Furthermore, additional chain elongation before the ω-oxidation step in the biosynthesis of these methyl-branched chain dicarboxylic acids would be expected to lead to methylazeleic and methylsebacic acids.

Finally, condensation of propionyl-CoA with malonyl-CoA and chain elongation by fatty acid synthetase followed by ω-oxidation would yield straight chain odd-numbered dicarboxylic acids. The occurrence of C_{15}-C_{17} straight chain fatty acids has been reported in hepatic (9) and erythrocyte (7) fat of two patients with propionic acidemia (7, 9) and in the glycerolipids of the nervous system of a patient with methylmalonic acidemia (12). These studies on the identification and quantitation of methyl-branched and straight chain dicarboxylic acids in amniotic fluid and urine in propionic and methylmalonic acidemia may contribute to explanations of the abnormal biochemistry of these disorders and to better understanding of the mechanisms of clinical illness.

Acknowledgments. We thank Dr. O. W. Jones for providing normal amniotic fluids and the many physicians who sent amniotic fluids from pregnancies at risk for the organic acidurias. We thank Dr. S. G. Kahler and Dr. Mace for providing the urine of the patients with propionic acidemia and methylmalonic acidemia, respectively.

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0031-3998/84/1811-1191$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 11, 1984
Printed in U.S.A.

Glucocorticoids Preferentially Increase Fetal Alveolar β-Adrenoreceptors: Autoradiographic Evidence

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ABSTRACT. To localize fetal rabbit lung β-adrenoreceptors before and after glucocorticoid treatment, light microscopic autoradiography was performed with the reversible radiolabeled β-adrenergic antagonist, [^{3}H]dihydroalprenolol, on day 26 of gestation. Autoradiograms of adult lung and fetal myocardium were also prepared. Examination of these autoradiograms showed densely labeled airways, alveoli, and myocardium. Specific labeling, defined as that prevented by incubation with l-propranolol (1 μM), was 90%. Analysis of grain counts in the fetus showed that airways were more densely labeled than alveoli ($p < 0.001$), labeling was increased by treatment ($p < 0.001$) and treatment increased alveolar ($p < 0.002$) but not airway labeling. Adult lungs were much more densely labeled than fetal, and fetal myocardial labeling was not altered by treatment. Adult untreated lung showed the same pattern as fetal untreated lung with airways being more densely labeled than alveoli ($p < 0.001$). To validate estimates of

Received April 27, 1983; accepted November 29, 1983.
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This work was supported by National Institutes of Health Grant HUHD 24056.
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relative β-adrenoreceptor concentration derived from autoradiograms, comparisons to determinations of β-receptor concentration from scintillation counting of lung section digests and from previously performed radioligand binding studies, using membranes prepared from whole lung homogenates, were made. There is excellent agreement between estimates of relative receptor concentration and specific binding derived from the counting of autographic grains and both scintillation counting of lung section digests and previously performed radioligand binding of lung particulate. In all preparations, specific binding was (90%), increased with glucocorticoid treatment in fetal lung (50–100%), was greater in concentration in adult compared to fetal lung (7–10-fold), and did not increase in fetal myocardium with treatment. We conclude that β-adrenoreceptors can be localized within the fetal lung with this autoradiographic method and that airways and alveoli contain β-adrenoreceptors, with airways containing more than alveoli in both fetal and adult lung. Also, there is a preferential increase in fetal alveolar β-adrenoreceptors after glucocorticoid treatment. This increase is not present in either airway or myocardial β-adrenoreceptors and is consistent with the known maturational effect of glucocorticoid treatment on fetal alveolar function. (*Pediatr Res* 18:1191–1194, 1984)

Abbreviation

DHA, dihydroalprenolol

Glucocorticoids have been shown to accelerate fetal lung devlopment in humans and experimental animals by both direct and indirect effects (2). One such an indirect effect may be to increase β-adrenergic sensitivity of the fetal lung. Previously, our laboratory and others have demonstrated that in membrane particulate prepared from fetal rabbit lung homogenate there is an increase in β-adrenoreceptor concentration during gestation (6, 8, 12, 15) which is temporally related to increasing fetal free plasma glucocorticoid concentration (11). This increase in β-adrenoreceptor concentration can be precociously produced in the fetus by administration of glucocorticoid to the pregnant doe (6). It is known that β-adrenergic stimulation of the fetal lung activates important alveolar functions necessary for the adaptation of that organ to function in its air-breathing role. These functions include the resorption of alveolar fluid (14) and the synthesis and release of surface active material (9). Also, during gestation there is an increase in the responsiveness of the fetal lung to stimulation by β-adrenergic agents (9) which may be explained by increased lung β-adrenoreceptor concentration. Because the lung is a structurally complex organ, changes in β-adrenoreceptor concentration previously described in lung homogenates might not reflect changes in alveolar β-adrenoreceptors which mediate neonatal lung adaptations. To localize β-adrenoreceptors in the fetal lung and to discriminate changes in their concentration in different lung structures after glucocorticoid treatment, we performed autoradiography of fetal rabbit lung sections with the reversible β-adrenergic antagonist [^{3}H] dihydroalprenolol.

MATERIALS AND METHODS

Time-bred pregnant New Zealand White rabbits were obtained from LIT, Inc., Billings, MT, DHA (23 Ci/mmol) was from New England Nuclear Corporation, Boston, MA, and other reagents and chemicals were from commercial sources. Fetal rabbits, either control or those exposed for 24 h to β-methasone (0.17 mg/kg; Celestone Soluspan, Schering Inc., Kennelworth, NJ) by maternal intramuscular injection, were delivered by hysterotomy and sacrificed by cooling on ice for 7–10 min at day 26 of gestation. The fetal trachea was exposed, a plastic catheter was placed in its lumen, and 1 ml of a 30% solution of cryostat imbedding medium (OCT Compound Ames Co., Elkhart, IN) was injected to preserve lung architecture. Fetal lungs were then immediately dissected free from the thorax and quick frozen in liquid nitrogen. Cryostat sections 8-μm thick of lung and heart were mounted on gelatin-coated glass slides (3, 16). Sections from control adult rabbit lungs were similarly prepared. These slides, containing two to four sections each of inflated lung were incubated in 50 mM Tris-HCl buffer, pH 7.4, with 1.7 nM DHA at 25°C for 20 min. This concentration of DHA, approximately equal to the dissociation constant of DHA for fetal lung β-adrenoreceptors (6) will occupy approximately one-half of these receptors. Bound DHA was separated from free by washing in a bath of 50 mM Tris-HCl buffer at 4°C for 10 min with two gentle agitations. In preliminary experiments with adult lung, this reduced nonspecific binding without decreasing specific binding (3). Drying was accomplished with compressed air at 4°C to minimize off-diffusion of bound radioligand. Adjacent sections were incubated similarly but with l-propranolol at 1 μM, which is approximately 100 times the inhibition constant of l-propranolol for stereoselective DHA binding in whole lung membrane particulates (7). Nonspecific binding was defined as binding of DHA not prevented by 1 μM l-propranolol. In previous experiments with fetal lung particulate, we found that at these concentrations of DHA and l-propranolol, binding of DHA competed by l-propranolol was virtually all (>96%) from a high affinity stereoselective DHA-binding site. To determine optimum incubation conditions, relative receptor concentrations, and the proportion of specific binding, incubated sections from each group were removed from the slides, placed in tissue solvent (Protosol, New England Nuclear) for 12 h and counted by scintillation photometry at 52% efficiency. Vials containing scintillant (Hydroflor, New England Nuclear) and Protosol were weighed before and after tissue was added to determine tissue wet weight. Other glass-mounted lung sections were apposed with dry emulsion-coated glass coverslips and stored at 4°C for 10–12 weeks. Coverslips were developed (D 19, Kodak) and tissue sections were stained with 2% cresyl violet.

Although the resolution of this technique does not allow localization of grains to different cell types, the specific grain counts in different lung areas could be determined by counting total autoradiographic grains in each area and subtracting nonspecific grain counts in the same area of adjacent sections. Counts were performed in lung areas corresponding to airway alveoli, and myocardium. Three areas of alveoli, airway, and myocardium were counted in three sections each of two fetuses in control and two fetuses in treated groups. Similarly, grain counts were performed from autoradiograms obtained from two adult animals. Data analysis was by analysis of variance for fetal lung grain counts and by t test for adult lung and fetal myocardial grain counts. Dark field photomicrographs were made from emulsion-coated coverslips and bright field photomicrographs from stained lung sections immediately beneath the coverslips.

RESULTS

Specific DHA binding to fetal and adult lung sections (1.7 nM DHA with or without 1 μM l-propranolol as described in "Materials and Methods") quantitated by scintillation photometry of lung section digests showed a significant increase in DHA binding of treated *versus* control lung sections (3.2 ± 0.6 fmol/mg wet weight *versus* 1.7 ± 0.5 fmol/mg wet weight; $n = 4$; $p < 0.01$ by unpaired t test). Scintillation counting of adult lung sections showed specific DHA binding (11.6 fmol/mg wet weight), seven times that present in the control fetal preparation. Light microscopy of the autoradiograms showed grains labeling airways, alveoli (Fig. 1), and heart (not shown). Two-way analysis of variance of fetal lung section grain counts (Table 1) showed

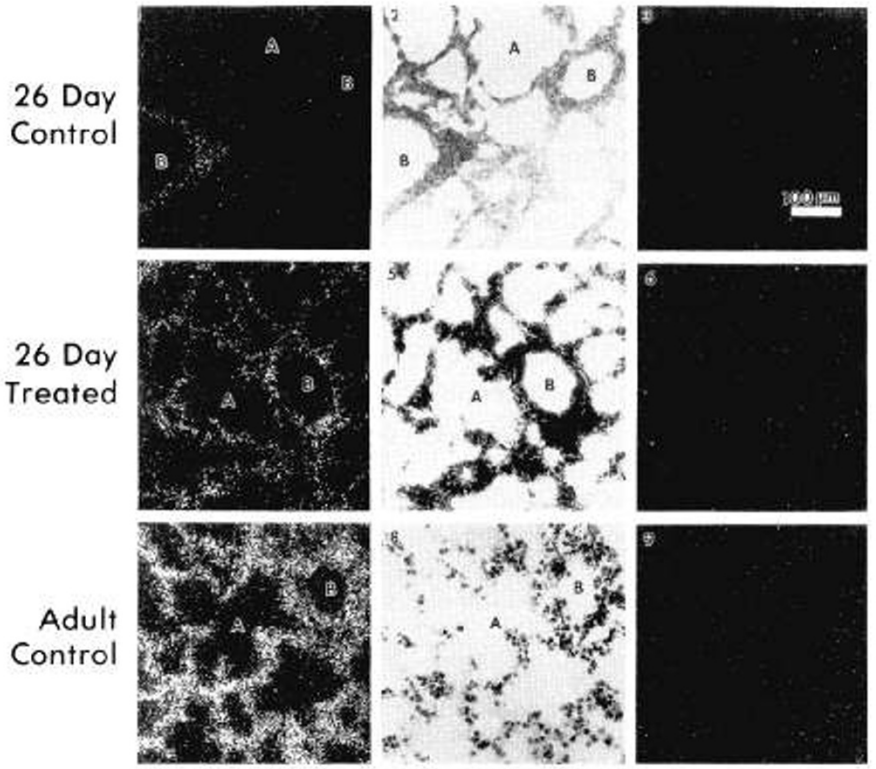

Fig. 1. Autoradiograms of DHA binding to sections of fetal and adult rabbit lung. *1–3*, 25-day control fetal rabbit lung: *1*, dark field photomicrograph of autoradiogram; *2*, bright field photomicrograph of the lung section beneath *1*, showing labeling of a bronchiole (*B*) and lesser labeling of alveoli (*A*); *3*, dark field photomicrograph of an autoradiogram showing nonspecific labeling in the presence of 1 μm l-propranolol in the same lung area of an adjacent section. 4–6, 25-day glucocorticoid fetal rabbit lung: *4*, dark field; *5*, bright field; and *6*, nonspecific dark field photomicrographs showing an increase in alveolar labeling compared to control lungs. *7–9*, adult control rabbit lung: *7*, dark field; *8*, bright field; and *9*, nonspecific dark field photomicrographs showing dense labeling of both bronchioles and alveoli.

Table 1. *Specific autoradiographic grain counts of [^{3}H]dihydroalprenolol binding to sections of fetal and adult rabbit lung**

	Specific grain counts/1000 μm^2	
	Control	Steroid treated
26-day fetal		
Alveoli	18.5 ± 3.8	39.2 ± 8.5
Airways	43.4 ± 7.2	54.2 ± 6.8
Heart	61.2 ± 10.5	56.7 ± 10.3
Adult		
Alveoli	162.7 ± 27.5	
Airways	228.7 ± 23.5	

* Data are the means ± SD of three areas from three separate slides in two animals. Two-way analysis of variance showed, in fetal lung, a significant effect of treatment ($p < 0.001$, treated labeling greater than control), location ($p < 0.001$, airway labeling greater than alveolar), and a significant combined effect of treatment and location ($p < 0.002$, alveolar but not airway labeling increased by treatment). Unpaired Student's *t* test showed no difference after treatment in myocardial labeling ($p = 0.6$) and airway labeling greater than alveolar in adult ($p < 0.001$).

significant effects of treatment and location and a significant combined effect of treatment on location for alveolae but not airways. Thus, treatment increased total labeling, airway labeling was greater than alveolar, and treatment increased alveolar but not airway labeling. By contrast, there was no increased specific labeling of myocardium with treatment (Table 1). Control adult lung which was very densely labeled (Fig. 1), had the same pattern as fetal lung, with airways more densely labeled than alveoli (Table 1).

DISCUSSION

[^{3}H]Dihydroalprenolol has been shown in studies using membrane particulate from whole rabbit lung (6, 8, 12, 15) and in lung sections of adult ferret (3) to bind saturably, with high affinity and stereoselectively to lung β-adrenoreceptors. While it is difficult to precisely estimate β-receptor concentration using autoradiographic technique, in these experiments there is excellent agreement between specific grain counts and determination of β-receptor concentration obtained by other means. The estimate of increased lung β-receptor concentration after glucocorticoid treatment in the fetus using this autoradiographic method agrees with scintillation counting of fetal lung section digests; an increase of approximately 60–80%. Additionally, we have reported previously that membrane particulates prepared from fetal lung after glucocorticoid treatment show a similar increase in β-adrenoreceptor concentration (6). The lack of increased specific labeling of myocardium in this autoradiographic study parallels the unchanged concentration of myocardial β-receptors prepared from membrane particulates of fetal rabbit heart after glucocorticoid treatment (6). Furthermore, the much greater specific autoradiographic labeling of adult lung sections compared to fetal lung sections is in excellent agreement with the 7-fold greater concentration determined by scintillation counting of adult lung section digests and by prior determinations of β-receptor concentration in lung membrane particulate prepared from adult lungs (6, 8). The estimate of specific binding of approximately 90% derived by subtracting grains counted in the presence of l-propranolol from total grains in the same lung area in adjacent sections with this autoradiographic technique agrees well with determinations of specific binding using scintillation counting of lung section digests and those using membrane particulate prepared from whole rabbit lung. Thus, the estimation of relative β-receptor concentration by the technique of grain counting appears to be valid.

Glucocorticoids have many effects on the structure, function, and biochemical constitution of the fetal lung (2, 13). Glucocorticoid treatment reduces neonatal respiratory problems in humans and in other mammals delivered prematurely (1, 10). Although this therapy reduces neonatal respiratory morbidity, the molecular basis by which it improves alveolar function is as yet unclear. While glucocorticoids increase the synthesis of surface active material by type II alveolar cells (5), there is evidence that surface active material, necessary for alveolar stability, may not appear in a functional form in the alveolus without β-adrenergic stimulation (4, 7). Because glucocorticoids also act to increase β-receptor concentration, and because the stimulation of lung β-receptors activate important alveolar functions, a major glucocorticoid action to enhance alveolar function may be through an indirect effect on alveolar β-adrenergic receptor concentration. An increase in β-adrenergic concentration in the alveolus may be responsible for the enhanced alveolar responsiveness to β-adrenergic stimulation which has been shown to occur during gestation (9). It was unclear from earlier studies of whole lung homogenate whether the increase in β-adrenergic receptors seen near term or after glucocorticoid treatment reflected an increase in the functionally important β-adrenoreceptors, those in the alveolus. Morphologic studies alone cannot directly link increased alveolar β-receptor concentration to enhanced alveolar β-adrenergic sensitivity. However, the demonstration of increased alveolar β-receptor concentration after glucocorticoid treatment could provide an important link between glucocorticoid treatment and enhanced fetal lung maturity. These autoradiographic data clearly show that there is a preferential increase in fetal alveolar β-receptors after glucocorticoid treatment; airway and myocardial β-receptors are not increased. Although airways and alveoli contain many β-adrenoreceptors, their concentration in airways is greater in both fetal and adult lung. However, the large contribution of alveolar membranes to whole lung membrane particulate implies that the alveolus is the primary source of β-receptors in whole lung membrane particulate. Thus, prior studies using membrane particulate prepared from whole lung homogenate, which demonstrated increased lung β-receptor concentration after glucocorticoid treatment, reflected increased alveolar β-receptor concentration. The mechanism by which glucocorticoids increase the concentration of some populations of fetal β-receptors and not others is not known. However, it is clear from these preliminary studies that this differential action has both organ-specific, lung *versus* heart, and tissue-specific, alveoli *versus* airway, components.

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