(10 mM Tris and 0.5 mM Dithiothreitol (Sigma Chemical Co., St.Louis, MO 63178) at pH 7.4) was added, the homogenizers kept on ice. The homogenate was then spun at 220,000 × g for 30 min at 2 °C in a Sorvall OTD-65 ultracentrifuge. The supernatant was collected as the cytosol extract; when not being subjected to an assay, this extract was stored frozen at −75 °C or kept on ice.

Estrogen receptor assay. The method used was a modification of that of DeSombre et al.[10]. The modifications used were as follows: In place of CI-628, 2.5 M Diethylstibestrol was used to prevent ^{3}H-estradiol binding to receptors. Sucrose gradients were spun at 340,000 × g (49,000 rpm on SW 60 rotor).

β-hCG radioimmunoassay. Most of the reagents used were obtained from Serono Labs, Inc. (Serono Labs, Inc., Braintree, MA 02184). The procedure uses β-subunit specific rabbit antibody and ^{125}I-hCG as a tracer. Bound-free separation was achieved by the use of polyethylene glycol. The standard is a purified hCG preparation referenced against the second international standard for hCG supplied in lyophilized form with BSA added. Positive and negative hCG control sera were also utilized. Normal male serum was used as a protein equalizer for standards and samples.

Peroxidase-anti-peroxidase tissue staining. Rabbit anti-hCG, goat anti-rabbit IgG, and horseradish peroxidase-rabbit anti-peroxidase complex were obtained commercially (Cappel Labs., Inc., Cochranville, PA 19330). The staining procedure used was similar to those described by others[11,12] for a triple-bridge indirect stain.

9 of 65 (table) tumor extracts studied contained β-hCG in measurable amounts by RIA. The relationship between β-hCG occurrence and estrogen receptor content may appear that tumors with insignificant quantities of estrogen receptor are more likely to produce hCG, χ^2 analysis indicates a relatively high probability of a false conclusion ($\chi^2 = 1.722$, $0.1 > \alpha > 0.5$). All tumors found by RIA to contain β-hCG were also found to be immunoperoxidase positive in formalin fixed tissue. The positive staining was localized within the cytoplasm and, in some cases, on the surface of the malignant cells; surrounding normal cells remained unstained.

We have demonstrated β-hCG production in approximately 14% of the malignant breast tumors examined. This rate of occurrence corresponds well with at least 1 previous report involving serum hCG levels[5]. In consideration of serum measurements, the possibility arises of hCG production by the pituitary or other endocrine organs, whether tumor-related or not, being misinterpreted as production by the tumor. The use of the immunoperoxidase technique has shown by the localization of the hCG that the tumor cells may indeed be producing the hCG measured by RIA, rather than the hormone appearing in the tumor via serum contamination. Measurement of tumor hCG may be a much more sensitive indicator of tumor β-hCG production, since the hCG is not greatly diluted by the circulatory system.

Some studies of hCG production by certain cancer types have suggested a relationship between tumor hCG production and response to specific types of therapy[2]. The production of this hormone may prove to be of prognostic value and aid in the selection of proper post-operative therapy for the breast cancer patient.

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Antigenic correlation between rat brain synaptic vesicles and rat bone marrow B lymphocytes[1]

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Summary. Rabbit anti-rat brain synaptic vesicle serum reacted with thymocytes and B lymphocytes in cytotoxicity and immunofluorescence assays. Quantitative absorption analysis revealed that this antiserum contained antibodies specific for antigenic determinants on the surface membrane of a subpopulation of rat bone marrow B lymphocytes.

The antigenic relationship between the rat brain and thymocytes is well established[2,3]. It has been suggested that brain cells and B lymphocytes share common antigenic determinants[4-6]. We provide here further evidence on the antigenic correlation between rat brain synaptic vesicles and a subpopulation of rat bone marrow B lymphocytes.

Materials and methods. The thymuses were obtained from normal Wistar rats and the femoral bone marrow from rats thymectomized at birth and irradiated 2 weeks later with 700 r. Donor rats aged 6 weeks were perfused with saline before removal of the thymus and bone marrow.

The synaptic vesicle (SV) fraction[7] from the rat brain was homogenous, as shown by electron microscopy. The problem of purity of materials used for the preparation of anti-brain sera was discussed elsewhere[8]. Rabbits were immunized with 18 mg of SV as described previously[5]. Anti-SV sera were absorbed with rat erythrocytes, liver-cell membranes, and glutaraldehyde-treated IgM and IgG. In order to remove antibodies reacting with haemopoietic stem cells, anti-SV sera were also absorbed with rat fetal liver[9]. Anti-SV sera thus absorbed yielded titers between 1:512 and 1:1024 in quantitative complement-fixation reaction, and produced 2 precipitin lines in 0.8% agarose.

Thymocytes were obtained from fresh rat thymuses, and dead cells and erythrocytes removed by centrifugation in a bovine serum albumin solution[10], and thymocytes were

purified on an Isopaque-Ficoll gradient. The viability was determined by trypan blue exclusion, and the morphology by examining smears of cells stained with May-Gruenwald and Giemsa. This procedure yielded approximately 99% of thymocytes.

Anti-rat thymocyte sera (ATS) were prepared in rabbits, and absorbed with rat erythrocytes, liver-cell membranes and insolubilized IgM and IgG, and SV. In addition, ATS were absorbed with 8.5×10^8 rat bone marrow cells. ATS thus prepared reacted in cytotoxicity and immunofluorescence only with thymocytes, and were employed for purification of B cell suspension.

For the preparation of B lymphocytes, the bone marrow cell suspension was passed through a column packed with glass beads[11] to remove granulocytes, immature cells and large unidentified cells. Since a confusion in interpretation of results can ensue if B lymphocytes are not identified, the cytotoxic elimination of T marker-bearing lymphocytes from the preparation of B cells was employed. For this purpose, B cells were incubated with ATS (10^8 cells/0.5 ml of ATS) for 1 h at 37 °C, then 0.3 ml of guinea-pig serum (absorbed with rat erythrocytes, thymocytes and bone marrow cells) were added, and the mixture reincubated for 1 h at 37 °C. Dead cells were separated by pelleting on an Isopaque-Ficoll gradient. This procedure yielded a bone marrow cell fraction which contained 82% B lymphocytes (> 10 µm in diameter). EAC rosetting[12] and identification of surface Ig molecules[13] revealed that bone marrow B lymphocytes contained about 42% cells bearing receptors for complement and about 36% cells carrying Ig receptors.

For indirect immunofluorescence staining[13] of thymocytes and B lymphocytes, rat thymocytes were exposed to rabbit ATS or anti-SV, and rat B lymphocytes to anti-SV. Thus sensitized cells were treated with a sheep fluorescein-conjugated anti-rabbit Ig. Several controls were set up, including the 'blocking' test.

Results. The cytotoxicity of anti-SV (unabsorbed with rat bone marrow cells) for thymocytes disappeared at a dilution of 1:512. The highest cytotoxic index was about 45% with B cells from normal rats and somewhat lower with B cells from thymectomized-irradiated rats. Immunofluorescence analysis of thymocytes and B lymphocytes exposed to anti-SV (unabsorbed with rat bone marrow cells) revealed that anti-SV induced specific staining of about 99% of thymocytes (1144–1204 counted cells/preparation) from normal rats, and about 72% of B lymphocytes (1136–1162 counted cells/preparation) from thymectomized-irradiated rats.

Thus, cytotoxicity and immunofluorescence assays demonstrated an antigenic correlation between rat brain synaptic vesicles, thymocytes and B lymphocytes. Absorption of anti-SV with B cells removed completely its activity for B cells and only moderately reduced its activity for thymocytes. However, absorption of anti-SV with 80 mg of SV eliminated cytotoxicity and immunofluorescence for both thymocytes and B lymphocytes. In order to evaluate the relationship between shared antigens recognized on thymocytes and B lymphocytes by anti-SV, a quantitative absorption analysis was performed: anti-SV was absorbed with increasing numbers of thymocytes and checked back each time with thymocytes and B lymphocytes. The plateau-type curves were obtained with B lymphocytes both in cytotoxicity (figure 1, A) and immunofluorescence (figure 1, B) tests, thus indicating that the absorption of anti-SV with different

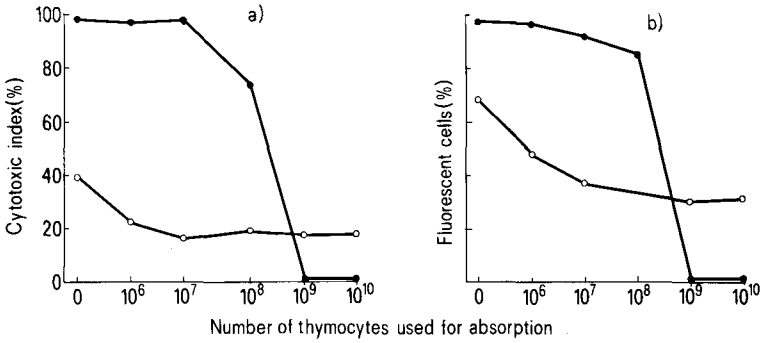

Fig. 1. Cytotoxic activity (A) and indirect immunofluorescence (B) of rabbit anti-SV serum absorbed with increasing number of rat thymocytes and checked back each time for cytotoxicity and immunofluorescence against thymocytes from normal rats (●), and bone marrow B lymphocytes from thymectomized-irradiated rats (○). Note that antiserum absorbed with 10^9–10^{10} thymocytes yielded negative results with thymocytes and positive results with B lymphocytes.

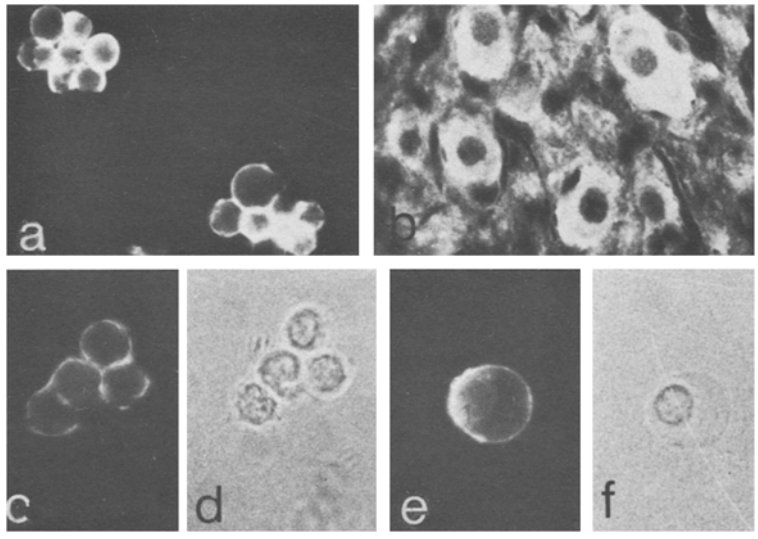

Fig. 2. *a* Intense ring-type specific fluorescence of rat thymocytes after exposure to rabbit anti-SV serum and fluorescein-conjugate × 800. *b* Immunofluorescence microphotograph of a section of the rat pons cerebri exposed to anti-SV and conjugate. Specific fluorescence is concentrated in the neuronal cytoplasm, whereas fibrillar structures exhibit less intense fluorescence. Note negative shadows of neuronal nuclei. × 500. *c* 4 rat B lymphocytes exposed to anti-SV serum absorbed with 10^{10} rat thymocytes. Cells displayed discontinuous membrane fluorescence. × 800. *d* The same 4 cells in ordinary light. *e* 1 plasma cell treated with rabbit anti-SV serum absorbed with 10^{10} rat thymocytes. Note the accumulation of fluorescein-conjugated molecules on the nuclear pole of the cell. *f* The same rat plasma cell in ordinary light. × 800.

numbers of thymocytes rendered anti-SV serum specific only for the rat bone marrow B lymphocytes.

In immunofluorescence assays, thymocytes exhibited brilliant green fluorescence of the ring-type (figure 2, a). The brain sections treated with anti-SV[5] showed an accumulation of specific fluorescence in the nerve-cell body, whereas the network of fibres exhibited a less intense fluorescence (figure 2, b). The neuronal nuclei, pia, choroid plexus, cilia of the ependymal lining and brain blood vessels remained virtually unstained. B lymphocytes exposed to anti-SV serum absorbed with 10^9–10^{10} thymocytes displayed a discontinuous specific staining of the cell membrane (figure 2, c and d). Anti-SV thus absorbed stained also cells of the plasmacytic series, and this fluorescence was characterized by a concentration of fluorescein-conjugate in the region of the excentric nucleus (figure 2, e and f).

Discussion. The most important finding made in this study concerns the common antigenic markers of rat brain cells (i.e. synaptic vesicles) and B lymphocytes. Thus, anti-SV absorbed with 10^9–10^{10} thymocytes reacted only with rat B lymphocytes in cytotoxicity and immunofluorescence tests, although being completely inactive for thymocytes. It should be argued that this B-specific anti-SV serum can also act on lymphohaemopoietic stem cells of the rat bone marrow. This possibility was excluded by absorbing anti-SV serum with the rat fetal liver[9]. These results also suggest that the rat bone marrow contains a 'null' subpopulation of B lymphocytes[11] which cannot be detected with properly absorbed rabbit anti-SV serum[14].

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Passive transfer of resistance in rabbits infested with adult *Ixodes ricinus* L.: Humoral factors influence feeding and egg laying[1]

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Summary. Partial immunity against the bites of ♀♀ *I. ricinus* was transferred to normal rabbits by inoculating immune serum from resistant animals. Transferred humoral factors diminished the weight of the ticks' blood meal by 29% and increased the feeding period by about 1 day in comparison with ectoparasites engorged on controls. They provoked also the failure of egg laying by ♀♀ *I. ricinus*. Only 55% of ticks fed on treated rabbits laid eggs (94% on controls). The immunological state of immune serum donors or recipients was studied and the IgG and homocytotropic specific anti-*I. ricinus* antibodies were identified. The immediate hypersensitivity of rabbits' skin was also controlled.

In a previous paper, it was found that rabbits acquired progressively a resistance against the bites of ♀♀ *I. ricinus* as a result of repeated infestations by this tick[2]. This 'immunity' was reflected by increased mean feeding time, inadequate blood meals and poor oviposition. Thus, after 4 tick infestations, only 25% of all ticks fed normally, and as high as 84% of females failed to lay eggs. It was also shown that transfer of immune sera to normal rabbits affected the feeding of ticks; females ingested 25% less blood than did ticks fed on control rabbits[3].

In the present study, an attempt was made to increase the transferred resistance by inoculating larger volumes of immune serum. This treatment not only influenced the amount of ingested blood but also the feeding time and the production and laying of eggs. Efforts were made to identify and evaluate the anti-*I. ricinus* antibodies (IgG class or homocytotropic), and, finally, to determine the rabbits' skin sensitivity against allergens prepared from ♀♀ *I. ricinus*.

Table 1. Effects of humoral factors on feeding of ♀♀ *I. ricinus*

	On control rabbits n = 34	On treated rabbits n = 44
Mean weight of fed ♀♀ (mg)	231 ± 73	165 ± 81
Mean feading time (h)	173 ± 20	190 ± 27

n = Number of fed ♀♀ *I. ricinus*.

Table 2. Immunological state of donors and recipients of immune serum

	Control rabbits number				Treated rabbits number				
	1	2	3	4	5	6	7	8	9
IgG titre (day 4)	0	0	0	0	1/80	1/80	1/80	1/80	1/80
IgG titre (infestation end)	1/40	1/20	1/20	1/20	1/160	1/80	1/80	1/80	1/160
Homocytotropic antibodies titre (day 4)	0	0	0	0	1/1	1/3	1/1	1/1	1/3
Homocytotropic antibodies titre (infestation end)	0	0	0	0	1/3	1/9	1/3	1/3	1/9
Skin's sensibility (infestation end)	−	−	−	−	+	+	+	+	+

Transferred immune serum: IgG titre = 1/640; homocytotropic antibodies titre = 1/81; + = positive cutaneous test; − = negative cutaneous test.