Effects of oxytocin, peptides Ib and II, and of insulin on the formation of $^{14}CO_2$ and lipogenesis from [1-^{14}C] glucose by rat epididymal adipose tissue in vitro

Substance added (concentration)	No. of experimente	Radioactivity[a] CO_2[b]	Incorporated into total lipids[b]
None (control)	13	9.23 (5.94–17.4)	15.5 (7.17–22.4)
Oxytocin (0.66 µg/ml)	6	23.6[d] (12.3–30.9)	45.6[c] (7.82–61.2)
Peptide Ib (0.66 µg/ml)	5	32.7[d] (22.6–38.7)	40.5[d] (26.5–56.7)
Peptide II (1.5 µg/ml)	6	10.9 (6.23–24.5)	20.9 (8.99–38.3)
Peptide II (15 µg/ml)	5	45.4[d] (37.3–51.3)	66.5[d] (43.4–80.8)
Insulin (1 mU/ml)	5	28.1[d] (27.9–34.1)	49.9[d] (37.6–55.8)

[a] Thousands of cpm/100 mg tissue. [b] Top: median, below (in parentheses): minimum and maximum values. Statistical significance of the differences from the control values determined by the Mann-Whitney test[19]. [c] $P = 0.02$, [d] $P < 0.002$.

absorbed during 30 min. Total lipids were extracted with chloroform-methanol (2:1, v/v) and the extract was washed as described by FOLCH et al.[18]. The radioactivity of samples dissolved in toluene scintillation fluid (SLT 31, Tesla, Czechoslovakia) was measured with a scintillation spectrometer (Tracerlab, Chicago, USA). Synthetic oxytocin (purified by countercurrent distribution) and the peptides Ib and II were available from earlier work; crystalline insulin (Novo, Denmark) was used for comparison.

The results in the Table confirm the insulin-like effect of oxytocin on rat epididymal adipose tissue in vitro. Moreover, the thioether analogue of oxytocin (Ib) is seen to be about as active as oxytocin in enhancing carbon dioxide formation and lipogenesis from glucose by this preparation. The peptide II also shows this insulin-like effect but its potency (per mole) is only about $^1/_{10}$ of that of oxytocin.

It can be concluded that, like other biological effects of oxytocin, its 'insulin-like' action on adipose tissue does not functionally involve the disulphide bond but is associated with more general features of its molecular architecture. A closer structural approach to the disulphide-bridged region of the insulin chain A as in II does not enhance, but rather decreases the 'insulin-like' action on the epididymal fat pad. This finding raises further doubts about the possibility that the 'insulin-like' effects of oxytocin are due to its structural resemblance to a part of the insulin molecule. On the other hand, the correlation between the 'insulin-like' effect and the oxytocin activity of peptides related to the neurohypophysial hormones[3] also breaks down for peptide II since it possesses no detectable uterotonic activity[20].

Zusammenfassung. Es wird mittels synthetischer Peptide (Ib, II) bewiesen, dass eine Disulphidgruppe für die «insulinähnliche» Wirkung von mit dem Oxytocin verwandten Peptiden am Fettgewebe der Rattenepididymis nicht notwendig ist. Somit wird ein von Disulphidaustausch abhängiger Wirkungsmechanismus ausgeschlossen.

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[18] J. FOLCH, M. LEES and G. H. STANLEY, J. biol. Chem. *226*, 497 (1957).
[19] S. SIEGELS, *Nonparametric Statistics for the Behavioral Sciences* McGraw-Hill Book Co., Inc., New York 1956).
[20] Determined by Dr. I. KREJČÍ, Research Institute for Pharmacy and Biochemistry, Prague.

Inhibition of Large Distal Tooth Formation in Male Medaka, *Oryzias latipes*, by Estradiol

In the male medaka, a tooth carp (*Oryzias latipes*), large distal teeth on the maxillae and mandible appear when the medaka is young, at the stage when total body length is about 22 mm. The number of large teeth gradually increases to about 6 in each jaw by the time total body length reaches 30 mm[1]. The females have no large distal teeth during the growth stage at 22–30 mm total body length. However, the large distal teeth were formed in young female medakas by giving male sex hormone[2]. The present study deals with an inhibitory action of female sex hormone, estradiol, on the formation of the large teeth in the young male.

Forty young male medakas (15–17 mm), bred in the laboratory at Nagoya, were allotted to 4 equal groups. Group 1 (controls) were given a standard diet[3] which contains shrimp powder, toasted whole barley flour, yeast and green tea; groups 2, 3 and 4 were given 10, 50 and 250 µg of estradiol-17β[4] per g of standard diet, respectively, for 3 months (from 15 June to 15 September). Total body length at the end of that time was 25–29 mm. For observation of the teeth, jaws of the medakas were treated with 2% NaOH for several h, stained by 0.1% alizarin S, and preserved in glycerine.

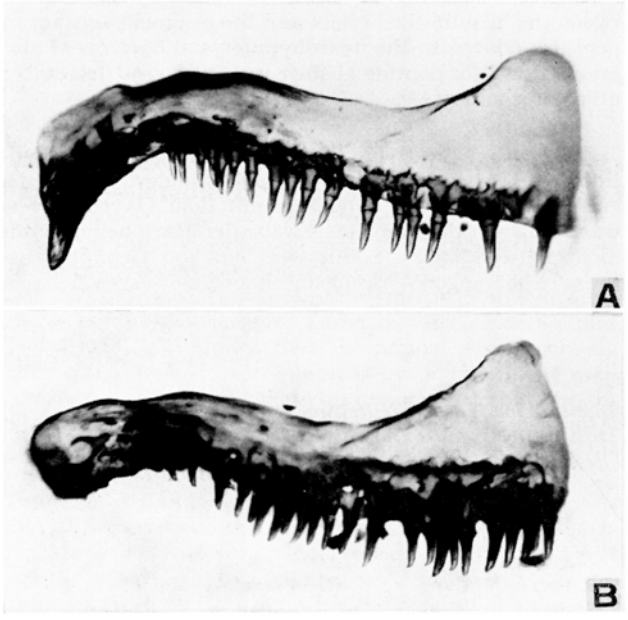
No large teeth were formed in group 3 (Table and Figure B), whereas all male fish in the control group had 2–7 large distal teeth (Figure A). The papillar process on the male anal fin, the most prominent secondary sexual characteristic, disappeared in fish of group 3. In group 2, 3 fish had the female anal fin type and no large distal teeth, while the other 7 fish has 1–5 large distal teeth, indicating that 10 µg estradiol/g of standard diet was not enough to inhibit the formation of the large teeth in male medakas. The disappearance of papillar processes in the anal fin parallels the inhibition of large teeth formation. The fish in group 4 did not grow well and some died, suggesting that the dosage of estradiol was too high.

Estradiol has a female-inducing action on medaka fry and 100% sex-reversal in genetic males can be obtained at a dosage level of 10 µg/g diet[5]. In the present experi-

[1] K. TAKEUCHI, Zool. Mag., Tokyo *75*, 236 (1966).
[2] K. TAKEUCHI, J. dent. Res. *46*, 750 (1967).
[3] T. YAMAMOTO, J. exp. Zool. *137*, 227 (1958).
[4] Estradiol-17β was obtained from Sigma Chemical Company.
[5] T. YAMAMOTO and N. MATSUDA, Gen. Comp. Endocr. *3*, 101 (1963).

Large distal tooth formation in male medaka

Group	Diet	No.	Total body length (mm)		Anal fin type			No. of large teeth			
					♂	♀	♀	Maxillae		Mandible	
			Mean	(Range)				Mean	(Range)	Mean	(Range)
(1)	Standard diet	10	26.9	(25–29)	10	0	0	2.3	(2–4)	1.7	(0–4)
(2)	10 μg estradiol/g standard diet	10	26.8	(25–28)	5	3	2	1.2	(0–3)	1.3	(0–4)
(3)	50 μg estradiol/g standard diet	10	25.8	(25–27)	0	10	0	0	(0)	0	(0)



Teeth on right maxillary bone of 25 mm male medaka fed with standard diet (A) and 50 μg of estradiol/g of standard diet (B). Large distal tooth is seen at left end of the bone in (A).

ment, 50 μg of estradiol/g of diet was necessary to suppress the manifestation of male sex characteristics. This may be interpreted as an antagonism between exogenous estrogen and endogenous male sex hormone liberated from the testis.

In the normal male medaka, large teeth begin to develop when total body length is about 20 mm, erupt from oral epithelium at 22 mm and formation is complete at 23 mm. Body length of the medakas was 15–17 mm at the beginning of the experiment, a stage well in advance of the development of large teeth.

Estradiol had no effect on the shape of the small teeth on the main part of the maxillae as shown in the Figure[6].

Zusammenfassung. Junge Männchen von *Oryzias latipes* haben im Ober- und Unterkiefer grosse Eckzähne, die den weiblichen Tieren fehlen. Verfütterung von 50 μg Östradiol/g Normalfutter hemmt die Bildung dieser Zähne völlig, 10 μg nur unvollständig.

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Chromosome Numbers in Five Species of Pentatomidae Reut (Hemiptera - Heteroptera)

The family status of the Plataspidae, Acanthosomatidae, Cydnidae and Scutelleridae have been recognized by SOUTHWOOD and LESTON, and also KERZNER and JACZEWSKI[1]. However, following KIRKALDY[2], several authors, such as STICHEL and WAGNER[3], put the last-mentioned family in the Scutellerinae of the Pentatomidae. Based on cytological findings all these families are characterized by having 12 chromosomes with the usual $XX:XY$ determination and caryotype comparable with that of the pentatomid bugs. Their chromosome number is distinct from the pentatomid type which may be characterized by the presence of 14 chromosomes.

The present paper deals with the chromosomes of some Pentatomoidea, excluding the Pentatomidae.

Materials and methods. The materials used were as follows: Plataspidae: *Coptosoma scutellatum* Geoffr., Acanthosomatidae: *Elasmucha grisea* L., Cydnidae: *Legnotus picipes* Fall., Scutelleridae: *Phimodera lapponica* Zett. and *Eurygaster testudinaria* Geoffr. They were collected in Lublin and Olsztyn provinces.

Aceto-orcein testes squashes were made and figures have been drawn with the aid of camera lucida. × 2400.

Observations. (1) *Coptosoma scutellatum* Geoffr. This species, like some others belonging to Plataspidae[4], has 12 chromosomes. In the spermatogonial metaphase (Figure 1), 2 *M*-chromosomes could be distinguished from the remaining ones. They are distinctly larger than the 8, nearly of the same size, autosomes and the *X* and *Y* com-

[1] T. R. E. SOUTHWOOD and D. LESTON, *Land and Water Bugs of the British Isles* (F. Warne and Co. Ltd., London and New York 1959). – I. M. KERZNER and T. L. JACZEWSKI, in *A Key to the Insects of European Part of Soviet Union* (Nauka Press, Moskow, Leningrad 1964), vol. 1.

[2] G. W. KIRKALDY, *Catalogue of the Hemiptera (Heteroptera)* (F. L. Dames, Berlin 1909), vol. 1.

[3] W. STICHEL, *Illustrierte Bestimmungstabellen der Wanzen. II Europa* (Hermsdorf, Berlin 1955–1962). – E. WAGNER, in *Die Tierwelt Mitteleuropas* (Quelle Meyer, Leipzig 1961), vol. 4.

[4] S. MAKINO, *An Atlas of the Chromosome Number in Animals* (Iowa State College Press, Ames 1951). – G. K. MANNA, Proc. Int. Congr. Ent. *2*, 919 (1958).