Table 2. EFFECT OF AN OVULATION-INHIBITING DOSE OF ICI 46,474 ON CONCENTRATIONS OF PITUITARY LH IN ADULT RATS

Group	Stage of cycle at autopsy	Inhibition of ovulation (%)	Pituitary LH* concentration (95% CL)	Total LH content (μg/gland)	Relative potency (95% CL)
Control	Pro-oestrus	—	2·49 (1·64–3·76)	22·8	100
Control	Oestrus	0	1·00 (0·66–1·51)	11·8	40 (27–59)
46,474†	Oestrus	100	1·70 (1·13–2·55)	18·7	68 (46–101)

CL, Confidence limits.
* In μg equivalents of NIH–LH–S11 per mg wet pituitary (assay by OAAD).
† 0·5 mg/kg, given orally, at 1700 h on the day before pro-oestrus.

with 5 mg/kg of ICI 46,474—that is, ten times the minimum effective dose—it was restored by the same dose (25 μg) of LH (group 5). Furthermore, inhibition of ovulation by the compound (0·5 mg/kg, given orally) could be prevented by concomitant injection of a single subcutaneous dose of oestradiol benzoate (400 μg/rat; group 6). The oestrogen by itself did not affect the rate of ovulation (group 7). When ovulation was blocked by giving the minimum effective dose of the antagonist at 1700 h on the day before pro-oestrus, there was no decrease in pituitary LH between pro-oestrus and oestrus as there was in control rats (Table 2).

These results indicate that ICI 46,474 does not reduce the sensitivity of the ovaries to LH, but that it blocks ovulation by preventing the ovulatory surge of LH from the pituitary—probably by interfering with the discharge, or action, of LRF. The fact that inhibition of ovulation is obtained with an antagonist of oestrogen and can be prevented by simultaneous administration of excess oestrogen suggests that this interference stems from an interruption of oestrogen feedback. It is fully consistent with the hypothesis that the train of events that culminates in ovulation is usually set in motion by positive feedback of ovarian oestrogen.

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Oral Contraceptives and Copper Metabolism

IT has been known for many years[1] that serum copper is increased during the later stages of pregnancy. Exogenous oestrogens produce an increase in both serum copper and the copper-binding globulin ceruloplasmin[2] and it is therefore not surprising that all of the popular oral contraceptives containing potent synthetic oestrogens have been reported to increase serum copper and ceruloplasmin[3–7].

A recent development in oral contraception is the daily administration of a continuous small dose of a synthetic progestogen alone without added oestrogen. Because chronic increases of ceruloplasmin have been suspected as being implicated in the aetiology of certain side-effects of oral contraceptives—notably migraine and chloasma—it seemed worthwhile to investigate the effects of one of these new products on forms of blood copper.

Six normal healthy young women received 0·3 mg daily of norethisterone acetate (a product available under the 'SH–420C' Schering code name). As a control, two other similar healthy young women received the combined 21-day preparation 'Minovlar' (1·0 mg norethisterone acetate + 0·05 mg ethinyl oestradiol). Blood samples were taken by venepuncture after an overnight fast, serum was collected and copper was determined by the diethyldithiocarbamate method[8] and ceruloplasmin by the p-phenylenediamine method[9]. Results of these determinations are shown in Table 1.

Table 1. EFFECTS OF ORAL CONTRACEPTIVES ON SERUM COPPER AND CERULOPLASMIN

Treatment	Duration (months)	Mean values ± S.D. Serum copper (μg/100 ml.)	Serum ceruloplasmin (μg/100 ml.)
None	0	121 ± 15	31 ± 4
'SH–420C'	2 to 4	128 ± 20	33 ± 6
'Minovlar'	3 to 4	235 ± 25	84 ± 5

It is clear that the oestrogen-containing product 'Minovlar' produced a marked increase in serum copper and ceruloplasmin, but that 'SH–420C' had no significant effect. The progestogen component of 'SH–420C', norethisterone acetate, is known to produce in man oestrogenic metabolites[10,11], but the extent of this production and its biological effect are still obscure. Our data indicate that, at the dose level used, no significant amount of oestrogenic substances could have been formed. Indeed, the use of changes in serum ceruloplasmin offers a novel approach to the detection of oestrogenic effects in the human.

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Deferred Action of Juvenile Hormone

WHEN some insect eggs are treated with a synthetic analogue of juvenile hormone the resulting larvae grow normally, but much later their metamorphosis may be inhibited, so that they develop into intermediates or even extra larval instars[1,2]. This has been interpreted by Riddi-

ford and Williams[1] as indicating "that the hormonal materials are able to interfere with the programming or latent storage of information for postembryonic development". An alternative explanation is that there is an excess of juvenile hormone in the insect at metamorphosis, a situation which could have two causes: either the embryonic brain or corpora allata could have been directly affected by the hormone analogue so that secretion of juvenile hormone was abnormally prolonged[2,3] or the applied hormone could simply have persisted throughout growth until metamorphosis.

To help decide between these three hypotheses we have treated eggs of the milkweed bug, *Oncopeltus fasciatus*, with a juvenile hormone analogue and exchanged larval integument between individuals in the treated and untreated groups. The results demonstrate that there is enough hormone in the treated insects to affect the graft, and that the excess is probably a consequence of the persistence of the analogue used to treat the embryos.

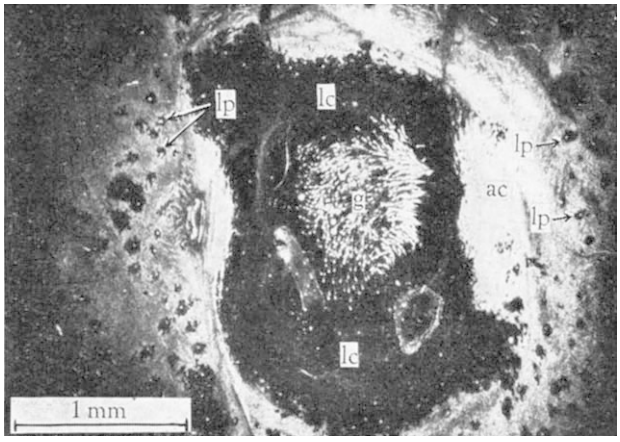

Fig. 1. The tergal cuticle of a larval/adult intermediate treated with hormone analogue when an embryo, showing grafted patch of sternal cuticle (g). The graft was taken from an untreated insect and transplanted in the fifth instar. Note that the host forms mostly adult cuticle (ac) but there are patches of larval cuticle (lp) located around the bristles. The graft has developed bristles characteristic of a larval/adult intermediate in predominantly larval cuticle. Larval cuticle (lc) is formed near the junction of graft and host by both tissues.

At 29° C embryonic development of *Oncopeltus* lasts approximately 120 h. Eggs 72–96 h old were treated with 5 or 10 μg of "synthetic juvenile hormone" obtained from Calbiochem. This preparation is a crude mixture of compounds obtained by treating farnesoic acid with ethanolic hydrogen chloride[4,5]. When 5 μg of this material was applied topically to late fourth or early fifth stage larvae they invariably developed into perfect sixth stage larvae. The hormone analogue was dissolved in octane or acetone; a volume of 0·25 μl. of solution was applied to each egg; the egg was suspended on the needle for 1 min to facilitate thorough evaporation of the solvent and then placed in a tube with milkweed seeds and a wet cotton plug.

After hatching, the larvae in a group were transferred to refrigerator boxes for mass rearing. The eggshells, and in some experiments the early larval skins, were discarded. Controls treated with acetone or octane developed normally.

Two groups of treated eggs developed normally up to the fifth stage, and then moulted into larval/adult intermediates. Larval individuals of these affected groups were used as donors and hosts in the grafting experiments, in which pieces of sternal integument (cuticle, epidermis and adhering fat body) were transferred to the tergites, where the grafted cells could be identified[6]. In control grafting experiments between normal insects, complete metamorphosis of both host and graft always occurred.

The affected insects had normal adult wings and genitalia, but around each abdominal bristle there was a little patch of larval integument (lp, Fig. 1). Such insects had earlier been obtained by topical application of synthetic juvenile hormone analogues, but not by injection of hormone or by implantation of corpora allata[7,8]. This local effect was thought to be the consequence of the greater permeability to oils of bristle cuticle[9]. In some areas all bristles were affected, which, because most bristles develop during larval life, suggests that the time of action of the hormone analogue could not have been at the egg stage.

The grafting experiments were carried out on young fourth and fifth stage larvae. There were three cases where integument was grafted onto individuals which, as a result of treatment when still embryos, developed into larval/adult intermediates. In each of these cases the graft was also affected, and its cells secreted the cuticle of an intermediate (Fig. 1). Moreover, around the border between graft and host both types of cells formed a band of completely larval integument, probably because of the greater sensitivity of dividing and regenerating cells to juvenile hormone[10]. In all five examples of the reverse experiment, where integument taken from insects which were effectively treated when still embryos was transplanted onto untreated larvae, the metamorphosis of both host and graft cells was complete. Probably the small amount of transferred juvenile hormone was diluted in the host.

We think that these experiments unequivocally rule out the hypothesis that juvenile hormone analogue can interfere with the programming of the embryonic cells. Moreover, the restriction of larval patches to areas around bristles seems to suggest that the hormone analogue enters the cells externally from the cuticle, rather than the haemolymph, and this speaks against the hypothesis that the corpora allata, as a result of treatment of the egg, fail to cease production of juvenile hormone in the fifth stage larva. All our results suggest that the hormone analogue persists throughout larval development, at least some of it in the cuticle; we suspect that at each moult cycle some analogue may pass through the moulting fluid from the old to the nascent cuticle. Two other facts support this idea. (i) Some genetically marked *Oncopeltus* were reared with the treated groups of insects and showed traces of juvenile cuticle on their sternites, presumably obtained by coming into contact with the cuticle and cast skins of treated larvae. (ii) Treatment of second stage larvae with the same dose of hormone analogue also resulted in larval/adult intermediates of the same kind as obtained by treatment of the egg.

These results provide no information about the action of the true juvenile hormone(s) of insects but suggest that some of the deferred actions of the hormone analogues[1,2,11] can be explained by the persistence of these stable molecules.

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Altitude Effect on the Biological Half Life of Caesium in Man

When humans reach a high altitude there is a marked reduction in the rate of urinary excretion of aldosterone with a slow return to normal[1]. These changes account for the parallel decrease and increase in rates of salivary and urinary excretion of potassium and sodium respectively. A difference of about a factor of three in the ratio of salivary sodium to potassium has been observed for an altitude as low as 2,400–3,000 m compared with values near sea level. Because of the marked chemical similarity between potassium and caesium, a transitory change in the rate of excretion of caesium might be expected to accompany a change in altitude. and we have observed such an effect in a volunteer who was on an exchange visit from Harwell (altitude 100 m) to Los Alamos (altitude 2,200 m).

At the time of the oral administration of rather less than 0·2 μCi of caesium-134 he had been at Los Alamos for 11 months so that the rate of secretion of aldosterone could have stabilized after his arrival at 2,200 m. Retention was followed for 130 days by body radioactivity measurement, starting 15 days after ingestion; the subject then left Los Alamos and returned to Harwell where measurements of body radioactivity were continued after 38 days. The equipments at Los Alamos and Harwell were intercalibrated from the results of measurements of a second subject containing caesium-134 from Harwell while on a brief visit to Los Alamos.

During the interval between 15 and 396 days after ingestion the body content of caesium decreased from 132 nCi to 7·4 nCi; a non-linear weighted least squares fit to a single exponential term indicated an effective half life of $89{\cdot}0 \pm 0{\cdot}3$ days and an intercept of $149{\cdot}1 \pm 0{\cdot}6$ nCi. Systematic departures of the data from the line of "best" fit were observed, however, when a plot was prepared of the percentage deviations from the fitted curve against time (upper half of Fig. 1).

The data were re-analysed by the least squares method to give values for the effective half lives in three different periods, with the results shown in Table 1. The percentage deviations from the lines of "best" fit are shown in the lower half of Fig. 1 and no systematic trends are apparent; the points scatter fairly evenly around zero, and approximately two thirds of them lie between the lines representing ±1 standard error (the abrupt change in these lines at 340 days resulted from an increase in measurement time).

Table 1. ANALYSIS OF DATA BY LEAST SQUARES METHOD

Period	Time after intake (days)	No. of observations	Initial and final ^{134}Cs contents, nCi		Intercept, nCi	Effective half life, days
1	15–144	29	132	49	$148{\cdot}0 \pm 0{\cdot}3$	$90{\cdot}7 \pm 0{\cdot}3$
2	182–217	10	36	26	185 ±9	77 ±2
3	238–396	23	23	7·4	138 ±4	92 ±1

It seems that when the subject returned to near sea level the effective half life was reduced for about 3 months. The effective half life for period 3 is not significantly different from that for period 1. When we corrected for the radioactive decay of caesium-134 with a half life of 747 days,

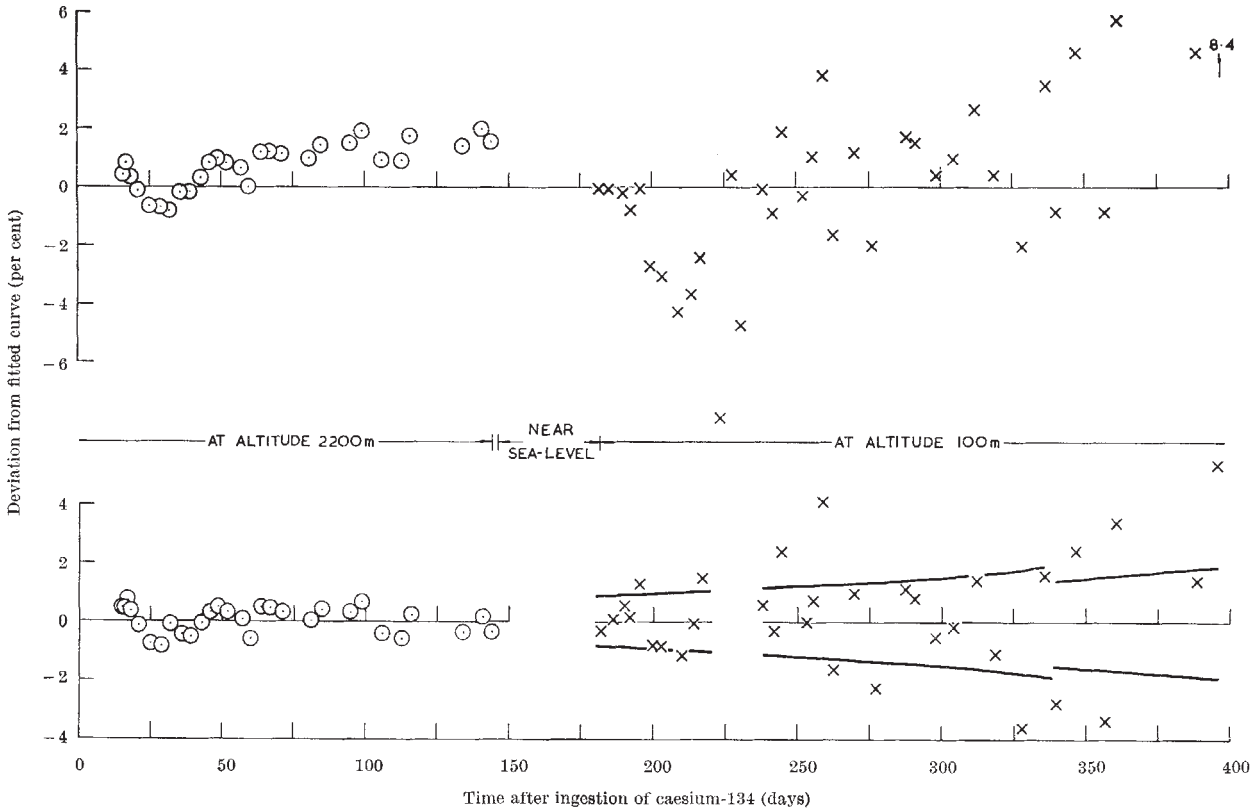

Fig. 1. Deviations of measured points (15–396 days after ingestion) from fitted curves, as a percentage, for a single exponential (upper half) and for three separate exponentials (lower half). Measurements made at Los Alamos (○) and Harwell (×).