JH-I and JH-II in male accessory sex glands (ASG) and in the bursa copulatrix (BC) after copulation. Both juvenile hormones were labelled biosynthetically in the male before copulation

Mating Pair	Organ preparation Type of organ	Time after copulation (days)	JH-I (dpm)	JH-II (dpm)
1	ASG	Copulation interrupted	nil[a]	nil[a]
	BC	Copulation interrupted	9600	1600
2	ASG	0 days	2300	200
	BC	2 days	850	nil[b]
3	ASG	0 days	1000	nil[b]
	BC	2 days	10,100	2500
Control	ASG	Unmated male 2 days old	16,400	1800

[a] nil ≤ 60 dpm total between 2 adjacent fractions; [b] nil ≤ 600 dpm total between 2 adjacent fractions.

The transfer of JH from the male to the female Cecropia during mating is similar in many respects to the transfer of cantharidin between the males and females of *Lytta vesicatoria* (Spanish flies). The adult female *L. vesicatoria* is unable to produce any cantharidin but is known to contain this compound[16]. During copulation the cantharidin in the ASG of the male is transferred with the seminal materials to the female[15]. The function of the transferred JH remains unknown. Allatectomy clearly demonstrates that JH plays no role in reproductive processes of the Cecropia silkmoth[17,18].

1 Research was supported by a grant from the National Science Foundation (PCM72-01892) and Organized Research Funds from Texas A&M University.
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Effect of experimental diabetes on estradiol binding by the anterior pituitary and hypothalamus in ovariectomized rats

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Summary. In diabetic rats, ^{3}H-estradiol binding by the cytosol and nuclei of the anterior pituitary was lower than normal. Changes in affinity and receptor numbers were demonstrated by Scatchard analysis. Protein synthesis in diabetic pituitaries, however, was in the normal range.

Induction of diabetes in female rats is followed by abnormalities in their reproductive function, manifested by alterations of the sex cycle, persistent anestrus and impaired fertility[1-3]. These changes were ascribed partly to primary ovarian disease[4], although recent work has focused on alterations in the regulation of ovarian activity by the central nervous system (CNS) and in the central action of steroids in diabetes. In this latter context, Denari and Rosner[5] first demonstrated a reduction in estrogen uptake in the CNS and anterior pituitary (AP) obtained from diabetic animals, whereas Gentry et al.[6] reported decreased nuclear uptake, but no changes in whole tissue uptake or cytoplasmic binding of estrogens in CNS areas and AP from diabetic rats. In this report, we confirm the changes in nuclear uptake[6], and show in addition that cytosol binding of estradiol in vitro is consistently lower in the AP, but not in the hypothalamus, from ovariectomized, streptozotocin-diabetic rats. These changes in AP estrogen binding are not due to impaired protein synthesis.

Materials and methods. Adult Wistar rats were ovariectomized and 1 week afterwards, diabetes was induced by the injection of 90 mg/kg of streptozotocin i.v., prepared as described by Junod et al.[7]. The animals were used 1 month after diabetes induction, at which time glycosuria was > 2% and glycemia measured over 400 mg%.

The estradiol binding assay was performed as described by Weisenberg et al.[8]. After decapitation of the animals, the AP and hypothalamus were homogenized in 0.010 M Tris

buffer, pH 7.4, containing 0.0015 M EDTA, 0.002 M mercaptoethanol, and centrifuged at 105,000 × g for 60 min at 0 °C. Cytosol (0.2 ml) was incubated in triplicate with ^{3}H-estradiol (2.2×10^{-9} M) at 30 °C for 20 min, after which bound and free steroid fractions were separated on Sephadex LH-20 columns. The void volume, corresponding to the bound steroid, was collected in counting vials, and the radioactivity determined after overnight extraction into 10 ml of scintillation fluid (Omnifluor 4 g, toluene 1 l) at 83% efficiency for tritium. Parallel incubations were carried out with a 1000-fold excess of non-radioactive estradiol, to correct for nonspecific binding. Results were expressed as fmoles bound/mg protein. Proteins were determined by the method of Lowry et al.[9], using bovine serum albumin as standard.

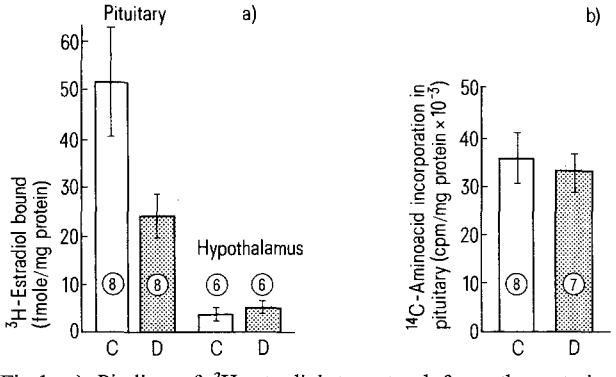

Fig. 1. a) Binding of ^{3}H-estradiol to cytosol from the anterior pituitary and hypothalamus in control (C) and diabetic (D) rats. Encircled figures represent the number of experiments. b) ^{14}C-Amino acid incorporation into proteins from anterior pituitaries of control (C) and diabetic (D) rats. Encircled figures represent the number of animals studied. $p < 0.05$, versus control rats.

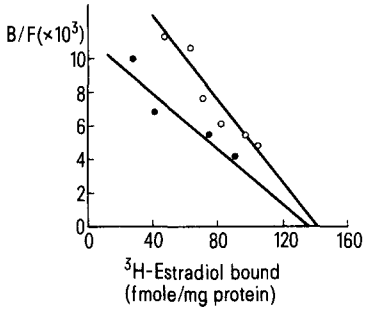

Fig. 2. Scatchard plot analysis of ^{3}H-estradiol binding to cytosol from anterior pituitaries in control ovariectomized rats (○) and diabetic rats (●). In controls, equilibrium association constant (Ka) was 1.2×10^8/M and maximal binding capacity 143 fmoles/mg protein (for the slope, $r = 0.91$, $p < 0.01$). In diabetics, Ka was 0.8×10^8/M and maximal binding capacity 138 fmoles/mg protein (for the slope, $r = 0.88$, $p < 0.05$).

Uptake by cell nuclei of ^{3}H-estradiol by anterior pituitary and hypothalamus in control and diabetic rats

Fraction	Anterior pituitary		Hypothalamus	
	Control	Diabetic	Control	Diabetic
Cytoplasmic	4.7	3.5	2.1	1.3
Nuclear: KCl-extractable	26.9	5.3	18.1	15.4
Nuclear: ethanolextractable	64.0	55.8	39.1	22.3

Results expressed as fmoles/mg protein.

In vivo uptake by cell nuclei was studied after administration of 10 μCi/150 g of ^{3}H-estradiol i.v. into ether-anesthetized animals. 1 h afterwards the rats were reanesthetized and perfused with 60 ml of 0.9% NaCl through the heart[10]. The AP and hypothalamus were collected from a group of 3 rats and pooled, and nuclear isolation was performed as described by McEwen and Zigmond[11]. The tissues were homogenized in 0.001 M phosphate buffer, pH 6.5, containing 0.003 M $MgCl_2$, 0.32 M sucrose and 0.25% Triton X-100. The homogenate was centrifuged for 10 min at 850 × g and 0 °C, and purified nuclei were isolated from the crude nuclear pellet[11].

The purified nuclear fraction was extracted with 0.4 M KCl at 0 °C, and after centrifugation, the residual radioactivity retained by nuclei was extracted with ethanol (Mc Clusky et al.[12]). Radioactive determinations were performed on the supernatant from the original 850 × g centrifugation (cytoplasmic fraction) and on the KCl extract, by extraction of the radioactive material with 10 ml scintillation fluid. The ethanol extract was evaporated into counting vials and scintillation fluid added. Results were expressed as fmoles steroid present in the fraction/mg protein.

Incorporation of a ^{14}C-amino acid mixture into total AP proteins was assessed as follows: the tissue was incubated for 120 min at 37 °C in 1 ml of Krebs Ringer bicarbonate glucose buffer under 95% O_2/5% CO_2 atmosphere with 400,000 cpm of the substrate. A detailed description of the method used for incubatioin, purification of the proteins and measurement of radioactivity has been published elsewhere[13].

(2,4,6,7)-^{3}H-estradiol (sp.act. 98 Ci/mmole), ^{14}C-amino acid mixture, and Omnifluor were purchased from New England Nuclear. Sephadex LH-20 was obtained from Pharmacia. Streptozotocin was a kind gift from Dr R. Houssay, Upjohn Laboratories.

Results and discussion. Figure 1 shows the results of the ^{3}H-estradiol receptor assay employing cytosol from the AP and hypothalamus. 8 different assays were carried out for the AP and 6 for the hypothalamus, which showed that the diabetic group bound significantly less ^{3}H-estradiol than the control rats in the AP, whereas no changes were found in the hypothalamus. That this reduction in binding was not due to a deleterious (and nonspecific) effect of diabetes upon protein synthesis in the AP was demonstrated by the results of incorporation of a ^{14}C-amino acid mixture into AP proteins, which were similar in the control and diabetic groups (figure 1).

Parameters for equilibrium binding of ^{3}H-estradiol in AP were investigated by the addition of increasing concentrations of estradiol (760–7600 fmoles) to a series of tubes containing cytosol from pooled AP of 20 control or 20 diabetic rats. The results were analyzed according to Scatchard and are shown in figure 2. In the AP from diabetic rats, there was a change in the affinity and also in binding capacity of the estrogen, which explained the reduced binding observed in the ^{3}H-estradiol receptor assay reported in figure 1.

Finally in vivo uptake of ^{3}H-estradiol by nuclei was studied. The results of the table show the amount of estradiol (in fmoles/mg protein) remaining in the cytoplasm after nuclear precipitation plus that incorporated into 2 nuclear fractions. Diabetic rats had less radioactivity in the cytoplasmic fraction from AP. Nuclear radioactivity extractable with 0.4 M KCl, representing macromolecular bound ^{3}H-estradiol[12], was markedly reduced in the AP from diabetics, as opposed to normal controls. Ethanol-extractable radioactive material, which was presumably bound to chromatin[14], was reduced to a lesser extent, and a slight

decrement in all these fractions was observed in the hypothalamus. These results suggest that in diabetic rats the receptors for estradiol were decreased in both cytoplasmic and nuclear fractions.

Our results thus support the view of Gentry et al.[6], in that uptake of ^{3}H-estradiol was reduced in AP nuclei of diabetic rats, but we also demonstrated in this gland a cytosol receptor defective in number, in affinity for the steroid, and probably in its translocation step from cytoplasm into the nuclear compartment.

The impaired binding of estradiol in the AP may be involved in the abnormalities in reproductive function described in experimental diabetes[1-4]. It is known that estradiol action on the AP is necessary for the responsiveness of the gonadotrophs to LHRH[15], and along this line, Kirchick et al.[16] demonstrated that in diabetic rats, the preovulatory LH surge is lost, presumably due to a decreased sensitivity of the hypothalamus and the AP to estradiol. This in turn would explain the anovulation of female diabetic rats.

Although little has been contributed to the knowledge of the regulation of estradiol-receptor interaction at the level of the AP, this binding reaction may be modulated by the hypothalamus. This conclusion is based upon recent demonstrations by us that rats bearing hypothalamic lesions bound considerably less estradiol in the AP[8], which would suggest that diabetic animals had this feature in common with rats with median eminence destruction. It is possible, therefore, that a defective hypothalamus lies behind the receptor changes of the AP described in the present report. However, other pathogenic factors cannot be excluded, as the changes in diabetes are not exclusive for estradiol. Decrements in binding and response to androgens and corticosteroids by the brain and AP of diabetic animals have been demonstrated by others as well as by our own laboratory[5,10,17,18].

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PRO EXPERIMENTIS

A simple apparatus to investigate the orientation of walking insects

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Summary. A simple apparatus to record the track and angular position of walking insects without any servo-mechanism is described. Recordings of fast running *Cicindelae* are presented.

To investigate the orientation of walking insects towards external stimuli it is often desirable to hold the animals in a constant position relative to the environment. This can be achieved by constraining them on the vertex of a sphere while they are running. Usually one lets the animals run upon a sphere which is counterrotated by a servo-mechanism[1-3]. We have built an appartus which records the translatory component of the animals' movements without any servo-mechanism and yet restricts their locomotion very little. Other non-servo ball type machines have been reported[4,5] which turned out to be of limited use.

The basic idea is to make the ball very light in weight and to let it float in an air stream in such a way that the ball can be handled, turned and moved easily by the animal itself. Figure 1 shows the essential parts of the apparatus: the ball used is a hollow sphere of Styropor® (polystyrene foam) of reasonable dimensions for the animal. It swims in an 'air cup', a hollow hemisphere of a slightly larger diameter than the ball (difference about 2 mm). Through a small hole in the bottom of the cup a weak air stream enters and supports the ball with nearly no friction and keeps it off the inner surface of the hemisphere in a surprisingly stable position. Since the diameter of the 'air cup' is slightly larger than that of the ball the cross-section of the airstream enlarges from the inlet to the equator of the cup and, consequently, the stream velocity decreases rapidly. The ball can easily be supported by an extremely weak air stream which does not cause any detectable air current on top of the ball in addition to the air flow usually present in the laboratory. Owing to Bernoulli-forces, increasing the velocity of the air stream merely results in a small change of the ball's position in the cup, and the ball will be more rigidly fixed in its position.

Good hollow spheres can be produced by boiling Styropor balls (available in toy shops) in an accurately-made metal mould for 3 min to give them the shape of a sphere of well defined diameter. Afterwards they are cut into 2 halves using a thin hot wire and hollowed out by hand by means of a hot wire loop; the 2 hollow halves are then glued together by a 2-component glue. Balls of 2 different sizes