Abnormalities in the development and reproduction of Blattella germanica (L.) (Dictyoptera: Blattellidae) treated with insect growth regulators with juvenile hormone activity

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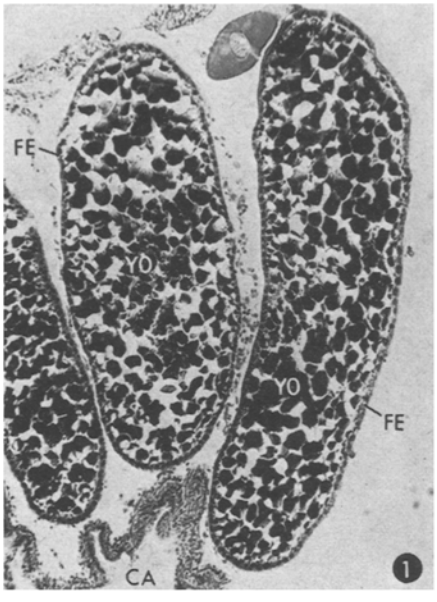
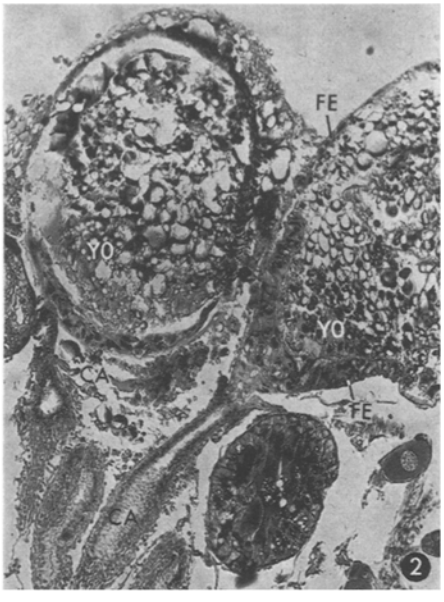
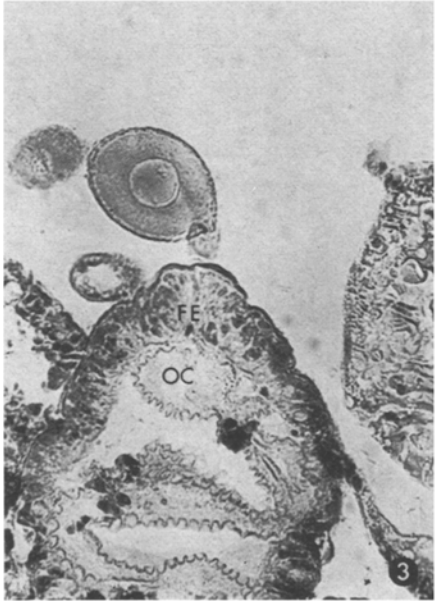
Summary. Laboratory populations of German cockroach reared on food treated with R-20458 (75 ppm) or Ro 20-3600 (3000 ppm) became completely sterile and eventually extinct. The hypertrophied oocytes had excessively laid chorion, and the hypertrophied accessory sex glands contained excessive amounts of protein.

Several synthetic insect growth regulators (IGRs) with juvenile hormone activity have been tested in recent years against various insect pests in the hope of developing safer means of insect control[1]. We tested 3 such chemicals on the German cockroach, Blattella germanica (L.), and found significant adverse effects on certain aspects of metamorphosis and reproductive physiology[2]. We describe now the gross effects of 2 IGRs on the growth and development of the laboratory populations, and in some detail, the nature of abnormalities in the female reproductive system.

Materials and methods. Undiluted technical grades of the IGRs[3,4] were applied topically (1.5–4 µl/nymph), or by injection (0.33 µl/nymph). For treating the food pellets[5]







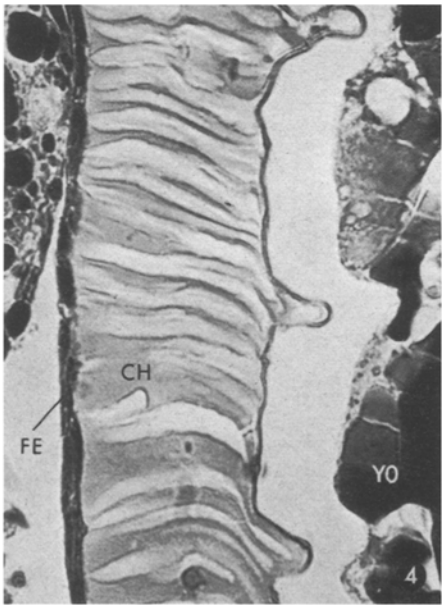


Fig. 1. Well-developed basal oocytes in normal adult. Note the well-defined follicular epithelium (FE) and yolk (YO). CA = calyx. ×60. Fig. 2. Hypertrophied basal oocytes in adultoid. Note the distorted follicular epithelium (FE) and irregularly deposited yolk (YO). Ro 20-3600 (3000 ppm) in food pellets. ×40. Fig. 3. Atrophied basal oocyte in an adultoid. Note the thick follicular epithelium (FE) and the shrunken oocyte contents (OC). Ro 20-3600 (1.5 µl) topical. ×180. Fig. 4. Basal oocyte (a portion) of an adultoid showing the follicular epithelium (FE), chorion (CH) and yolk (YO). Ro 20-3600 (1.5 µl) topical. ×170.

(10–10,000 ppm), the IGRs were dissolved in excessive acetone, and dried with constant shaking under an exhaust hood.

Results and discussion. The nymphs reared on treated food lost the capacity to molt, died in the process of molting, or metamorphosed into sterile adults. For example, 50 first-instar nymphs reared on food pellets treated with R-20458 (75 ppm) either died as nymphs or metamorphosed into sterile adult (= adultoid) population (table 1). Ro 20-3600 produced similar effects but at a much higher dose (3000 ppm) than R-20458 (75 ppm), probably due to the relative instability of Ro 20-3600. In a 4-day exposure to treated food, younger nymphs exhibited greater tolerance than the older nymphs, presumably due to the normal high titers of juvenile hormone in the younger instars. The older the nymphs at treatment, the lesser their capacity to undergo subsequent molts (table 2).

The development of ovarioles and accessory sex glands was affected to varying degrees in individual adultoids. The epithelium of the hypertrophied oocytes was thin, distorted, and not easily discernible; the oocytes were apparently filled with yolk but somewhat irregularly (figure 2). The epithelium of the atrophied oocytes was very thick and conspicuous, but the oocyte contents were reduced and shrunken (figure 3). In many hypertrophied oocytes there was a thick (non-staining) zone of chorion between the follicular epithelium and the yolk (figure 4). Histochemical tests[6] for detection of carbohydrate-protein complexes and glycogen (polysaccharides) did not reveal any qualitative differences in the oocyte contents between the normal and hypertrophied oocytes. In the latter, however, the staining for carbohydrate-protein complexes was less intense and uneven. Development of the left accessory sex gland was inhibited in some cases and in a few others hypertrophy resulted. In the latter situation, histochemical tests revealed that there had been excessive secretion of protein into the lumen of the tubules (figure 6).

Table 1. Development of German cockroach populations on food pellets treated with R-20458[a]

Treatment	No. of cockroaches[b]	Remarks
Control	346	Normal adults and nymphs
R-20458, 50 ppm	127	Adultoids, normal adults and nymphs
R-20458, 75 ppm	36	Adultoids, no nymphs
R-20458, 150 ppm	23	Adultoids, no nymphs

[a] Mean of 3 replications. Each culture jar initially contained 50 freshly hatched nymphs. [b] Surviving population at the end of 5-month period.

Table 2. Toxic effects of IGR on Blattella germanica nymphs

Characteristic	Nymphal instar[a] I	II	III	IV	V	VI
Mortality (%)[b]	50	50	60	78	100	100
Mean age at death[c]	2	9	4	18	14	16
Metamorphosis (%)[d]	48	28	14	0	0	0

[a] Nymphs were reared on treated food pellets (R-20458 2%) for 4 days, and thereafter maintained on untreated food; 10–20 nymphs studied under each instar. [b] Due to loss of capacity to molt and eventual death. [c] Days after exposure to the treated food. [d] Percent of individuals that have successfully undergone subsequent molts to adult stage.

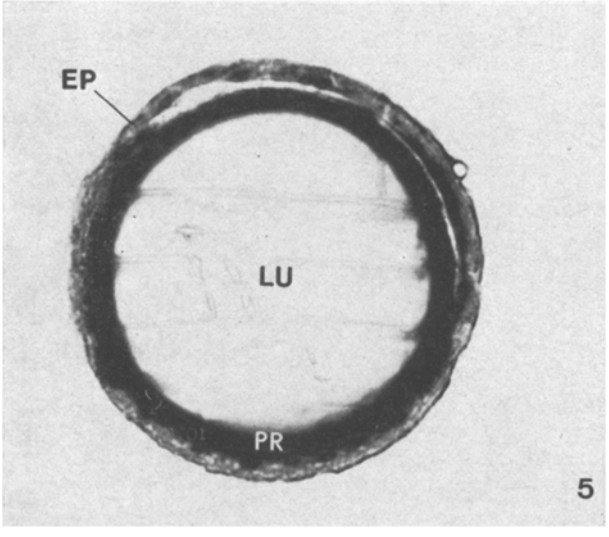


Fig. 5. Well-developed tubule of left accessory sex gland showing protein layer (PR) in the lumen (LU) of normal adult female. ×530.

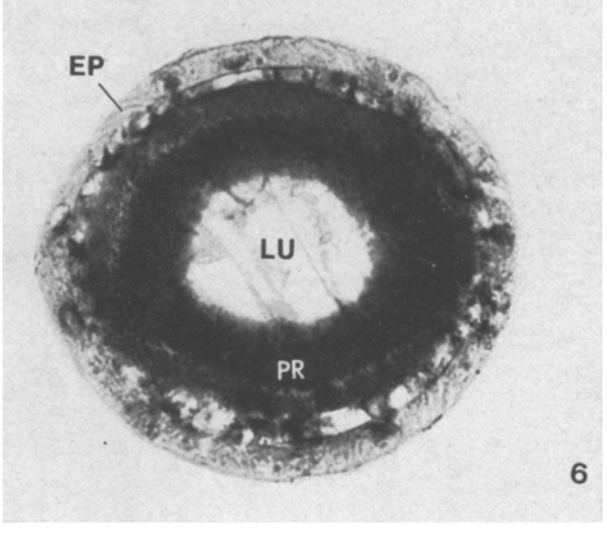


Fig. 6. Hypertrophied tubule of left accessory sex gland showing excessive secretion of protein (PR) in the lumen of adultoid. R-20458 (100 ppm) in food pellets. ×310.

1 G. B. Staal, Ann. Rev. Ent. *20*, 417 (1975).
2 Y. T. Das and A. P. Gupta, Experientia *30*, 1093 (1974).
3 6,7-epoxy-3,7-dimethyl-1-(*p*-ethylphenoxy)-2-octene (R-20458). Generous gift from Stauffer Chemical Co., Mountainview, California, USA.
4 6,7-epoxy-3-methyl-7-ethyl-1-(3,4-methylenedioxyphenoxy)-2-octene (Ro 20-3600). Generous gift from Hoffmann-La Roche, Nutley, New Jersey, USA.
5 Little Friskies® cat food, Carnation Co., Los Angeles, California, USA.
6 A. G. E. Pearse, Histochemistry, Theoretical and Applied, 2nd ed. Little-Brown, Boston 1960.

In B. germanica, although the ovaries appear to be capable of vitellogenesis during the last nymphal instar itself[2,7,8], it does not occur until after the adult emergence when the juvenile hormone reappears and acts as a gonadotropin. Last instar nymphs treated in the present study developed, upon metamorphosis, abnormal (hypertrophied and atrophied) ovaries (figures 1–4), presumably due to the simultaneous action of molting hormone and juvenile hormone. For normal vitellogenesis, therefore, the ovaries appear to depend on the presence of juvenile hormone and absence of molting hormone[9,10].

7 P. Masner and W. Hangartner, Experientia *29*, 1550 (1973).
8 P. Masner, W. Hangartner and M. Suchy, J. Insect Physiol. *21*, 1755 (1975).
9 The situation is comparable to that of Nauphoeta cinerea in which the ovaries are capable of vitellogenesis in the last nymphal instar (B. Lanzrein, J. Insect Physiol. *20*, 1871 (1974)).
10 In Periplaneta americana, the ovaries are incompetent for vitellogenesis in the last nymphal instar (A. Girardie, J. Insect Physiol. *8*, 199 (1962)), and appear to require the presence of molting hormone and absence of juvenile hormone for their future vitellogenic activity in the adult (W. J. Bell and G. R. Sams, J. Insect Physiol. *21*, 173 (1975)).

Effect of vincristine on glucose-induced insulin secretion in man

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Summary. 60 min after the injection of therapeutic doses of vincristine for cancer chemotherapy, there is a reduction of the total (40%) and of the acute phase (43%) areas of insulin secretion induced by a 5-g i.v. glucose load, and the constant of glucose utilization is reduced by 25%. No differences are observed after 3 5-g i.v. glucose loads given at hourly intervals in control subjects.

The participation of the microtubular-microfilamentous system in insulin release has been documented in experimental animals, utilizing isolated pancreatic islets in vitro and rat pancreases in vivo[2–6]. Various agents that influence the system can modify insulin secretion induced by different stimuli[7]. Among these, citochalasin-B[8–11] has a defined action on the microfilaments and colchicine[12–15] interferes with the microtubules; vincristine[10–16] and vinblastine[6–7] disrupt the microtubules and, only partially, the microfilamentous systems.

The aim of this study was to investigate the influence of vincristine (VCR) on insulin release in man, utilizing therapeutic doses for cancer chemotherapy.

Material and methods. 8 patients (4 males and 4 females) suffering from various neoplastic diseases were studied. They required chemotherapy and were at their first treatment. As a control group, 9 patients (5 males and 4 females) without neoplastic diseases were studied. No patient presented a metabolic disease neither were they treated with steroids.

After an overnight fast, each subject was rapidly injected 5 g glucose i.v.[17], 3 times at 1 h by intervals. In the treated group, 1.4 mg/m² of VCR were injected i.v. immediately before the second glucose load. Samples for blood glucose and insulin were drawn from the opposite arm at the following times: 30, 15, 0 min before and 3, 5, 10, 30, 60 min after each load. An additional sample was drawn immediately after VCR in treated patients. Studies were concluded before 13.00. Blood glucose was determined by the glucose-oxidase method and blood insulin by double antibody radioimmunoassay[18].

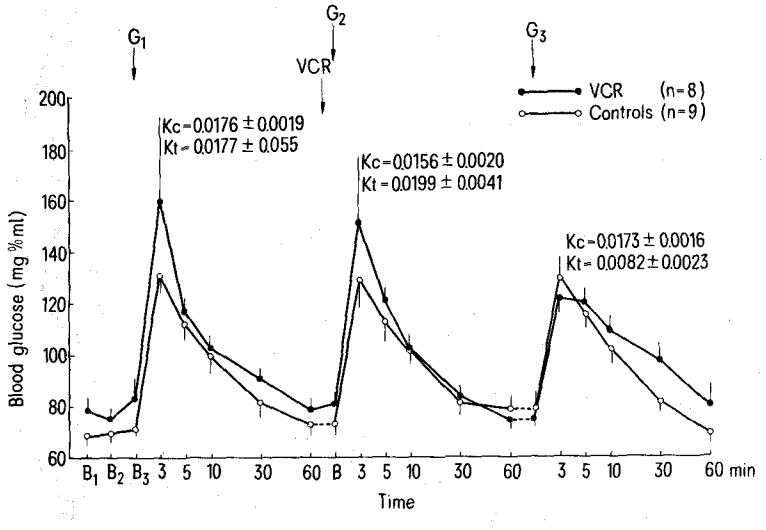

Fig. 1. Glycemias and constants of glucose utilization (K) after 3 5-g i.v. glucose loads (G_1, G_2, G_3). In treated patients 1.4 mg/m² of vincristine (VCR) were injected immediately before the second load. Vertical bars indicate ± SEM. KtG_3 vs KtG_1, $p < 0.05$; KtG_3 vs KcG_3, $p < 0.05$.