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BINDING OF GLUCOCORTICOIDS IN FETAL TISSUES

S. SOLOMON and D. K. H. LEE
Departments of Biochemistry and Experimental Medicine, McGill University and the University Clinic, Royal Victoria Hospital, Montreal, Quebec, Canada

SUMMARY

Glucocorticoids enhance the activity of a number of enzyme systems in fetal tissues. In the rabbit fetal lung [^{3}H]-dexamethasone binds to cytosol and nuclear receptors with a high degree of specificity which is not competed for by other hormones or metabolites. The cytosol [^{3}H]-dexamethasone–receptor complex sediments at 4 *s* in sucrose density gradients in the presence of 0.6 M KCl and at approximately 7–8 *s* in the absence of salt, whereas the nuclear complex sediments at approximately 4–5 *s* in the presence or absence of 0.4 M KCl. At 37°C the saturation of nuclear binding sites is reached with 5×10^{-8}–1×10^{-7} M [^{3}H]-cortisol or with 1×10^{-8} M [^{3}H]-dexamethasone. There is an increase in the uptake of [^{3}H]-cortisol by lung nuclei (c.p.m./mg DNA) with advancing gestation, beginning on day 20 and reaching a maximum on day 28–30. A lower uptake is observed in lung nuclei of the newborn, the one-month-old and the adult rabbit. The maximum uptake of [^{3}H]-cortisol by fetal lung nuclei correlates well with the concentration of surface-active phospholipids extracted from lungs of rabbit fetuses.

[^{3}H]-Dexamethasone binds to macromolecules in the cytosol and nuclei of fetal rabbit small intestine. The binding is specific for steroids with glucocorticoid potency. There is a parallelism between the increase in the total number of glucocorticoid binding sites in the cytosol and nuclei and the increase in tissue weight up to term. The concentration of binding sites (pmol/mg protein or DNA) is maximum at day 25 to 26 of gestation, followed by a decrease to adult levels within a few days of birth. Alkaline phosphatase activity is first detectable on day 25 of gestation and increases rapidly thereafter. There seems to be a temporal relationship between the development of the fetal rabbit small intestine and the level of glucocorticoid receptors in the cytosol and nuclei.

INTRODUCTION

Following on the classic work of Moog[1] it has been well established that glucocorticoids play an important role in the maturation of a number of fetal tissues. Glucocorticoids are also known to induce a large number of enzymes in developing tissues. It has been postulated that the initial stage in the action of glucocorticoids is binding to specific cytoplasmic sites, here referred to as receptors [2, 3]. Following on the binding to specific receptors the hormones, and particularly glucocorticoids, can trigger in target cells specific changes in macromolecular synthesis. The exact mechanism of how this is accomplished in fetal tissues remains to be elucidated. During development the action of a hormone such as glucocorticoid is the end point of several complex events such as the stage of maturation of the tissue and whether the process under control is stimulated during fetal life or in the postnatal period [4]. Several model systems have been employed to study the action of glucocorticoids during development. These have included the embryonic neural retina where glucocorticoids induce glutamine synthetase [5], fetal rat liver [6], and chick embryonic liver [7]. In our laboratory, we have used the rabbit fetal lung, fetal small intestine and fetal kidney as model systems for studying glucocorticoid action. In this discussion, we will confine ourselves to the rabbit fetal lung and fetal small intestine.

The fetal lung was chosen as a model system because it was shown that glucocorticoids could accelerate lung maturation and the synthesis and release of pulmonary surfactant in the fetal lamb [8] and rabbit [9]. Glucocorticoids were shown to have the ability to mature the fetal small intestine and to induce alkaline phosphatase in this process [1].

EXPERIMENTAL

All of the methods used in the studies to be described have been published in detail [10–15].

RESULTS AND DISCUSSION

Glucocorticoid receptors in fetal lung. Because cortisol binds to corticosteroid binding globulin (CBG) in plasma, we turned to the use of [^{3}H]-dexamethasone in studying cytoplasmic binding in rabbit fetal lung as this corticosteroid is not bound to CBG. Initially we determined the saturation kinetics using both [^{3}H]-cortisol and [^{3}H]-dexamethasone. Using rabbit fetal lung nuclei we determined that with [^{3}H]-cortisol in the medium in the concentration

range of 5×10^{-8}–1×10^{-7} M saturation of the nuclear binding sites is attained while with [^{3}H]-dexamethasone saturation of the binding sites is realized with a concentration of approximately 1×10^{-8} M [16]. In addition, it was also shown that most of the binding is specific for both cytosol and nuclear binding macromolecules [11, 12]. A Scatchard plot of the specific binding data for fetal lung cytosol was linear and the apparent dissociation equilibrium constant for the binding at 0°C was estimated to be 3.8×10^{-9} M [12]. This indicated a single class of receptor sites with high affinity for dexamethasone and from the intercept with the abscissa it was possible to estimate that the concentration of glucocorticoid receptor sites in lung cytosol was between 0.4 and 0.6 pmol per mg protein for rabbit fetuses at 28 days of gestation, when these measurements were performed. As is shown in Fig. 1, the [^{3}H]-dexamethasone macromolecule complex in lung cytosol sediments at about 7 *s* in sucrose gradients at low ionic strength. In sucrose gradients containing 0.4 M KCl (Fig. 1,b) the 7 *s*-dexamethasone–macromolecule complex sediments at approximately 4 *s*, which is in keeping with the behaviour of other steroid receptors described [3]. The [^{3}H]-dexamethasone macromolecule complex prepared with purified nuclei from rabbit fetal lung at day 28 of gestation, sediments at 4–5 *s* in sucrose gradients containing no KCl or the presence of 0.4 M KCl (Fig. 2).

The nature of the [^{3}H]-dexamethasone macromolecular complex from rabbit lung cytosol prepared at day 28 of gestation was studied in several ways [12]. Heating of the lung cytosol at 60°C for 5 min completely prevented binding with [^{3}H]-dexamethasone. Addition of p-chloromercuribenzoate (1 mM) to lung cytosol had the same effect while addition of N-ethylmelamide (1 mM) reduced the binding to [^{3}H]-dexamethasone by 50%. Treatment with Pronase led to a release of radioactivity from the complex but treatment with RNase and DNase was without effect [12].

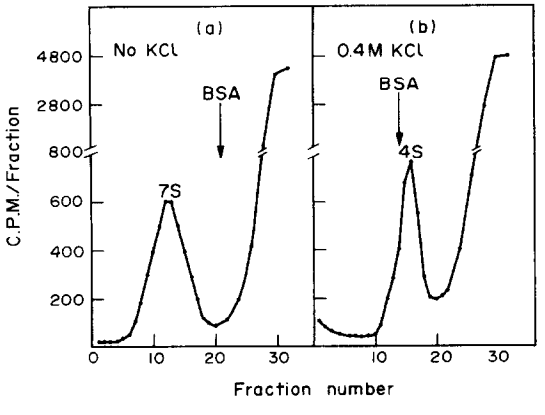

Fig. 1. Effect of KCl on the sedimentation rate of [^{3}H]-dexamethasone-binding components of lung cytosol. Gradients without KCl (a) were 10 to 30% sucrose and those containing 0.4 M KCl (b) were 5 to 20% sucrose. *BSA*, bovine serum albumin. From [12].

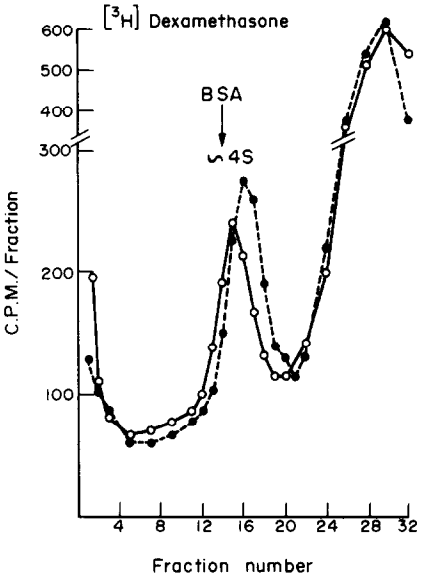

Fig. 2. Sucrose density gradient patterns of nuclear extracts from rabbit fetal lung. The tissue was incubated in Eagle's HeLa medium containing 2.5×10^{-8} M [^{3}H]-dexamethasone for 2 h at 37°. Purified nuclei were prepared and extracted with 0.01 M Tris–0.0015 M Na_2 EDTA–0.6 M KCl buffer pH 8.5. Aliquots (0.2 ml) of the nuclear extract were applied on 5 to 20% sucrose gradients prepared in 0.01 M Tris–0.0015 M Na_2 EDTA buffer, pH 7.6 without (○—○) or without (●---●) 0.4 M KCl. Centrifugation was performed at 297,000 × *g* at 2° for 16 h. From [11].

Similar results have been obtained with purified fetal lung nuclei [11] except that 23% of the radioactivity was released with DNase and 17% with RNase compared to 80% released with Pronase. These results indicate that the dexamethasone binding complex is a protein in nature and also point to a need for intact sulfhydryl groups in the interaction of the hormone with the cytosol and nuclear binding components.

Specificity of glucocorticoid binding. The [^{3}H]-dexamethasone bound to the fetal lung cytosol receptor is transferred to the nucleus in the same manner as has been described for other steroid receptor systems [3]. One of the ways to assess the specificity of the binding of a hormone to a receptor is to determine the competition of a variety of other steroid hormones and metabolites for the binding site. Table 1 contains such data for [^{3}H]-cortisol binding to purified rabbit fetal lung nuclei and competition for the binding sites by a 10-fold excess of non-labeled steroids added to the incubation medium. Testosterone, estradiol-17β, progesterone and aldosterone do not compete for the binding of [^{3}H]-cortisol, neither do the reduced metabolites of cortisol or 17α-hydroxyprogesterone, act as competitors. The ability to compete for the [^{3}H]-cortisol binding sites is proportional to the glucocorticoid potency of the steroids tested which are shown in Table 1 with two exceptions. These are cortisone and 21-deoxycortisol. At 37°C cortisone is converted to cortisol by rabbit fetal lung nuclei but the reverse reaction does not occur [17].

Table 1. Effect of nonlabeled steroids on nuclear uptake of [^{3}H]-cortisol in rabbit fetal lung

Competing steroid	Nuclear uptake of [^{3}H]-cortisol
Testosterone	100
Progesterone	100
Tetrahydrocortisol	100
Estradiol-17β	98
Aldosterone	95
3β,11β,17α,21-Tetrahydroxy-pregn-5-en-20-one	81
17α-Hydroxyprogesterone	81
Allotetrahydrocortisol	78
Deoxycorticosterone	74
11β-Hydroxyprogesterone	53
Corticosterone	47
11-Deoxycortisol	44
21-Deoxycortisol	34
Cortisone	25
Cortisol	19
6α-Methylprednisolone	13
9α-Fluorocortisol	9
Fluocinolone	8
Triamcinolone acetonide	8
Dexamethasone	7

From [11]

One-gram aliquots of rabbit fetal lung were incubated in 10 ml of Eagle's HeLa medium containing 1×10^{-7} M [^{3}H]-cortisol with or without a 10-fold excess of nonlabeled steroid at 37° for 2 h. Each value is the average of six determinations.

At 0°C, 21-deoxycortisol but not cortisone is still able to compete for the [^{3}H]-cortisol binding sites in fetal lung nuclei and it is unlikely that 21-hydroxylation can occur under these conditions. It is possible that 21-deoxycortisol is an antiglucocorticoid in fetal lung and a similar observation has been reported for this steroid in rat thymus [18] where it inhibits the binding of cortisol to the cytosol receptor and blocks the effect of cortisol on glucose uptake. Similar specificity data involving competition for [^{3}H]-dexamethasone binding to the lung cytosol receptor has been obtained [11].

Glucocorticoid binding as a function of gestation. Figure 3 shows the uptake of [^{3}H]-cortisol by fetal rabbit lung nuclei and fetal liver nuclei as a function of gestation, in the newborn, the month-old rabbit and in the adult. The [^{3}H]-cortisol uptake by lung nuclei reaches a maximum at about 28–30 days of gestation, falls slightly in the newborn and stays constant in the one-month-old and in the adult rabbit. The [^{3}H]-cortisol uptake by lung nuclei is 4–5 times higher than the uptake by liver nuclei pointing to the fetal lung rather than the liver, in this species, as the prime target for glucocorticoids. The maximum [^{3}H]-cortisol uptake by fetal lung nuclei at 28–30 days of gestation correlates well with the time when optimum amounts of surface active phospholipids can be extracted from rabbit fetal lungs [19, 20]. The increase of [^{3}H]-cortisol uptake by fetal lung nuclei with advancing gestation was not observed with [^{3}H]-dexamethasone uptake to lung cytosol as a function of gestation.

Glucocorticoid receptors in fetal tissues. Ballard and Ballard[21] described the [^{3}H]-dexamethasone binding to the cytoplasmic fraction of human fetal lung. Some of their data for the human fetus is shown in Table 2. It is apparent from this data that the highest uptake of [^{3}H]-dexamethasone is to fetal lung cytosol followed by the small intestine, liver and kidney. On the basis of mg DNA, muscle has a high cytoplasmic uptake of glucocorticoid.

Specific binding of [^{3}H]-dexamethasone has been found in the cytosol fraction of all fetal tissue examined. The concentration of [^{3}H]-dexamethasone required for the saturation of specific binding sites of the cytosol fractions examined ranged between 1×10^{-8} and 3×10^{-8} M. In all instances the Scatchard plots of the data were linear and the apparent dissociation constant of [^{3}H]-dexamethasone binding was in the range of 1.5×10^{-9}–8×10^{-9} M. The concentration of specific binding sites for [^{3}H]-dexamethasone in the cytosols of the different fetal tissues studied is shown in Table 3. This data is calculated as pmol of bound steroid per mg of cytosol protein or per mg of tissue DNA. On the basis of DNA, the amount of bound [^{3}H]-dexamethasone was 3–5 times higher for placenta, kidney, lung and skin than for liver, thymus and brain. Muscle, small intestine and heart showed intermediate concentrations.

Comparison of fetal and adult rabbit tissues. In Figure 4 are shown the sucrose gradients of cytosols prepared from fetal tissues of rabbits at 28–30 days gestation and adult females (2.3–3.1 Kg). With the

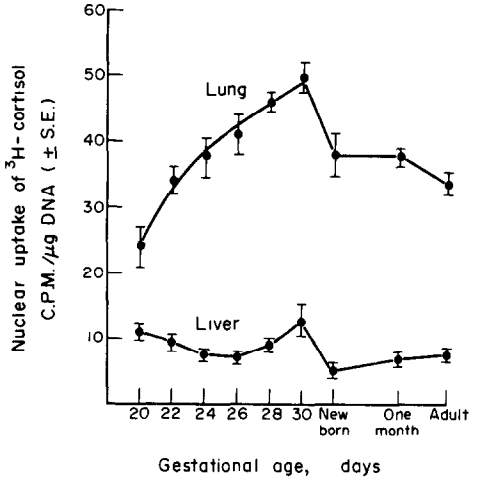

Fig. 3. Changes in uptake of [^{3}H]-cortisol (per unit DNA) by lung and liver nuclei of the developing rabbit fetus and of newborn and adult rabbits. Each point represents the mean of 5–6 determinations. For each determination, lung from all fetuses of the same litter (5–10 fetuses) were pooled, the tissue was minced and a 1 g aliquot of the minces was incubated in 10 ml Eagle's HeLa medium containing 1×10^{-7} M ^{3}H-cortisol for 2 h at 37° C. From [10].

Table 2. Occurrence of cytoplasmic receptor in various tissues of a 16-wk gestation human fetus: site concentrations and equilibrium dissociation constants

Tissue	Receptor site concentration		Dissociation constant
	pmol/mg protein	pmol/mg DNA	nM
Lung	0.21	0.78	5.7
Small intestine	0.16	0.64	5.6
Liver	0.13	0.23	4.3
Kidney	0.13	0.60	3.1
Heart	0.07	0.27	—
Muscle*	0.06	0.68	5.5
Skin*	0.015	0.25	- -

* Obtained from upper leg.
From [21]

exception of adult heart and muscle, the cytosol fraction of all tissues studied contained macromolecules which bound [^{3}H]-dexamethasone and the complexes sedimented at 7–8 *s*. This indicates that similar dexamethasone receptors are present in different rabbit tissues during both fetal life and in the adult. The cytosol of liver, kidney, small intestine and spleen has a binding complex which sedimented at 4 *s* in addition to the 7–8 *s* complex suggesting that there are differences in the nature of the glucocorticoid binding of the different rabbit tissues.

Binding of glucocorticoids to rabbit fetal small intestine. Following the incubation of [^{3}H]-cortisol or [^{3}H]-dexamethasone with minces of rabbit fetal small intestine taken from fetuses on day 28 of gestation, the subcellular distribution of radioactivity found is shown in Table 4. Most of the radioactivity from [^{3}H]-cortisol and [^{3}H]-dexamethasone is in the nuclei or in the cytoplasmic fraction. When cytosols of the fetal rabbit small intestine were subjected to sucrose gradient centrifugation in the absence of KCl, two peaks of radioactive material were found sedimenting at approximately 4 *s* and 7 *s* (Fig. 5a). In the presence of 0.4 M KCl, the [^{3}H]-dexamethasone complex sediments only at 4 *s* (Fig. 5b). In the absence of salt and with the addition of a 10-fold excess of non-labeled dexamethasone, the amount of radioactivity bound to the 4 *s* and 7 *s* components were greatly reduced (Fig. 5a). This indicates that dexamethasone binds to both 4 *s* and 7 *s* components in the fetal small intestine and that there is a limited number of binding sites in the cytosol from the fetal small intestine.

We were able to demonstrate that the binding components in the cytosol and nuclei were proteins in nature and that the binding was specific by doing competition studies similar to those described for the fetal lung in Table 1.

In order to study the translocation of the [^{3}H]-dexamethasone–macromolecular complex from the cytosol to the nucleus minces of fetal small intestine were incubated with [^{3}H]-dexamethasone at 0°C for 90 min. Then the minces were transferred to a steroid-free medium and incubated at 37°C for 0,5 and 60 minutes. Then cytosol fractions and purified nuclei were prepared and submitted to sucrose density gradient centrifugation and the results are shown in Fig. 6. At the time of transfer (designated as 0 time) almost all of the bound radioactivity was found in the cytosol. When the temperature was raised to 37°C and the minces incubated in the steroid-free medium for 5 min, there was a rapid fall of bound radioactivity in the cytosol which did not change at 60 min and a concomittent rapid increase in bound radioactivity in the purified nuclei. At 60 min the nuclei had most of the bound radioactivity which was present as a 4 *s* complex whereas in the cytosol from whence it came had both 7 *s* and 4 *s* complexes.

Correlation of dexamethasone binding and alkaline phosphatase activity. Alkaline phosphatase activity reflects the development of the fetal small intestine and is known to increase with advancing gestation [22]. In order to compare the cytosol binding of [^{3}H]-dexamethasone with alkaline phosphatase activity we measured specific binding and then made Scatchard plots to measure the K_D and the number of glucocorticoid binding sites. The data shown in Fig. 7 were expressed in two ways: as a function of the total weight of intestine (Fig. 7A) and on the basis of mg of cytosol protein (Fig. 7B). The specific dexamethasone binding sites expressed as pmol per total weight of fetal small intestine was low at day 21 and increased after day 23 of gestation until term (Fig. 7A). The increase in the number of binding sites closely parallels the increase in the weight of the fetal small intestine shown in Table 5. When the concentration of binding sites was expressed as pmol per mg cytosol protein, there was an increase which attained a maximum value on about day 25 of gestation and was followed by a gradual decrease to

Table 3. Concentration of specific [^{3}H]-dexamethasone-binding sites in cytosols of fetal tissues

Tissue	Concentration of binding sites*	
	pmoles/mg protein	pmoles/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

* Calculated from Scatchard plots of binding data. Each value is the mean ± S.E.M. of six determinations and is expressed as picomoles of specifically bound [^{3}H]-dexamethasone per mg of cytosol protein or per mg of tissue DNA.
From [13].

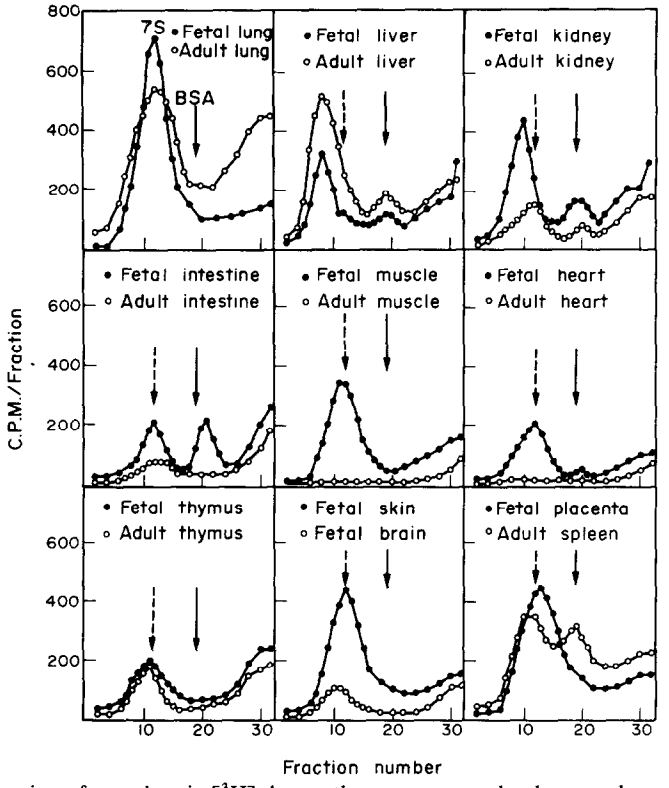

Fig. 4. Sedimentation of cytoplasmic [^{3}H]-dexamethasone-macromolecular complexes of various fetal and adult rabbit tissues in sucrose gradients. Cytosols prepared from different tissues were incubated for 2 h at 0° with 1×10^{-8} M [^{3}H]-dexamethasone. Following incubation, the samples were mixed briefly with charcoal and centrifuged at 900 × *g* for 5 min to remove the unbound labeled steroid. Aliquots of the supernatants (0.2 ml) were layered on 10 to 30% sucrose gradients prepared in Tris-EDTA buffer and centrifuged for 16 h at 297,000 × *g* at 2 in a Spinco SW 56 rotor. Fractions were collected from the bottom of the tubes and assayed for radioactivity. *Solid arrows*, the sedimentation position of bovine serum albumin (*BSA*, 4.6 *s*); *broken arrows*, the 7 *s* region of the gradients. From [13].

adult levels a few days after birth (Fig. 7B). Similar data was obtained when the concentration of binding sites was expressed as pmol/mg DNA. In Table 5, it is seen that the cytosol protein increased linearly

Table 4. Subcellular distribution of radioactivity in fetal small intestine following incubation with [^{3}H]-dexamethasone

Subcellular fractions	% of Total Radioactivity	
	[^{3}H]-Cortisol	[^{3}H]-Dexamethasone
Purified nuclei	18 ± 3.2	13 ± 2.1
Mitochondria	5 ± 0.5	0
Microsomes	2 ± 0.4	3 ± 1.3
Cytosol	75 ± 2.7	84 ± 3.1

Half-gram portions of minced small intestine from fetal rabbits of 28–30 days of gestation were incubated with 20 nM of [^{3}H]-dexamethasone or [^{3}H]-cortisol in 10 ml of Eagle's basal medium at 37° for 2 h. Various subcellular fractions were then prepared. The contents of radioactivity for each fraction was determined and is corrected for non-specific uptake by running parallel experiments with the same amount of labeled hormone plus a 1000-fold excess of non-labeled steroid. Each value represents the mean ± S.E.M. of 5 determinations.

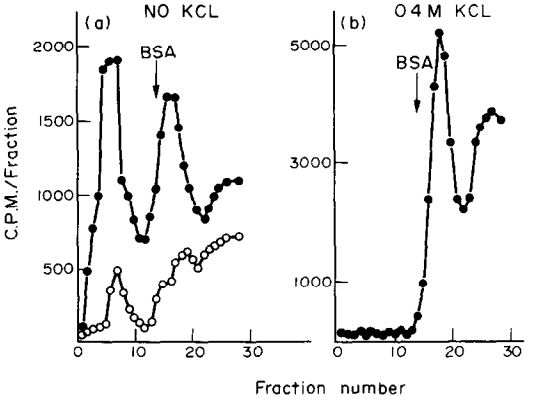

Fig. 5. Sedimentation profile in sucrose density gradients of cytoplasmic [^{3}H]-dexamethasone–receptor complexes of small intestine from fetal rabbits of 27–29 days gestation. Cytosol was incubated at 4°C with 10 nM [^{3}H]-dexamethasone in the absence (closed circles) or presence (open circles) of 100 nM of non-labeled dexamethasone. The [^{3}H]-dexamethasone–receptor complexes were first separated from the free steroids by the charcoal method. Aliquots of the charcoal supernatant (0.2 ml) were then layered on 10–30% sucrose gradients prepared in Tris-EDTA buffer without (a) or with (b) 0.4 M KCl. The gradients were centrifuged at 297,000 *g* at 4°C for 16 h. Fractions were collected from the bottom of the tubes. BSA, bovine serum albumin. From [15].

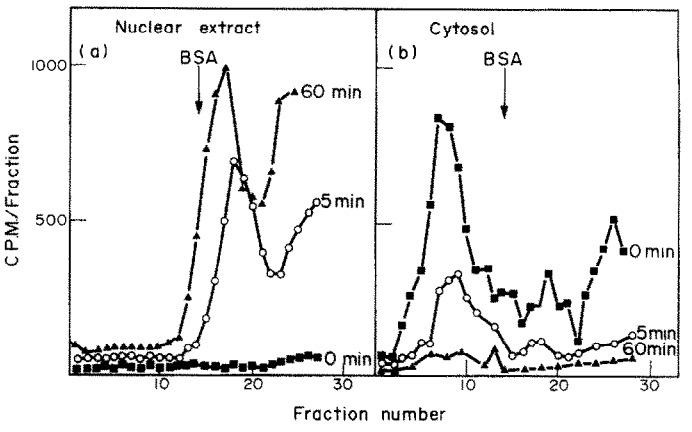

Fig. 6. Sedimentation profiles in 10–30% sucrose density gradients of bound radioactivity in the cytosol and nuclear extracts of samples incubated in the steroid-free medium at 37° for 0, 5, and 60 min. Experimental details are given in the text.

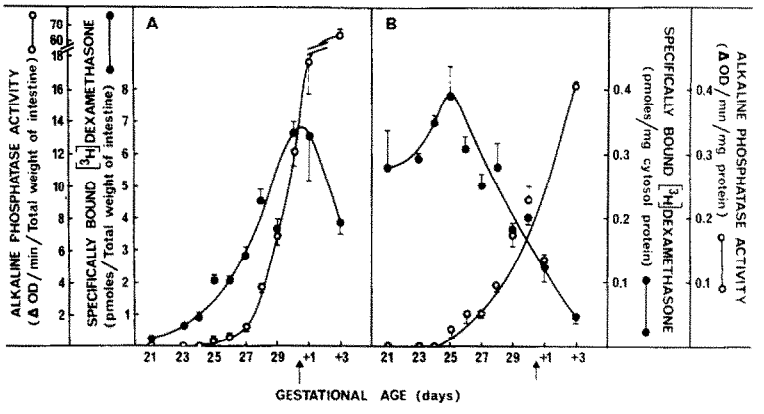

Fig. 7. Changes in the number of specific cytosol dexamethasone binding sites and alkaline phosphatase activity in the small intestine during development. Rabbits from 21 days of gestation to 3 days after birth were used. Specific [^{3}H]-dexamethasone binding in the cytosol from the small intestine and alkaline phosphatase were measured as described previously [15]. The number of determinations for each point (mean and S.E.M.) is the same as that for cytosol protein and DNA determinations given in Table 5. Arrows denote the time of birth. Part A expresses the data on the basis of intestinal wet weight while Part B expresses the data per mg protein.

Table 5. Changes in intestine weight, cytosol protein, and DNA in the fetal rabbit intestine during development

Age days	Intestine weight g/intestine	Cytosol protein mg/g tissue	DNA mg/g tissue
Gestation of fetuses			
21	0.04 ± 0.002* (38)†	21.4 ± 2.2* (5)‡	2.3 ± 0.50* (5)‡
23	0.07 ± 0.002 (42)	29.0 ± 1.0 (10)	6.2 ± 0.86 (10)
24	0.09 ± 0.002 (61)	28.4 ± 0.5 (9)	8.1 ± 0.73 (9)
25	0.16 ± 0.006 (14)	35.1 ± 2.1 (6)	7.6 ± 1.24 (6)
26	0.18 ± 0.004 (18)	35.1 ± 1.5 (7)	5.1 ± 0.73 (7)
27	0.24 ± 0.010 (19)	41.8 ± 2.7 (12)	7.5 ± 0.60 (12)
28	0.39 ± 0.016 (23)	44.0 ± 4.6 (12)	7.8 ± 0.36 (12)
29	0.53 ± 0.010 (30)	46.5 ± 1.5 (15)	7.5 ± 0.29 (15)
30	0.63 ± 0.020 (16)	53.6 ± 2.2 (9)	8.1 ± 0.21 (9)
Newborn			
1	0.99 ± 0.060 (7)	57.3 ± 5.9 (7)	7.3 ± 0.23 (7)
3	1.62 ± 0.040 (4)	56.7 ± 1.8 (10)	6.8 ± 0.23 (10)

* Mean ± S.E.M.
† Number of fetal or newborn rabbits used for intestine weight determination.
‡Number of duplicate determinations done for cytosol protein and DNA.

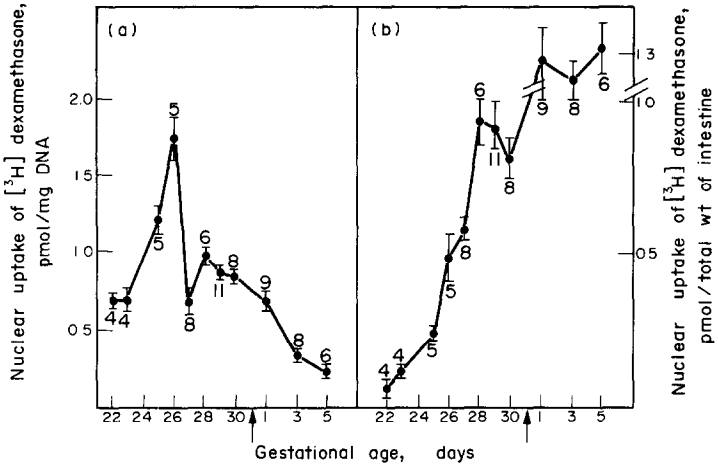

Fig. 8. Changes in the nuclear uptake of [^{3}H]-dexamethasone by fetal small intestine with gestation. Tissues were obtained from fetal or newborn rabbit of various ages as indicated. *In vitro* nuclear uptake of [^{3}H]-dexamethasone was measured as described previously [15]. The nuclear uptake is expressed as (a) pmol of [^{3}H]-dexamethasone/mg DNA and (b) pmol of [^{3}H]-dexamethasone/total weight of intestine. The number of determinations is shown above or below each value (mean ± S.E.M.). Arrows indicate the day of birth.

but the DNA leveled off at day 24 of gestation. The K_D of the binding of dexamethasone to the cytosol receptors in the fetal small intestine throughout the period of gestation studied remained unchanged, the value obtained varied from 2.0–4.5 nM. Alkaline phosphatase was measurable on day 25 of gestation and the enzyme activity continued to increase after birth (Figure 7 A and B). When alkaline phosphatase was first detected on day 25, the fetal small intestine had the highest concentration of dexamethasone binding sites expressed on per mg protein. Thereafter, the enzyme activity continued to increase even though the concentration of binding sites was decreasing.

A similar study was done comparing the specific binding to purified nuclei and the data is shown in Fig. 8. In this figure the specific binding was calculated as pmol per mg DNA (Fig. 8a) or as pmol per total weight of small intestine (Fig. 8b). There was a rapid increase in the nuclear uptake of [^{3}H]-dexamethasone from day 22 of gestation which reached a peak on day 26 and was followed by a biphasic decline to a low level five days after birth (Fig. 8a). When the uptake was calculated on the basis of the weight of the whole intestine, there was a gradual increase in the specific binding which reached a peak value at about day 28 but increased further after birth and plateaued up to day 5 postnatally. The pattern observed here is almost identical to that seen in Fig. 7 for the specific binding to the cytosol receptor and suggests that the nuclear binding follows closely on the changes in receptor concentration in the cytosol.

It is clear that specific glucocorticoid binding sites are present in rabbit fetal small intestine before alkaline phosphatase activity can be measured in this tissue. This temporal relationship suggests that the availability of the relatively high concentrations of glucocorticoid receptors may be needed to trigger the maturation process, leading to the induction of an enzyme such as alkaline phosphatase. Further increases in alkaline phosphatase activity with advancing gestation was not paralleled by an increase in glucocorticoid receptor concentration implying that enzyme synthesis, once triggered, does not depend on enhanced binding. This phenomena has been observed in several other systems such as the chick neural retina [5,23] in the rat uterus [24, 25]. In the small intestine of the suckling rat, maximum concentration of cytoplasmic glucocorticoid binding sites were found on day 10 of postnatal life [26]. In this system the time lag between the maximum glucocorticoid receptor concentration and changes in enzyme activity may depend on the time of migration of enterocytes in moving from the base into the tip of the villus of the small intestine [27].

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REFERENCES

1. Moog F.: The influence of the pituitary–adrenal system on the differentiation of phosphatase in the duodenum of the suckling mouse. *J. exp. Zool.* **124** (1953) 329–346.
2. Jensen E. V., Numata M., Brecher P. I. and DeSombre E. R.: Hormone–receptor interaction as a guide to biochemical mechanism. In: *The Biochemistry of Steroid Hormone Action* (Edited by R. M. S. Smellie). Academic Press, London (1971) pp. 133–159.

3. Raspé G.: Steroid hormone receptors. *Advances in the Biosciences* 7 (1970). Pergamon Press, Oxford.
4. Greengard O.: Enzyme differentiation in mammalian liver. *Science* **163** (1969) 891–895.
5. Moscona A. A.: Hydrocortisone mediated regulation of gene expression in embryonic neural retina: induction of glutamine synthetase. *J. steroid Biochem.* **6** (1975) 633–638.
6. Feldman D.: Ontogeny of rat hepatic glucocorticoid receptors. *Endocrinology* **95** (1974) 1219–1227.
7. Strittmatter C. F.: Cytoplasmic receptor protein for etiocholanolone in chick embryo liver. *Enzyme* **15** (1973) 24–39.
8. De Lemos R. A., Shermata D. W., Knelson J. H., Kotas R. and Avery M. E.: Acceleration of appearance of pulmonary surfactant in the fetal lamb by administration of corticosteroids. *Am. Rev. Resp. Dis.* **102** (1970) 459–461.
9. Kotas R. V., Fletcher B. D., Torday J. and Avery M. E.: Evidence for independent regulators of organ maturation in fetal rabbits. *Pediatrics* **47** (1971) 57–64.
10. Giannopoulos G., Mulay S. and Solomon S.: Cortisol receptors in rabbit fetal lung. *Biochem. biophys. Res. Commun.* **47** (1972) 411–418.
11. Giannopoulos G., Mulay S. and Solomon S.: Glucocorticoid receptors in lung—II. Specific binding of glucocorticoids to nuclear components of rabbit fetal lung. *J. biol. Chem.* **248** (1973) 5016–5023.
12. Giannopoulos G.: Glucocorticoid receptors in lung—I. Specific binding of glucocorticoids to cytoplasmic components of rabbit fetal lung. *J. biol. Chem.* **248** (1973) 3876–3883.
13. Giannopoulos G., Hassan Z. and Solomon S.: Glucocorticoid receptors in fetal and adult rabbit tissue. *J. biol. Chem.* **249** (1974) 2424–2427.
14. Giannopoulos G.: Variations in the levels of cytoplasmic glucocorticoid receptors in lungs of various species at different developmental stages. *Endocrinology* **94** (1974) 450–458.
15. Lee D. K. H., Stern M. D. and Solomon S.: Cytoplasmic glucocorticoid receptors in the developing small intestine of the rabbit fetus. *Endocrinology* **99** (1976) 379–388.
16. Giannopoulos G., Mulay S. and Solomon S.: Glucocorticoid receptors in rabbit fetal lung. *Excerpta Medica Inter. Congr.* Ser. **273** (1972) 1032–1037.
17. Giannopoulos G.: Uptake and metabolism of cortisone and cortisol by the fetal rabbit lung. *Steroids* **23** (1974) 845–853.
18. Munck A. and Brinck-Johnson T.: Specific and nonspecific physicochemical interactions of glucocorticoids and related steroids with rat thymus cells *in vitro*. *J. biol. Chem.* **243** (1968) 5556–5565.
19. Gluck L., Scribney M. and Kulovich M. V.: The biochemical development of surface activity in mammalian lung—II. The biosynthesis of phospholipids in the lung of the developing rabbit fetus and newborn. *Pediat. Res.* **1** (1967) 247–265.
20. Kotas R. V. and Avery M. E.: Accelerated appearance of pulmonary surfactant in the fetal rabbit. *J. Appl. Physiol.* **30** (1971) 358–361.
21. Ballard P. L. and Ballard R. A.: Cytoplasmic receptor for glucocorticoids in lung of the human fetus and neonate. *J. clin. Invest.* **53** (1974) 477–486.
22. Overton J. and Shoupe J.: Fine structure of the free surface in developing mouse intestinal mucosa. *J. exp. Zool.* **159** (1965) 195–201.
23. Lippman M. E., Wiggert B. O., Chade G. J. and Thompson E. B.: Glucocorticoid receptors. Characteristics, specificity, and ontogenesis in the embryonic chick neural retina. *J. biol. Chem.* **249** (1974) 5916–5917.
24. Clark J. H. and Gorski J.: Ontogeny of the estrogen receptor during early uterine development. *Science* **169** (1970) 76–78.
25. Sömjen G., King R. J. B., Kay A. M. and Lindner H. R.: Nuclear binding of oestradiol-17β and induction of protein synthesis in the rat uterus during postnatal development. *Biochem. J.* **136** (1973) 25–33.
26. Henning S. J., Ballard P. L. and Kretchmer N.: A study of the cytoplasmic receptors for glucocorticoids in intestine of pre- and post-weanling rats. *J. biol. Chem.* **250** (1975) 2073–2079.
27. Herbst J. J. and Koldovsky O.: Cell migration and cortisone induction of sucrase activity in jejunum and ileum. *Biochem. J.* **126** (1972) 471–476.

DISCUSSION

Farrell. Have you ever examined fetal lung for the possibility of regional differences with respect to corticosteroid receptors? The reason I ask this question is that there is now sound evidence indicating that the upper or cephalad lobes reach a differentiated state prior to the time that the lower lobes show the presence of surfactant. Could this be due to increased uptake of steroid hormone, occurring before the lower lobes are stimulated?

Solomon. Yes that is a good question. Actually we haven't addressed ourselves to that question. I want to leave some of the details to you. I don't want to say a word about the surfactin release from the lining of the fetal lung. But there is a problem here of compartmentalization. You see glucocorticoid receptors in fetal lung rather before you can detect surfactin in the lining. The body of the lungs seems to have glucocorticoid receptors. We have published a study on glucocorticoid in the rabbit fetal blood; it is present and seems to be made by the fetal adrenal. We have difficulty with compartmentalization from the maternal or fetal compartments. It is present in a free form. The free versus CBG bound fraction, the free increases in the fetal compartment towards term, in the maternal compartment it does not do so, there is no association, so if it is there it is in a right form, not bound to CBG at the right time. But it is there long before you can see surfactin activity at the lining of the lung, you see it in the body of the lung.

McEwen. You demonstrated the receptor activity in animals that had intact adrenal secretions. They had their own steroids circulating, and yet you got binding of labeled dexamethasone. Do you have any way of estimating to what extent those receptors were occupied by endogenous corticosterone?

Solomon. We have been washing them out.

McEwen. How so?

Solomon. In the *in vivo* experiments of course you are always faced with the endogenous. I have not gone into great detail but we started with *in vivo* experiments injecting the glucocorticoids directly into the rump of the fetus and then isolated the nuclear–cytosol fractions *in vitro* after the *in vivo* injection because we have done a whole lot of *in vitro* studies. It is always a problem because we know from the data published initially by Manson that the level of endogenous steroids markedly affects the binding of the steroid to the receptor on a protein basis.

McEwen. All right, let me say what I am driving at. We find in the brain that it is possible with [^{3}H]-dexamethasone to get the same labeling *in vivo* of cell nuclear receptor sites in intact animals as we obtain in adrenalectomized animals, as long as the intact animals have resting

corticosterone levels. If intact rats are stressed, there is a reduction in the amount of dexamethasone which binds. Perhaps there is a binding site for dexamethasone which normally responds only to high corticosterone levels, ie, a stress receptor. Recently, Neil Maclusky and Barbara Turner in my laboratory have been able to separate into two populations the glucocorticoid binding macromolecules of rat brain cytosol by means of isoelectric focusing. One of the populations prefers dexamethasone; the other, corticosterone. Is it possible that the same thing may exist in the fetal lung?

Solomon. It is. Giannopoulus two years ago published evidence for a specific dexamethasone receptor.

McEwen. And has he also been able to see a corticosterone receptor?

Solomon. Yes in the cytosol there are two separate receptors. There is the dexamethasone receptor which is separable with difficulty (of course all inductive backtracking) from the hydrocortisone. There is a definite separation in the two activities.

McEwen. And I think there is also evidence for this in kidney from Edelman's lab in San Francisco. Perhaps this is a general characteristic of glucocorticoid responsive tissues.

Pasqualini. In your data you showed that in the fetal lung cortisone is converted to cortisol. Do you have some data on the conversion of cortisol to cortisone?

Solomon. I must repeat that in the nuclei cortisone is converted to cortisol but not the reverse.

Pasqualini. And in the cytosol?

Solomon. In the cytosol, at the time that this was published, there wasn't any interest. So the cytosol wasn't done, if you remember the publication. But in the cytosol there is some back and forth.

Pasqualini. What is it mainly?

Solomon. It is mostly from E to F and not F to E. Do you know there is a big controversy on this subject? You and Egon Dicsfalucy published on the subject originally and not a lot of other people have published papers in this area in the human. If you want me to review what this story is, it is messy. Some people have confirmed and some people have not been able to confirm your original findings.

Pasqualini. I think that you must consider the animal species. We have demonstrated that in human fetus, cortisol is largely converted to cortisone which was confirmed by others. Furthermore, it is well known that in fetal human plasma the concentration of plasma cortisone is many times that of cortisol. This significant conversion of cortisol to cortisone was demonstrated also in fetal guinea pig but at the present we have not investigated other species.