the untreated pairs (table). However, comparatively low egg deposition was noted in I♂ × N♀ (at 2500 rad). Egg hatch in this treatment was 80, 12 and 10% in 1000, 2000 and 2500 rad respectively as compared to 90% in normal pairs (N♂ × N♀).

In treated females mated to normal males, eggs laying was low at 2000 rad and least at 2500 rad (table). At 1000 rad, females still deposited as many eggs as the normal with egg hatch of 85%. None of the eggs laid by treated females in 2000 and 2500 rad treatment could hatch.

Treated females mated to treated males behaved essentially similar to the previous combination, i.e. I♀ × N♂. Enough of eggs were laid by only 1000 rad treated insects with egg hatch of 78%. However, few eggs which were laid by insects treated at 2000, and after 2500 rad they did not hatch.

Hennebery et al.[5] have reported complete sterilization of females of *Epilachna varivestis* (Mexican bean beetle) at doses of 1, 4, 8 or 16 krad and of males at doses of 4, 8 or 16 krad. But in our study, sterilization of *E. vigintioctopunctata* in both sexes was achieved at minimum dose of 2000 rad at which in females infecundity is caused and in males 88% sterility has been caused (only 12% egg hatch in I♂ × N♀ in 2000 rad). At 2500 rad, although 90% sterility in males has been caused, at this dose longevity of adults is reduced. In conclusion, 2000 rad is a suitable dose of gamma radiation where both sexes are sterilized, and thus there is no need of sex separation before their release in fields, if field trials are to be conducted (as sex separation in large number is difficult).

Other important observations which we found in the present study were: There was no delay in the egg laying in treated and control insects. Egg laying started after 10 days of adult emergence in every combination. First 3–4 eggings laid by treated females in 2000 and 2500 rad were abnormal i.e. eggs laid were scattered (normally eggs are laid in cluster) and were abnormal in shape and shrunk in size. But later on eggs laid were normal and in clusters.

Adults treated at 2500 rad died within 36 days of their treatment as compared to more than 80 days in normal and other treated insects. One interesting fact which we noted was delayed death in females of 2000 rad treatment combinations. N♂ I♀, N♀ I♂ and I♂ I♀ in all these combinations females lived for 15–20 days longer than females treated at 1000 rad and those of normal pair. This observation was similar to the observations made by Brower[2]. He found that females of *Tribolium* treated at 5 krad of gamma radiation lived longer than the controls. He has discussed possibility of utilization of stored food reserves in egg laying by the controls which so died sooner than the females which were sterile.

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Ectopic human chorionic gonadotropin in breast carcinoma

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Summary. Immunoreactive human chorionic gonadotropin (hCG) was found in 9 of 65 surgically removed malignant breast tumors. Concentrations ranged from 5 to greater than 500 mIU hCG/g tumor. hCG was measured by a β-chain specific radioimmunoassay. In further study of these specimens, an immunoperoxidase staining technique was used to stain for hCG in formalin-fixed sections. The hCG was shown to be localized within the cytoplasm and on the surface of the malignant cells.

Human chorionic gonadotropin (hCG) is a well-characterized[1,2] and fully sequenced[3] glycoprotein hormone. Earlier reports of tumor-associated hCG utilized methods of detection which cross-react with other gonadotropic hormones, particularly luteinizing hormone[LH]; however, following the development of a radioimmunoassay[RIA] for hCG specific to the unique β-subunit[4], β-specific antisera have been used to demonstrate hCG in the serum present in some cases of breast carcinoma examined[5,6]. Some workers have shown hCG in the urine[7] and normal tissues[8,9] of apparently normal subjects; however, these studies involved concentration of extracts to achieve hCG levels sufficient for measurement. Thus, tumor and serum levels reported are significantly higher than those demonstrable in normal subjects.

Material and Methods. Tumor specimens. Tumors were surgically removed and a portion was formalin-fixed and parafin embedded. A 2nd portion was frozen in liquid nitrogen and stored at −75 °C for estrogen receptor assay. The cytosol extracts used for estrogen receptor assay were also used for β-hCG radioimmunoassay.

Cytosol extraction. Tumor samples were frozen in liquid N_2 and pulverized in a tissue crusher. All utensils used to handle tumor tissue and the parts of the crusher cells were kept well below freezing by using liquid N_2. After the tissue was completely crushed, it was placed in a glass vial, the liquid N_2 allowed to boil off, and the vial put into an ultrafreezer at −75 °C for storage. To extract the cytosol, crushed tissue was weighed into Kontes ground glass homogenizers and 4 volumes (ml/g tissue) of buffer

Relationship of estrogen receptor content to the presence of hCG in cytosol extract

	Number hCG-positive	Number hCG-negative	Percent positive
Estrogen receptor positive	2	22	8.3
Estrogen receptor negative	6	26	18.8
Borderline estrogen receptor	1	8	11.1
Total	9	56	13.8

(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