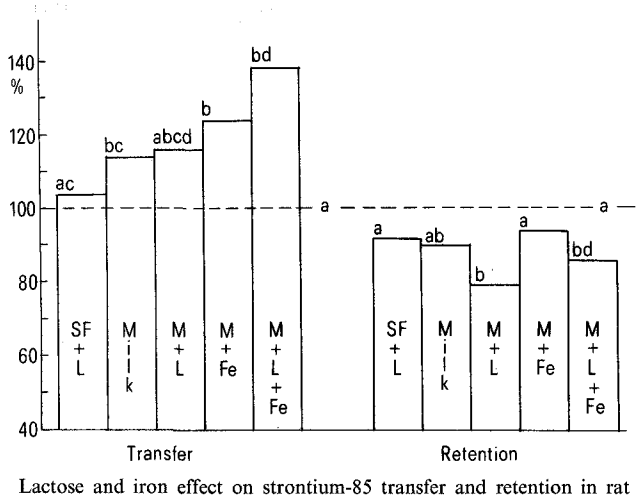
treatment, etc. Differences in these parameters could explain the inconsistency of the available literature data on the lactose effect[10–16].

Equally unexpected was the stimulatory effect of iron, either alone or (even more) in combination with lactose, on radiostrontium transfer. We confirmed that the low iron content of milk is the cause of its enhancing effect on transduodenal iron and manganese transport and their intestinal uptake[3,5]. Apparently, the iron-strontium interaction differs from the interrelationship of iron with some other ions[17–19].

The effects of experimental diets on strontium-85 retention in the intestinal wall were completely different. The retention was never enhanced, and with 2 diets it was significantly inhibited, by 14 and 23% (fig., right-hand part). This would imply either a) that there exists a diet-activated mechanism acting differently upon the transfer through and retention in the intestinal wall, or b) that the increased transfer is (partly) a concomitant result of a more efficient strontium release from the intestinal wall. Since a significant enhancement of strontium transfer does not always coincide with an equivalent inhibition of strontium retention (as, for instance, in the case of pure and iron-fortified milk) the assumption b) does not seem to be correct.

The fact that a 3-day, in vivo, pretreatment had a belated effect on the in vitro results suggests some (ir)reversible (possibly histobiochemical) changes in the intestinal mucosa provoked by the diets. Notwithstanding the caution necessary in extrapolating data from animal and in vitro experiments to humans, our results indicate that a certain carefulness should be exercised when fortifying cow's milk with iron.



Lactose and iron effect on strontium-85 transfer and retention in rat ileum. Data are expressed as percentages of the control (100% = standard food, SF). M, milk. L, 15% lactose; Fe, 10.3 mg Fe per 100 ml milk. [a,b,c,d]The results without a common superscript are significantly different ($p < 0.05$).

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Estrogens in insects

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Summary. Insects representing 5 different orders contain androgen and estrogen-like substances as determined by radioimmunoassay. Estradiol and estriol have been identified by gas chromatography-mass spectrometry. The presence of these steroids in insects suggests that the vertebrate sex hormones have an ancient evolutionary history.
Key words. Insect hormones; estradiol; estriol; evolution; sex hormones.

A basic tenet of insect physiology is that insects lack sex hormones[3]. Sex determination and differentiation are generally assumed to be strictly genetically based. There are, however, scattered indications in the literature of the existence of sex hormones[4,5]. Already over 50 years ago Loewe et al.[4] reported that extracts of a butterfly from Java (Attacus atlas) caused estrus in castrated mice.

Material and methods. As part of a project aimed at throwing additional light on the hormonal basis of insect reproduction we have tested various insects for the presence of estrogens and androgens. The insects were ground in 0.1 M sodium phosphate buffer (w:v; 1:4), pH 7.0 and then homogenized. The mixture obtained was centrifuged at 10,000 × g for 30 min; the supernatant was removed, the pellet was resuspended in buffer,

recentrifuged and the resulting supernatant combined with the first. The combined supernatants were then centrifuged at 105,000 × g for 1 h to provide the cytosolic preparation. The cytosol was extracted with methylene chloride which was dried and evaporated and the residue dissolved in 1.0 ml methylene chloride for radioimmunoassay[6]. The 17 β-estradiol RIA antisera cross-reacts 0.5% with estrone and 0.1% with estriol, while the testosterone RIA antisera cross-reacts 9.5% with 5α-dihydrotestosterone, 24.0% 19-nortestosterone, 6.0% 11α-hydroxytestosterone, 42.0% 11α-hydroxytestosterone, and $< 0.02\%$ 17β-estradiol. No cross-reactivity was observed with 20-hydroxyecdysone at concentrations ranging from 10 pg/ml to 10 ng/ml.

For identification of the steroids by mass spectrometry[7], 2400 7-day-old female flesh flies *(Sarcophaga bullata)* were processed in the same manner as described for the RIA. Further purification of the material was accomplished by thin layer chromatography on silica gel with hexane/ethyl acetate (1:1). The estrogen bands were scraped and eluted with methylene chloride. The final methylene chloride solution was evaporated under nitrogen and the residue dissolved in pyridine. The trimethylsilyl ether derivatives were prepared by addition of hexamethyldisilizane/trimethylchlorosilane (2:1) to the pyridine solution and heating to 50 °C for 16 h. The identification of estradiol and estriol in the samples was confirmed by the GC retention times of the derivatized estrogens and comparison of the MS spectra with those obtained from standard samples. Figures 1 and 2 represent comparisons of the mass spectra of trimethylsilyl ether derivatives of estradiol, E_2-$(OTMS)_2$, and of estriol, E_3-$(OTMS)_3$, respectively, obtained from flesh flies with those published in the mass spectra collection of the U.S. Environmental Protection Agency-National Institutes of Health. As we have not yet identified androgens by GC-MS the term 'androgens' in this publication is meant to imply androgen-like substances identified by RIA alone.

Results and discussion. The flesh fly *Sarcophaga bullata,* which was examined most extensively contains both androgens and estrogens in all developmental stages tested (table 1). Highest activity for both steroids was observed in 3-day-old adult males. Androgen and estrogen levels were consistently higher in males than in females at each age of adult life. To test the possibility that the steroids may have originated from the food source, a 10-g sample of the larval food (beef liver) was also extracted and analyzed. No activity could be detected by RIA, thus implying that the androgens and estrogens are synthesized by the flies, presumably from steroid skeltons obtained from the food.

Androgens and estrogens appear to be widely distributed in insects. In addition to flies, 4 other species that were examined by RIA contain these steroids (table 2). Only in samples of the milkweed bug did we fail to detect androgens. Schildknecht has found that various steroids, including testosterone and estradiol, are present in high (µg range per insect) concentrations in adult dytiscid beetles where they serve a role in chemical defense[8]. In the present publication we report much lower concentrations (pg range per insect) indicating a possible different role for these constituents.

The identification of estrogens and androgens in several insect species does not necessarily imply that these compounds serve as sex hormones. If these steroids do play a role in insect reproduction, one should not presume that their function is necessarily identical to that in vertebrates. The presence of androgens and estrogens in both male and female insects at levels of

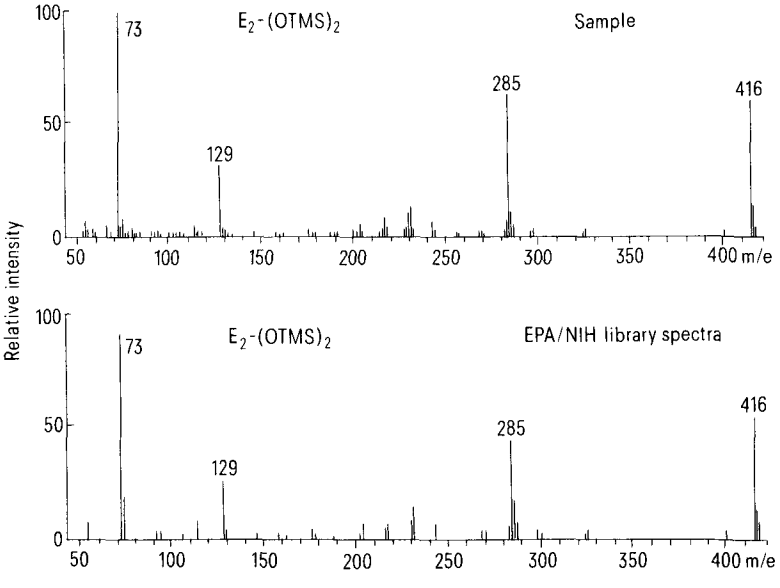

Figure 1. Comparison on the mass spectrum of di-trimethylsilyl ether of estradiol, E_2-$(OTMS)_2$, obtained from GC-MS of *Sarcophaga bullata* extracts with that published for the same compound in the U.S. Environmental Protection Agency-National Institute of Health Library Spectra Collection.

Table 1. Radioimmunoassay results on *Sarcophaga bullata* (flesh flies)*: Amount of steroids measured per insect

Stage	Sex	Number	Total weight (g)	Androgen (pg/insect)	Estrogen (pg/insect)
Third instar larva (feeding phase)	Mixed	50	5.41 (0.05)	16.67 (6.96)	45.13 (31.09)
Pupae	Mixed	50	4.20 (0.05)	69.33 (22.93)	24.73 (8.69)
Pharate adults (red eye stage)	Mixed	50	5.43 (0.01)	39.33 (19.64)	54.23 (19.17)
Adults, 3-day	Male	50	3.39 (0.27)	116.00 (22.54)	168.70 (27.37)
Adults, 3-day	Female	50	3.89 (0.19)	96.67 (40.17)	101.30 (23.68)
Adults, 7-day	Male	50	4.43 (0.03)	106.00 (37.17)	44.45 (11.34)
Adults, 7-day	Female	50	4.84 (0.04)	24.02 (10.07)	14.17 (2.07)
Adults, 10-day	Male	50	3.50 (0.30)	90.67 (8.51)	86.67 (26.62)
Adults, 10-day	Female	50	4.46 (0.34)	10.04 (0.57)	68.82 (22.09)

*The flesh flies were reared at 25 °C and were fed as larvae on beef liver and sugar as adults[11]. ≠ Mean (± SE), n = 3.

Table 2. RIA results for various insects: Amount of steroid measured per insect*

Insect	Stage	Sex	Number	Total weight (g)	Androgen (pg/insect)	Estrogen (pg/insect)
Periplaneta americana	Nymph	Male	15	9.93	153.3	30.9
(cockroach)	Nymph	Female	15	10.23	160.0	84.6
	Adult	Male	10	7.20	1000.0	86.0
	Adult	Female	10	10.23	100.0	33.0
Manduca sexta	Egg	Mixed	2895	3.39	3.5	1.5
(tobacco hornworm)	Larva	Female	1	4.70	10900.0	11250.0
	Pupae	Mixed	5	9.49	340.0	456.2
	Adult	Female	7	8.69	1128.6	657.7
	Adult	Male	6	7.88	1650.0	2729.8
Tenebrio molitor	Larva	Mixed	50	9.53	6.0	7.4
(mealworm)	Adult	Mixed	19	1.72	184.2	94.8
Oncopeltus fasciatus	Nymph	Mixed	100	3.64	0.0	6.3
(milkweed bug)	Adult	Male	75	2.89	0.0	9.4
	Adult	Female	75	3.72	0.0	2.5

* Insects were obtained from colonies maintained in the Department of Entomology at OSU. The cockroach *(Periplaneta americana)* colonies were maintained on dog biscuits (Milkborne, Nabisco Corp.), the tobacco hornworms *(Manduca sexta)* were fed on an artificial diet and milkweed bugs *(Oncopeltus fasciatus)* were fed milkweed seeds. All the above species were reared at 25 ± 1 °C.

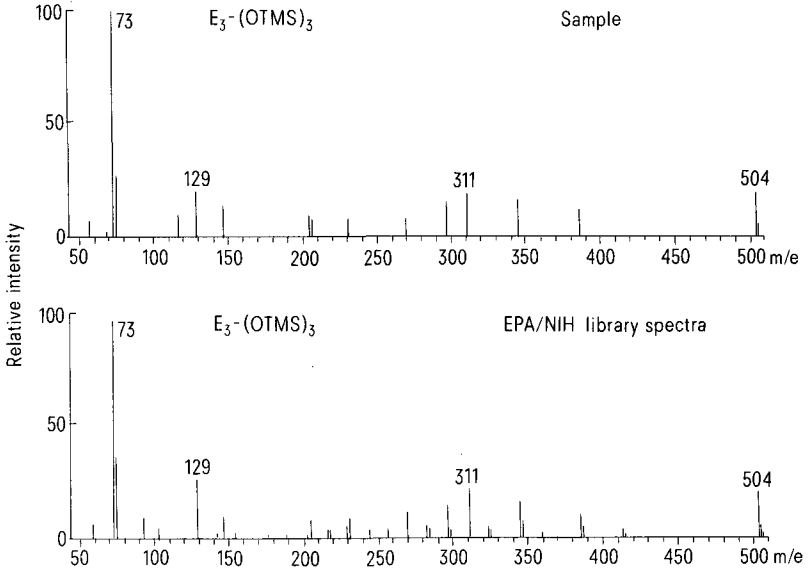

Figure 2. Comparison of the mass spectrum of tri-methylsilyl ether of estriol, E_3-(OTMS)$_3$, obtained from GC-MS of *Sarcophaga bullata* extracts with that published for the same compound in the U.S. Environmental Protection Agency-National Institute of Health Library Spectra Collection.

the same order of magnitude already indicates an important difference from the mammalian pattern. Ratios of androgens to estrogens may prove crucial, but since our present assays are specific for testosterone and estradiol, the overall amounts of closely related steroids are still unknown. Though the occurrence of these steroids is well documented for most vertebrate groups there are few reports suggesting the presence of androgens and estrogens among invertebrates[9]. Their discovery in insects indicates an ancient evolutionary history for the mammalian sex hormones.

The presence of androgens and estrogens in insects may provide a raison d'être for the wide distribution of steroidal, as well as non-steroidal, estrogenic and androgenic constituents of plants[10]. These secondary plant metabolites may offer a chemical defense against not only mammalian herbivores but also against an abundant array of plant feeding insects.

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