these animals is not as developed or specialized as in the mammal.

It is also worthy of note that, while in animals treated with 3 antigen doses, the number of antibodies circulating becomes greater than in those treated with 2 doses, the ratio between the various types of antibody producing cells does not change.

Research is in progress to establish the percentage of antibody producing cells out of the total number of cells extracted from the spleen, and whether this percentage varies with the antigen dose. Moreover, by using other pairs of antigens (red cells of newts and lizards, of lampreys and trout etc.), it will be interesting to see if the mixed ‚rosette' percentage varies.

Riassunto. Si è studiata la natura delle cellule immunologicamente competenti della milza di *X. laevis* Daud. Mediante la tecnica della immuno-cito-aderenza, si è potuto constatare che tali cellule estratte da milze di animali immunizzati con globuli rossi di pollo e coniglio, rispondono ad uno solo o ad ambedue gli antigeni, che la percentuale delle cellule che reagiscono sia contro le emazie di coniglio che di pollo è di molto superiore a quella dei mammiferi e che tale percentuale non varia al variare delle dosi di antigene somministrato.

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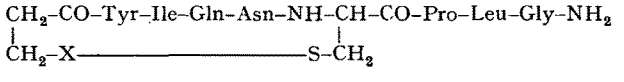
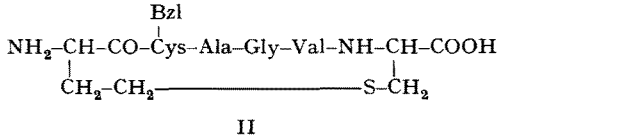
'Insulin-Like' Action of an Oxytocin Analogue Lacking a Disulphide Group, and of a Cystathionine Peptide Related to a Sequence of Insulin, on Rat Epididymal Adipose Tissue in vitro

Like insulin, oxytocin and some synthetic analogues of the neurohypophysial hormones stimulate glucose uptake[1], glucose oxidation[2–7], lipid[2–5] and protein[8] synthesis by rat epididymal adipose tissue in vitro. MIRSKY and PERISUTTI[4] have shown that the action of oxytocin on adipose tissue in vitro is inhibited by pretreatment with sulphydryl-blocking reagents and concluded that the disulphide bond is a necessary (though not a sufficient[3]) requirement for 'insulin-like' action on this tissue[4]. This was in line with earlier conclusions about the functional role of the disulphide bond in the action of neurohypophysial hormones on the amphibian bladder and mammalian kidney[9]. However, it has recently been shown[10–13] that a synthetic oxytocin analogue[14] Ib isosteric with deamino-oxytocin[15] (Ia), but with the disulphide group replaced by a methylene thioether grouping, shows the typical effects of oxytocin on the uterus[10,12], mammary gland[12], vascular smooth muscle[12], mammalian kidney[11–13] and amphibian membranes[11,13]. We have now used this analogue to examine the functional significance of the disulphide bond for the action of oxytocin on rat epididymal adipose tissue.

A structural feature shared by the insulins and by oxytocin is the presence of a hexapeptide sequence bridged by a disulphide bond, and it has been suggested[3,6] that their qualitatively similar effects on adipose tissue may be associated with this particular feature. We have therefore extended our study to a cystathionine peptide[16], II, related to the disulphide-bridged sequence A (6–11) of ovine insulin.



Ia X = S Ib, X = CH_2



II

The 'insulin-like' activity of the peptides was evaluated from their ability to stimulate $^{14}CO_2$ formation and ^{14}C incorporation into total lipids from [1-^{14}C] glucose by rat epididymal tissue pieces in vitro. The tissue was taken from male Wistar rats (100–120 g body weight) after an overnight fast. Tissue samples (80–100 mg) were incubated in 5 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 12.5 μmoles of unlabelled glucose, 1 μCi of [1-^{14}C] glucose (Radiochemical Centre, Amersham, England), and 10 mg of bovine serum albumin (Fraction V, Armour, Chicago, USA) at 37 °C in a Dubnoff-type metabolic shaker for 2 h. The buffer was equilibrated with 95% O_2 and 5% CO_2. At the end of the incubation period, 0.5 ml of 50% aqueous ethanolamine[17] was injected through the stopper into the central compartment and 0.5 ml 2.5 *N* H_2SO_4 into the incubation medium in the main compartment of the incubation vessel. The $^{14}CO_2$ evolved was

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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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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-

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