Autoradiographic demonstration of estrogen uptake by the armadillo pituitary gland[1]

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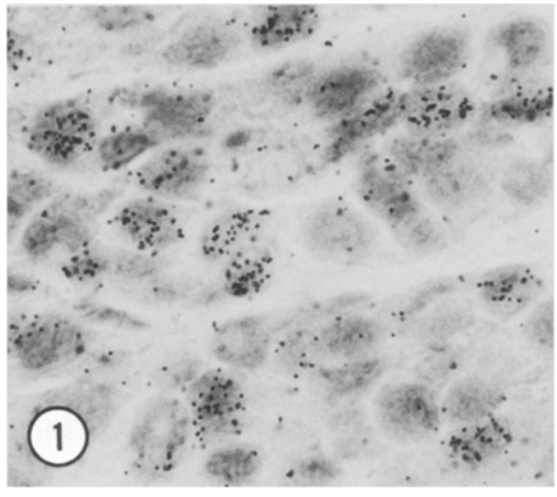
Summary. Adult female armadillos were injected with tritiated estradiol. The pituitary gland was removed and processed for autoradiography. Nuclear uptake and retention of the steroid was found in 28.8% of the cells in the pars distalis and 3.7% of the pituicytes.

Target cells for estradiol have been demonstrated in the pituitary gland of neonatal[2], sexually immature[3] and adult rats[3] using autoradiography. The studies have shown radiolabelled estradiol to be sequestered primarily by cells located in the pars distalis. The number of labelled cells varies depending upon the age as well as the reproductive state of the animal[2,3]. Localization of the steroid was not demonstrable in either the partes intermedia or nervosa in the neonate[2]. In contrast, radiolabelled cells were present in the latter region of the pituitary in pregnant rats and in animals with delayed implantation[4]. Moreover, 'ectopic' cells residing at the border between the pars intermedia and the pars nervosa have also been shown to be target cells for estradiol[3,4].



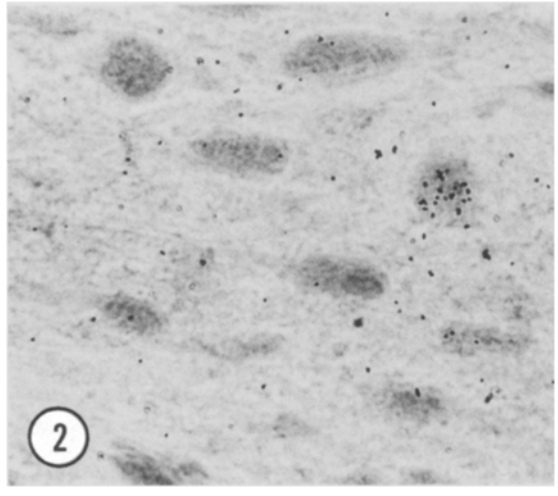


Fig. 1 and 2. Autoradiograms of the armadillo pituitary gland from an animal injected with ^{3}H-estradiol. Localization of the steroid can be observed over the nuclei of cells in the pars distalis (fig. 1) and the pars nervosa (fig. 2). Exposure time: 3 months. Hematoxylin and eosin. × 985.

Autoradiographic studies have identified cells which possess estrogen binding sites in the pituitary glands of a number of other vertebrate species[4,5]. In general, these observations are in good agreement with what has been reported in the rat. The present study was initiated to determine whether any differences exist in the pattern of uptake and retention of radiolabelled estradiol by the pituitary gland of a monestrous, monovulating species[6], the armadillo, as compared to other mammals.

Methods. 4 adult female nine-banded armadillos, *Dasypus novemcinctus,* were caught in the wild in south Texas during the month of December and housed in a temperature-controlled room having a 14:10 light:dark cycle. 3 animals were injected intracardially with 1.4 µg/kg b.wt of [2,4,6,7-^{3}H]-estradiol 17β (101 Ci/mmole). The remaining armadillo received an intracardial injection of the tritiated steroid along with 140 µg/kg b.wt of the unlabelled estrogen. All 4 animals were sacrificed 90 min after the injections. The pituitary gland was rapidly removed from each armadillo, mounted on brass tissue holders coated with minced liver and frozen in liquified propane[7]. 4-µm-thick sections were cut at −35 °C in a Harris Wide-Range cryostat, mounted on slides coated with Kodak NTB-2 emulsion and exposed at −15 °C for 3 months. Following this, the slides were photographically developed, fixed and stained with hematoxylin and eosin[8].

Results. Autoradiographic localization of estradiol was found in both the pars distalis and the pars nervosa of the 3 armadillos that were injected with the tritiated steroid. The distribution of the labelled cells in the pars distalis (fig. 1) was heterogeneous and varied from regions in which only 6% of the cells contained steroid binding sites to areas in which 75% were labelled. After counting 80 randomly selected regions containing a total of 1706 cells, it was found that only 28.8% of the parenchymal cells displayed silver grains over their nuclei.

A smaller percentage of nuclei (3.7%) within the pars nervosa (fig. 2) concentrated the radioactive steroid. In addition, clusters of labelled and unlabelled cells which resembled those present in the pars distalis were found within the connective tissue septum that separated the 2 major lobes. Moreover, a few fibroblast nuclei within this zone sequestered the tritiated steroid.

In the animal that was injected with both the radiolabelled and the unlabelled steroid, no uptake was found by nuclei in any of the cells in the hypophysis or by the fibroblasts present in the connective tissue septum.

Discussion. The results of the present study clearly demonstrate the existence of target cells for estradiol in the armadillo pituitary gland. Within the pars nervosa, a small but significant number of cells concentrated the steroid; however, the degree of nuclear labelling was less than that noted in the cells of the pars distalis. It is presumed that these labelled cells are pituicytes rather than 'ectopic' cells from the pars distalis or cells originating from the pars intermedia[3,4] because the partes distalis and nervosa in the armadillo are the only major lobes in this species and they are separated from each other by a well-developed connective tissue septum[10]. The rat and opossum have also been

reported to contain pars nervosa cells which take up estrogen[4]. As in the armadillo, the functional significance of these findings is unknown.

The number of anterior lobe cells which possess binding sites for estradiol appears to vary considerably from one species to another. A total of 28.8% of the cells in the armadillo bound the tritiated steroid as compared to 17.2% of the pars distalis cells in the adult ovariectomized rhesus monkey[5] and 86% in the mature, castrated rat[3]. Nuclei of a 'relatively small' number of cells in the hypophyses of the intact tree shrew and castrated squirrel monkey were labelled after exposure to the radioactive estrogen[4] while considerably more cells concentrated this hormone in the pituitary glands of the opossum[4] and guinea-pig[9]. The reason for the high degree of interspecies variation in the number of target cells for estradiol is unclear. It may be partially related to the amount of endogenous estrogens which are available to occupy binding sites within the pars distalis at the time the test animal is given the radiolabelled steroid[4]. On the other hand, there may be a phylogenetic relationship to the number of estrogen-sensitive cells; however, considerably more species need to be examined in order to validate this premise.

It has recently been shown that the pituitary cells which concentrate estradiol also secrete luteinizing hormone, follicle-stimulating hormone, growth hormone, prolactin, thyrotrophin and corticotrophin[11]. Studies are currently underway in our laboratory to determine which of these hormones reside in cells that possess estrogen binding sites in the pars distalis of the armadillo.

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PRO EXPERIMENTIS

A simple method of cannulating the portal vein and obtaining multiple blood samples in the rat[1]

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Summary. A simple and rapid technique for cannulating the portal vein in the rat is described. Multiple blood samples can be obtained or alternatively substrate may be infused through the cannula.

We describe a simple means of obtaining sequential blood samples from the portal vein of rats after directly cannulating the vein. Prior descriptions have been more complex[3], or require repeated insertions of a needle[4] or, a cannula has to be inserted through and ligated into a tributory of the portal vein (splenic vein[5], pyloric vein[6]), or a venous shunt has to be constructed[7]. Our method is also readily adaptable to sampling of blood from other (small) blood vessels or to infusion of substrate.

Method. Rats weighing 250–300 g are suitable. After anesthesia a midline abdominal incision is made from the symphysis pubis to the xyphoid process. From the xyphoid process the incision is extended cephalad and laterally on both sides superficially to the bony thoracic cage so that the total incision is Y-shaped. A straight hemostat is clamped to the flap of skin and muscle on each side close to the junction of the thoracic and abdominal tissues. Each flap of tissue is rolled around the hemostat and pulled laterally (this has the effect of raising up the abdominal content and eases further access). The liver is then gently everted over the sternum, the duodenum is freed, reflected to the (rat's) left side and the portal vein is exposed. A 0.5–1-cm segment of portal vein is teased free from the mesentery. Then a curved hemostat (concave dorsally) is slipped under the freed portion of the portal vein and opened just sufficiently to place slight tension on the vein (fig.). With the bevel facing dorsally the point of a 23-gauge needle is partially inserted in a cephalad direction into the wall of the portal vein. The needle is then lifted slightly ventrally to act as a guide for the immediate introduction of the bevelled end of a 13–15 cm long PE 50 polyethylene cannula (Intramedic polyethylene tubing, inside diameter 0.585 mm × outside diameter 0.965 mm, Clay-Adams, Division of Becton Dickinson & Co. Parsippany, N.J. 07054) filled with normal saline and connected to a variable speed infusion pump. The cannula is threaded 3–4 mm into the portal vein by sliding it along the underside of the needle (side nearest to the portal vein). The needle is now quickly removed. The puncture site is immediately sealed and the cannula is simultaneously fixed into place with 1 drop of a cyanoacrylate ester cement (Super Glue 3, Woodhill Permatex, Cleveland, Ohio 44128). Normal saline is continually infused via the cannula at a slow rate (1 ml/h). Duodenum and liver are replaced and the abdominal incision is clamped shut. The cannula is further anchored by applying 1 drop of cyanoacrylate ester cement to the skin at the point where the cannula exits from the abdominal cavity. With practice total operating time, after induction of anesthesia is less than 15 minutes. We allow an equilibration period of 15 min after clamping the abdominal cavity shut before commencing experiments. Pilot studies have shown that this period is adequate for measuring intestinal transport.

Samples of portal vein blood are obtained by temporarily disconnecting the cannula from the pump. Portal blood