glia. These effects were reversed by injection of the brain extract (table). Injection of brain extract into sham-operated mussels resulted in lowering of blood sugars and a rise in foot muscle glycogen.

Discussion. The results indicate the possible presence, in the cerebral ganglia of the mussel *L. marginalis*, of a neurohormonal substance that has a regulatory effect on carbohydrate metabolism. The changes in carbohydrate levels induced by ablation and subsequent injection of the extract into ablated and sham-operated mussels suggest that the factor is hypoglycemic, having an activity similar to vertebrate insulin.

Effect of ablation of cerebral ganglia and injection of cerebral ganglionic extract on the carbohydrate levels in the hemolymph and foot muscle of the mussel *L. marginalis*. The values are mean ± SD for 10 animals. p-Values significant, * < 0.01

	Time	Total blood sugar mg/100 ml Mean ± SD	Foot glycogen g/100 g dry tissue Mean ± SD
Normal mussels	–	40.1 ± 8.7	11.0 ± 0.2
Sham operated controls	1h	41.2 ± 4.2	11.2 ± 0.5
	2h	42.1 ± 5.8	11.8 ± 0.6
	3h	41.0 ± 6.5	11.2 ± 0.8
Ablated mussels	1h	57.0 ± 5.6*	10.3 ± 2.8*
	2h	58.0 ± 4.2*	9.5 ± 4.3*
	3h	58.2 ± 2.0*	9.1 ± 4.1*
Ablated mussels injected with phosphate buffer pH 7.4 (control)	30 min	59.0 ± 3.4	8.8 ± 2.8
	1h	58.9 ± 4.9	9.2 ± 2.7
Ablated mussels injected with foot muscle extract (control)	30 min	60.2 ± 2.9	8.7 ± 3.2
	1h	59.5 ± 3.8	8.9 + 3.5
Ablated mussels injected with brain extract	30 min	47.0 ± 0.7*	9.3 ± 2.8*
	1h	38.6 ± 9.2	10.7 ± 2.8
Sham operated mussels injected with brain extract	30 min	35.4 ± 7.5*	12.8 ± 1.2*
	1h	33.8 ± 6.5*	13.4 ± 0.8*
Sham operated mussels injected with phosphate buffer (pH 7.4) (control)	30 min	41.8 ± 6.5	11.0 ± 1.1
	1h	42.1 ± 5.6	10.7 ± 0.9
Sham operated mussels injected with foot muscle extract (control)	30 min	40.5 ± 3.8	10.8 ± 2.2
	1h	41.5 ± 7.2	11.5 ± 3.8

The physiological role of neurosecretion in pelecypod molluscs is poorly understood, although there is some evidence for the presence of neurosecretory cells in the central ganglia of a few pelecypods[1-3]. Since several gastropod and pelecypod molluscs have been found to have carbohydrate-oriented metabolism[11-13], molluscan tissues should be able to synthesize and store carbohydrate as glycogen, and utilize it under stress. This is possible, perhaps under the aegis of hormonal substances. Vertebrate insulin was found to cause hypoglycemia and glycogen synthesis in the tissues of the clam *Meretrix costa*[13], indicating that molluscan tissue is responsive to insulin. In a search for the source of a hypoglycemic factor in molluscan tissues, the gut wall of pelecypods *Anodonta cygnea, Unio pictorum* and *Ostrea edulis* was found to produce a factor similar in activity to that of vertebrate insulin[5,6,15,16]. The present work has revealed yet another source for a hypoglycemic factor. This opens up possibilities for regulation of carbohydrate metabolism at different levels by multiple hormones. However, a generalization is not possible at this stage as further probing into other sources in molluscan tissues is needed.

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0014-4754/84/121423-02$1.50 + 0.20/0

Nuclear progestin receptor in the chimpanzee sex skin[1]

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Summary. We describe for the first time the presence of a nuclear progestin receptor in the sex skin of the chimpanzee, which is possibly involved in the anti-estrogen action of progestins in the sexual swelling.
Key words. Chimpanzee; sex skin; progestin receptor; anti-estrogen action.

The sex skin of the chimpanzee undergoes a cyclic fluctuation in its degree of swelling[3]. The degree of sexual swelling has been correlated with the concentration of the nuclear estrogen receptor in the tissue, which characterizes the chimpanzee sex skin as an estrogen target tissue[4]. Since progestins inhibit estrogen-induced swelling, an anti-estrogen action has been suggested for progestins in the sexual swelling of the chimpanzee[4].

As a preliminary step to determine whether or not this anti-estrogen action of progestins is a receptor-mediated action of the steroid, we investigated the presence of the nuclear progestin receptor in the chimpanzee sex skin.

Materials and methods. Sex skin tissues (0.7–1.8 g) biopsied from chimpanzees at various reproductive stages were homogenized at 0–4 °C in 4 vol. of TEDG (10 mM Tris-HCl, 1 mM EDTA, 12 mM monothioglycerol, 10% (v/v)

glycerol, pH 7.5) after extensive washing in the same buffer. Preparation of the cytosol and nuclear extract was identical to that previously described except that KCl-TED (TEDG plus 0.5 M KCl, pH 7.5) was used for the extraction of the nuclear receptor[4].

The nuclear progestin receptor was quantitated according to a Scatchard analysis[5] after a 20-h incubation of 100 µl aliquots of the nuclear extract with 0.5-5 nM ^{3}H-R5020 (85 Ci/mmole, New England Nuclear) in the presence or absence of a 100-fold excess of unlabeled R5020. A 100-fold excess of cortisol was present in all assay tubes to inhibit binding of ^{3}H-R5020 to corticosteroid binding globulin (CBG)[6]. Subtraction of binding in the presence of a 100-fold excess of unlabeled R5020 as well as ^{3}H-R5020, from that in the presence of ^{3}H-R5020 alone, shaved the specific binding of the tritiated steroid. Separation of bound from unbound ^{3}H-steroid was accomplished by treatment with 0.05% dextran T70 and 0.5% acid-washed activated charcoal in TEDG, pH 7.5 (DCC) at 0-4 °C for 10 min.

Sucrose gradient analysis of the nuclear progestin receptor was made on the nuclear extract of sex skin tissues prepared from an estrogen-treated (mestranol 200 µg per day for 20 days) animal which had been ovariectomized more than 10 years before. Prior to extraction of nuclear receptor, tissues were minced and incubated at 25 °C for 1 h with 10 nM ^{3}H-R5020 with or without a 100-fold excess of unlabeled R5020 or cortisol in KRH buffer[7]. The nuclear extract thus prepared was treated with DCC prior to layering onto sucrose gradients. Procedures for sucrose gradient analysis were described previously[4].

Protein was quantitated by the method described by Bradford[8].

Results and discussion. Sedimentation profile of nuclear ^{3}H-R5020 binding is presented in figure 1. A single peak of bound radioactivity sedimenting at approximately 3.5 S is identifiable. This peak was abolished when tissues were incubated in the presence of excess unlabeled R5020 but was unaffected with excess cortisol; it was not demonstrable in the nuclear extract from the same tissue when incubation with ^{3}H-R5020 was done for 2 h at 0-4 °C following, but not prior to, the preparation of the extract, suggesting that translocation of cytosolic receptors into the cell nucleus is required (data not shown). Cytosols prepared from an animal in the periovulatory phase were incubated at 0-4 °C for 2 h with 10 nM ^{3}H-R5020 with or without a 100-fold excess of various unlabeled steroids and were submitted to sucrose gradient analysis. As shown in figure 2, 2 peaks of bound radioactivity, sedimenting at 8 and 4 S, were present when the cytosol was incubated with ^{3}H-R5020 alone. While the 4 S peak was not affected in the presence of excess unlabeled R5020 and progesterone, the 8 S peak showed the steroid specificity expected for progestin receptors. Thus this 8 S peak was considered to represent the specific progestin binding, whereas the 4 S peak

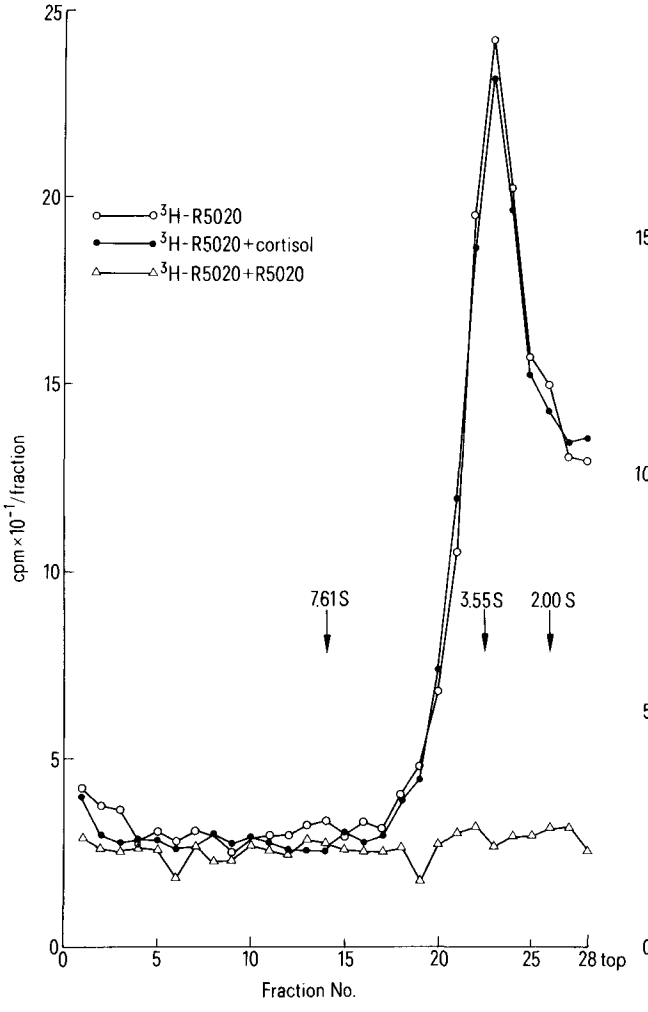

Figure 1. Sucrose density gradient profiles of ^{3}H-R5020 binding in chimpanzee sex skin nuclear extract. Nuclear extracts were prepared after incubation of minced tissues obtained from an estrogen-treated ovariectomized animal for 1 h at 25 °C with 10 nM ^{3}H-R5020 with or without a 100-fold excess of unlabeled R5020 or cortisol.

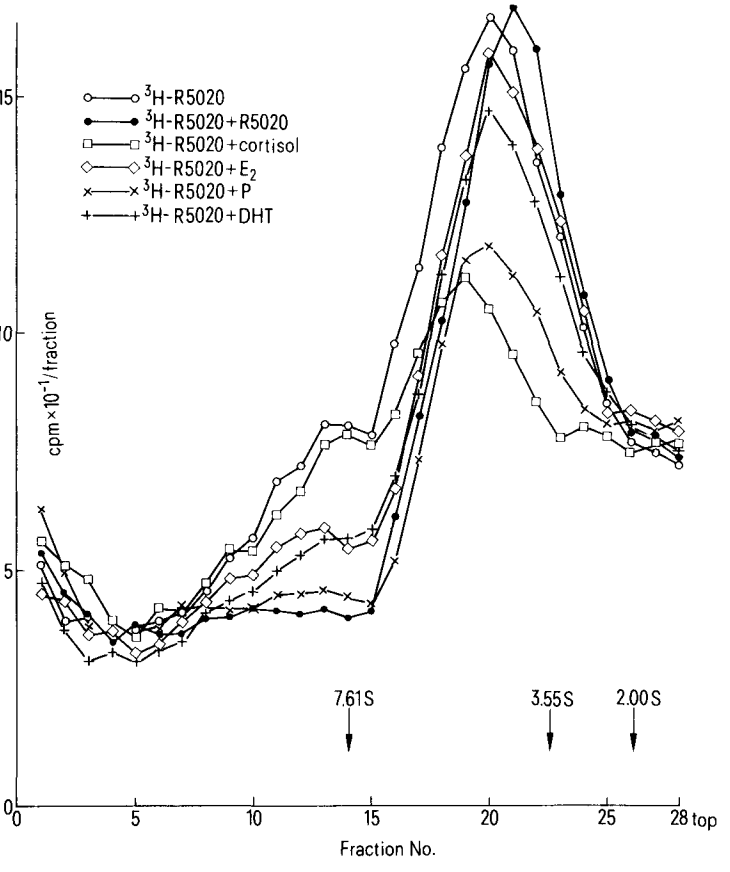

Figure 2. Sedimentation profiles of ^{3}H-R5020 binders in chimpanzee sex skin cytosol. Cytosols of sex skin from an animal in the periovulatory phase were incubated for 2 h at 0-4 °C with 10 nM ^{3}H-R5020 and a 100-fold excess of various unlabeled steroids. E_2, 17β-estradiol; P, progesterone; DHT, dihydrotestosterone.

might reflect non-receptor-related progestin binders including CBG.

Scatchard analysis yielded the concentration of binding sites of 167 fmoles per mg protein and the dissociation constant of 1.0 nM for the nuclear extract prepared from an animal in the early luteal phase (fig. 3).

The present study indicates the presence of a nuclear progestin receptor in the sex skin of the chimpanzee. The nuclear progestin binding component in the chimpanzee sex skin has both a high affinity and a limited capacity for progestin binding and the steroid specificity expected for a progestin receptor. Furthermore evidence was provided for the possible nuclear translocation of the cytosol receptor. According to our current understanding of steroid hormone action[9], demonstration of nuclear receptor is imperative for any given tissue to be characterized as the target of hormone action. Previous studies on the progestin receptor in the sex skin were limited to the cytosol prepared from the sex skin of the Japanese monkey[10].

The chimpanzee sex skin has not been analyzed for the progestin receptor. In the light of the results of the present study, it may well be that the anti-estrogen action of progestin in the sexual swelling of the chimpanzee is effected through a progestin receptor system present in the tissue. This study provides the basis for further evaluation of the physiological role of the progestin receptor in the sexual swelling of the chimpanzee.

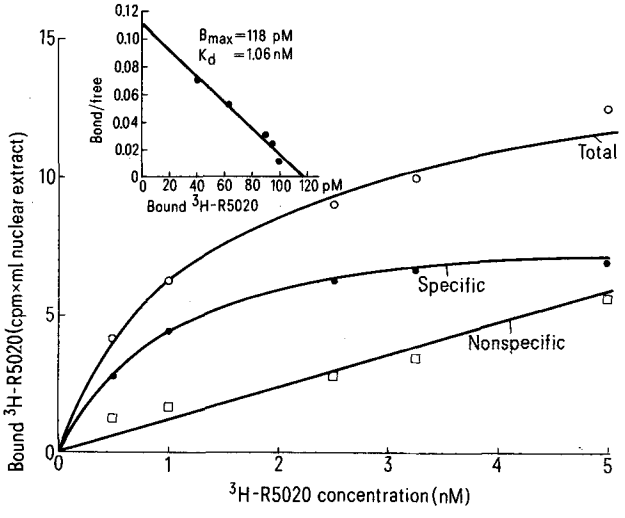

Figure 3. Saturation curve for ^{3}H-R5020 binding to sex skin nuclear extract from a chimpanzee in the early luteal phase. Corresponding Scatchard plot is shown in the inset. B_{max}, concentration of receptor; K_d, dissociation constant.

1 This work was supported by NIH grants RR-00165 and HD-13083 to the Yerkes Primate Research Center, Emory University.

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0014-4754/84/121424-03$1.50 + 0.20/0

The role of phosphoenolpyruvate in insulin secretion: the effect of L-phenylalanine

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Summary. Incubation of rat islets with phenylalanine increased the tissue content of phosophoenolpyruvate, both in the presence and in the absence of glucose. At the same time, L-phenylalanine neither stimulated nor inhibited insulin release. It is unlikely that insulin secretion is tightly coupled to the availability of phosphoenolpyruvate in rat islets.

Key words. Rat islets; islets, rat; insulin secretion; phosphoenolpyruvate; phenylalanine.

The substrate site model[1], or fuel hypothesis[2], for the stimulation of insulin release by glucose proposes that the secretory process is triggered by one or more intracellular metabolites. One candidate for this role is phosphoenolpyruvate (PEP). This has been reported to stimulate islet cell adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] activity[3], and a membrane protein kinase[4]; it also inhibits Ca^{2+} uptake into islet mitochondria[5] in a process, involving an ATP translocase[6], similar to that in other tissues[7,8]. PEP also causes release of insulin from suspensions of isolated granules and plasma membranes[9,10]. In addition, its concentration in whole islets has been shown to correlate with the tissue content of 3':5'-cyclic AMP[11] and with insulin secretion elicited by glucose and glyceraldehyde[12].

The content of PEP is islets is dependent on the relative activities of the glycolytic sequence to enolase [2-phospho-D-glycerate hydro-lyase, EC 4.2.1.11], phosphoenolpyruvate carboxykinase [GTP: oxaloacetate carboxylase (transphosphorylating), EC 4.1.1.32] and the type M_2 pyruvate kinase [ATP: pyruvate 2-O-phosphotransferase, EC 2.7.1.40][13]. This last activity is strongly inhibited by L-phenylalanine[13], a fact which was exploited in the work described in this paper to examine the possible relationship between PEP concentration and insulin secretion.

Materials and methods. The sources of materials were as given previously[13,14].

Preparation and incubation of islets. Islets were isolated from fed Sprague-Dawley rats (male, 150–200 g)[15] and were incubated as described elsewhere[16]. Briefly, batches of 10 islets were pre-incubated for 15 min at 37°C in 100 µl portions of medium[17]. Medium was then replaced with fresh medium containing appropriate additions; incubation was continued for a further 60 min. 10 µl portions were stored at −20°C for insulin assay; the remaining contents were frozen in liquid N_2, then were acidified with HCl. Islets were disrupted by sonication and the resulting supernatants were neutralized before assay of ATP and PEP.

Assay procedures. Insulin[18] and ATP[19] were assayed by stan-