marked with some powdered carbon. These rats and the other 12 rats which served as controls were closed with sutures. 8 days after operation all animals were sacrificed by decapitation. In a previous study the time interval was determined as a conservative amount of time that endothelium would require to regenerate[7]. In the tissue preparation the aortae were perfused with Hank's solution, excised, cut longitudinally and then pinned to a piece of cork. The silver staining procedure was that of Geissinger[8]. Specimens were dehydrated in a graded series of alcohols and allowed to air-dry. They were then glued to studs coated with a thin layer of gold and viewed in a Hitachi HHS-2R scanning electron microscope.

Endothelial cells in the control specimens appeared as described in normal tissues by others[4,5] (figure 1). The silver lines, denoting intercellular boundaries, appeared as raised structures. This observation was probably due to air-drying of the specimens which caused shrinkage.

Experimental tissues exhibited a considerably different pattern to the silver lines. The shapes of the cells took many forms. Some appeared to be elongated like control cells but most cells were more rounded or polygonal. The pattern of the intercellular boundaries of the regenerated tissues was markedly different as a result of variability in cell shape (figure 2). There was definitely no distinct orientation of the cells as was observed in the control tissues whose cells paralleled the long axis of the blood vessel. The significance of the orientation and shapes of the cells is not known.

1 The author would like to acknowledge the technical assistance of Mr William Daniels and supply from the MRC grant No. MT-1011.

2 H.W. Florey, J.C.F. Poole and G.A. Meek, J. Path. Bact. *77*, 625 (1959).
3 C. Garbarsch and B. Collatz Christensen, Angiologica *7*, 365 (1970).
4 B. Collatz Christensen and C. Garbarsch, Angiologica *9*, 15 (1972).
5 R. Gottlob and H.F. Hoff, Vasc. Surg. *1*, 92 (1967).
6 Henryk T. Malczak and Robert C. Buck, Am. J. Path. *86*, 133 (1977).
7 Henryk T. Malczak, Thesis, University of Western Ontario, 1976.
8 H.D. Geissinger, J. Microsc. *95*, 471 (1972).

Sterilization of solanaceous hadda, *Epilachna vigintioctopunctata* F. by irradiating pupae using gamma radiation

J.K. Gupta and A. Kumar[1]

Department of Bio-Sciences, Himachal Pradesh University, Simla-171 005 (India), 1 November 1978

Summary. Sterility in males and females was observed in the resulting adults when 3-4-day-old pupae of solanaceous hadda, *Epilachna vigintioctopunctata* F. were exposed to gamma radiation doses of 2000 rad and 2500 rad. However, at 2500 rad longevity of treated insects was adversely affected.

Ionizing radiations have been extensively used for the control of insect pests of stored commodities[2,3]. Knipling[4] has shown sterile male technique to have great potentials in the control of certain insect pests. One of the most important prerequisites in the use of this technique is the determination of the minimum effective dosage of radiation required for the control. The present paper reports sterilization studies made on solanaceous hadda, *E. vigintioctopunctata* F. (Coleoptera; Coccinellidae), a serious pest of solanaceous crops.

Materials and methods. Hadda beetles were reared in the controlled conditions of temperature (30 ± 1 °C), photoperiod (17 h light: 7 h dark) and relative humidity (70%). Fresh potato leaves were fed to the beetles. The beetles used in the present study belong to the 5th generation reared in the laboratory. 3-4-day-old pupae were exposed to Co-60 (gamma source) at radiation doses of 0 (control), 1000, 2000 and 2500 rad. The dose rate of Co-60 gamma source was 52 rad/sec. After irradiation, pupae were allowed to eclose. 4 pairing combinations were made at all the doses of treatment viz. a) N (normal) ♂ × N♀, b) I (irradiated) ♂ × N♀, c) I♀ × N♂ and d) I♂ × I♀. Pairs were placed singly in 150-ml plastic jars. At each cross 5 pairs were maintained. Egg laying and egg hatching was recorded daily. Total number of eggs laid and hatching percentage has been considered a parameter of sterility.

Results and discussion. Normal females mated to males treated at 1000 and 2000 rad deposited eggs little less than

Total number of eggs laid (by 5 pairs) and the viability of eggs laid by hadda beetle, exposed to gamma radiation in pupal stage

Mating combination (dosage rad)	Total number of eggs Deposited (by 5 pairs)	Hatched	Hatching % age
a) Normal male × normal female (N♂ × N♀)	2509	2255	90
b) Irradiated male × normal female (I♂ × N♀)			
1000	2400	1920	80
2000	2090	250	12
2500	505	50	10
c) Irradiated female × normal male (I♀ × N♂)			
1000	2600	2210	85
2000	723	0	0
2500	80	0	0
d) Irradiated male × irradiated female (I♂ × I♀)			
1000	2325	1820	78
2000	230	0	0
2500	75	0	0

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.

1 Acknowledgment. Financial help to one of us (J.K.G.) by C.S.I.R. in the form of J.R.F. is gratefully acknowledged. Authors are thankful to Prof. P.N. Srivastava and Dr A.R. Rao, School of Life Sciences, J.N.U. Delhi for providing radiation facility.

2 J.H. Brower, J. stored Prod. Res. *11*, 223 (1975).
3 P.B. Cornwell, ed. in: The Entomology of radiation disinfectation of grain, p. 236. Pergamon, Oxford 1966.
4 E.F. Knipling, J. econ. Ent. *48*, 459 (1955).
5 T.J. Hennebery, F.F. Smith and W.L. McGovern, J. econ. Ent. *57*, 813 (1964).

Ectopic human chorionic gonadotropin in breast carcinoma

A. Castro, P. Buschbaum, M. Nadji, W. Voigt, S. Tabei and A. Morales

Hormone Research Lab., Department of Pathology, University of Miami School of Medicine, Miami (Florida 33101, USA), 23 March 1979

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