Role of Adenosine 3',5'-Monophosphate in Maturation of Fetal Lungs

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Extract

Fetal lung cyclic AMP phosphodiesterase, cyclic AMP, phosphatidyl choline, and incorporation of precursors into phosphatidyl choline were measured in rabbits after maternal administration of hydrocortisone phosphate and aminophylline. Both agents inhibited lung phosphodiesterase activity and augmented cyclic AMP concentrations (Table 1). Aminophylline administration was associated with a significant increase in lung saturated phosphatidyl choline (Table 2). Incorporation of [¹⁴C]choline and [³H]methionine was increased by both aminophylline and hydrocortisone (Table 3).

Speculation

Aminophylline, which inhibits cyclic AMP phosphodiesterase as its primary mode of action, has effects similar to hydrocortisone on incorporation of labeled precursors into phosphatidyl choline. Cyclic AMP, therefore, may be a mediator of maturation of lung phosphatidyl choline synthesis and/or secretion in the fetus, and pharmacologic agents which enhance cyclic AMP levels in the lungs may also enhance synthesis of phosphatidyl choline.

Idiopathic respiratory distress syndrome of the newly born usually occurs in infants with deficiency of pulmonary surfactant at the alveolus (2, 8, 47). Many investigative efforts, in consequence, have been directed toward study of maturation of surfactant which appears to consist of several surface-active lipoproteins (12, 28-30). The single component of surfactant found in the highest concentration (and the best studied) is a saturated phosphatidyl choline, dipalmitoyl lecithin, which accounts for approximately 50% of total lung lipids (18) and approximately 40% of lung surface-active material (12). Two *de novo* biosynthetic pathways for phosphatidyl choline have been identified. The choline incorporation pathway, *pathway I*, probably accounts for more than 90% of newly synthesized lecithin in all mammalian species, both fetal and postnatal, studied to date (16, 17). *Pathway II*, the phosphatidyl ethanolamine methylation pathway, has also been confirmed in fetal animals but probably accounts for only minimal amounts of newly synthesized lecithin (16, 17). In addition to these *de novo* pathways, restructuring of the lecithin molecule through the lysolecithin pathway is an important, although less well studied, mechanism by which saturation of the acyl groups occurs (1, 24).

Saturated phosphatidyl choline can be isolated from fetal lungs in most species during the last half of gestation, with rapidly increasing concentrations appearing at approximately 90% of gestation (16, 18). During this period increasing surface forces in the lung can be measured, indicating that surface-active compounds are present in the alveolar spaces (7, 8).

The mechanisms regulating synthesis of phosphatidyl choline are poorly understood. All of the enzymatic activities necessary for its production by both pathways have been identified in fetal lungs, however (18). In addition, phosphatidic acid, the most important intermediate compound in glycerolipid synthesis, can be synthesized in fetal lungs (18). The rate-limiting step in synthesis of

phosphatidyl choline appears to be the final reaction with conversion of CDP-choline to choline, involving choline phosphotransferase activity (17). This enzyme, in addition, appears to be stimulated selectively when lung maturation is accelerated by corticosteroids (19). Accelerated rates of phosphatidyl choline synthesis in response to glucocorticoid administration have now been reported in a number of species including fetal sheep, rabbits, and subhuman and human primates, associated in some studies with increased surface forces (14, 15, 17, 19, 27, 33, 34, 38, 39, 44, 49, 50). Increasing fetal serum concentrations of cortisol near term and increased amniotic fluid cortisol, associated with advancing gestation and maturation of fetal lungs, indicate that increased output of cortisol by fetal adrenal glands may be one mechanism by which normal maturation of pulmonary surfactant occurs (18, 20). Thyroid hormone administration has likewise been reported to stimulate lung phosphatidyl choline synthesis in fetal rabbits (58) as well as in adult, thyroidectomized rats (47). In addition, disruption of the thyroid-pituitary-adrenal axes by fetal decapitation results in a delay of pulmonary maturation (5, 6, 11).

Although not studied to date in lungs, glucocorticoids enhance concentrations of cyclic AMP in various tissues by inhibition of cyclic AMP phosphodiesterase (36, 41, 43, 53). Thyroid hormones also enhance concentrations of cyclic AMP in target tissues through stimulation of adenylate cyclase (37). Lung tissue studies of cyclic AMP metabolism have to date focused on responses to catecholamines, histamine, prostaglandin E₁, and aminophylline, all of which augment cyclic AMP concentrations (9, 13, 31, 32, 45, 46). Adenylate cyclase has, in addition, been measured in human and rabbit lung homogenates (3, 42). In the fetal rabbit, by 21 days of gestation adenylate cyclase activity is high and can be stimulated by epinephrine (3). In fetal rabbit lung slices by *day 21* cyclic AMP can be identified and its concentration enhanced by histamine and catecholamines (46). We undertook the present study to determine whether a relationship could be established between surfactant production and cyclic AMP metabolism in fetal lung tissue.

MATERIALS AND METHODS

New Zealand albino rabbits with a single 3-hr mating period were studied in pairs. In 10 pairs, hydrocortisone phosphate, 1.95 mg/kg/day, was injected three times daily into the study doe (intramuscularly) on *days 24-26* of gestation, with the control doe receiving equal volumes of saline at the same times. In 7 pairs, aminophylline, 6.25 mg/kg/day divided into two doses, was infused intravenously at a rate of 2.5 mg/min between *days 24* and *27* of gestation; control does received intravenous infusions of equal volumes of saline.

On *day 26* the does were anesthetized with sodium pentobarbital; using sterile techniques we exposed the uterus through a midline abdominal incision. [³H]methionine (15 μ Ci) and [¹⁴C]choline (2.5 μ Ci) were dissolved in 0.1 cc 0.15 M NaCl and injected into fetal hindlimbs through the intact uterus. Specific activities of [³H]methionine and [¹⁴C]choline were 1.0 Ci/mmol and 58.0 mCi/mmol, respectively.

Eighteen hours after the surgical procedure, the does were again anesthetized with sodium pentobarbital and killed with intravenous administration of a saturated solution of KCl. The fetuses were rapidly removed and their tracheas clamped to prevent respiration. They were immediately exsanguinated by decapitation, the lungs removed, washed free of excess blood with cold normal saline, and divided into three portions. Two portions of each lung were immediately placed on Dry Ice and the third portion placed in a saline bath at 4°. Fetal lung tissue was pooled so that approximately equal weights of upper and lower lobes were in each portion. The frozen samples were assayed for cyclic AMP content and cyclic AMP phosphodiesterase activity as described below. Those samples which were placed in cold saline were lyophilized at -30° and later analyzed for lipid content as described below. In order to assess the stability of cyclic AMP, some lungs were immediately immersed in liquid nitrogen. A comparison was made of the cyclic AMP content of lungs prepared by the two methods. At the time of study, hydrocortisone had last been administered 18 hr previously. Aminophylline, which has a shorter duration of action, was administered 1 hr before the study.

Cyclic AMP in lung tissue was measured by the method of Gilman (22) and expressed as picomoles per mg tissue, wet weight. Aminophylline content of lung tissue was measured by the method of Schack and Waxler (52) and expressed as micrograms per g tissue, wet weight. Cyclic AMP phosphodiesterase was measured according to the method of Russell *et al.* (51) and activity was expressed as picomoles of cyclic AMP hydrolyzed per mg protein; protein was determined by the method of Lowry (40).

Lipid analysis of lung tissue was performed by extraction of lipids from the lyophilized specimens according to the methods of Folch *et al.* (21) using a Polytron homogenizer under a stream of nitrogen. The extracted lipids were then separated into neutral and phospholipid fractions using silicic acid (Bio-Rad, Bio-Sil, HA-325 mesh) column chromatography. The phospholipid fraction was further fractionated into the phosphatidyl ethanolamine and phosphatidyl choline components by thin layer chromatography on silica gel H-coated plates and developed in chloroform-methanol-water in the proportions 65:25:4. These components were then assayed for ³H and ¹⁴C content as well as total phospholipid concentration by weight (using a Cahn electrobalance) and by phosphorus content according to the method of Bartlett (4). The maximum counting efficiencies of the Nuclear Chicago Isocap 300 scintillation counter for the ³H/¹⁴C-double label program were 25% and 50%, respectively. Efficiency was determined from the quench curve derived for this program by use of an external standard. Raw counts per min data obtained for ³H and ¹⁴C were corrected to disintegrations per min in this counting program by using the observed efficiency values for each sample. The calculated disintegrations per min were then used to determine the level of labeled substrate incorporation into the lipids and were expressed in the tables as picomoles of substrate incorporated into

phosphatidyl choline. The computed specific activities represent incorporation in terms of moles of methionine and choline into these phospholipids over the 18-hr interval.

The phosphatidyl choline components were subsequently converted to their respective fatty acid methyl esters using 0.5 N HCl in methanol; the reactions were carried out in 5-ml ampoules sealed under nitrogen at 80° for 4 hr. The resultant fatty acid methyl esters were then washed and extracted into heptane for injection into a gas-liquid chromatograph (Perkin-Elmer model 80) fitted with an ethylene glycol succinate column. Characterization and quantifying of individual fatty acid methyl ester components were performed by referencing against known standards obtained from Applied Science Laboratories. The relative proportion of saturated:total phosphatidyl choline was thereby computed. Weights and crown-rump lengths were determined from randomly chosen fetuses in each litter.

Comparisons of control vs. study fetal lungs were made for cyclic AMP phosphodiesterase activity, cyclic AMP content, phospholipid content, saturated phosphatidyl choline content, and phosphatidyl choline specific activity using Student's *t*-test (55).

RESULTS

Aminophylline concentrations in pooled fetal lungs from 8 litters 1 hr after maternal administration of the agent averaged 2.80 ± 0.49 (SD) $\mu\text{g/g}$ tissue.

In two litters, not otherwise part of this study, lung cyclic AMP concentrations were no different between lungs which were excised rapidly and placed on Dry Ice (mean value 0.107 pmol/mg tissue) and those which were placed immediately in liquid nitrogen (mean value 0.092 pmol/mg tissue). Therefore, cyclic AMP results from lungs treated by both methods are analyzed together.

Lung cyclic AMP phosphodiesterase activities and cyclic AMP concentrations are shown in Table 1. Aminophylline and hydrocortisone both inhibited phosphodiesterase activity to a highly significant degree ($P < 0.001$); a higher concentration of lung cyclic AMP was present in the litters treated with these pharmacologic agents ($P < 0.001$). Responses of cyclic AMP phosphodiesterase and cyclic AMP to hydrocortisone and aminophylline were equal.

Concentrations of lung total and saturated phosphatidyl choline are shown in Table 2, expressed both as percentage of total lung lipids and as milligrams per g lung tissue. Differences of total phosphatidyl choline among the three groups were not significant, and saturated phosphatidyl choline in the control and hydrocortisone-treated groups were not different. Lungs from the litters treated with aminophylline, however, had a higher percentage of saturated phosphatidyl choline ($P < 0.01$), as well as an absolute increase of saturated phosphatidyl choline content ($P < 0.025$), than the other groups.

Incorporation of [³H]methionine and [¹⁴C]choline into saturated phosphatidyl choline is shown in Table 3. In all three groups of litters choline incorporation accounted for the majority of newly

Table 1. Lung cyclic AMP phosphodiesterase (picomoles of cyclic AMP hydrolyzed per mg protein) and cyclic AMP (picomoles per mg tissue) in fetal rabbits after maternal administration of aminophylline (A), hydrocortisone phosphate (HC), or normal saline (control)

	Phosphodiesterase			Cyclic AMP		
	Control	A	HC	Control	A	HC
Mean	26.1	18.8	18.2	0.190	0.386	0.342
SD	3.24	2.55	2.42	0.051	0.111	0.102
Number of litters	15	8	8	11	6	7
Significance						
Control vs. aminophylline		<0.001			<0.001	
Control vs. hydrocortisone		<0.001			<0.001	
Aminophylline vs. hydrocortisone		NS ¹			NS	

¹ Not significant.

Table 2. Lung phosphatidyl choline, total and saturated (mean $\pm$ SD), expressed as percentage of total lung lipid, and as milligrams per g lung tissue in fetal rabbits after maternal administration of aminophylline (A), hydrocortisone phosphate (HC), or saline (control)

	Total lung lipid, %		Tissue, mg/g	
	Total	Saturated	Total	Saturated
Control	52.5 $\pm$ 7.22	25.8 $\pm$ 3.71	5.2 $\pm$ 0.99	1.3 $\pm$ 0.34
A	58.3 $\pm$ 12.87	31.6 $\pm$ 4.92	6.4 $\pm$ 1.66	2.0 $\pm$ 0.83
HC	54.5 $\pm$ 7.75	28.8 $\pm$ 3.60	6.0 $\pm$ 2.07	1.8 $\pm$ 0.69
Significance				
Control vs. aminophylline	NS ¹	<0.01	NS	<0.025
Control vs. hydrocortisone	NS	NS	NS	NS
Aminophylline vs. hydrocortisone	NS	NS	NS	NS

¹ Not significant.

Table 3. Incorporation of [¹⁴C]choline and [³H]methionine, expressed as picomoles per mg lung phosphatidyl choline, incorporated into saturated phosphatidyl choline in fetal rabbits after maternal administration of aminophylline (A), hydrocortisone phosphate (HC), or saline (control)

	[¹⁴ C]Choline			[³ H]Methionine		
	Control	A	HC	Control	A	HC
Mean	92.6	116.8	131.9	4.3	5.4	5.4
SD	12.86	13.17	26.44	1.01	1.03	0.88
Number of litters	14	7	7	14	7	7
Significance						
Control vs. aminophylline		<0.001			<0.025	
Control vs. hydrocortisone		<0.001			<0.025	
Aminophylline vs. hydrocortisone		NS ¹			NS	

¹ Not significant.

synthesized saturated phosphatidyl choline. Aminophylline and hydrocortisone significantly increased incorporation of both [³H]methionine and [¹⁴C]choline into phosphatidyl choline (*P* values all <0.025).

A recent report by Karotkin and his coworkers has demonstrated that prolonged (5–10 days) administration of phenobarbital is associated with delay of pulmonary maturation (26). Although we anesthetized our does with pentobarbital during the laparotomy and again just before death, we used a very low dose of barbiturate which was identical in both our control and study groups.

DISCUSSION

Although maternal administration of glucocorticoids has been associated with accelerated pulmonary maturation in sheep, subhuman primates, and human infants (14, 15, 38, 39), only direct fetal administration of the agents in rabbits has been associated with this response (33, 44). When large doses of cortisol, 10–30 mg/kg/day, were injected into pregnant rabbit does, there was a high incidence of fetal wastage and intrauterine growth retardation (44). The much smaller doses of glucocorticoid reported in this study resulted in no fetal demise, and biochemical evidence of accelerated synthesis of phosphatidyl choline was present.

Hydrocortisone and aminophylline administered to pregnant rabbits in this report had similar effects upon cyclic AMP phosphodiesterase, lung cyclic AMP concentrations, and acceleration of precursor incorporation into phosphatidyl choline. In several reports, lung cyclic AMP responses to catecholamines, histamine and aminophylline have been reported, but no previous correlations with phosphatidyl choline synthesis have been made, other than a single report that dibutyl-cyclic AMP administration to adult rats results in an increased lamellar body synthesis and secretion in alveolar type II cells (56). In fetal rabbits lung

cyclic AMP is responsive to catecholamine and histamine stimulation by 21 days of gestation (46). Adenylate cyclase activity also is high in the same tissue by 21 days and can also be stimulated *in vitro* by epinephrine (3). Surface properties of fetal rabbit lung, however, develop somewhat later, and mature pressure-volume relationships are normally not observed until 28–29 days of gestation (33). This development of mature surface forces is accompanied by increased production of lung surface-active materials and their increased secretion into the alveolar spaces (27, 33). Current information indicates that glucocorticoids accelerate lung maturation by increasing the activity of choline phosphotransferase, the final enzyme in the synthesis of lecithin by the choline incorporation pathway (17, 19). The effect of glucocorticoids upon secretion into alveolar spaces is not known. Because glucocorticoids exert some metabolic effects in other tissues through inhibition of cyclic AMP phosphodiesterase and consequent increase of tissue cyclic AMP concentrations (36, 41, 43), we thought it possible that cyclic AMP might in part mediate accelerated surfactant development observed in fetuses treated with glucocorticoids. We have shown that aminophylline, a pharmacologic agent with one major mode of action, inhibition of phosphodiesterase, produced responses in fetal lungs that were almost identical to those of glucocorticoids, both with respect to phosphodiesterase and cyclic AMP, and also with respect to increased incorporation of labeled precursors into phosphatidyl choline. We demonstrated the same dramatic increase in incorporation of choline which has previously been reported. In addition, we observed a definite increase in incorporation of methionine, which is in contrast to previous reports of Farrell and Zachman (19), who noted no response of *pathway II*, and Russell *et al.* (50), who reported a decreased incorporation of methionine into lecithin in fetuses receiving glucocorticoids. The differences among these results cannot be explained, but physiologically they are of minor

importance since the phosphatidyl ethanolamine methylation pathway contributes little to the total pool of phosphatidyl choline. Our data did not show a significant increase of lung phosphatidyl choline in the cortisone-treated litters, in contrast to the findings of Farrell and Zachman (19), although increased saturated phosphatidyl choline was present in the aminophylline-treated litters. It is possible that, by reporting phosphatidyl choline concentration per mg lung wet weight, we are masking changes which are present but of lesser magnitude than changes of lung water content which occur with cortisone administration. In addition, our methodology for determining saturated phosphatidyl choline using gas-liquid chromatography may give values different from the less specific method of acetone precipitation. Finally, the trend toward increased phosphatidyl choline in the hydrocortisone group was present and with a larger number of trials might be significantly different from controls. Our results indicate that aminophylline and glucocorticoids may accelerate lung maturation through a common pathway, inhibition of phosphodiesterase and augmentation of lung cyclic AMP.

Since Liggins and Howie (39) reported decreased morbidity from respiratory distress syndrome and improved survival of infants delivered preterm whose mothers had received high dose β -methasone treatment for 24 hr or more before delivery, this approach has been advocated by some as a means of decreasing the incidence of respiratory distress syndrome. Potential toxicity of maternal corticosteroid administration exists. Mothers with eclampsia treated with β -methasone may have an increased fetal mortality when compared with control mothers (39). Animal studies indicate that corticosteroids may compromise placental function to the detriment of the fetus (57) and, in addition, inhibit lung growth, although this effect is reversible with age (10, 35). The reduction in cell number in the brain accompanying glucocorticoid administration to the newborn rodent, however, does not appear to be reversible (25), and may be accompanied by disturbances of myelination, synaptic growth, and locomotor ability (54). Moreover, Gross *et al.* (23), have shown that corticosteroid administration to fetal rabbits inhibits fatty acid synthetase and acetyl-CoA carboxylase activity in the lung. These animal studies are not comparable with antenatal human administration of glucocorticoids, either in duration or dose of the agents used. They do suggest, however, that antenatal administration of corticosteroids may be accompanied by deleterious effects and point up the need for searching for alternative means for prevention of neonatal respiratory distress syndrome. Our observations suggest that aminophylline or other agents which augment tissue cyclic AMP should be studied for potential effectiveness in the prevention of respiratory distress syndrome.

SUMMARY

Since aminophylline and hydrocortisone have similar effects upon lung cyclic AMP phosphodiesterase, cyclic AMP, and incorporation of choline and methionine into phosphatidyl choline in fetal rabbits, it appears that cyclic AMP mediates fetal lung surfactant synthesis. Because maternal administration of glucocorticoids to accelerate lung maturation is not without risk, it seems reasonable to look for other agents, with less risk, that may be equally effective in accelerating maturation of fetal lungs. Aminophylline or other agents which inhibit cyclic AMP phosphodiesterase activity should be studied, but because of widespread effect of cyclic AMP, may present the same risk to the fetus as glucocorticoids.

REFERENCES AND NOTES

1. Abe, M., Akino, T., and Ohno, K.: The formation of lecithin from lysolecithin in rat lung supernatant. *Biochim. Biophys. Acta*, **280**: 275 (1972).
2. Avery, M. E., and Mead, J.: Surface properties in relation to atelectasis and hyaline membrane disease. *Amer. J. Dis. Child.*, **97**: 517 (1959).
3. Barrett, C. T., Sevanian, A., and Kaplan, S. A.: Adenylate cyclase activity in immature rabbit lung. *Pediatr. Res.*, **8**: 244 (1974).
4. Bartlett, G. R.: Phosphorus assay in column chromatography. *J. Biol. Chem.*, **234**: 466 (1959).
5. Blackburn, W. R., Kelly, J. S., Dickman, P. S., Travers, H., and Lopata, M. A.: The role of the pituitary-adrenal-thyroid axes in lung differentiation. II. Biochemical studies of developing lung in anencephalic rats. *Lab. Invest.*, **28**: 352 (1973).
6. Blackburn, W. R., Travers, H., and Potter, D. M.: The role of the pituitary-adrenal-thyroid axes in lung differentiation. I. Studies of the cytology and physical properties of anencephalic fetal rat lung. *Lab. Invest.*, **26**: 306 (1972).
7. Brumley, G. W., Chernick, V., Hodson, W. A., Normand, C., Fenner, A., and Avery, M. E.: Correlations of mechanical stability, morphology, pulmonary surfactant, and phospholipid content in the developing lamb lung. *J. Clin. Invest.*, **46**: 863 (1967).
8. Brumley, G. W., Hodson, W. A., and Avery, M. E.: Lung Phospholipids and surface tension correlations in infants with and without hyaline membrane disease and in adults. *Pediatrics*, **40**: 13 (1967).
9. Butcher, R. W., and Baird, C. E.: Effects of prostaglandins on adenosine 3',5'-monophosphate levels in fat and other tissues. *J. Biol. Chem.*, **243**: 1713 (1968).
10. Carson, S. H., Taeusch, H. W., and Avery, M. E.: Inhibition of lung cell division after hydrocortisone injection into fetal rabbits. *J. Appl. Physiol.*, **34**: 660 (1973).
11. Chiswick, M., Ahmed, A., Jack, P., and Milner, R.: Control of fetal lung development in the rabbit. *Arch. Dis. Childhood*, **48**: 709 (1973).
12. Clements, J. A.: Composition and properties of pulmonary surfactant. In: C. A. Villee, D. V. Villee, and J. Zuckerman: *Respiratory Distress Syndrome*, p. 77 (Academic Press, New York, 1973).
13. Collins, M., Palmer, G. C., Baca, G., and Scott, H. R.: Stimulation of cyclic AMP in the isolated perfused rat lung. *Res. Commun. Chem. Pathol. Pharmacol.*, **6**: 805 (1973).
14. de Lemos, R. A., and McLaughlin, G. W.: Induction of the pulmonary surfactant in the fetal primate by the intrauterine administration of corticosteroids. *Pediatr. Res.*, **7**: 425 (1973).
15. 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. *Amer. Rev. Resp. Dis.*, **102**: 459 (1970).
16. Epstein, M. F., and Farrell, P. M.: The choline incorporation pathway: Primary mechanism for *de novo* lecithin synthesis in fetal primate lung. *Pediatr. Res.*, **9**: 658 (1975).
17. Farrell, P. M.: Regulation of pulmonary lecithin synthesis. In: C. A. Villee, D. B. Villee, and J. Zuckerman: *Respiratory Distress syndrome*, p. 311 (Academic Press, New York, 1973).
18. Farrell, P. M., and Avery, M. E.: Hyaline membrane disease. *Amer. Rev. Resp. Dis.*, **111**: 657 (1975).
19. Farrell, P. M., and Zachman, R. D.: Induction of choline phosphotransferase and lecithin synthesis in the fetal lung by corticosteroids. *Science*, **179**: 297 (1973).
20. Fencel, M. de M., and Tulchinsky, D.: Total cortisol in amniotic fluid and fetal lung maturation. *N. Engl. J. Med.*, **292**: 133 (1975).
21. Folch, J., Lees, M., and Sloan Stanley, G. H.: A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**: 497 (1957).
22. Gilman, A. G.: A protein binding assay for adenosine 3',5'-cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.*, **67**: 305 (1970).
23. Gross, L., Rooney, S. A., and Warshaw, J. B.: The influence of cortisol on the enzymes of fatty acid synthesis in developing mammalian lung and brain. *Pediatr. Res.*, **9**: 752 (1975).
24. Hallman, M., and Raivio, K.: Studies on the biosynthesis of disaturated lecithin of the lung: The importance of the lysolecithin pathway. *Pediatr. Res.*, **8**: 874 (1974).
25. Howard, E.: Reductions in size and total DNA of cerebrum and cerebellum in adult mice after corticosterone treatment in infancy. *Exp. Neurol.*, **22**: 191 (1968).
26. Karotkin, E. H., Cashore, W. J., Kido, M., Redding, R. A., Douglas, W., Stern, L., and Oh, W.: Pharmacological induction and inhibition of lung maturation in fetal rabbits. *Pediatr. Res.*, **9**: 397 (1975).
27. Kikkawa, Y., Kaibara, M., Motoyama, E. K., Orzalesi, M. M., and Cook, C. D.: Morphologic development of the fetal rabbit lung and its acceleration with cortisol. *Amer. J. Pathol.*, **64**: 423 (1971).
28. King, R. J., and Clements, J. A.: Surface active material from dog lung. I. Method of isolation. *Amer. J. Physiol.*, **223**: 707 (1972).
29. King, R. J., and Clements, J. A.: Surface active materials from dog lung. II. Composition and physiological correlations. *Amer. J. Physiol.*, **223**: 715 (1972).
30. King, R. J., Klass, D. J., Gikas, E. G., and Clements, J. A.: Isolation of apoproteins from canine surface active material. *Amer. J. Physiol.*, **224**: 788 (1973).
31. Klainer, L. M., Chi, Y.-M., Freidberg, S. L., Rall, T. W., and Sutherland, E. W.: Adenyl cyclase. IV. The effects of neurohormones on the formation of adenosine 3',5'-phosphate by preparations from brain and other tissues. *J. Biol. Chem.*, **237**: 1239 (1962).
32. Klainer, M. A., Orange, R. P., Koopman, W. J., Austen, K. F., and Laraia, P. J.: Cyclic adenosine 3',5'-monophosphate in human lung. *Biochim. Biophys. Acta*, **252**: 160 (1971).
33. Kotas, R. V., and Avery, M. E.: Accelerated appearance of pulmonary surfactant in the fetal rabbit. *J. Appl. Physiol.*, **30**: 358 (1971).
34. Kotas, R. V., Fletcher, B. D., Tarday, J., and Avery, M. E.: Evidence for independent regulators of organ maturation in fetal rabbits. *Pediatrics*, **47**: 57 (1971).
35. Kotas, R. V., Mims, L. C., and Hart, L. K.: Reversible inhibition of lung cell

- number after glucocorticoid injection into fetal rabbits to enhance surfactant appearance. *Pediatrics*, 53: 358 (1974).
36. Lavin, N., Rachelefsky, G., and Kaplan, S. A.: Inhibition of cyclic-AMP phosphodiesterase in human lymphocytes by physiological concentrations of hydrocortisone. *Horm. Metab. Res.*, 7: 253 (1975).
 37. Levey, G. S., and Epstein, S. E.: Myocardial adenylyl cyclase: Activation by thyroid hormones and evidence for two adenylyl cyclase systems. *J. Clin. Invest.*, 48: 1663 (1969).
 38. Liggins, G. C.: Premature delivery of foetal lambs infused with glucocorticoids. *J. Endocrinol.*, 45: 515 (1969).
 39. Liggins, G. C., and Howie, R. N.: A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics*, 50: 515 (1972).
 40. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265 (1951).
 41. Manganiello, V., and Vaughan, M.: An effect of dexamethasone on adenosine 3',5'-monophosphate content and adenosine 3',5'-monophosphate phosphodiesterase activity of cultured hepatoma cells. *J. Clin. Invest.*, 51: 2763 (1972).
 42. Menon, K. M. J., Giese, S., and Jaffe, R. B.: Hormone- and fluoride-sensitive adenylyl cyclases in human fetal tissues. *Biochim. Biophys. Acta*, 304: 203 (1973).
 43. Moskowitz, J., and Fain, J. N.: Stimulation by growth hormone and dexamethasone of labeled cyclic adenosine 3',5'-monophosphate accumulation by white fat cells. *J. Biol. Chem.*, 245: 1101 (1970).
 44. Motoyama, E. K., Orzalesi, M. M., Kikkawa, Y., Kaibara, M., Wu, B., Zigas, C. J., and Cook, C. D.: Effect of cortisol on the maturation of fetal rabbit lungs. *Pediatrics*, 48: 547 (1971).
 45. Palmer, G. C.: Characteristics of the hormonal induced cyclic adenosine 3',5'-monophosphate response of the rat and guinea pig lung *in vitro*. *Biochim. Biophys. Acta*, 252: 561 (1971).
 46. Palmer, G. C.: Cyclic 3',5'-adenosine monophosphate response in the rabbit lung—adult properties and development. *Biochem. Pharmacol.*, 21: 2907 (1972).
 47. Redding, R. A., Douglas, W. H. J., and Stein, M.: Thyroid hormone influence upon lung surfactant metabolism. *Science*, 175: 994 (1972).
 48. Reynolds, E. O. R., Orzalesi, M. M., Motoyama, E. K., Craig, J. M., and Cook, C. D.: Surface properties of saline extracts from lungs of newborn infants. *Acta Paediat. Scand.*, 54: 511 (1965).
 49. Rooney, S. A., Gross, L., Motoyama, E. K., and Warshaw, J. B.: Effects of cortisol and thyroxine on fatty acid and phospholipid biosynthesis in fetal rabbit lung. *Physiologist*, 17: 323 (1974).
 50. Russell, B. J., Nugent, L., and Chernick, V.: Effects of steroids on the enzymatic pathways of lecithin production in fetal rabbits. *Biol. Neonate*, 24: 306 (1974).
 51. Russell, T. R., Terasaki, W. L., and Appleman, M. M.: Separate phosphodiesterases for the hydrolysis of cyclic adenosine 3',5'-monophosphate and cyclic guanosine 3',5'-monophosphate in rat liver. *J. Biol. Chem.*, 248: 1334 (1973).
 52. Schack, J. A., and Waxler, S. H.: An ultraviolet spectrophotometric method for the determination of theophylline and theobromine in blood and tissues. *J. Pharmacol. Exp. Ther.*, 97: 283 (1949).
 53. Senft, G., Schultz, G., Munske, K., and Hoffman, M.: Effects of glucocorticoids and insulin on 3',5'-AMP phosphodiesterase activity in adrenalectomized rats. *Diabetologia*, 4: 330 (1968).
 54. Shapiro, S.: Some physiologic, biochemical and behavioral consequences of neonatal hormone administration: Cortisol and thyroxine. *Gen. Comp. Endocrinol.*, 10: 214 (1968).
 55. Snedecor, G. W., and Cochran, W. G.: *Statistical Methods*, Ed. 6 (Iowa State University Press, Ames, Iowa, 1967).
 56. Stahlman, M. T., Gray, M. E., Lieu, S., and Chytil, F.: The role of cyclic AMP in lamellar body synthesis and secretion. *Pediat. Res.*, 8: 470 (1974).
 57. Wellman, K. F., and Volk, B. W.: Fine structural changes in the rabbit placenta induced by cortisone. *Arch. Pathol.*, 94: 147 (1972).
 58. Wu, B., Kikkawa, Y., Orzalesi, M. M., Motoyama, E. K., Kaibara, M., Zigas, C. J., and Cook, C. D.: The effect of thyroxine on the maturation of fetal rabbit lungs. *Biol. Neonate*, 22: 161 (1973).
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Aspartylglucosaminuria
4-L-aspartylglycosylamine amido hydrolase
glycoprotein

leukocytes
lymphocytes
plasma

Enzymatic Diagnosis and Carrier Detection of Aspartylglucosaminuria Using Blood Samples

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Extract

The activity of the glycoprotein degrading lysosomal hydrolase, 4-L-aspartylglycosylamine amido hydrolase (AADGase, EC.3.5.1.26), was measured in plasma, buffy coat leukocytes, and separated lymphocytes (Ficoll separation) from 16 patients with aspartylglucosaminuria (AGU), 29 obligate heterozygotes, and 30 control subjects. In lymphocytes the AGU patients had unmeasurable or minimal AADGase activity with a mean of 3.9 U. The obligate heterozygotes showed AADGase activities ranging from 5 to 69 U with a mean of 34.1 U. Enzyme activities in the control group ranged from 91 to 243 U with a mean of 127.9 U, and were clearly separated from the values of the heterozygotes.

In leukocytes the AGU patients had unmeasurable enzyme activity and obligate heterozygotes had enzyme levels closely similar to those in the lymphocytes from the same individuals. The

AADGase activity in the leukocytes of the control group displayed a much wider variation than in the lymphocytes, ranging from 22 to 132 U with a mean of 70.7 U.

In plasma the AGU patients had undetectable AADGase activity. The mean enzyme level of obligate heterozygotes was 72.2 U and that of control individuals 107.2 U, but the overlap between the groups was extensive. The results indicate that homozygous deficiency of AADGase, i.e., aspartylglucosaminuria, can be reliably diagnosed using plasma, leukocytes, or separated lymphocytes. For carrier detection only separated lymphocytes allow a satisfactory differentiation between heterozygous and normal individuals.

A group of 31 siblings of verified AGU cases and 11 children of identified carriers, whose spouses had normal AADGase activity, were investigated using the lymphocyte assay. The observed and expected frequencies (on the basis of Mendelian probabilities) were closely similar, suggesting that the lymphocyte assay can be used reliably for carrier detection.