

$\beta$ -cells from inhibition by catecholamine inactivation. It is worth mentioning that epinephrine elicited a significant hyperglycemic response in *B. conchonus*  $\frac{1}{2}$ –1 h post injection (blood glucose:  $83.5 \pm 3.6$  mg% control;  $193.8 \pm 17.6$  mg% and  $223.5 \pm 2.4$  mg% after  $\frac{1}{2}$  and 1 h, respectively)<sup>24</sup>, which might have triggered an enhanced insulin secretion. Collateral histological examination of the pancreatic islets in the fish<sup>24</sup> revealed degranulation and vacuolation in the  $\beta$ -cells suggesting an enhanced secretory activity.

Compared to the warm blooded animals, bony fishes, like other poikilothermic animals, seem to have a low sensitivity to the catecholamines, which may be related to a lower metabolic rate. In addition, a slow degradation of the administered amine in these animals cannot be overlooked. These considerations, together with the i.m. route of administration and consequently the slower distribution of the amine, might be responsible for a delayed action of epinephrine in *B. conchonus*.

- 1 N.K. thanks the U.G.C. for the award of a research fellowship.
- 2 Lundholm, L., Mohme-Lundholm, E., and Svedmyr, N., Biological basis of medicine. Academic Press, New York 1968.
- 3 Farkas, T., Biochim. biophys. Acta 4 (1969) 237.
- 4 Larsson, A., Gen. comp. Endocr. 20 (1973) 155.
- 5 Lipshaw, L. A., Patent, G. J., and Foa, P. P., Hormone Metab. Res. 4 (1972) 34.
- 6 Zlatkis, A., Zak, B., and Boyle, A. J., J. Lab. clin. Med. 41 (1953) 486.
- 7 Novak, B., J. Lipid Res. 6 (1965) 431.

- 8 Zilversmit, D. B., and Davis, A. K., J. Lab. clin. Med. 35 (1950) 155.
- 9 Oser, B. L., Hawk's Physiological Chemistry. McGraw Hill, New York 1965.
- 10 Rosenthal, H. L., Pfluke, M. L., and Buscaglia, S., J. Lab. clin. Med. 50 (1960) 318.
- 11 Thorpe, A., and Ince, B. W., Gen. comp. Endocr. 23 (1974) 29.
- 12 Riggs, T. R., Biochemical action of hormones. Academic Press, New York 1970.
- 13 Bilinski, E., and Lau, Y. C., J. Fish. Res. Bd Can. 26 (1969) 1857.
- 14 Bilinski, E., Fish in Research. Academic Press, New York 1969.
- 15 Farkas, T., Prog. Biochem. Pharmac. 3 (1967) 314.
- 16 Ince, B. W., and Thorpe, A., Gen. comp. Endocr. 27 (1975) 144.
- 17 Leibson, L. G., Plisetskaya, E. M., and Mazina, T. I., Zh. Evol. biokhim. Fiziol. 4 (1968) 121.
- 18 Genes, S. G., Usp. Fiz. Nauk. 6 (1975) 92.
- 19 Gerich, J. E., Charles, M. A., and Grodsky, G. M., A. Rev. Physiol. 38 (1976) 353.
- 20 Plisetskaya, E. M., Leibush, B. N., and Bondareva, V., The evolution of pancreatic islets. Pergamon Press, Oxford 1976.
- 21 Ince, B. W., and Thorpe, A., Gen. comp. Endocr. 33 (1977) 453.
- 22 Porte, D. Jr, Graber, A. L., Kuszuya, T., and Williams, R. H., J. clin. Invest. 45 (1966) 228.
- 23 Minick, M. C., and Chavin, W., Comp. Biochem. Physiol. 44 (1973) 1003.
- 24 Khanna, N., Studies on the carbohydrate, protein and lipid metabolism in a freshwater teleost, *Barbus conchonus*. Ph. D. Thesis, Kumaun University, Naini Tal 1983.

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## The effect of lactose and iron on strontium absorption<sup>1</sup>

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**Summary.** In rats fed on a milk diet with or without the addition of lactose and/or iron the transileal strontium-85 transfer was higher by 14–38% and the intestinal strontium retention lower by 6–23% than in control rats fed on standard laboratory food.  
**Key words.** Rat ileum; milk diet; strontium absorption; lactose, dietary; iron, dietary.

The stimulatory effect of a milk diet on the absorption of some ions from the intestinal tract<sup>2–6</sup> could be explained by the high lactose and low iron content of milk. Bearing in mind the role of radiostromium in internal contamination, we studied the effect of lactose and iron on strontium absorption from the rat's ileum.

**Materials and methods.** Strontium transport was determined on ileal segments taken from female 5-week-old rats by the in vitro method of the 'everted intestinal sac'<sup>7</sup>. There were altogether 60 animals in the experiment and two, 4 cm long, ileal segments were cut out from each rat. Before the experiment all the animals were on a standard diet with 1.2% calcium and 0.8% phosphorus. They were divided into 6 equal groups according to the diet they were fed on for 3 consecutive days: 1. Standard laboratory food (SF) + drinking water (control); 2. SF + 15 g lactose (L) added to 100 ml of drinking water; 3. milk (M) (pasteurized cow's milk containing 140 mg Ca and 95 mg P/100 ml); 4. M + L; 5. M + Fe (10 mg Fe was added – as FeSO<sub>4</sub>·7 H<sub>2</sub>O – to 100 ml of milk); 6. M + L + Fe. On the 4th day all animals were killed by decapitation and ileal everted sacs prepared by the experimental procedure described before<sup>7</sup>. The composition of the medium was as follows (mM/l): 135 NaCl, 11 KCl, 0.05 SrCl<sub>2</sub>, and 10 mM sodium phosphate buffer, pH 7.4. Strontium-54 (Radiochemical Centre, Amersham, England) in an almost carrier-free form was added

to the mucosal solution in the form of chloride. The activity was adjusted to about 300 KBq strontium-85 per 100 ml of the solution. After a 45-min incubation of the samples, the amount of radiostromium retained in the solution inside (S) and outside (M) the ileal sac and in the intestinal wall was determined in an automatic well-type scintillation counter (Nuclear Chicago, USA).

**Results and discussion.** The results were calculated as S/M (serosal over mucosal) activity ratios for strontium transport, and as percentages of the initial mucosal solution activity for its intestinal retention. To make comparison between the groups easier the results for experimental groups are presented in the figure, as percentages of the control values. In all groups fed on the experimental diets for 3 days, the mean values for strontium transfer were higher than in the stock diet-fed controls (fig., left part). They were significantly higher (by 14, 24 and 38%) for the following diets: milk, milk + Fe and milk + L + Fe. The addition of lactose alone to either food or milk did not produce significant changes. This is rather surprising since, according to Armbricht and Wasserman<sup>9</sup> a dose of lactose even 2.5 times lower than ours increased the permeability (for calcium) of the absorptive intestinal cell by over 60%. This difference may be attributed to alteration of several items of the experimental set-up, such as animal species, segment of the intestine studied, dose of lactose, duration of pre-

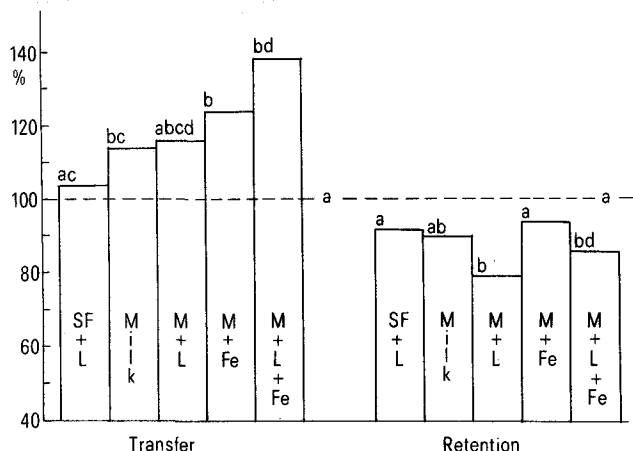
treatment, etc. Differences in these parameters could explain the inconsistency of the available literature data on the lactose effect<sup>10-16</sup>.

Equally unexpected was the stimulatory effect of iron, either alone or (even more) in combination with lactose, on radio-strontium 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<sup>3,5</sup>. Apparently, the iron-strontium interaction differs from the inter-relationship of iron with some other ions<sup>17-19</sup>.

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. <sup>a,b,c,d</sup>The results without a common superscript are significantly different ( $p < 0.05$ ).

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- Lengemann, F.W., Comar, C.L., and Wasserman, R.H., *J. Nutr.* 61 (1957) 571.
- Gruden, N., *Nutr. Rep. int.* 14 (1976) 515.
- Hafer, Y.S., and Kratzer, F.H., *Poultry Sci.* 55 (1976) 918.
- Gruden, N., *Nutr. Rep. int.* 19 (1979) 69.
- Bell, R.R., and Spickett, J.T., *Fd Cosmet. Toxic.* 19 (1981) 429.
- Wilson, T.H., and Wiseman, G., *J. Physiol., Lond.* 123 (1954) 116.
- Gruden, N., *Toxicology* 5 (1975) 163.
- Armbrecht, H.J., and Wasserman, R.H., *J. Nutr.* 106 (1976) 1265.
- Pansu, D., Bellaton, C., and Bronner, F., *J. Nutr.* 109 (1979) 508.
- King, B.D., Lassiter, J.W., Neathery, N.W., Miller, W.J., and Gentry, R.P., *J. Anim. Sci.* 50 (1980) 452.
- Anonymous, *Nutr. Rev.* 40 (1982) 116.
- Flanagan, P.R., Chamberlain, M.J., and Valberg, L.S., *Am. J. clin. Nutr.* 36 (1982) 823.
- Bushnell, P.J., and DeLuca, H.F., *J. Nutr.* 113 (1983) 365.
- Cochet, B., Jung, A., Griessen, M., Bartholdi, P., Schaller, P., and Donath, A., *Gastroenterology* 84 (1983) 935.
- Andrieux, C., and Sacquet, E., *Reprod. Nutr. Dévelop.* 23 (1983) 259.
- Ragan, H.J., *Proc. Soc. exp. Biol. Med.* 150 (1975) 36.
- Flanagan, P.R., Haist, J., and Valberg, L.S., *J. Nutr.* 110 (1980) 1754.
- Nielsen, F.H., and Shuler, T.R., *Biol. Trace elem. Res.* 3 (1981) 245.

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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 radio-immunoassay. Estradiol and estril 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; estril; evolution; sex hormones.

A basic tenet of insect physiology is that insects lack sex hormones<sup>3</sup>. 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<sup>4,5</sup>. Already over 50 years ago Loewe et al.<sup>4</sup> 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  $10,000 \times g$  for 30 min; the supernatant was removed, the pellet was resuspended in buffer,