11. N. Kefalides, *Biochem. Biophys. Res. Commun.* **45**, 226 (1971).
12. K. A. Piez, E. A. Eigner, M. S. Lewis, *Biochemistry* **2**, 58 (1963).
13. D. Layman, E. B. McGoodwin, G. R. Martin, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 454 (1971); G. Bellamy and P. Bornstein, *ibid.*, p. 1138.
14. K. A. Piez, G. R. Martin, P. Bornstein, *Biochemistry* **5**, 3813 (1966).

15. Publication No. 582 of the Robert W. Lovett Memorial Group for the study of Diseases Causing Deformities. Supported by NIH grants AM 3564 and AM 12785 and by the National Foundation–March of Dimes grant CRBS-229. R.L.T. is a fellow of the Helen Hay Whitney Foundation; A.H.K. is Cecil L. Russell Fellow of the Arthritis Foundation.

23 June 1972; revised 25 September 1972 ■

Induction of Choline Phosphotransferase and Lecithin Synthesis in the Fetal Lung by Corticosteroids

Abstract. *Rabbit fetuses 23 to 24 days of gestation were injected with either 9-fluoroprednisolone acetate or saline. Three days later the lungs of steroid-treated animals showed a significant increase in lecithin concentration and cholinephosphotransferase activity. In addition, lung slices from these animals incorporated more [^{14}C]choline into lecithin. The rise in enzyme activity and [^{14}C]choline incorporation was blocked by prior treatment of fetuses with cycloheximide but not by treatment with actinomycin D. It is proposed that the corticosteroids induce de novo synthesis of the lung enzyme, which in turn leads to increased synthesis of lecithin through the choline incorporation pathway. Furthermore, it appears that the site of regulation involves translation of messenger RNA.*

Respiratory distress syndrome is a developmental disease of prematurely delivered animals characterized by progressive atelectasis of the lungs in association with a marked reduction in the concentration of surface-active lecithin, that is, surfactant (*1*). At the present time only supportive measures such as administration of oxygen and alkali are available therapeutically. Current evidence suggests that there are two principal pathways for pulmonary lecithin biosynthesis—incorporation of cytidine diphosphocholine (CDP-choline) into 1,2-diglyceride and trimethylation of phosphatidyl ethanolamine. The former pathway appears to develop late in gestation, and its deficiency in the premature animal may well be etiologically related to the respiratory distress syndrome (*2*).

Kotas and Avery (*3*) and Motoyama *et al.* (*4*) reported that rabbit fetuses treated with corticosteroids showed evidence of increased pulmonary maturation as determined by pressure-volume curves obtained during deflation, bubble stability ratios, and surface tension properties of lung extracts. These results are consistent with the proposal that glucocorticoid administration leads to earlier development of alveolar surface-active phospholipid. However, the above factors provide a relatively crude assessment of the capability of fetal lung for lecithin biosynthesis.

Using a biochemically directed approach, we examined the effect of corticosteroids on surface-active lecithin by assaying the compound directly and by measuring the incorporation of isotopic precursors into lecithin by lung slices. The capacity of hydrocortisone to induce synthesis of several enzymes involved in gluconeogenesis has been documented (*5*). We postulated that a similar mechanism might be operative for the lecithin biosynthetic pathway, and in order to assess this possibility the activities of four enzymes in two pathways were also examined. These included choline kinase (E.C. 2.7.1.32) and choline phosphotransferase (E.C. 2.7.8.2) of the choline incorporation pathway and methionine adenosyltransferase (E.C. 2.5.1.6) and phosphatidyl ethanolamine methyltransferase of the methylation pathway. Only choline phosphotransferase was elevated.

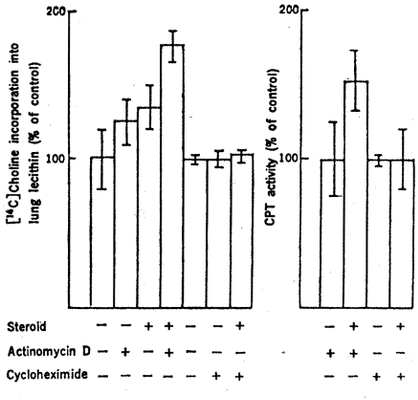

Fig. 1. Effects of actinomycin D and cycloheximide on the corticosteroid-associated changes in choline phosphotransferase (*CPT*) activity and in lecithin synthesis in fetal rabbit lung. The latter was assayed as incorporation of [^{14}C]choline into lecithin by lung slices. Each group contains two to four rabbit fetuses. Standard errors of the mean are indicated.

Pregnant rabbits 23 to 24 days of gestation, for which conception time was known within 2 hours, were anesthetized with barbiturates and subjected to laparotomy. The fetuses were injected intramuscularly through the transparent uterine wall with 50 μg of 9-fluoroprednisolone acetate as Predef (*6*) or with saline. Three days later, unless otherwise noted, the animals were delivered by cesarean section, and breathing was prevented by the application of a ligature around the neck. The lungs were rapidly removed, blotted free of blood, and extracted by the technique of Folch *et al.* (*7*) for lecithin analysis. The latter was performed after thin-layer chromatography by measuring the char density of spots sprayed with sulfuric acid. Fresh lungs were also sliced in a Stadie-Riggs microtome and incubated for 1 to 2 hours at 37°C in a Krebs-Ringer bicarbonate solution containing 1 μc of either [^{14}C]choline or [^{14}C]methionine. Incorporation of radioactivity was subsequently measured in either lipid extract or isolated lecithin. In the case of [^{14}C]choline incorporation, thin-layer chromatography demonstrated that more than 99 percent of the radioactivity was found in lecithin. Choline phosphotransferase activity was measured in lung homogenates at *p*H 8.5 by incubation of dipalmitin (1 m*M*) with CDP-[^{14}C]choline. The assay system also contained 0.1*M* tris(hydroxymethyl)aminomethane hydrochloride, 5 m*M* $MgCl_2$, and 0.006 percent Triton X-100. Radioactivity of isolated lecithin served as a measure of enzyme

Table 1. Synthesis of lung lecithin in the rabbit fetus after injection of corticosteroid or saline. The lecithin fraction precipitated by cold acetone is represented as surface-active lecithin (*9*). Incorporation of isotopic precursors by lung slices is given as counts per minute in lecithin per milligram of wet lung per hour of incubation. Standard errors of the mean are given. The numbers of animals are in parentheses. Abbreviations: Met., methionine; Chol., choline.

Lecithin (milligrams per gram of dry lung)		Incorporation of	
Total	Surface-active	[^{14}C]Met.	[^{14}C]Chol.
		NaCl	
70 ± 3 (17)	59 ± 2 (17)	72 ± 8 (5)	1043 ± 114 (6)
		Steroid	
96 ± 7* (15)	86 ± 2* (15)	69 ± 11 (5)	1589 ± 167† (6)

* $P < .005$. † $P < .05$.

activity. In all experiments a saturating concentration of dipalmitin was employed; however, in early experiments on rabbits 3 days after surgery, nonsaturating amounts of CDP-choline were used. Determination of the apparent Michaelis constant ($3 \times 10^{-5} M$) permitted use of a saturating concentration of CDP-choline (1 mM) in later measurements. In all cases velocity was proportional to protein concentration in the ranges employed for these assays.

The results of lung lecithin analyses are presented in Table 1. Values for saline-injected rabbit fetuses agreed with those for fetuses of comparable gestation who did not undergo surgery, and also agreed with the data of Gluck *et al.* (*8*). Steroid-treated fetuses showed a statistically significant ($P < .005$) elevation in lung lecithin from 70 mg per gram of dry lung (control value) to 96 mg per gram of dry lung. The latter figure is even higher than the lecithin content in lungs of full-term rabbit fetuses (*8*). Fractionation of isolated lecithin by precipitation with cold acetone revealed that the lecithin synthesized is precipitable by acetone and therefore is presumably surface-active (*9*).

Although the analyses of lung lecithin were consistent with a net increase in biosynthesis after corticosteroid injection, it was important to directly demonstrate an elevated rate of synthesis and to determine which pathway was involved. Incorporations of [^{14}C]choline and [^{14}C]methionine into lecithin by lung slices are shown on Table 1. Whereas there were no differences in methylation, choline incorporation occurred at a significantly greater rate in lung exposed to corticosteroid as compared to control lung. The 150 percent increase in choline incorporation was noted in lung slices both 6 and 72 hours after steroid administration.

Choline phosphotransferase activity was significantly increased in the steroid-treated rabbit fetuses both 6 and 72 hours after administration of the compound. Of 32 rabbit fetuses examined, the mean increase (counts per minute in lecithin per milligram of protein per minute) was from 211 ± 23 for controls to 299 ± 27 for the Predef-treated animals ($P < .05$). Similar changes in choline incorporation by lung slices and in choline phosphotransferase activity were also observed after injection with hydrocortisone; however, for an equivalent response approximately 50 times as much steroid (by weight) was required.

Prior treatment of the rabbit fetuses with actinomycin D (*10*) (1 μg per gram of body weight, given intraperitoneally 30 minutes before either steroid or saline) did not block the steroid-mediated rise in choline incorporation and enzyme activity (Fig. 1). In contrast, a paradoxical effect of actinomycin D was observed; choline incorporation was higher in animals first treated with the antibiotic as compared with those first treated with intraperitoneal saline. Such a phenomenon was noted for cortisol induction of tyrosine aminotransferase (E.C. 2.6.1.5) (*11*). In contrast, prior treatment with cycloheximide (*12*) (7 μg/g, given as described for actinomycin D) abolished the corticosteroid effects.

Numerous biochemical effects of hydrocortisone in the whole animal have been noted, including induction of phenylethanolamine methyltransferase in the adrenal medulla (*13*) and of several hepatic enzymes involved in gluconeogenesis (*5*). It is conceivable that the observed elevation in lecithin biosynthesis might be secondary to increased production of another metabolite such as epinephrine or glucose. However, the concurrent and proportional rise in choline phosphotransferase activity, along with inhibition of both effects by cycloheximide, suggests that the increased lecithin production requires de novo synthesis of this enzyme. Moreover, since actinomycin D did not prevent the proposed induction in doses that are effective in other systems (*13*), it would appear that the corticosteroid effect is mediated on the level of messenger RNA translation. Further studies will be required to substantiate this impression.

Extrapolations to the human fetus and newborn may be hazardous. However, these observations suggest that hydrocortisone may be beneficial as a specific therapy in the respiratory distress syndrome.

PHILIP M. FARRELL
Pediatric Metabolism Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, Bethesda, Maryland 20014
RICHARD D. ZACHMAN
Department of Pediatrics, University of Wisconsin, Madison 53705, and *St. Mary's Hospital Medical Center, Madison 53715*

References and Notes

1. G. W. Brumley, W. A. Hodson, M. E. Avery, *Pediatrics* **40**, 13 (1967).
2. L. Gluck, M. V. Kulovich, A. I. Eidelman, L. Cordero, A. F. Khazin, *Pediat. Res.* **6**, 81 (1972).
3. R. V. Kotas and M. E. Avery, *J. Appl. Physiol.* **30**, 358 (1971).
4. E. K. Motoyama, M. M. Orzalesi, Y. Kikkawa, M. Kaibara, B. Wu, C. J. Zigas, C. D. Cook, *Pediatrics* **48**, 547 (1971).
5. G. Weber, R. L. Singhal, N. B. Stamm, E. A. Fisher, M. A. Mentendiek, *Advan. Enzyme Regul.* **2**, 1 (1964); E. C. C. Lin and W. E. Knox, *Biochim. Biophys. Acta* **26**, 85 (1957).
6. Predef is the registered trade name of the Upjohn Company (Kalamazoo, Michigan) for 9-fluoroprednisolone acetate, a potent glucocorticoid with activity 50 times greater than that of hydrocortisone, as measured by liver glycogen deposition in the rat.
7. J. Folch, M. Lees, G. H. S. Stanley, *J. Biol. Chem.* **226**, 497 (1957).
8. L. Gluck, M. Sribney, M. V. Kulovich, *Pediat. Res.* **1**, 247 (1967).
9. L. Gluck, E. K. Motoyama, H. L. Smits, M. V. Kulovich, *ibid.*, p. 237.
10. Actinomycin D was a product of Merck Sharp & Dohme, West Point, Pennsylvania.
11. G. M. Tomkins, T. D. Gelehrter, D. Granner, D. Martin, H. H. Samuels, E. B. Thompson, *Science* **166**, 1474 (1969).
12. Cycloheximide was obtained from Sigma Chemical Co., St. Louis, Missouri.
13. R. J. Wurtman and J. Axelrod, *J. Biol. Chem.* **241**, 2301 (1966).
14. We thank E. B. Olson and A. Chung for assistance in the lecithin and enzyme assays. Supported by grant 1336966 from the John A. Hartford Foundation. Send reprint requests to R.D.Z.

17 July 1972; revised 5 October 1972

Rickettsia-like Bacterium Associated with Pierce's Disease of Grapes

Abstract. *A pleomorphic bacterium was observed by electron microscopy in grape plants infected with Pierce's disease. The organism was located in xylem tissue, and its occurrence was closely associated with symptoms of Pierce's disease. The bacterium resembled a rickettsia in morphology, in its failure to grow on cell-free media, and in its sensitivity to tetracycline antibiotics.*

Pierce's disease, a killer of grapevines (*Vitis* species), is one of the principal factors limiting grape production in Florida (*1*). The pathogen has long been considered a virus, but the successful suppression of the development of symptoms with tetracycline antibiotics in 1970 indicated that this may not be the case (*2*). We now report that an obligately parasitic bacterium resembling a rickettsia is constantly associated with the disease.

'Thompson Seedless' grape plants in their second year of growth were used