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EARLY EVENTS IN THE ACTION OF GLUCOCORTICOIDS IN DEVELOPING TISSUES*

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SUMMARY

The interaction of glucocorticoids with developing tissues has been examined. Saturable, high affinity, glucocorticoid-specific binding sites (receptors) have been detected in several fetal and mature tissues of the rabbit and other species. Extensive studies in fetal rabbit lung indicate that nuclear uptake and retention of glucocorticoids in the tissue involves a multi-step mechanism in which the steroid first associates with an extranuclear receptor protein. Subsequently, the steroid-receptor complex is activated to a form which can enter the nucleus where it interacts with acceptor sites in the chromatin. The nuclear acceptor sites appear to involve DNA and do not seem to be specific for target tissues.

Fetal lungs of various species contain glucocorticoid receptors long before the normal appearance of surfactant in alveolar spaces. In some species the pulmonary receptor could be detected only during fetal life suggesting a maturation-dependent responsiveness of lung to glucocorticoids. In addition to its ability to bind and retain glucocorticoids, the fetal rabbit lung is very efficient in activating cortisone to cortisol. Inactivation of cortisol to cortisone is a minor reaction.

The failure of glucocorticoids to induce tyrosine aminotransferase in fetal rat liver does not appear to be due to the absence of receptors in this tissue. Glucocorticoid receptors are present in fetal rat liver and their concentration increases after birth reaching mature levels by the 5th postnatal day.

A complex pattern of glucocorticoid interaction with adult liver nuclei was observed. Both high affinity and low affinity binding sites were detected. The high affinity sites do not appear to be homogeneous since only a small fraction is extractable with 0·4 M KCl. In addition, a fraction of these sites is released by Triton X-100 suggesting an interaction of glucocorticoids with nuclear membranes. In contrast, fetal rat liver nuclei appear to contain only a single class of high affinity sites and no evidence for the presence of low affinity sites or for glucocorticoid interaction with nuclear membranes was obtained.

Comparative studies suggest that the glucocorticoid receptors of fetal and adult rat liver may not be identical. Evidence for this includes differences in the relative affinity of cortisol and corticosterone for the binding sites of fetal and adult liver cytosol as well as differences in the dissociation constants and sedimentation coefficients of the steroid-receptor complexes.

INTRODUCTION

Glucocorticoids are known to be involved in the regulation of maturation and biochemical differentiation of a variety of fetal tissues. Enhanced morphologic and cellular maturation of the small intestine [1] and lung [2], increased glycogen deposition and enhanced elimination of hepatic hematopoietic cells [3, 4], increased rate of accumulation of exportable pancreatic enzymes [5], prolongation of beating of cardiac cells [6], increased collagen content in cartilage [7], enhanced keratinization of skin [8], and induction of a variety of enzymes including glycogen synthetase in liver [9], alkaline phosphatase in small intestine [10], glutamine synthetase in retina [11], glycerol phosphate dehydrogenase in brain [12] and choline phosphotransferase in lung [13], are some of the effects observed after exposure of fetal tissues to glucocorticoids *in vivo* or *in vitro*.

An important characteristic of the action of glucocorticoids in developing tissues is that hormone responsiveness is tissue specific. Moreover, the ability of a particular tissue to respond to the hormone and the type of response evoked depends on the state of tissue maturation [14]. Thus a tissue may respond to the hormone only after a certain stage of development and the ability to respond may be present long before the normal ontogenic response occurs [14]. In addition, the biochemical end point of response to the same hormone may be completely different at different stages of tissue maturation [14].

The mechanism(s) by which glucocorticoids exert their effects in developing tissues are largely unknown. Among the fundamental questions that need to be answered are: do glucocorticoids influence fetal tissues by acting directly and what are the factors which determine competence of a tissue to respond to the hormones? In the light of present knowledge, one of these factors may be the ability of the tissue to recognize and retain the hormone by a mechanism involving a specific hormone receptor [15]. The absence of the receptor may be partly responsible for the inability of a tissue to respond to glucocorticoid prior to a certain period of development. Alternatively, the receptor may be present

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but the enzymic metabolic machinery required for manifestation of an overt metabolic response may be absent. The ability of hormone administration to evoke a certain response prematurely may be explained by the presence of the receptor prior to the period at which the response normally occurs and which is normally determined by other factors such as the availability of effective hormone levels. Qualitative differences in hormone responses in different tissues or in the same tissue at different stages of maturation may be due to differences in the nature of the receptor present or to differences in the nature of the cells according to their state of differentiation.

The present report describes some of the work carried out in our laboratory in the last few years in an attempt to answer some of the above questions.

EXPERIMENTAL

The methods used in these studies have been described in detail previously, and the reader is referred to these publications [16–20].

RESULTS AND DISCUSSION

Fetal lung: a target tissue for glucocorticoids

In recent years it has been recognized that glucocorticoids may play an important role in the development of the lung *in utero*. Administration of glucocorticoids to mammalian fetuses at appropriate stages of gestation accelerates morphological development of the lung and causes precocious appearance of pulmonary surfactant [2, 21]. Endocrine ablation such as fetal decapitation inhibits normal lung maturation and results in decreased levels of surfactant in fetal lungs [22]. It has been suggested that a principal action of glucocorticoids in fetal lung may be the induction of key enzymes involved in the biosynthesis of surface-active phospholipids. This is supported by the finding that rabbit fetuses injected with glucocorticoid show elevated levels of lung choline phosphotransferase activity, an enzyme involved in lecithin biosynthesis by the CDP-choline pathway [13].

To investigate whether the observed effects of glucocorticoids on the developing lung are direct, we undertook extensive studies on the uptake, retention and subcellular distribution of glucocorticoids in this tissue using the rabbit fetus as a model [16, 17]. Twenty minutes after the administration of labeled cortisol to rabbit fetuses *in utero*, the major fractions of radioactivity in the fetal lung are present in the cytosol (60%) and nuclear fractions (20%). Virtually all of the nuclear radioactivity represents unmetabolized cortisol and most of it can be extracted with 0·4 M KCl solutions and shown to be bound to macromolecules. That fetal lung nuclei contain a limited number of binding sites for glucocorticoids is indicated from the observation that administration of an excess of nonlabeled cortisol almost completely inhibits nuclear uptake of the labelled hormone [17].

Further studies *in vitro* [16, 17] confirmed and extended the above observations. After incubation of fetal lung slices with labeled cortisol or dexamethasone at 37°C, it was demonstrated that the tissue contains a limited number of nuclear binding sites (about 6000 sites per nucleus) which are saturated with low hormone concentrations (1×10^{-8} M dexamethasone and 5×10^{-8} M cortisol). Fetal lung cytosol also contains saturable binding sites with a high affinity (Kd ~ 4×10^{-9} M) for dexamethasone. Both the cytoplasmic and nuclear binding sites are highly specific for glucocorticoids. Steroids without glucocorticoid activity do not affect binding, but glucocorticoids compete with dexamethasone for the binding sites, the order of binding affinity being approximately proportional to their glucocorticoid potency.

The high affinity and steroid specificity of the binding sites present in fetal lung cytosol and nuclei are characteristics appropriate to glucocorticoid receptors responsive to circulating levels of the hormones. These findings coupled with the known effects of glucocorticoids on lung maturation and surfactant synthesis or release [2, 13, 21] provide strong evidence that the fetal lung is a target tissue for glucocorticoids.

Properties of glucocorticoid receptors of fetal lung

Binding of glucocorticoids to lung cytosol and nuclei is abolished by proteolytic enzymes but not by nucleases indicating that the steroid-binding site is at least partly protein in nature. The integrity of sulfhydryl groups is critical since binding is inhibited by SH blocking agents [16]. When examined in solution, the glucocorticoid receptors are highly thermolabile and their binding activity is completely destroyed by brief heating at 37°C. Glucocorticoid has a stabilizing effect upon the binding protein since the steroid–protein complex is much less thermolabile than the protein in the free state [16]. The cytoplasmic dexamethasone–protein complex sediments at 7S in low-salt solutions and at 4S in the presence of 0·4 M KCl [16]. The nuclear complex sediments at 4S in both low- and high-salt solutions [17]. These properties distinguish unequivocally the glucocorticoid receptor of fetal lung from serum corticosteroid-binding globulin.

Mechanism of specific glucocorticoid uptake by fetal lung nuclei

Figure 1 shows that during incubation of fetal lung slices with dexamethasone at 37°C, the receptor content of the cytosol is depleted concomitantly with an increase in nuclear uptake of the hormone. The rates of receptor depletion from the cytosol and of nuclear hormone uptake are related to hormone concentration. Uptake of dexamethasone by nuclei is highly temperature-dependent since only small amounts of hormone are associated with the nuclei

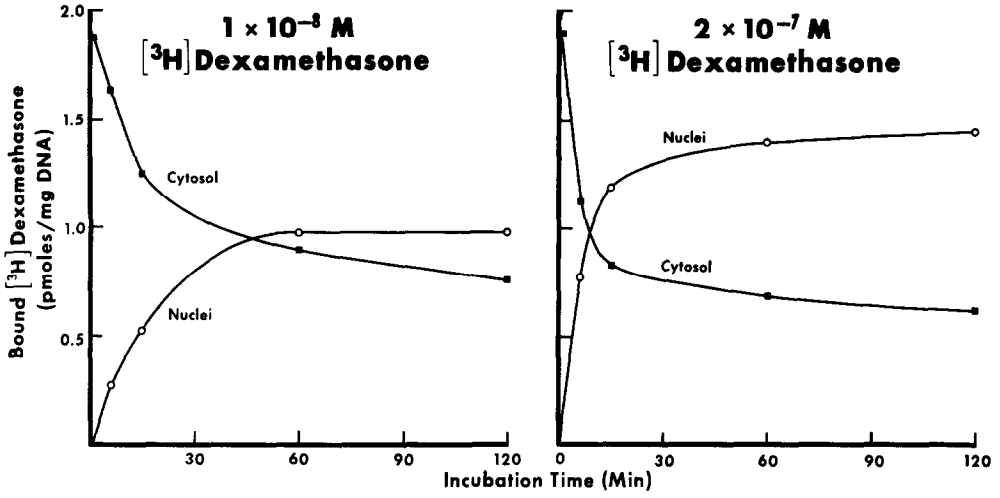

Fig. 1. Relationship between loss in the capacity of the cytosol to bind [^{3}H] dexamethasone and nuclear uptake of the hormone after incubation of fetal rabbit lungs at 37°C for various time periods with 1 × 10^{-8} or 2 × 10^{-7} M [^{3}H]-dexamethasone. The binding capacity of the cytosol was estimated after the addition of excess [^{3}H] dexamethasone to saturate the binding sites. All values are corrected for nonspecific binding.

after a 2 h incubation of lung slices at 0°C (not shown). These observations are compatible with the hypothesis that a separate nuclear receptor for glucocorticoids does not exist in fetal lung cells. Rather, the nuclear steroid–receptor complex may arise by a hormone- and temperature-dependent transfer of the cytoplasmic complex into the nucleus.

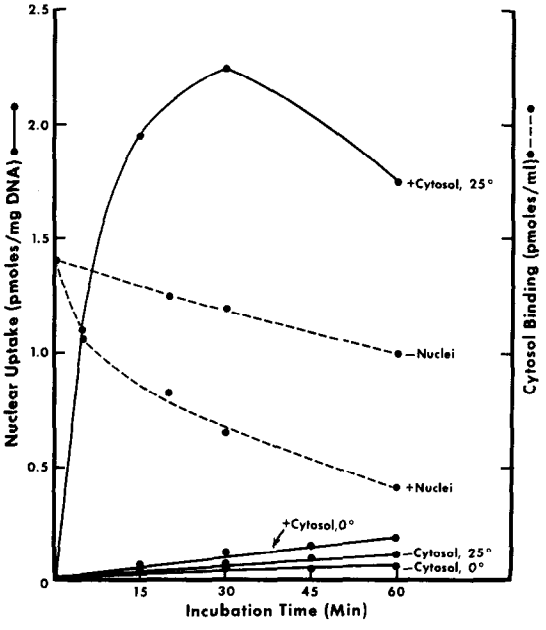

Fig. 2. Requirement of cytosol for specific uptake of [^{3}H]-dexamethasone by isolated fetal rabbit lung nuclei and its dependence on temperature. Lung nuclei were incubated at 0 or 25°C for the time periods indicated either with cytosol or with buffer which had been preincubated with [^{3}H] dexamethasone alone or together with competing steroid. Following incubation, the nuclei were reisolated by centrifugation and the total nuclear radioactivity as well as bound radioactivity in the supernatant were assayed. Solid line, nuclear uptake; broken line, specific binding in cytosol after incubation with or without nuclei at 25°C.

This concept is further supported by observations in cell-free preparations (Fig. 2). Incubation of isolated lung nuclei with dexamethasone at 0 or 25°C does not result in significant specific hormone uptake. If, however, the nuclei are incubated with dexamethasone in the presence of lung cytosol, specific uptake of the hormone is observed. This phenomenon is highly temperature dependent since the rate of hormone uptake is very slow at 0°C but is drastically accelerated by raising the temperature to 25°C. Concomitantly with the accumulation of the hormone in the nuclei the amount of hormone-receptor complex remaining in the cytosol is decreased.

Although uptake of dexamethasone by isolated nuclei in the presence of cytosol is very slow at 0°C (Fig. 2), the rate of nuclear uptake at 0°C is highly enhanced provided that the cytosol–hormone mixture is heated for 30 min at 25°C before mixing with the nuclei (Fig. 3a). Preheating the nuclei at 25 or 37°C has no effect on the rate of nuclear binding at 0°C (not shown). These results indicate that the cytoplasmic dexamethasone-receptor complex must first be activated before it can bind to nuclei. Activation of the complex is temperature dependent but its subsequent binding to the nuclei is relatively independent of variations in temperature. A similar activation of the cytoplasmic complex is observed after precipitation with ammonium sulfate or after brief exposure to 0·4 M KCl (Fig. 3b and c).

Based on the above observations, the following three-step mechanism for the specific uptake of glucocorticoids by fetal lung nuclei is proposed: (i) after entry into the cell, the hormone binds specifically to a cytoplasmic receptor protein. The complex formed is inactive in the sense that it cannot bind to nuclei; (ii) the second step involves activation of the complex to a form which has an enhanced

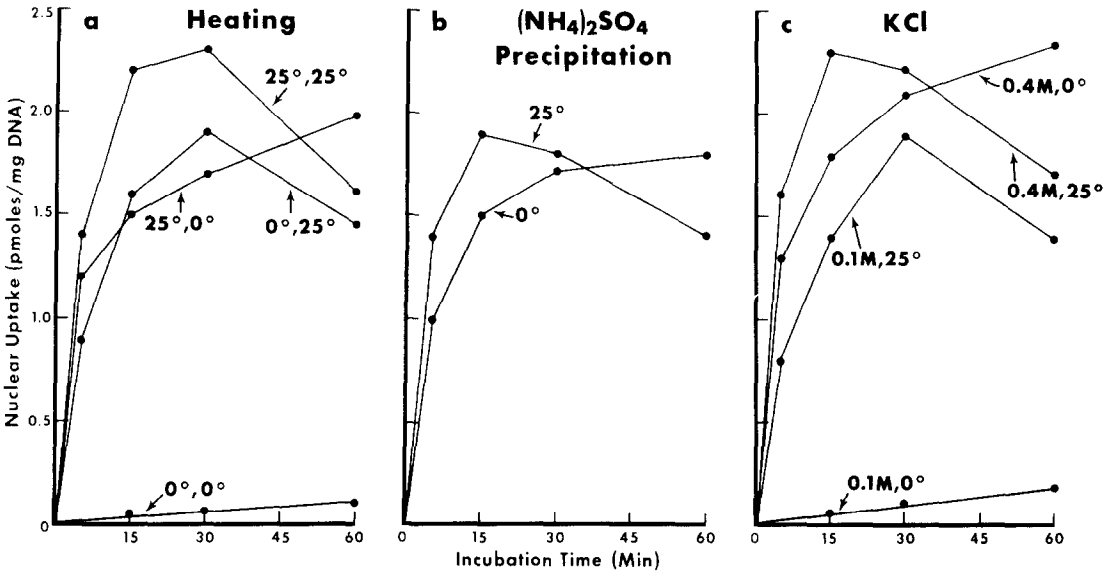

Fig. 3. Activation of [^{3}H]-dexamethasone-receptor complexes of fetal rabbit lung by heating or by exposure to high ionic strength. [^{3}H]-Dexamethasone cytosol mixtures were treated as follows: (a) incubated for 30 min at 0 or 25°; (b) precipitated with ammonium sulfate at 33% saturation followed by solubilization of the precipitate and desalting by gel filtration; and (c) exposed to 0·1 or 0·4 M KCl for 3 h at 0°C following which the concentration of KCl in all samples was adjusted to 0·1 M. Aliquots from each preparation (a, b or c) were mixed with lung nuclei and incubated for various time periods at 0 or 25°C. The nuclei were reisolated by centrifugation, washed, and assayed for radioactivity. All values were corrected for nonspecific binding. The first of each pair of temperatures shown in (a) is the incubation temperature of the hormone–cytosol mixture; the latter is the incubation temperature after addition of the nuclei. The first number of each curve shown in (c) is the molarity of KCl to which the hormone–cytosol mixture was exposed and the second number is the incubation temperature after addition of the nuclei.

affinity for the nuclei. This step is highly dependent on temperature and ionic strength and does not require the nuclei; and (iii) finally, the third step involves translocation of the activated complex into the nucleus where it is retained by binding to hypothetical "acceptor sites". The latter sites seem to involve DNA since the nuclear steroid-receptor complex is released by DNase (not shown).

Formation of cortisol from cortisone in fetal rabbit lung

In addition to its ability to retain specifically glucocorticoids, the fetal rabbit lung is efficient in converting the biologically-inactive cortisone to the biologically-active cortisol. Conversion of cortisol to cortisone is much less extensive (Table 1). The high efficiency of fetal lung to metabolize cortisone to cortisol is further indicated by the finding of similar amounts of cortisol bound to nuclear receptors after incubation of fetal lungs at 37°C with either steroid (Table 1).

Ontogeny of glucocorticoid receptors in fetal lungs of the rabbit and other species

Glucocorticoid receptors have also been detected in the lungs of rat, guinea pig and human fetuses. The pulmonary receptors have been detected as early as the 21st day of gestation in the rabbit, the 19th day of gestation in the rat, the 35th day of gestation in the guinea pig and the 9th week of gestation in the human (Table 2). The earliest time of their appearance in all these species is not known because of technical limitations for detection at earlier stages. The results, however, show that the glucocorticoid receptors are present in the fetal lungs of the species examined long before the appearance of surfactant in the alveolar lining and are compatible with the ability of exogenous glucocorticoids to cause precocious appearance of pulmonary surfactant [2, 21].

Although glucocorticoid receptors were demonstrated in fetal lungs of all species examined, they could not be detected in the lungs of mature rats, mice, guinea pigs and hamsters (Table 2). The only

Table 1. *In vitro* uptake and metabolism of cortisone and cortisol by fetal rabbit lung

Precursor	Metabolite	% Conversion	Nuclear uptake (pmol/mg DNA) Total	Bound
Cortisol	Cortisone	10–14		
	Cortisol	68–75	1·60	0·65
Cortisone	Cortisol	64–71	1·32	0·59
	Cortisone	12–17		

Table 2. Cytoplasmic glucocorticoid receptors in lungs of different species at various developmental stages

Species	Developmental stage	Specific [^{3}H]-dexamethasone binding (pmol/mg protein)
Rabbit	Fetus, 21 days	0·45 ± 0·03
	Fetus, 28–30 days	0·52 ± 0·04
	Adult	0·27 ± 0·05
Rat	Fetus, 19·5–21·5 days	0·22 ± 0·04
	Newborn,	0·05 ± 0·02
	2 Days after birth	0·02 ± 0·01
	Adult	0·01 ± 0·01
Guinea pig	Fetus, 35–45 days	0·04 ± 0·01
	Adult	0·01 ± 0·01
Human	Fetus, 9–10 weeks	0·07 ± 0·01
	Fetus, 15–17 weeks	0·18 ± 0·04
Hamster	Adult	0·02 ± 0·01
Mouse	Adult	0·01 ± 0·01

exception was the rabbit in which significant amounts of the receptor could be detected in lungs during adult life. In the rat, the pulmonary glucocorticoid receptor became undetectable shortly after birth (Table 2). These observations suggest that the mammalian lung may be responsive to glucocorticoids only during the period of cellular maturation and biochemical differentiation. As such, the responsiveness of the lung to glucocorticoids is a developmental phenomenon.

Glucocorticoid receptors in other fetal tissues

Besides the lung, several other tissues of the rabbit fetus contain specific binding sites for glucocorticoids (Table 3). On a per DNA basis, the concentration of the receptors is 3–5 times higher in placenta, kidney, lung and skin than in liver, thymus and brain. Intermediate concentrations are present in muscle, heart and small intestine. Differences in the concentration of receptors in various tissues, however, are difficult to interpret since these studies do not permit estimation of the receptor in specific cell types. Nevertheless, these findings suggest that glucocorticoids may have a fairly general role as growth or maintenance hormones with a broader tissue specificity than was previously anticipated.

Ontogeny of glucocorticoid receptors in rat liver

Administration of glucocorticoids to rat fetuses fails to cause an increase in tyrosine aminotransferase activity in the fetal liver but hepatic enzyme activity increases markedly when the steroids are administered to postnatal rats [23]. It has been reported [24] that dexamethasone binding activity is virtually absent in liver cytosol of fetal rats but increases to adult levels 1–2 days after birth. This suggested that the reason for the failure of glucocorticoids to induce tyrosine aminotransferase in fetal rats is either that the receptor is absent from the fetal liver or that its binding activity is masked by endogenous steroids.

Our own studies on the interaction of glucocorticoids with cytosol of the developing rat liver are illustrated in Fig. 4. In agreement with the results of others [24], we could not detect specific binding of dexamethasone in liver cytosol of fetuses sacrificed immediately after birth. However, significant dexamethasone binding activity is detectable in liver cytosol of fetuses at the 20th day of gestation. The binding sites of fetal liver cytosol have high affinity (Kd $\sim 5 \times 10^{-9}$ M) for dexamethasone and become saturated at a hormone concentration of about 4×10^{-8} M. High affinity binding sites for dexamethasone are also detectable in liver cytosol of postnatal

Table 3. Specific binding of [^{3}H]-dexamethasone in cytosols of fetal rabbit tissues

Tissue	Specific [^{3}H]-dexamethasone binding (pmol/mg protein)	(pmol/mg DNA)
Kidney	0·46 ± 0·04	1·83 ± 0·10
Lung	0·51 ± 0·05	1·62 ± 0·13
Fetal placenta	0·25 ± 0·04	1·78 ± 0·15
Skin	0·23 ± 0·02	1·25 ± 0·13
Muscle	0·21 ± 0·02	0·95 ± 0·09
Small intestine	0·17 ± 0·01	0·80 ± 0·07
Heart	0·19 ± 0·02	0·69 ± 0·11
Liver	0·17 ± 0·02	0·49 ± 0·05
Brain	0·08 ± 0·01	0·41 ± 0·06
Thymus	0·22 ± 0·03	0·35 ± 0·04

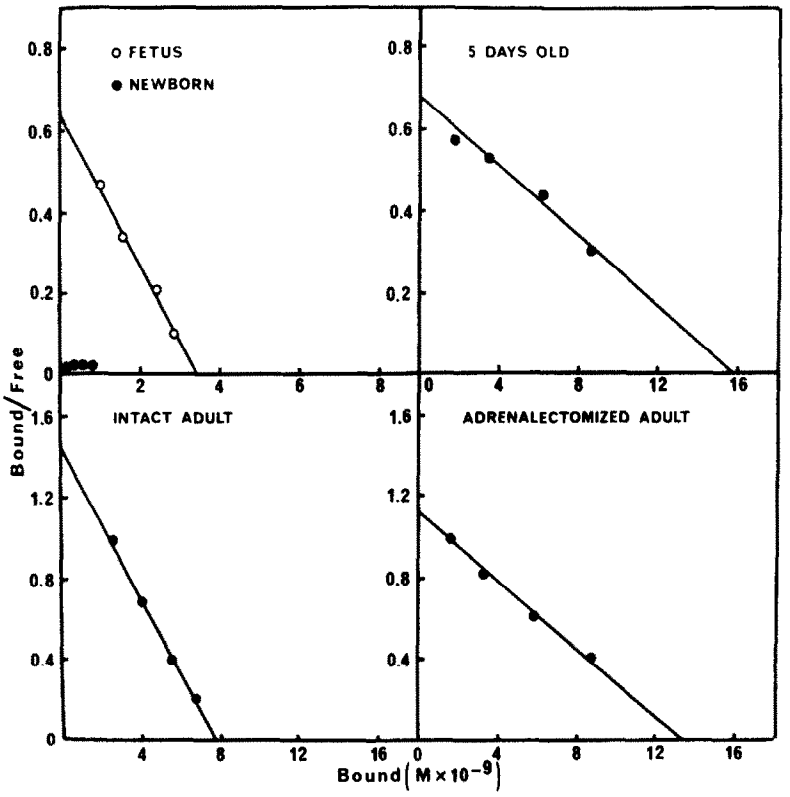

Fig. 4. Specific binding of [^{3}H]-dexamethasone to cytosol of the developing rat liver. Cytosols were incubated for 4 h at 0°C with increasing concentrations of [^{3}H]-dexamethasone in the presence or absence of competing steroid. Specifically bound hormone was estimated by a charcoal assay [16] and the data were plotted by the Scatchard method.

rats (Fig. 4) and their concentration increases shortly after birth reaching higher than adult levels on or before the 5th day (Table 4). In the adult animal, binding activity is increased significantly after adrenalectomy.

Specific binding sites for dexamethasone could also be detected in liver cytosol of fetuses older than 20 gestational days but they became undetectable by the method used 6–24 h before parturition and for a short time after parturition (not shown). The receptors could be detected again within 1–2 h after birth and their levels continue to rise thereafter. Thus hepatic glucocorticoid receptors could not be detected only during a short period before and after parturition. Since this period is characterized by a marked elevation in the levels of corticosteroids in the fetal circulation [25], the failure to detect specific binding in liver cytosol is most probably due to masking of the binding sites by endogenous steroids. This is further suggested by the finding of specific, saturable, dexamethasone-binding sites in liver nuclei of newborn rats (Fig. 5) despite the failure to detect binding of the hormone in the cytosol of the same livers. Indeed, the concentration of nuclear binding sites is higher in newborn liver than in liver of 20-day fetuses (Fig. 5) whose cytosol contained detectable glucocorticoid receptors. Since it is generally accepted that nuclear steroid receptors in target tissues originate from the cytoplasmic receptor [15], these results can best be explained by the formation of cytoplasmic dexamethasone-receptor complexes during tissue incubation at 37°C through an exchange of receptor–bound endogenous corticosterone with dexamethasone, the latter having a higher

Table 4. Concentration of cytoplasmic dexamethasone receptors in the developing rat liver

Age	Specific [^{3}H]-dexamethasone binding (pmol/mg protein)	(pmol/mg DNA)
19-Day-old fetus	0·17	0·76
Newborn	0·00	0·00
5 Days after birth	0·62	6·4
Adult (intact)	0·28	4·7
Adult (adrx)	0·67	9·6

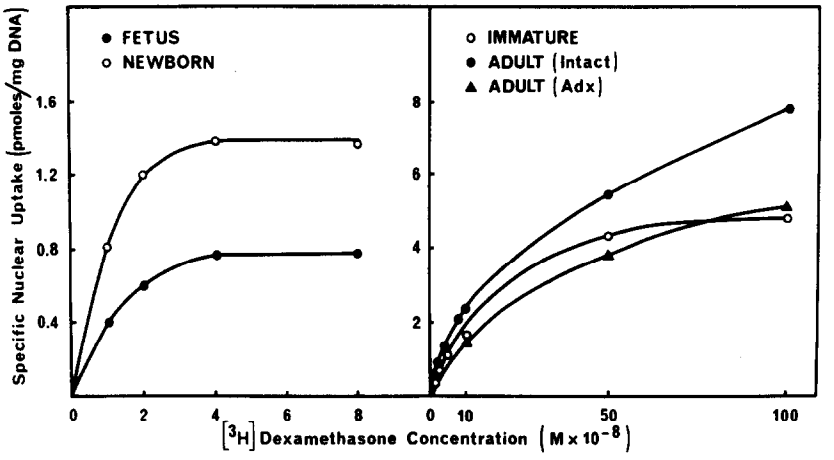

Fig. 5. Specific uptake of [^{3}H]-dexamethasone by nuclei of the developing rat liver. Liver slices were incubated in Eagle's HeLa medium for 2 h at 37°C with increasing concentrations of [^{3}H]-dexamethasone. Purified nuclei were prepared and assayed for radioactivity [17].

affinity than corticosterone for the receptor sites. The dexamethasone-receptor complexes thus formed would then be transferred into the nucleus.

The above observations indicate that the failure of glucocorticoids to increase tyrosine aminotransferase in rat fetuses *in utero* is not due to the absence of steroid receptors in fetal liver. This conclusion is supported by the finding that glucocorticoids can increase manifold tyrosine aminotransferase activity of rat fetal liver in organ culture provided that the explants have been subjected to a preliminary incubation in the absence of steroid [26]. Recently, it was shown that there are large differences in the ability of fresh and aged explants to convert cortisol to inactive metabolites [27]. In fresh explants (non responsive) 75% of added cortisol was converted to inactive metabolites while in aged explants (hormone responsive) 80% of cortisol was recovered unaltered. Therefore control of response may in part be achieved by a mechanism which controls the levels of active steroid within the cell. Such a mechanism is unlikely to be totally responsible for the failure of glucocorticoids to increase enzyme activity in fetal liver *in utero* since fetal corticosteroids reach high levels near term [25] and the metabolic activity of the liver increases with age [28]. Additional hormonal or other inhibiting factors may also be involved.

The postnatal increase in the concentration of cytoplasmic receptor sites (Table 4) is accompanied by a similar age-related increase in specific uptake of glucocorticoids by liver nuclei (Fig. 5). The nuclear binding sites of fetal and newborn liver are saturated at a dexamethasone concentration near 4×10^{-8} M. However, saturation of the nuclear binding sites of adult liver is not reached even at a dexamethasone concentration as high as 1×10^{-6} M. Although this apparent difference between fetal and adult nuclei in their affinity for dexamethasone may be due to an increased inactivation of the steroid in the adult tissue, the physiological significance of the nuclear binding sites of adult liver may be questioned particularly since synthetic glucocorticoids are known to be more resistant to metabolic inactivation than natural corticosteroids [29].

It is, however, possible that the binding sites of adult liver nuclei are not homogeneous and they may include both high and low affinity sites. This hypothesis is supported by the observations shown in Fig. 6. Brief suspension of purified nuclei prepared from adult liver exposed to increasing concentrations of dexamethasone results in release of a substantial fraction of the hormone. The amount of hormone in the nuclear wash increases linearly throughout the range of dexamethasone concentrations (4×10^{-8} to 1×10^{-6} M) to which the tissue was exposed. Thus adult liver nuclei appear to contain a class of low affinity sites for dexamethasone which are not saturated with hormone concentrations up to 1×10^{-6} M. These sites, however, are saturable since they were estimated after correction for non-specific binding by the use of an excess (1×10^{-4} M) of nonlabeled dexamethasone. Association of dexamethasone with washed purified nuclei approaches saturation at hormone concentrations between 5×10^{-7} and 1×10^{-6} M and, therefore, represents binding to nuclear sites having a higher affinity for the hormone. On the basis of extractability of nuclear dexamethasone with Triton X-100 or with 0·4 M KCl solutions, these high affinity sites appear to be heterogeneous. A significant fraction (~20%) of nuclear dexamethasone is released by Triton X-100 suggesting an interaction of the hormone with nuclear membranes. Of the remaining dexamethasone associated with the nuclei after treatment with Triton X-100 only about half is extractable with 0·4 M KCl suggesting the presence of more than one type of steroid binding site on the chromatin.

The interaction of dexamethasone with fetal liver nuclei shows a pattern significantly different from that described for the adult liver (Fig. 6). No evidence for the presence of low affinity sites or for

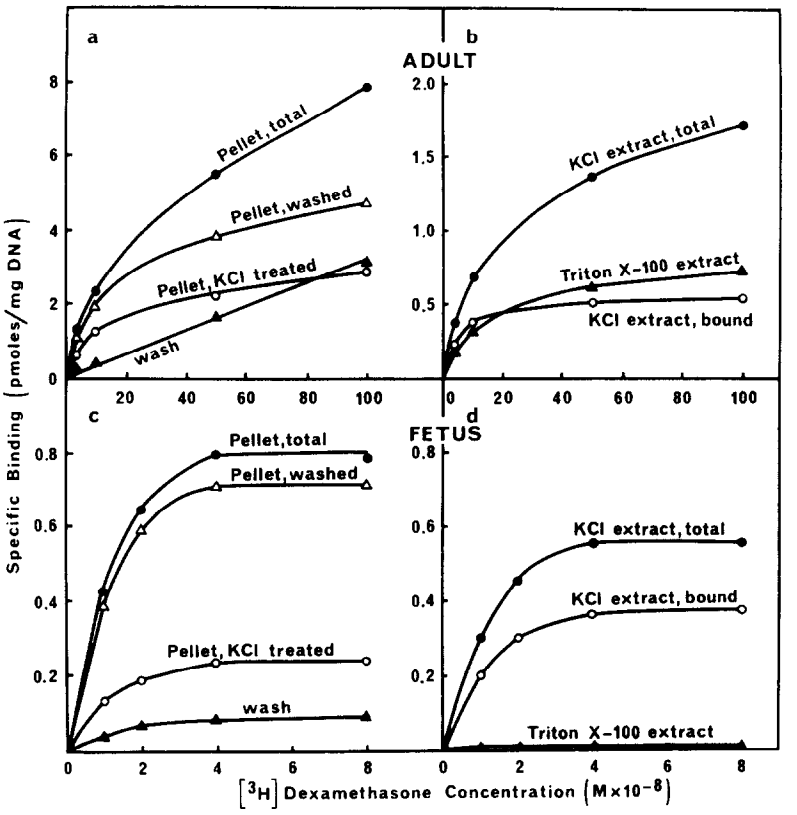

Fig. 6. Evidence suggesting heterogeneity of nuclear binding sites of adult rat liver and comparison with nuclear sites of fetal rat liver. Liver slices were incubated in Eagle's HeLa medium for 2 h at 37°C with increasing concentrations of [^{3}H] dexamethasone and purified nuclei were prepared as described earlier [17]. Nuclei were suspended briefly (5 min) in buffered 0·25 M sucrose and reisolated by centrifugation. The washed pellet was extracted either with 0·4 M KCl or with 0·2% Triton X-100. Radioactivity remaining in pellet after extraction with KCl or with Triton X-100 was extracted with ethanol. Bound hormone in KCl extracts was estimated by gel filtration. Panels a and c show the radioactivity present in purified nuclear pellets before any treatment (total), after washing with sucrose (washed) or after extraction with KCl (KCl extracted) as well as the radioactivity released from the nuclei by washing with sucrose (wash). Panels b and d show the radioactivity extracted with 0·4 M KCl (total) or with Triton X-100 and the radioactivity in KCl extracts that was excluded from Sephadex G-50 (bound).

hormone interaction with nuclear membranes was obtained. Most of the nuclear dexamethasone is extractable with 0·4 M KCl and a major fraction of the extracted hormone is excluded from Sephadex G-50. Thus in contrast to adult liver nuclei, fetal liver nuclei appear to contain a single class of binding sites for dexamethasone. It is noteworthy that, although the total nuclear binding capacity of adult liver is at least ten times higher than that of fetal liver (Fig. 5), the amount of protein-bound dexamethasone extractable with 0·4 M KCl is similar at both developmental stages (Fig. 6).

Comparison between glucocorticoid receptors of fetal and adult rat liver

Apart from the observed differences in the interaction of dexamethasone with fetal and adult liver nuclei, the following observations suggest that glucocorticoid receptors of fetal and adult rat liver may not be identical.

a. Cytoplasmic dexamethasone-receptor complexes of adult liver have a higher dissociation constant at 0°C than complexes of fetal liver (Table 5). This difference does not appear to be due to endogenous steroids since it persists in the adult rat after adrenalectomy.

b. Fetal steroid-receptor complexes sediment in sucrose gradients somewhat slower than complexes of adult liver (Table 5).

c. Cytoplasmic receptors of fetal and adult liver differ in their relative affinity for cortisol and corticosterone. The fetal receptors have a significantly higher affinity for corticosterone than for cortisol.

Table 5. Dissociation constants and sedimentation coefficients of cytoplasmic dexamethasone-receptor complexes of fetal and adult rat liver

	K_d (at 0°C) (M)	Sedimentation coefficient*
Fetal	5×10^{-9}	7S
Adult	$1{\cdot}4 \times 10^{-8}$	7·5S

* Estimated by centrifugation in 5–20% sucrose gradients of low ionic strength.

Table 6. Inhibition of [^{3}H]-dexamethasone binding to cytoplasmic and nuclear sites of fetal and adult rat liver by cortisol and corticosterone

Competitor (M)	% Inhibition of [^{3}H]-dexamethasone* binding			
	Cytoplasmic		Nuclear	
	Fetal	Adult	Fetal	Adult
Cortisol				
(2×10^{-7})	58	38	42	5
(2×10^{-6})	89	77	86	23
(2×10^{-5})	92	85	92	60
(1×10^{-4})	—†	—	—	85
Corticosterone				
(2×10^{-7})	73	34	26	0
(2×10^{-6})	91	73	78	10
(2×10^{-5})	92	83	88	39
(1×10^{-4})	—	—	—	83

* 2×10^{-8} M.
† Not examined.

In adult liver, both hormones have approximately the same affinity for the receptor sites (Table 6).

It is therefore possible that subtle differences in the nature of hepatic glucocorticoid receptors may be partly responsible for the maturation-dependent qualitative differences in tissue responsiveness to glucocorticoids.

It is well known that cortisol is a much more potent glucocorticoid than corticosterone *in vivo*. However, corticosterone has a similar or higher affinity compared to cortisol for the cytoplasmic receptor sites of rat liver (Table 6). Thus the greater biological potency of cortisol does not appear to be due to its higher affinity for receptor sites. Other factors such as differences in the ability of liver to inactivate cortisol and corticosterone may be responsible for the relative biological activity of the two steroids *in vivo*. This is supported by the observation that cortisol is more efficient than corticosterone in inhibiting the *in vitro* nuclear uptake of dexamethasone in intact fetal or adult liver slices at 37°C (Table 6). Therefore higher levels of circulating corticosterone may be required for intracellular accumulation of sufficient amounts of unaltered hormone due to its rapid inactivation in the liver.

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