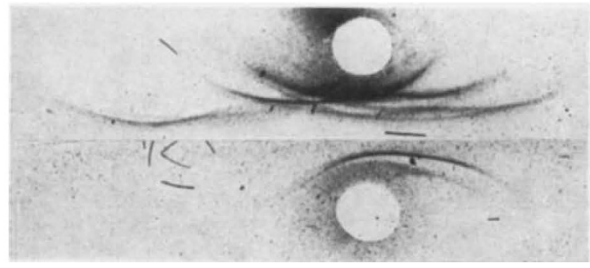

Fig. 2. Immunoelectrophoresis of normal chicken serum (top) and the concentrated antibody from a PAS–BSA column eluted by *p*H 3 0·5 molar acetate buffer (bottom). Precipitin lines developed with rabbit-antichicken serum.

migrated in the globulin region but appeared to be of high molecular weight. The finding of several peaks of activity indicates that the BSA antigen molecule probably has more than one antigenic determinant as has been shown for human serum albumen[3].

It was found that only the material from the *p*H 3 eluate could be coupled to red cells and used to detect antigen. Estimates of antibody yield from this fraction were from 12–20 per cent of the total adsorbed haemagglutinating antibody. The amount of material needed was 1–3 mg protein/0·1 ml. of 50 per cent cells. The sensitized cells could detect 1 μg/ml. to 10^{-4} μg/ml. BSA with maximal agglutination at 10^{-2} μg/ml. BSA. The range could be increased into the prozone region at concentrations greater than 1 μg/ml. BSA by removing the supernatant fluid after the cells had settled and resuspending them in diluent containing antibody. An added advantage of the latter step was the greater amount of agglutination that occurred over the whole detectable range.

Attempts were made to carry out similar procedures with Bryan standard strain Rous sarcoma virus (BS RSV). Moloney *T*2 preparations[7] were prepared in the normal way from 60 g of confluent chorio-allantoic membrane. The final pellet (containing 10^6–10^8 pock forming units (P.F.U.)) was taken up into 5 ml. of 1/15 molar *p*H 7 phosphate buffer which was added to 2 g of diazotized PAS and the coupling carried out as usual. Preliminary experiments had shown that neutralizing antibody could be regained from virus-antibody complexes by simple centrifugation after dissociation at *p*H 3 even though viral infectivity was destroyed at this *p*H. It was difficult, however, to remove contaminating serum proteins from the virus-antibody complex by this method. The washed PAS-virus complex adsorbed 70–80 per cent of the neutralizing activity from 5 ml. of serum. After exhaustive washing to remove serum proteins, neutralizing activity could be recovered by reducing the *p*H to 3. The eluted material had similar components to that seen in the BSA antibody eluted at this *p*H. The yield of antibody as measured by neutralization[8] was 1 per cent or less, which when concentrated to the original serum volume gave a protein concentration in the region of 0·5 mg/ml. Several eluates were concentrated and pooled to provide sufficient material for coupling over the range of 1–3 mg protein/0·1 ml. of 50 per cent red cells. No agglutination was observed when these cells were tested against 10^4 P.F.U. or less of BS–RSV.

Several factors may explain this last result. The antibody titre of the starting serum can affect the yield of suitable material[2]. The ratio of non-specific protein to actual antibody protein is probably high as a result of the low protein yield per serum volume[9]. The use of strong buffers may also have been deleterious to the antibody[8]. Improved yields of antibody may be obtained by using better virus preparations, either in virus content or purity, for coupling to the PAS columns.

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Antibody Synthesis *in vitro* by the Rabbit Vagina against Diphtheria Toxoid

EXPERIMENTS concerned with local antibody production have resulted in a growing list of tissues where antibody synthesis has been demonstrated. The reproductive tract has been examined by several workers[1–5], whose results have suggested that antibodies may be produced locally by the uterus, cervix and/or vagina. A review of the literature, however, has failed to uncover an experiment where *de novo* synthesis or antibody by the reproductive tract was clearly demonstrated. The present report presents evidence for the *in vitro* synthesis of antibody by the vagina of the rabbit after the vagina was exposed directly to the antigen *in vivo*. Attempts to sensitize the uterus by intrauterine injection of antigen, however, have apparently failed.

Mature, virgin, female, New Zealand white rabbits were immunized with an alum precipitated diphtheria toxoid (60 Lf/ml.) by the following three methods. (1) Rabbits were vaginally sensitized after tranquillization with 'Innovan', by inserting into the vagina a series of cotton plugs soaked with the antigen. (2) A second group received an injection of 0·5 ml. of diphtheria toxoid in combination with and without Freund's incomplete adjuvant into each horn of the uterus, after exposing this organ through a midventral incision. (3) A third group was immunized systemically by either an injection of 0·5 ml. of antigen into each hind footpad or 1·0 ml. intravenously in addition to the footpad injection (Table 1). After a minimum of three weeks each rabbit received a booster injection by the same route. Three days following the booster each rabbit was exsanguinated. The popliteal lymph nodes, uterus and vagina were removed aseptically for the *in vitro* experiment.

The *in vitro* culturing technique, as developed by Stavitsky[6], was adapted for use as follows: the tissues were cut into fragments 2–3 mm in size and distributed into 16 × 125 mm culture tubes, 3–5 fragments per tube. The culture tubes, containing 3 ml. of Eagle–Earle's medium with 10 per cent foetal calf serum and 100 μ/ml. of penicillin, were incubated at 37° C on a rotating drum (12 r.p.h.).

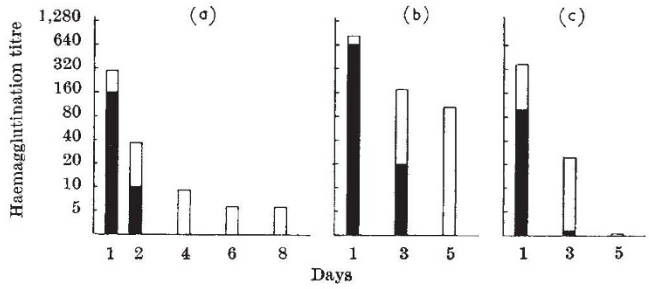

Fig. 1. *In vitro* antibody synthesis by the vagina of rabbit numbers (*a*) 1, (*b*) 4 and (*c*) 2, sensitized by intravaginal cotton plugs soaked with diphtheria toxoid. □, Geometric mean antibody titre after *in vitro* cultivation; ■, geometric mean antibody titre from tubes containing an inhibitor to antibody synthesis.

After 24 h the culture media were poured off, saved for antibody assay, and fresh media were added. Thereafter the media were changed approximately every 48 h.

To detect net *in vitro* synthesis, at least one tube from several (6–12 tubes) of a particular tissue type contained an inhibitor to antibody synthesis, either potassium cyanide ($6{\cdot}5 \times 10^{-3}$ molar) or 2,4-dinitrophenol (10^{-3} molar).

All antibody titres were determined using Stavitsky's modification[7] of the Boyden haemagglutination test[8], in which tannic acid treated sheep erythrocytes were coated with diphtheria toxoid. In each case specificity of the titre was confirmed by simultaneous haemagglutination inhibition tests and the use of tannic acid treated sheep erythrocytes that had not been coated with antigen. Serum samples were inactivated and absorbed with washed sheep red cells before assaying.

The degree to which the intravaginal method of immunization was effective as judged by the serum titre indicate that substantial sensitization had occurred *in vivo* in three of the rabbits (Table 1). The serum titres of these vaginally sensitized rabbits compare favourably to the systemically immunized ones.

The vaginal tissue from three of the rabbits that had been immunized by an intravaginal cotton plug demonstrated a net synthesis of antibody *in vitro*. (One rabbit failed to become sensitized, developed only a low serum titre, and antibody could not be detected after *in vitro* cultivation.) The results from those three rabbits is illustrated in the histograms of Fig. 1, where net antibody synthesis is represented by the difference between the geometric mean titre in the inhibited (solid bar) and non-inhibited (open bar) tubes. The maximum antibody titre was obtained on day 1 at about 640 and declined thereafter. There was, however, an increase in net antibody synthesis demonstrable after the first day.

Systemically immunized rabbits, while synthesizing considerable antibody from popliteal lymph nodes (Fig. 2*a*) and spleen, failed to produce *in vitro* antibody by the uterus or vagina. As a result of the high serum titres in

Table 1. IMMUNIZATION OF RABBITS WITH DIPHTHERIA TOXOID (D.T.) AND RESULTS OF *in vitro* CULTIVATION

The uterus in every case failed to synthesize detectable antibody *in vitro*.

Rabbit No.	Method of immunization*	Total No. of injections	Interval between injections in days	Serum titre	*In vitro* synthesis by: vagina	cervix	popliteal lymph node	spleen
1	Vag.	6	8,3,2,37,1	20,480	+	±	±	
2	Vag.	6	1,6,1,22,1	81,920	+	±	+	
3	Vag.	4	1,1,80	640	−	−	−	
4	Vag.	2	37	162,840	+	±	+	
	Ut.	2†	30					
5	Ut.	2‡	35	640	−	−	−	
6	Ut.	2	29	640	−	−	−	
7	F + IV	2§	8 mo.	20,480			+	+
8	F	3	1,24	5,120		−	+	+
9	F + IV	2	35	1,310,720	−		+	+
10	F + IV	3	48,5 mo.	1,310,720	−	−	+	+

* Vag., Intravaginal injection, Ut., Intrauterine; F, Footpad; IV, intravenous.
† The first injection consisted of D.T. in combination with Freund's incomplete adjuvant.
‡ Both injections consisted of D.T. in combination with Freund's incomplete adjuvant.
§ The first injection was into the footpads only.
+, Net *in vitro* synthesis; −, No *in vitro* synthesis; ±, Suggestive *in vitro* synthesis.