jugates (table 2) demonstrates that such conjugates are not or only weakly detected with our RIA.

These findings are very different from those in *Locusta migratoria* where the majority of ecdysteroids in the ovary are present in the form of *Helix*-hydrolyzable conjugates of ecdysone (50 µM) and of 2-deoxy-ecdysone (100 µM) bound to vitellin and outnumbering the corresponding free compounds 50 to 100-fold[9,13]. Only traces of ecdysteroids are observed in the hemolymph[14] and in this insect hydrolysis of the ecdysteroid conjugates in the eggs appears to be the source of the free ecdysteroids observed in early embryonic development[9]. In embryos of the cockroach *Nauphoeta cinerea* we have also observed a peak of 20-hydroxy-ecdysone before dorsal closure[12], when the prothoracic glands are not yet differentiated. For this species experiments using *Helix pomatia* enzymes reveal that the increase in 20-hydroxy-ecdysone before dorsal closure cannot be due to hydrolysis of *Helix*-hydrolyzable conjugates only[6,12]. We assume that ecdysteroid conjugates not susceptible to hydrolysis by *Helix* enzymes are formed in *Nauphoeta,* or that the free 20-hydroxy-ecdysone observed before dorsal closure does not originate from conjugate hydrolysis. On the basis of earlier investigations[6,7,12] and of the data shown here, we suppose for the cockroach *Nauphoeta cinerea* that the follicle epithelium of near-mature oocytes produces 20-hydroxy-ecdysone which plays a role in inducing chorion formation and which to some extent is released into the hemolymph, possibly to inactivate the corpora allata[7,8]. In addition part of the 20-hydroxy-ecdysone seems to enter the ooplasm in free form and as a conjugate of unknown identity, resistant to hydrolysis by *Helix pomatia* enzymes, which might give rise to the free 20-hydroxy-ecdysone observed before dorsal closure.

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2 To whom correspondence and reprint requests should be addressed.
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Somatomedin-C in zinc deficiency

Z.T. Cossack

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Summary. A significant decrease in the activity of somatomedin-C (SM-C) was observed due to zinc deficiency. SM-C activity correlated significantly with b.wt gain and with the status of zinc in rats. The present findings provide an insight into the mechanism by which zinc promotes growth and development.

Recently, it has become apparent that nutritional status may be an important factor that affects the activity of somatomedin. Low serum somatomedin with associated growth failure despite elevated growth hormone (GH) was first noted in children with Laron-type dwarfism[1]. Similar findings have been reported in children with marasmus[2] and kwashiorkertype malnutrition[3-4]. When these children were refed, somatomedin levels increased toward normal[5]. An inverse relationship between dietary fat and plasma somatomedin was observed in rats[6]. Moreover, the levels of somatomedin increased with increasing levels of protein[6-7] even though no change occurred in the levels (conc.) of GH. Increasing levels of protein in the diet may also mean additional micronutrients such as zinc. Poor growth in male villagers, subsisting on diet low in animal protein, has been related to zinc deficiency[8]. The present study was conducted to investigate the effect of zinc deficiency and supplementation on the activity of plasma somatomedin-C (SM-C) and to correlate these levels with the status of zinc in 4 different groups of rats: zinc deficient, pairfed control, continuously pairfed control, and ad lib-fed control group.

Materials and methods. White male rats of the Holtzman strain (Holtzman Company, Madison, Wis.) were housed individually in stainless steel cages. Initial body weight of rats ranged from 95 to 100 g. The rats were fed a standard diet (Purina rat chow, St. Louis, MO) for 4 days after which they were randomly allotted into 4 dietary treatment groups: zinc deficient (ZD), pair-fed (PF) control, continuously pair-fed (CPF) control, and ad lib-fed (ad lib) control. ZD group received (ad libitum) a semi-purified diet based on egg white solids[9] containing 1.2 ppm of zinc and 18% crude protein. The PF group received the basal diet which was supplemented with $ZnSO_4$ to provide 100 ppm of zinc. The amount of daily food given to the PF group matched the intake of their counterparts of the ZD group. The CPF group received the same amount of food daily from the zinc supplemented diet (100 ppm). The feeding pattern in this group simulated that of the ZD group. For this purpose, food was placed in a grooved auto-rotating feeders. A complete cycle of the feeder required 24 h. The ad lib-fed group received the zinc supplemented diet ad libitum. Distilled deionized water was offered ad libitum. Daily food intake and

body weight were recorded throughout the experimental period which lasted 14 days after which the rats were killed by decapitation. Rats were mildly anesthesized with ether and blood was drawn by heart puncture. The blood was collected in plastic tubes free of zinc, immediately centrifuged (2000 × g) for 10 min and plasma was separated and kept at −60 °C. Tibia and liver were treated as previously described[19] for the determination of zinc. Zinc in plasma was determined according to an established procedure[11]. The activity of SM-C was assayed using the radioimmunoassay (RIA) of Furlanetto and co-workers[12]. Data were analyzed using Student's t-test[13].

Results and discussion. The growth rate of the ZD rats decreased significantly compared to PF ($p < 0.001$); CPF ($p < 0.001$) and ad lib-fed group ($p < 0.0000$). In addition, the levels of zinc in plasma, liver and tibia decreased significantly in ZD rats compared to the other experimental groups (table I). This is consistent with previous findings in zinc deficient rats[9–10]. The activity of SM-C followed the same trend as b.wt gain and the levels of zinc in tissues when all experimental groups were compared. The activity of SM-C was found to correlate highly with b.wt gain ($r = 0.96$, $p < 0.001$) as shown in figure 1. This is in agreement with what was reported in growing rats[7]. Moreover, a high correlation was observed between SM-C activity and zinc concentration in the tibia ($r = 0.79$, $p < 0.005$) (fig. 2). Zinc level in the tibia is known to be a good indicator of zinc status in rats[14]. The decrease in SM-C activity in ZD rats is consistent with previous report by van den Brande and co-workers[15] in malnourished children. Similar findings, using rats, are in support of the present data[6,7,16]. Growth retardation is a prominent feature of zinc deficiency observed in man and animals. Poor growth in male villagers, subsisting on diet low in animal protein, has been related to zinc deficiency[8]. Cheruvanski and co-workers[17] showed that zinc administration to zinc deficient children increased their growth rate and zinc administration significantly increased the growth response of GH therapy in these children. On the other hand, Root et al.[18], indicated that the association of zinc deficiency and low serum levels of GH suggests that the depressed growth may be due, in part, to GH deficiency. However, GH did not increase weight gain in intact zinc deficient rats and zinc supplementation restored the growth of hypophysectomized zinc deficient rats[19] indicating that zinc may play an independent role in promoting growth. Further studies are needed to elucidate the zinc and GH interrelationship. The present data showed that the activity of SM-C was depressed due to dietary zinc deficiency. Somatomedins are known of their capacity to stimulate thymidine incorporation into cultures of human glial cells and fibroglasts[20]. Moreover, thymidine kinase has been shown to be a zinc dependent enzyme[21]. Thus, the effect of zinc is synergistic with somatomedin-C and zinc could be a limiting factor in promoting growth and development.

In conclusion, the present study has demonstrated the effect of dietary zinc on the level of SM-C in plasma. Growth retardation associated with zinc deficiency in children could be related to the adverse effect of zinc deficiency on the generation of SM-C, which can be reversed by zinc supplementation.

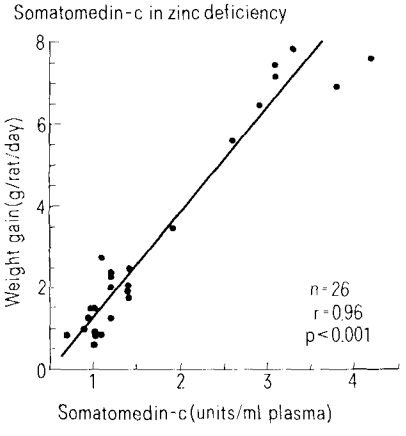

Figure 1. The correlation between the activity of SM-C and body weight gain.

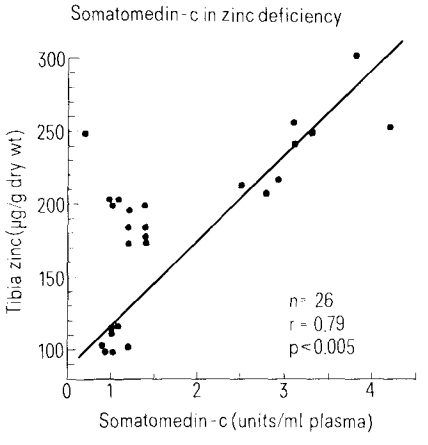

Figure 2. The correlation between the activity of SM-C and the level of zinc in the tibia.

Food intake, b.wt gain, zinc in plasma, liver and tibia and plasma somatomedin-C (mean ± SD)*

	Zinc deficient	Pair-fed control	Continuous pair-fed control	Ad-libitum fed control
Food intake (g/rat/day)	8.36 ± 1.70	8.36 ± 2.0	8.36 ± 0.00	16.37 ± 2.18
Body weight gain (g/rat/day)	0.92 ± 0.36[a]	2.27 ± 0.53[b]	1.95 ± 0.60[b]	7.45 ± 0.60[c]
Zinc in:				
Plasma (µg/100 ml)	65.67 ± 13.05[a]	193.60 ± 29.90[b]	188.8 ± 37.70[b]	180.30 ± 12.50[b]
Liver (µg/g dry wt)	98.46 ± 7.63[a]	140.20 ± 17.50[b]	136.40 ± 10.40[b]	139.68 ± 7.90[b]
Tibia (µg/g dry wt)	106.01 ± 6.99[a]	197.70 ± 5.50[b]	200.50 ± 3.14[b]	258.52 ± 24.04[c]
Somatomedin-C (unit/ml plasma)	1.03 ± 0.09[a]	1.40 ± 0.12[b]	1.29 ± 0.15[b]	3.40 ± 0.43[c]

* Means not having common superscripts are significantly different at 0.005 level of significance.

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Do luteinized unruptured follicles secrete progesterone in mature female rats?[1]

S. Plas-Roser, M.T. Kauffmann and C. Aron[2]

Institut d'Histologie, Faculté de Médecine, Université Louis Pasteur, F-67000 Strasbourg (France), 17 January 1983

Summary. Unruptured luteinized follicles were shown to secrete less progesterone than did postovulatory corpora lutea in cyclic female rats.

It is now well established that the antiinflammatory agent indomethacin can inhibit ovulation by interfering with the synthesis of prostaglandins in preovulatory follicles[3-6], thus causing the formation of luteinized unruptured follicles (LUF) in hCG-treated mature rabbits[7] and in prepubertal gilts injected with PMSG and hCG[8]. Moreover, Ainsworth et al.[8] reported that LUF, formed in the gilt at the expense of preovulatory ovarian follicles, which had failed to rupture under indomethacin treatment, could secrete physiological amounts of progesterone. Previous work in our laboratory which showed that LH was capable, when injected on the day of dioestrus 2, of advancing ovulation by 24 h in 4-day cyclic female rats[9-11], encouraged us to determine whether indomethacin could prevent ovulation from occurring in cyclic female rats early exposed to the ovulatory action of LH. Pilot experiments justified this hypothesis indicating that combined LH and indomethacin treatment resulted in the formation of LUF. Experiments whose results are reported here were then carried out to study whether LUF displayed luteal activity in the rat.

Material and methods. 3-4-month-old virgin female rats bred in our colony (strain WI) were used. The animals were housed in a light- (lights on 23.00-13.00 h) and temperature- (22-24 °C) controlled room and given free access to food pellets and water. Following transfer from natural lighting conditions, the females were allowed a month to adapt to their new environment. Only those which had experienced 2 or 3 4-day cycles prior to experiments were used. Cycles consisted of dioestrus 1 and 2, prooestrus and oestrus.

The females were allocated to 2 groups. The first one served for the evaluation of the function of corpora lutea induced by LH on dioestrus 2 at 10.30 h. This corresponded to the clock-time (16.00-17.00 h) under natural lighting at which LH was either injected on dioestrus 2 in our preceding experiments or spontaneously released on proestrus in the WI strain of rats bred in our colony[12-14]. All the females were given a dose of 7.1 µg/100 g b.wt s.c. of LH M4 (LH M4 = 2.25 X NIH-LH-S3). Some of them received 5 mg/100 g b.wt i.v. of indomethacin by 6 h 30 min after LH administration. The 2nd group consisted of uninjected females which were used for the study of corpora lutea spontaneously developed during 4-day cycles. In all cases the females were killed by decapitation at 11.00 h, either on the expected day of estrus, in the LH-treated females or, on dioestrus 1, that is at the time of the highest activity of the corpora lutea[15], in the females running natural cycles. The ovaries were removed for histological examination. Postovulatory corpora lutea (POCL) and LUF were counted in each female. An ovulation coefficient (OC) was computed by dividing the number of POCL by the whole number of POCL and LUF. The mean number of POCL and LUF and a mean OC was calculated in each experimental procedure. Blood was collected for the determination of progesterone using a previously described RIA[16]. The data were analyzed by one-way analysis of variance and Scheffe's test. Progesterone values were studied following logarithmic transformation.

Results and discussion. As shown in the table the total number of corpora lutea comprising of both POCL and LUF did not differ in the rats with a natural cycle and in the LH-treated females ($F^2_{31} = 0.46$; NS). The number of LUF per animal appeared to be greater in LH + indomethacin-treated females than in those receiving LH only ($p < 0.05$). This was confirmed by comparing OC in both groups of animals: OC value was significantly lower in the former than in the latter ($p < 0.05$). Regarding the corpus luteum's function by 48 h after either LH release in natural cyclers or LH injection in treated animals we observed that blood progesterone concentration differed in the 3 groups of animals ($F^2_{31} = 17.78$; $p < 0.001$), with the highest value in those with a natural cycle and the lowest value in LH + indomethacin-treated animals as compared to LH-treated females ($p < 0.05$).

These data first confirm previous findings[17,18], showing that the pool of follicles available for ovulation on the day of prooestrus is present in the ovary as soon as the morning of dioestrus 2. It is probably the reason why the number of follicles sensitive to LH treatment on dioestrus 2 appeared to be statistically comparable to that of follicles able to ovulate under physiological circumstances. The main point which emerges from our observations concerns the ability of indomethacin to prevent ovarian follicles from rupturing on early exposure to the ovulatory action of LH, thus causing the formation of LUF. This is in agreement with the above mentioned observations in the rabbit[7] and in the gilt[8]. However LUF were shown to secrete less progesterone in the rat than did LH-induced and spontaneously formed POCL, by contrast with the gilt[8] in which LUF developed normal progesterone secretory activity. Indeed we chose to study LUF's function at a stage expected to correspond with the time of the highest activity of POCL in animals with a natural cycle. New experiments are then needed to establish the pattern of the functions of LUF at various times after their formation.