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.

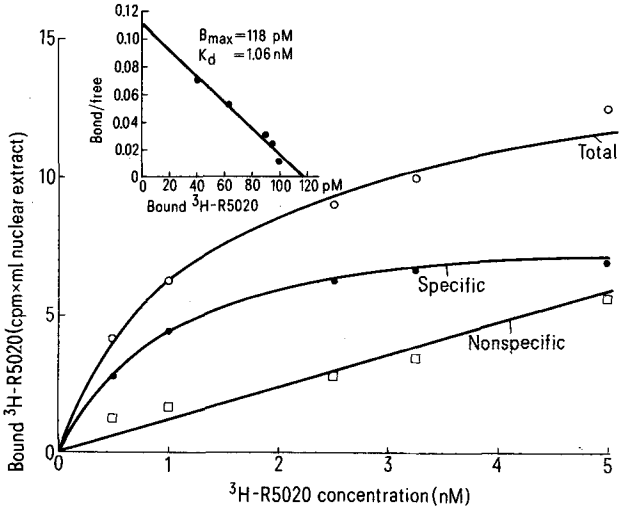

Figure 3. Saturation curve for ^{3}H-R5020 binding to sex skin nuclear extract from a chimpanzee in the early luteal phase. Corresponding Scatchard plot is shown in the inset. B_{max}, concentration of receptor; K_d, dissociation constant.

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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-

dard methods. PEP was measured as follows: 300 µl of each neutralized extract was mixed with 200 µl of charcoal suspension (de-fined Norit GSX, 50 mg/ml) in 50 mM triethanolamine HCl, 200 mM KCl, 10 mM $MgSO_4$, pH 7.4. After centrifugation (15,000 × g, 5 min, 4 °C), which was repeated if necessary, portions of supernatants were diluted to 500 µl with buffer (as above), containing 0.5 unit (final) of pyruvate kinase. Reaction was initiated by further addition of 10 µl of ATP-free ADP (10^{-4} M; ref. 20). After 60 min at 37 °C, ATP formation was assayed as above. PEP standards (0–60 pmol) were carried through the same procedure. The number of counts was linear with increasing amounts of PEP. Blank values were reproducible; they were always < 5% of counts at 60 pmol, and < 30% at 5 pmol. These blanks do not arise from ATP which is completely removed from extracts by charcoal treatment. PEP samples in extracts were found to be stable for at least 3 d at −20 °C.

Results and discussion. The mean ATP content of islets was 6.7 pmol/islet, a value comparable with those reported elsewhere[21,22].

In these experiments, the changes in islet PEP content with increasing glucose concentration were generally similar to those reported by Sugden and Ashcroft[12] but were not significant for the number of independent observations made (table). The absolute values for PEP content were also similar to those of Sugden and Ashcroft[12] but were greater than those noted elsewhere[23,24]. The inclusion of L-phenylalanine in the medium (with or without glucose) did not affect insulin secretion (table). This confirms the results of Panten and Langer[25] and, generally, those of Malaisse and co-workers[26,27] although these latter authors reported some effect at 8.3 mM glucose. Such a lack of effect is consistent with similar observations on glucagon secretion[28,29] and with the very limited extent to which phenylalanine is oxidized by islets[25].

10 mM L-phenylalanine caused substantial and significant increases in islet PEP content at all glucose concentrations tested. Lower concentrations were also effective, with 1.0 mM producing a 44% increase.

It is unlikely that these observations have any direct physiological significance. Phenylalanine concentrations in arterial blood are approximately 0.06 mM[30]; phenylalanine uptake in those tissues that have been examined is non-concentrative[31,32]. Alanine, which also inhibits islet pyruvate kinase[13], caused, at 10 mM, a small increase in islet PEP (1.04 ± 0.10 compared with 0.81 ± 0.07 pmol/islet in controls without glucose, $p < 0.05$). Insulin secretion remained unchanged in these experiments. This smaller effect of alanine may be attributable to the facts that this aminoacid is both a less effective inhibitor of pyruvate kinase than is phenylalanine[13] and is also readily metabolized in islets[33].

Hedeskov and Capito[23] have also noted that fructose, which is not an insulin secretagogue, can increase islet PEP contents. The evidence does not, therefore, support the contention that there is an *obligatory* coupling between PEP and insulin secretion. It does not, however, rule out the possibility that PEP plays a role during the primary phase of insulin secretion, since this may be masked, in batch incubations, by the larger secondary phase.

Effects of L-phenylalanine on content of phosphoenolpyruvate and insulin secretion in isolated rat islets of Langerhans

Glucose (mM)	L-Phenylalanine (mM)	Phosphoenol-pyruvate content (pmol/islet)	Insulin secretion (ng/h per islet)
0	0	0.81 ± 0.07	0.24 ± 0.03
0	10	1.71 ± 0.27*	0.37 ± 0.17
5.5	0	0.88 ± 0.08	2.60 ± 0.60
5.5	10	1.67 ± 0.15*	3.10 ± 0.32
8.3	0	0.95 ± 0.20	7.93 ± 1.51
8.3	10	1.55 ± 0.11*	8.67 ± 0.65
16.7	0	0.82 ± 0.03	17.4 ± 1.80
16.7	10	1.67 ± 0.43*	15.4 ± 2.50
0	0	0.63 ± 0.11	
0	0.05	0.77 ± 0.15	
0	1.0	0.91 ± 0.05†	
0	5.0	1.72 ± 0.16††	

Procedures were as described in Materials and Methods. In all cases, 10 islets were incubated together for 60 min, in triplicate. Results are means ± SEM from three independent observations. The significance of differences between means was determined by Student 's t-test. P (vs corresponding incubations without phenylalanine): * < 0.05 (paired test); † < 0.05, †† < 0.005 (unpaired test); all other differences are not significant.

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