similar surface constituent. The malignant cell lines HeLa (cervical cancer), HEp2 (laryngeal carcinoma), and 256 (rat sarcoma) were investigated under comparable conditions and demonstrated similar prepotential records. On the other hand, cultures of normal human embryonic heart and embryonic kidney rarely showed detectable prepotentials; normal monkey kidney and normal human lung cells (WI-38) seldom gave prepotential records except in cases in which the cells were rounded just prior to mitosis. This may have reflected an increase in cell surface chemicals during the G_2 phase of the cell cycle and the early mitotic process (*15*). Investigation of normal trophoblast and benign molar tumors of the trophoblast, as well as the malignant trophoblast, revealed prepotentials similar to those found in the malignant cell group. This reversion of malignant cell surface characteristics to that of a primitive trophoblast suggested a common biologic denominator between normal reproduction and cancer.

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References and Notes

1. G. Currie and K. Bagshawe, *Lancet* **1967-I**, 708 (1967).
2. J. Forrester, E. Ambrose, I. McPherson, *Nature* **196**, 1068 (1962).
3. R. Robinaux and S. Bogin, *Sanguine* **22**, 241 (1951).
4. G. Gasic and T. Beydak, *Biological Interactions in Normal and Neoplastic Growth* (M. J. Brennan and W. L. Simpson, London, 1961), p. 709.
5. W. R. Lowenstein, *Ann. N.Y. Acad. Sci.* **137**, 441 (1966).
6. K. Frank and M. C. Becker, *Physical Techniques in Biological Research* (Academic Press, New York, 1964), vol. 5, pp. 22–87.
7. R. Hertz, *Proc. Soc. Exp. Biol. Med.* **102**, 77 (1959).
8. R. A. Pattillo and G. O. Gey, *Cancer Res.* **28**, 1231 (1968).
9. R. A. Pattillo *et al.*, *Ann. N.Y. Acad. Sci.*, in press.
10. J. C. Garancis, R. A. Pattillo, R. O. Hussa, J. Schultz, R. F. Mattingly, *Amer. J. Obstet. Gynecol.*, in press.
11. A. Gottschalk, *Nature* **186**, 949 (1960).
12. G. Currie, *Trans. Rochester Trophoblast Conf. 4th* (1967), p. 162.
13. A. Martinez-Paloma and C. Brailovsky, *The Year Book of Cancer* (Year Book, Chicago, 1969), pp. 494–96.
14. A. Vorbrodt and H. Koprowski, *J. Nat. Cancer Inst.* **43**, 1241 (1969).
15. E. Mayhew, *J. Gen. Physiol.* **49**, 717 (1966).
16. We thank Dr. H. M. Kauffman, Dr. M. Rytel, A. C. F. Ruckert, J. Kurtz, J. Martin, and B. Stewart for technical assistance.

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Pulmonary Surfactant and Evolution of the Lungs

Abstract. *Pulmonary surfactant has been looked for and found in 11 representatives of four vertebrate classes. The amount of surfactant, estimated by quantitative spreading as a surface film, correlates well with alveolar surface area and with the amount of saturated, mainly dipalmitoyl, phosphatidylcholine in the lung parenchyma. The quantities of other phospholipids do not correlate well with alveolar surface area.*

The area of the alveolar surface of the lungs differs greatly among vertebrate species and is related to the rate at which oxygen is demanded by the individual. This relationship, clearly shown for mammals (*1*), also obtains for some lower vertebrates, such as the frog, the turtle, and the chicken (*2*). About 4.5 m^2 of respiratory surface is apparently needed for each millimole of oxygen absorbed per minute, and the required area is provided in the more active animals by subdivision of the lung into very small air spaces. Stabilization of this structure is attributed to the presence of a phospholipid-rich surfactant which can lower alveolar surface tension nearly to zero (*3*). The purposes of this investigation (*4*) were to determine whether the quantity of surfactant present in various lungs is appropriate to their respiratory surface area and whether their lipid composition reflects such a relationship. Previous studies did not quantify the surfactant (*5*, *6*) and did not reveal functionally significant differences (see *7*, *8*) in percentage composition of the lipids.

The lungs were excised from adult animals under anesthesia (guinea pig, rabbit, mouse, dog, sea lion, rat, chicken, turtle, and frog) or obtained fresh from the abattoir (cow) and immediately chilled in crushed ice. Human lung tissue, obtained during thoracotomy for bronchiogenic carcinoma and examined for absence of pathologic changes, was similarly processed. Weighed samples of parenchyma containing as little airway tissue as possible were homogenized thoroughly with 30 times their weight of 0.9 percent sodium chloride and centrifuged at 500*g* for 5 minutes to sediment fragments of connective tissue and cartilage. A small sample of the supernatant was dispersed in twice its volume of isopropyl alcohol (*9*) for demonstration of surfactant by efficient spreading on the surface of 0.9 percent sodium chloride in a surface balance similar to that described by Harkins and Anderson (*10*). Area was 350 cm^2 initially and was decreased at a constant rate of 4.5 cm^2/sec. Measurements were made at room temperature (24° to 26°C). The area occupied by the surface film when compressed so that surface tension fell to 12 dyne/cm (*11*) was taken as a measure of the amount of surface active material (SAM) in the sample. Multiplying this area by the ratio of the volume of the homogenate to the volume of the sample and by the ratio of the weight of the lung to the weight of the tissue sample gave the area coverable by surfactant in the whole lung. Other tissues (heart, liver, kidney) did not contain the surfactant as determined

Table 1. Correlation of surfactant, pulmonary surface area, and phospholipid concentrations in vertebrate lungs. Symbols: N, number of replicate lipid analyses; PE, phosphatidylethanolamines; SPH, sphingomyelins; LPC, lysophosphatidylcholines; PC_u, phosphatidylcholines containing one or more ethylenic bonds; PC_s, phosphatidylcholines containing no ethylenic bonds; A_{sp}, specific area; SAM, surface active material; *r*, correlation coefficient; *P*, probability of correlation. Phospholipid concentrations in milligrams per gram of fresh tissue, mean values. Standard errors of means averaged 9 percent of the means. Specific area in square centimeters per gram of fresh tissue. Surface active material in square centimeters of surface coverable at 12 dyne/cm by extracted surfactant per gram of fresh tissue. Each correlation coefficient gives the correlation between the component listed above it and A_{sp}.

Animal	N	PE	SPH	LPC	PC_u	PC_s	A_{sp}	SAM
Mouse	6	5.80	2.82	0.34	10.50	6.55	2985	9500
Guinea pig	4	6.49	3.46	.68	11.20	7.95	2811	9500
Rat	12	5.23	2.76	.35	10.33	5.00	2186	6400
Rabbit	10	5.54	3.45	.39	9.60	5.20	2091	7700
Sea lion	4	2.83	2.08	.14	5.10	6.90	1600	7900
Chicken	6	5.16	2.67	.25	6.58	4.72	1590	4700
Dog	4	4.49	4.10	.37	9.90	5.20	1378	8600
Man	2	1.41	2.46	.50	3.20	3.90	1253	6700
Cow	4	5.65	4.18	.22	9.30	2.30	831	4600
Turtle	6	2.28	1.50	.14	4.59	0.31	214	440
Frog	3	3.24	1.74	.25	8.97	.63	117	190
r		.65	.39	.58	.50	.90		.87
P		$<.05$	$>.2$	$>.05$	$>.1$	$<.01$		$<.01$

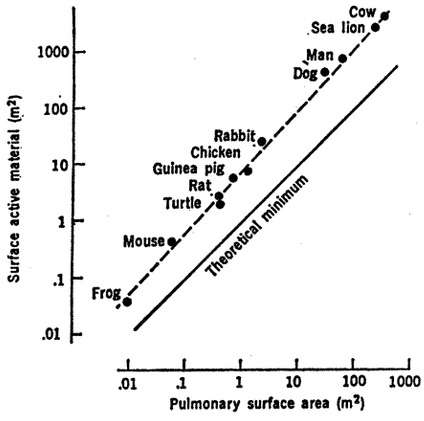

Fig. 1. Relationship of surfactant to alveolar area. Quantity of surfactant is expressed as the area coverable by extracted surface active material at a surface tension of 12 dyne/cm. The solid line indicates the amount of surfactant theoretically needed to cover the pulmonary surface with a single film. Each species appears to have a reserve.

by this test since they failed to give surface tensions as low as 12 dyne/cm. Purified surfactants, such as dipalmitoyl phosphatidylcholine and pulmonary surface-active lipoprotein from the dog (*12*) have been assayed with a precision of better than 5 percent by this method. We chose this technique, which does not incorporate any purification step, because of our unwillingness to make an a priori assumption about the chemical nature of the surfactants of various species. We cannot be sure that components of the tissue that are not directly related to the alveolar surfaces have been completely excluded from the film in the surface balance at 12 dyne/cm. Our method would define them, therefore, as surfactant. Unfortunately, no chemically specific quantitative method for pulmonary surfactant exists at present to test this possibility.

Similar samples of tissue were homogenized with a mixture of chloroform and methanol (2:1 by volume) to extract the lipids. The homogenates were separated and assayed by standard chromatographic and analytical methods (*13*). Special attention was given to isolation and estimation of saturated phosphatidylcholine (containing no ethylenic linkages) because of the prominence of this kind of substance in mammalian pulmonary surfactant (*14*). Unsaturated phosphatidylcholines were separated as mercuric acetate adducts by chromatography on silicic acid columns. An internal standard of dipalmitoyl phosphatidylcholine containing carbon-14 (synthesized in our laboratory by F. Rehbinder) was added to correct for losses during fractionation.

Values for alveolar surface area were culled from the literature for guinea pig, rabbit, mouse, dog, rat, cow, man, chicken, and frog (*1*, *15*). Area of the turtle lung was estimated on expanded, dried specimens by determination of net volume and average size of air space of the alveolated tissue. Area of sea lion lungs was calculated from pressure and volume measurements (*16*). For comparison of species, specific area (A_{sp}) was defined as the ratio of alveolar surface area to fresh weight of the lung.

The amount of surfactant estimated in these 11 kinds of lungs correlates well ($r = .99$, $P < .01$) with their alveolar surface area (Fig. 1). The wide range of lung size contributes to this correlation, but comparison of specific area and surfactant concentration in Table 1 confirms the correlation ($r = .87$, $P < .01$) with size removed as a factor, despite the crudeness of the estimates of area and surfactant.

Among the phospholipids assayed in these lungs only the concentrations of phosphatidylcholines containing no ethylenic linkages (PC_s) correlate well ($r = .90$, $P < .01$) with A_{sp} (Table 1). The fatty acid substituents of each PC_s fraction were determined by gas-liquid chromatography (*13*) in order to reveal any contamination with phosphatidylcholines containing unsaturated acids. These were absent, but in each animal species palmitic acid made up 93 to 99 percent of the saturated substituents of PC_s. Thus, the PC_s fractions are principally dipalmitoyl phosphatidylcholine, the predominant component of purified surfactant in several mammals. Not shown in Table 1 is the correlation between PC_s and SAM ($r = .93$, $P < .01$). These data make it likely that the pulmonary surfactants of nonmammalian vertebrates are also rich in dipalmitoyl phosphatidylcholine. Detailed analyses of purified surfactants from a variety of species are needed to test the generality of this conclusion.

This work confirms previous studies that did not show phylogenetic trends in the distribution of the major pulmonary phospholipid classes (*7*, *8*) and adds the observation that the content of dipalmitoyl phosphatidylcholine per unit weight of tissue varies widely among species. It does not confirm reports that surfactant is absent from the lungs of amphibia, reptiles, and birds (*5*, *8*) but does support those (*6*, *17*) that suggest its presence.

Despite the hazards of drawing conclusions about the evolution of vertebrate lungs from a comparison of present-day forms (*18*), one is tempted to suggest that the striking structural development of the lungs did not require obvious biochemical changes. The data presented here are consistent with the suggestion that components with putative functions at the alveolar surface remain in a fairly constant proportion to the respiratory surface area and that substances subserving cellular needs are related principally to the mass of the pulmonary tissue. If such a conclusion is valid it provides a simple example of the unity of biochemical organization in the biological world.

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References and Notes

1. S. M. Tenney and J. E. Remmers, *Nature* **197**, 54 (1963).
2. J. A. Clements, unpublished observations.
3. R. E. Pattle, *Nature* **175**, 1125 (1955); J. A. Clements, *Proc. Soc. Exp. Biol. Med.* **95**, 170 (1957).
4. Most of the work described here was summarized in lectures given at the 24th International Physiological Congr., Washington, D.C., 29 Aug. 1968, and at the Western Soc. for Clinical Research, Carmel, Calif., 30 Jan. 1969.
5. M. H. Klaus, O. K. Reiss, W. H. Tooley, C. Piel, J. A. Clements, *Science* **137**, 750 (1962).
6. R. E. Pattle and D. A. W. Hopkinson, *Nature* **200**, 894 (1963).
7. C. F. Baxter, G. Rouser, G. Simon, *Lipids* **4**, 243 (1969).
8. W. R. Harlan, Jr., J. H. Margraf, S. I. Said, *Amer. J. Physiol.* **211**, 855 (1966).
9. S. Ställberg and T. Teorell, *Trans. Faraday Soc.* **35**, 1413 (1939); D. O. Tinker and L. Saunders, *Chem. Phys. Lipids* **2**, 316 (1968).
10. W. D. Harkins and T. F. Anderson, *J. Amer. Chem. Soc.* **59**, 2189 (1937).
11. M. E. Abrams, *J. Appl. Physiol.* **21**, 718 (1966).
12. R. J. King and J. A. Clements, *Fed. Proc.*, in press.
13. D. F. Tierney, J. A. Clements, H. J. Trahan, *Amer. J. Physiol.* **213**, 671 (1967).
14. E. S. Brown, *ibid.* **207**, 402 (1964).
15. A. Krogh, *Skand. Arch. Physiol.* **15**, 328 (1904); E. Zeuthen, in *Biology and Comparative Physiology of Birds*, A. J. Marshall, Ed. (Academic Press, New York, 1960), p. 378.
16. J. A. Clements, in *Normal and Abnormal Respiration in Children*, 37th Ross Conference (1960), p. 39.
17. W. S. Tyler and J. Pangborn, *J. Cell Biol.* **20**, 157 (1964).
18. G. Hughes, in *Development of the Lung*, A. V. S. de Reuck and R. Porter, Eds. (Churchill, London, 1967), p. 64.
19. Since this paper was written, Fujiwara *et al.* have reported finding surfactant in turkey and rabbit lungs in proportion to estimated alveolar area [T. Fujiwara, F. H. Adams, M. Nozaki, G. B. Dermer, *Amer. J. Physiol.* **218**, 218 (1970)].
20. We thank O. I. Nissen for providing sea lion lungs and assisting in experiments with them. Supported in part by PHS grant HE-06285. Computing services were furnished under PHS grant FR-00122. J.A.C. is a career investigator of the American Heart Assoc.

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