bone to the blood was measured in the young chick. The isotope was given 6 days prior to the vitamin and was presumably located mainly in mature crystals when the ascorbic acid was given. Blood levels of ^{45}Ca were significantly elevated from 4 to 8 hours following administration of ascorbic acid. Twenty-four hours after this injection blood ^{45}Ca was approximately 25% less than controls. These results suggest that ascorbic acid, as used in these studies, had a bone salt mobilizing influence. Whether this effect was direct or indirect remains to be elucidated. If the mobilizing effect were direct, it would indicate that ascorbic acid has a more extensive role in skeletal physiology than previously thought.

1. Follis, R. H., Bull. Johns Hopkins Hosp. 89, 9 (1951).

2. Robertson, W. van B. and Hewitt, J., Biochim. Biophys. Acta 49, 404 (1961).

3. Gould, B. S. and Schwachman, H., Am. J. Physiol. 135, 489 (1942).

4. Bourne, G. F., "The Biochemistry and Physiology of Bone," Chap. 18. Academic Press, New York, 1956.

5. Thornton, P. A., "Third European Symposium on Calcified Tissues," p. 48. Springer, Berlin, 1966.

6. Ramp, W. K. and Thornton, P. A., Proc. Soc. Exptl. Biol. Med. 121, 1248 (1966).

7. McLean, F. C., Science 127, 451 (1958).

8. Thornton, P. A., Weber, C. W., and Moreng, R. E., J. Nutr. 69, 33 (1959).

9. Roe, J. H. and Kuether, C. A., J. Biol. Chem. 147, 399 (1943).

10. Schubert, J. and Lindenbaum, A., J. Am. Chem. Soc. 74, 3529 (1952).

11. Polin D., Sturkie, P. D. and Hunsaker, W., Endocrinology 60, 1 (1957).

12. Friberg, U. and Ringertz, N. R., Exptl. Cell Res. 6, 527 (1954).

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Qualitative Studies of the Protein in Fetal Lamb Lung Fluid* (32881)

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During fetal life the trachea, bronchi, and alveoli are filled with fluid which is produced by the lungs(1). It is believed that the physicochemical properties of this fluid are important during the onset of respirations at the time of delivery, particularly in regards to the presence or absence of substances influencing the surface tension at the air liquid interface.

Adams *et al.* (2,3) have described the fetal lung fluid and the presence of surface active material in the fluid of lambs near term before the first breath. A direct relationship between the presence at postmortem of hyaline membrane disease and decreased surface activity of lung extracts also exists both in man(4) and animal (5). Abrams(6) has isolated a surface active lipoprotein from homogenates of mammalian lungs with an α globulin mobility in agar gel electrophoresis.

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Although fibrinogen is not present normally in fetal lung fluid(1), it is one of the main constituents of the hyaline membrane(7). It seems plausible that under conditions of fetal distress, changes in the composition of the lung fluid may occur favoring the formation of the hyaline membrane. The purpose of these studies was to determine the protein fractions normally present in the lung fluid of fetal lambs before the onset of respiration.

Materials and Methods. Thirteen lambs, at different gestational ages, ranging in weight from 1.9 to 4.6 kg were delivered by cesarean section. Special precautions were taken to maintain the placental circulation intact(2).

Pregnant ewes were anesthetized with nembutal, and 100% oxygen was given by a respiratory pump through a tracheostomy. The rate and volume of the pump was adjusted in order to maintain a normal maternal arterial pH and pCO_2.

After shaving the abdomen, with the ewe

TABLE I. Results of Immunoelectrophoretic Analysis of Fetal Lamb Lung Fluid.

Status of fetus	No. of lambs	Pre-albumin/no.	Albumin/no.	α Globulin/no.	β Globulin/no.	γ Globulin/no.
Mature	9	3/9	9/9	9/9	2/9	4/9
Immature	4	1/4	4/4	4/4	2/4	2/4
Total	13	4/13	13/13	13/13	4/13	6/13

on her right side, the uterus was exposed through a midline incision. The uterine wall was sutured to the margins of the incision in order to prevent evisceration of the abdominal contents. The uterus was then incised, the fetus delivered and the fetal trachea exposed in the fashion previously described(2). A plastic cannula was inserted distally and secured with umbilical tape. The lung fluid was then collected from the open end of the cannula.

During the procedure, precautions were taken not to stress the ewe or the fetus. The state of the umbilical cord and the fetal heart were monitored at frequent intervals.

In order to appraise the condition of the ewe and the fetus, blood samples were drawn at the end of each experiment from the maternal carotid artery and the umbilical vein. These were analyzed for pH, pCO_2(8) and pO_2(9).

Nine of the fetuses were gestationally mature (135–145 days) and four were immature. Lung fluid was obtained in a similar fashion from five additional fetal lambs after rendering two ewes hypercarbic for 5 hours and three lambs acidotic for 3 hours. Hypercarbia was accomplished by administering a mixture of 5% CO_2 and 95% O_2 to the ewe. Acidosis was accomplished by intravenous infusion of 0.3 *N* HCl to the fetus for 3 hours.

Lung fluid. Immediately after collection, specimens were frozen without addition of preservatives and maintained at —20°C. Prior to qualitative study, they were thawed and the total protein was determined(10).

Immunoelectrophoresis. Immunoelectrophoresis (in agar gel) was performed on each sample with Scheidegger's micromodification (11). This procedure was repeated in pooled representative samples of each group following vacuum ultrafiltration concentration to approximately 3 gm/100 ml through a collodion sac(12).

Antisera. a. Preparation of specific antiserum. Rabbit antisheep lung fluid (ALF). Pooled lung fluid samples obtained from term fetal lambs used in earlier experiments were lyophilyzed and concentrated to a protein content of 1 gm/100 ml. They were then mixed with equal parts of complete Freund's adjuvant and injected into the footpads of white New Zealand rabbits (2 ml of the concentrate per rabbit). This procedure was repeated once 3 weeks later. Four weeks later, a schedule of weekly bleedings to determine serum antibody titer and intravenous injections was started and continued for 2 months when optimal antibody concentration was obtained.

b. Rabbit antisheep serum (ASS) and rabbit antisheep gamma globulin (AGG). Commercial antisera produced in rabbits by immunization with sheep whole serum and γ globulin, respectively, were obtained from Hyland Laboratories.

Cellulose acetate electrophoresis. Analysis of the electrophoretic components of the lung fluid proteins was performed on the concentrates using cellulose acetate as a supporting medium(13). Similar analysis was performed on maternal and fetal sheep sera.

Adult sheep plasma fractions. Albumin (FV), α globulins (FV-1), β globulins (FIII) and γ globulins (FII) were obtained from Hyland Laboratories, Los Angeles, California.

Results. The total protein concentration of the fetal lung fluid varied from 0.9 to 17.1 mg/100 ml. Although the plasma protein concentration of the fetuses increased with gestational age, no apparent change in lung fluid protein content was observed.

Immunoelectrophoresis of the fetal lung fluid revealed at least five protein fractions, all of which showed precipitin lines when reacted against the ALF and/or ASS as shown in Table I. Albumin and α globulin were invariably present, whereas prealbumin, β

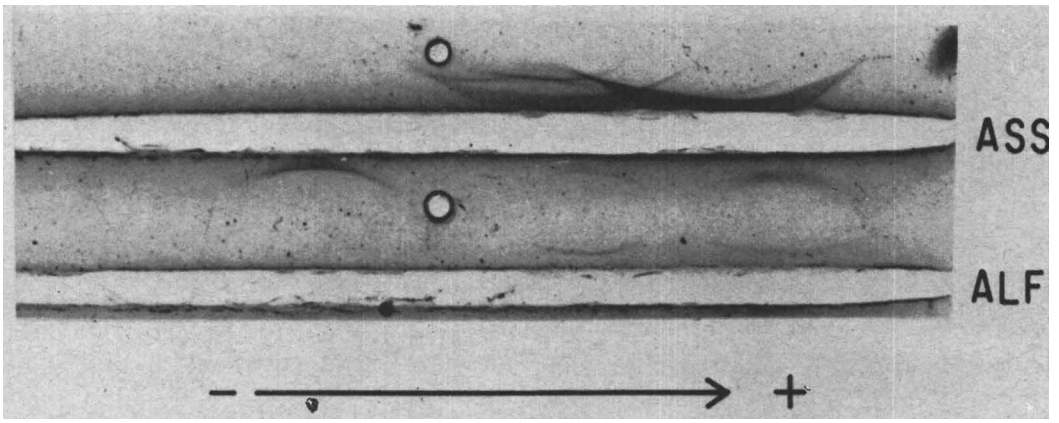

FIG. 1. Immunoelectrophoresis of fetal lamb serum, upper well, developed with rabbit antisera against whole sheep serum (ASS). In lower well, fetal lung fluid. Lower trough antiserum against fetal lung fluid (ALF). Notice lack of γ globulin in fetal serum whereas lung fluid contains a fraction with γ globulin mobility.

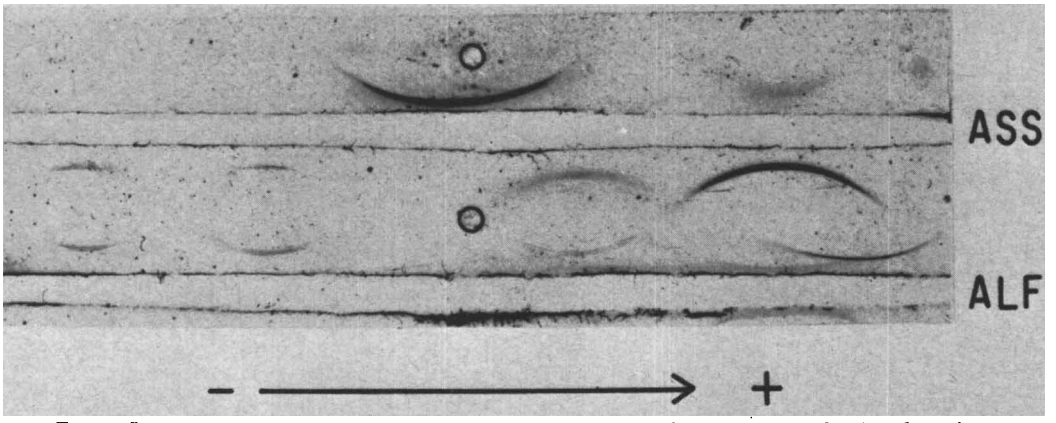

FIG. 2. Immunoelectrophoresis of concentrated fetal lung fluid, lower well, developed against specific antiserum (ALF), lower trough. Upper well, sheep β globulin (FIII). Upper trough, antiserum against whole sheep serum (ASS). The lung fluid shows fractions with prealbumin and γ globulin mobilities. No β globulin fraction is observed.

globulin and γ globulin were present in less than half of the samples. Fusion of the precipitin lines of fetal and maternal albumin seemed to indicate the identity of both proteins. Fractions with γ globulin and prealbumin mobility were detected only in lung fluid and not in fetal sera as shown in Figs. 1 and 2. Both ALF and ASS produced precipitin lines with sheep albumin, α globulin and β globulin. ALF did not react with sheep fibrinogen or γ globulin. Fetal lamb sera did not reveal the presence of γ globulin. The fetal lung fluid when analyzed unconcentrated by immunoelectrophoresis yielded visible lines in the γ globulin region against ASS only (Fig. 1). The same lung fluid when concentrated, yielded two lines against ALF in the region of immunoglobulin (Fig. 2). It is not known whether both of these precipitin lines correspond to immunoglobulin fractions. Presumably, one or both of these fractions may be immunoglobulin components secreted by the fetal lung. The lung fluid from acidotic fetal lambs invariably contained albumin, α globulin and γ globulin as shown in Table II.

Quantitative comparison of fetal lung fluid and sera by cellulose acetate electrophoresis showed the level of α_2 globulin per mg of

TABLE II. Results of Immunoelectrophoretic Analysis of Fetal Lamb Lung Fluid.

Status of fetus	No. of fetuses	Pre-albumin/no.	Albumin/no.	α Globulin/no.	β Globulin/no.	γ Globulin/no.
Postacidosis	3	1/3	3/3	3/3	2/3	3/3
Posthypercarbia	2	0/2	2/2	2/2	1/2	2/2
Total	5	1/5	5/5	5/5	3/5	5/5

albumin to be 5 to 10 times the value in the serum. Although the whole α fraction was high, α_2 was selected because it remains stable throughout the fetal life of the lamb(14).

Discussion. These studies describe the immunoelectrophoretic properties of the protein normally present in fetal lung fluid. Although not entirely analogous, human adult bronchial secretions have been extensively investigated. Atassi *et al.*(15) first reported the identification of seven components by electrophoresis. In the present study, five protein fractions were detected. One in particular, the γ globulin deserves some comment. Immunochemical studies of adult bronchial secretions have shown that γ A-globulin and albumin are the main plasma proteins identifiable in such material(16,17), although traces of most other plasma proteins have also been identified. These findings were confirmed by Biserte *et al.* (18,19) using chromatography on DEAE-cellulose in combination with electrophoresis in agar gel and immunoelectrophoresis, and further expanded by Masson *et al.* (20). It is therefore likely that the γ globulin fraction detected in fetal lung fluid is a polymeric γ A-globulin similar to that present in adult tracheal secretions(16,17,20). Since no γ globulin fraction can be detected by the usual techniques in fetal lamb plasma,[1] our studies add further support to the hypothesis that γ A-globulin is produced by the lungs as advocated by others(17,21,22). Additionally, our findings are suggestive of an early formation of a γ A-globulin fraction in some biological fluids, since it was present in immature fetuses, and preceded the appearance of a similar fraction in the plasma of both mature and immature fetal lambs.

[1] Although for all practical purposes the fetal lamb plasma is agammaglobulinemic(23,24), small amounts of γ globulin without a demonstrable antibody function have been described(25).

The albumin in human adult bronchial secretions is derived from the plasma(26). Based on the identical immunological reaction obtained in our study between the albumin fraction of fetal lung fluid and the fetal and maternal serum albumin, a similar origin can be postulated in the fetal lamb.

The presence of an α globulin in the fetal lung fluid was not an unexpected finding, since this fraction is prominent in the fetal plasma of all mammalian species including man(27), cow(28), goat, and sheep(14). Although its endogenous and/or exogenous origin cannot be determined at the present time, our preliminary data show an α_2/albumin ratio in the fetal lung fluid 5–10 times higher than fetal plasma. The latter suggests a partial production by the fetal lung, or less likely, a selective transport mechanism. The relative abundance of the α fraction in the lung fluid during fetal life, and its relationship with a similar fraction identified by Abrams(6) as the surface active lipoprotein, cannot be presently established. However, the establishment of this relationship and the possible implications with the respiratory distress syndrome warrant further studies.

Summary. The protein fractions present in fetal lung fluid were determined by means of cellulose acetate and immunoelectrophoresis. Proteins with identical electrophoretic mobility to that of sheep albumin and α globulins were detected in all; prealbumin, beta and gamma globulins were observed in some. An unidentified γ globulin other than γ G-globulin was shown to be present in fetal lung fluid and absent in fetal serum. The α_2 globulins in fetal lung fluid were found in a concentration which exceeds that of the serum. The local synthesis of both proteins in the lung is suggested.

1. Adams, F. H., J. Pediat. 68, 794 (1966).
2. Adams, F. H., Moss, A. J., and Fagan, L.,

Biol. Neonatorum 5, 151 (1963).

3. Adams, F. H. and Fujiwara, T., J. Pediat. 63, 537 (1963).

4. Avery, M. E. and Mead, J., Am. J. Diseases Children 97, 517 (1959).

5. Reynolds, E. O. R., Jacobson, H. N., Motoyama, E. K., Kikkawa, Y., Craig, J. M., Orzalesi, M. M., and Cook, C. D., Pediatrics 35, 382 (1965).

6. Abrams, M. E., J. Appl. Physiol. 21, 718 (1966).

7. Gitlin, D. and Craig, J. M., Pediatrics 17, 64 (1956).

8. Astrup, P. and Schroder, S., Scand. J. Clin. Lab. Invest. 8, 30 (1956).

9. Severinghaus, J. W. and Bradley, A. F., J. Appl. Physiol. 13, 515 (1958).

10. Kingsbury, F. B. and Clark, C. P., J. Lab. Clin. Med. 11, 891 (1926).

11. Scheidegger, J. J., Int. Arch. Allergy Appl. Immunol. 7, 103 (1953).

12. Burrows, S., Clin. Chem. 11, 1608 (1965).

13. Kaplan, A. and Johnstone, M., Clin. Chem. 12, 10 (1966).

14. Barboriak, J. J., Meschia, G., Barron, D. H., and Cowgill, G. R., Proc. Soc. Exptl. Biol. Med. 98, 635 (1958).

15. Atassi, M. Z., Barker, S. A., Houghton, L. E., and Mullard, K. S., Nature 196, 1269 (1961).

16. Anzai, T., Ibayashi, J., Carpenter, C. M., and Hyde, L., Am. Rev. Respirat. Diseases 88, 503 (1963).

17. Keimowitz, R. J., J. Lab. Clin. Med. 63, 54 (1964).

18. Biserte, G., Havez, R., and Cuvelier, R., Exposes Ann. Biochem. Med. 24, 85 (1963).

19. Biserte, G., Havez, R., Voisin, C., Delahousse, P., Cuvelier, R., and Gernez-Rieux, C., Lille Med. 6, 51 (1961).

20. Masson, P., Heremans, J. F., and Prignot, J., Biochim. Biophys. Acta 3, 466 (1965).

21. Askonas, B. A. and Humphrey, J. H., Biochem. J. 68, 252 (1958).

22. Tomasi, T. B., Jr. and Ziegelbaum, S. D., J. Clin. Invest. 42, 1552 (1963).

23. Richards, C. B. and Marrack, J. R., "Protides of the Biological Fluids," 10th Colloquim, Bruges, 1963.

24. Karcher, D., Bull. Soc. Roy. Zool. Anvers, 24, 1 (1962).

25. Sterzl, J., Kosta, J., Mandel, L., Riha, I., and Holub, M., "Mechanisms of Antibody Formation," Publishing House of Czechoslovak Academy of Sciences, Prague, 1960.

26. Steinmann, E. P., Fortschr. Hals-Nasen-Ohrenheilk 3, 40 (1956).

27. Bergstrand, C. G. and Czar, B., Scand. J. Clin. Lab. Invest. 8, 174 (1956).

28. Pedersen, K. O., Nature 154, 575 (1944).

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Absorption of Lactic Acid from an Isolated Intestinal Segment in the Intact Rat (32882)

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In certain disorders of digestion and absorption dietary carbohydrates are poorly absorbed and are degraded by intestinal bacteria. Some of the resultant products, principally organic acids, including lactic acid are excreted in the feces. These acids, because of their osmotic properties, have been implicated in the production of symptoms of disaccharide malabsorption (1–3).

Little is known about lactic acid absorption *in vivo*. This study was undertaken to determine the site of lactic acid absorption and the mechanism of its absorption, with special reference to the effect of intraluminal pH.

Materials and Methods. Male albino rats, 150–350 gm (Holtzman) were fasted 16–20 hours, anesthetized with ether, and through a midline incision a 20-cm loop of small intestine or colon was isolated. A glass cannula was sutured in place at each end of the segment. After a 20–ml saline wash, a test solution was perfused at room temperature by means of a pump at the rate of 5 ml for 15 min for 2 hours as described by Biggs and Davis(4). The perfusate consisted of various concentrations of sodium lactate with 1.0 μC of sodium lactate-1-^{14}C (4.5 mC/mmole) (New England Nuclear Corp.) adjusted to the desired pH with HCl and to approximately