PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 987–991 (1974)

Influence of Adrenalectomy on the Rat Lung[1] (37939)

R. A. RHOADES, L. N. GASSENHEIMER, AND W. R. BLACKBURN

Center for Air Environment Studies, Laboratory for Human Performance Research, and Department of Pathology, The Pennsylvania State University, University Park, Pennsylvania 16802

Recent studies have shown that hormones play an important role in lung function. Abnormal lung function has been associated with hyperthyroid individuals, which include a lower diffusing capacity and a decrease in both vital capacity and lung compliance (1, 2). Redding and coworkers (3) have reported that thyroid administration as well as thyroidectomy profoundly affect the morphologic characteristics of type II pneumocytes and the quantitative harvest of lung surfactant. However, Mason and coworkers (4) found no relationship between thyroid status and disaturated lecithin content in the lung, leaving open the question about the influence on pulmonary surfactant. Brody and Buhain (5) showed that growth hormone promoted lung growth by inducing cell hypertrophy, which resulted in a 20% increase in lung volume. Recently, Brody and coworkers (6) found individuals with hypopituitarism had reduced lung volumes and increased lung elastic recoil. Two lung volumes, in particular, functional residual capacity and residual volume, tended to be more severely affected with increased duration of the disease. These abnormalities seen in the lung were attributed to deficiency in growth hormone.

Another set of hormones, the adrenal corticosteroids, also are involved in lung function. Kotas and Avery (7) have shown that corticosteroid administration to the fetus accelerates lung development as well as surfactant synthesis. Blackburn and coworkers (8, 9) demonstrated that adrenal corticosteroids are involved in cell proliferation and differentiation in the lung, and adrenal deficiency may alter surfactant production. However, metabolic events underscoring these changes are poorly defined. Moreover, little information exists on the influence of adrenal hormones on the adult lung. The role that the adrenal hormones play is of particular interest since surfactant production and the structural integrity of the alveolar membrane are intimately related to lung metabolism.

As an initial approach to understanding adrenal deficiency on the adult lung, we examined the influence of adrenalectomy (ADX) on lung glucose utilization as well as surface tension measurments.

Methods. Male albino rats [adrenalectomized and (unoperated) control] were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA (Outbred CD Albino strain). Animals were housed individually in stainless steel cages in a room where temperature, relative humidity, and light were controlled at 22°, 40–60%, and 12-hr light–dark, respectively. All animals were supplied with food (Purina Laboratory Chow) and water *ad lib.*; the control group received tap water whereas the adrenalectomized animals received 0.9% NaCl in their drinking water.

Three weeks following the date of adrenalectomy, animals were sacrificed by intraperitoneal sodium pentobarbital injection (6 mg/100 g body wt) and rapid exsanguination via a carotid artery following thorocotomy. Blood was removed from the lungs by inserting a needle into the right ventricle and allowing the actively beating

[1] Supported in part by NIH Grant 1 RO1 HDO 6201 CAES No. 335-73.

heart to pump Krebs–Henseleit bicarbonate (KHB) buffer (10) through the pulmonary vasculature. The lungs were then excised, trimmed, and weighed to the nearest tenth of a milligram. Lung tissue slices were prepared using a Stadie–Riggs hand microtome and incubated in duplicate with each of three radioactive glucose substrates: glucose-1-^{14}C (sp act 4.7 μCi/μmole); glucose-6-^{14}C (sp act 3.95 μCi/μmole); and glucose-U-^{14}C (sp act 15 μCi/μmole). All isotopes were obtained from New England Nuclear Corp., Boston, MA. Incubation media in all cases consisted of KHB buffer, pH 7.4, containing 5 μmoles β-D-glucose/ml media and 3 μCi of the particular radioactive substrate/flask. Prior to incubation of tissue slices, the media in each flask was gassed with 95% O_2:5% CO_2 for 5 min, and the flask was placed in the reciprocating Dubnoff bath (90 strokes/min) at 37° for 10 min. Boiled tissue slices were incubated with each radioactive substrate and served as blanks.

The incubations of slices with glucose-1- and glucose-6-labeled substrates were carried out for 1 hr in 25 ml Kontes incubation flasks with syringe-cap stoppers fitted with polyethylene center wells. Incubations with glucose-U-^{14}C were carried out for 2 hr in 50 ml Kontes flasks fitted with side-arms which were capped during the course of the incubation period. Tissue slices were removed immediately at the end of the respective incubation period. In the case of glucose-1-^{14}C and glucose-6-^{14}C incubations, 0.2 ml Hydroxide of Hyamine (Rohm and Haas) was then injected into the center wells containing fluted Whatman No. 1 filter paper circles. For flasks containing glucose-U-^{14}C as the substrate, the caps on the side-arms were replaced by scintillation vials containing the filter paper circles and $^{14}CO_2$-trapping agent. $^{14}CO_2$ was then liberated from the incubation media by addition of 0.5 ml of 0.4 *N* H_2SO_4 and collected for 1 hr. At the end of this period, the center wells were placed in scintillation vials or the vials were removed from the side-arms and toluene scintillant prepared according to Buhler (11) was added to each $^{14}CO_2$ sample.

The lung slices removed following incubation were immediately placed in ice-cold saline, rinsed twice, blotted, and weighed to the nearest tenth of a milligram. In the case of glucose-U-^{14}C incubations, the lipids were extracted, separated into lipid classes, and phospholipids were hydrolyzed as previously described (3). All samples were counted in a Tri-Carb 3375 Scintillation spectrometer (Packard Instruments). Plasma glucose was determined by a direct *o*-toluidene method (12), and plasma corticosterone was determined by a spectrofluorometric procedure (13). Surface tension area-loops from crude-lung extracts were obtained from a modified surface tension balance (14). Approximately 0.60 g of lung tissue was homogenized with a Sorvall Omni-mixer in 30 ml of KHB. The material was then filtered through gauze and 25 ml of the filtered material placed in the surfactometer trough. The material was equilibrated for 10 min and then was cyclically compressed and expanded two times from 100 to 15% trough area; the second cycle was recorded. Duration of the full cycle was 6 min. Maximum (γ max) and minimum (γ min) surface tension and stability index ($\bar{s}$) were calculated from each curve. Student's *t* test was used for all tests of significance (15).

Results. Completeness of adrenalectomy was verified by measuring plasma corticosterone levels. Mean values are presented in Table I. Only one animal in the ADX group exhibited a high level of corticosterone and was eliminated. Mean body weights in the ADX group were significantly higher initially, although following the 3-week ADX period, mean body weights showed a significant 15% reduction below the controls. Both wet and dry weight in the ADX lungs were significantly higher. There was no significant difference in lung glycogen. Although mean glucose values were lower in the ADX groups, the difference was not statistically significant.

Table II shows the incorporation of glucose-U-^{14}C into lung slices. In the ADX group, there was a significant decrease of labeled glucose into total lipids (30%), phospholipids (31%), and oxidation of

TABLE I. Measurements Made on Adrenalectomized Rats.

	Control	Adrenalectomized
Body weight (g)		
Initial	188.8 ± 1.1[a]	212 ± 4.1*
	$N = 6$	$N = 8$
Final	400 ± 11.8	340.1 ± 9.6*
	$N = 6$	$N = 8$
Lung weight (g)		
Wet	1.530 ± 0.030	1.977 ± 0.122*
	$N = 6$	$N = 8$
Dry	0.2803 ± 0.005	0.3604 ± 0.021*
	$N = 6$	$N = 8$
Lung glycogen	0.0622 ± 0.0045	0.0690 ± 0.0049
(mg/100 mg wet lung)	$N = 6$	$N = 6$
Plasma corticosterone	13.5 ± 1.10	2.03 ± 0.59*
(μg/100 ml)	$N = 5$	$N = 7$
Plasma glucose	169.1 ± 6.05	159.5 ± 5.5
(mg/100 ml)	$N = 5$	$N = 7$

[a] Mean ± SE.
* Significantly different from control at 5% level.

glucose to CO_2 (26%). The C_1/C_6 ratio for the ADX group was also significantly lower, indicating less carbon flow through the hexose monophosphate shunt. Hydrolysis of the lung phospholipids revealed that the depressed incorporation of glucose-U-^{14}C was proportionally greater in the phospholipid fatty acids than in the phospholipid glyceride glycerol moiety. Surface tension properties in the ADX group (Table III) were normal in all respects to the controls.

Discussion. Glucose is readily taken up by the lung and is used for oxidation, glyceride synthesis, and synthesis of phospholipid fatty acids (16–18). Glucose also provides a source of NADPH via the hexose monophosphate shunt for reductive biosynthesis of fatty acids (16). Salisbury-Murphy *et al.* (19), using the C_1/C_6 ratio to assess the hexose monophosphate shunt, showed this pathway to be very active in the lung. The variety of metabolic pathways for glucose utilization underscores the importance of this substrate in overall lung metabolism. As seen in Table II, the preferential incorporation of glucose into phospholipids is consistent with the presence in the lung of high phospholipid levels and the rapid turnover of surfactant material.

In the present study, adrenal deficiency

TABLE II. Influence of Adrenalectomy on Glucose Utilization by Lung Slices.

	nmoles glucose-U-^{14}C-converted/100 mg/2 hr	
Metabolite	Control	Adrenalectomized
$^{14}CO_2$	438.4 ± 33.9[a]	326.5 ± 36.6*
$^{14}CO_2$, (C_1/C_6)	3.25 ± 0.19	2.71 ± 0.13*
Total lipid	133.2 ± 9.8	93.2 ± 9.9*
Neutral lipid and FFA	16.0 ± 1.1	13.4 ± 0.5
Phospholipid (PL)	112.6 ± 9.8	76.7 ± 9.0*
PL fatty acids	52.3 ± 4.1	36.3 ± 3.7*
PL glyceride glycerol	49.9 ± 2.1	35.9 ± 2.7*

[a] Mean ± SE ($N = 5$).
* Significantly different from controls ($P < 0.05$).

TABLE III. Effect of Adrenalectomy on Surface Tension.

	Adrenalectomized ($N = 8$)	Control ($N = 6$)
γ min	9.94 ± 0.68[a]	8.75 ± 0.67
γ max	37.94 ± 2.12	36.25 ± 1.82
$\bar{s}$	1.17 ± 0.03	1.22 ± 0.04

[a] Mean ± SE.

drastically altered glucose utilization. In particular, both glucose oxidation and glyceride synthesis were depressed. In addition, fatty acid synthesis was severely affected. The depressed fatty acid synthesis may be due, in part, to the decreased activity of the hexose monophosphate shunt, since this pathway supplies NADPH necessary for fatty acid synthesis.

Interpretation of the influence of adrenalectomy is complicated by the fact that both medullary and cortical hormones are removed. However, in the absence of exogenous epinephrine, ADX animals can be maintained indefinitely by the administration of corticosteroids (20, 21), which provides indirect proof that the disturbances in carbohydrate and lipid metabolism following ADX are due chiefly to the removal of the adrenal cortex. Of these hormones, the 11-oxy or 11-hydroxycorticosteroids are the most potent in their effects on carbohydrate and lipid metabolism. The lung appears to differ in some respects from other organs following corticosteroid insufficiency. First, lung glycogen remained relatively stable whereas glycogen in other organs is severely depleted (22). We have observed a similar phenomenon with lung glycogen during fasting. Following a 72-hr fast, lung glycogen was unaltered whereas liver glycogen was markedly decreased (18). Second, glucose utilization is not accelerated as seen in muscle and liver (21–23). The difference in metabolic response of the lung may be due to the duration of ADX as well as to corticosteroid insufficiency exerting an influence on specific enzymes.

The present study does not permit us to delineate the precise role in which adrenal insufficiency is exerting its effects on the lung. A direct effect of glucocorticoids on lung metabolism does not appear to be the mode of action, since, in this study, ADX leads to a decrease in glucose utilization, which is an effect opposite to that commonly seen in peripheral tissue following ADX. The increase in lung weight cannot be explained on the basis of pulmonary edema since there was no increase in lung water. The 30% increase in lung weight seen in Table I might be due, in part, to differences in body weights, since the ADX group had a higher initial body weight. To verify that this was not the case, we tested the proportionality of lung weight to initial body weight for the ADX group. Differences in initial body weights account for no more than one-third of the increase in lung weight, leaving at least a 20% increase in lung weight due to hormonal effects.

A possibility more consistent with our findings would be the interaction between adrenal hormones and another hormone. An attractive candidate would be growth hormone, which is known to promote organ growth through increased protein synthesis as well as inhibiting glucose utilization. Thus, an increase in amount of growth hormone or an increased sensitivity to normal secretion would account for both the higher lung weight and altered carbohydrate metabolism. Several points would tend to favor such an explanation. (a) Fasted ADX rats become hypoglycemic and low glucose levels stimulate the release of growth hormone (24). (b) Adrenalectomized animals are more sensitive to growth hormone (25). Brody *et al.* (5) observed that ADX potentiated an increase in lung weight with hypersecretion of growth hormone. (c) In other organs such as liver, there is a striking increase in amino acid incorporation into protein during the first 28 days following ADX (25).

From Table III, the physiologic properties of pulmonary surfactant do not appear to be affected by ADX. Thus, whether changes in glucose utilization and lung weight following ADX affects pulmonary ventilation remains to be determined.

Summary. Glucose-U-^{14}C, glucose-1-^{14}C, and glucose-6-^{14}C utilization were examined

in vitro in lungs from adrenalectomized (ADX) male rats. Lungs from rats killed 3 weeks following adrenalectomy showed a significant increase ($P < 0.05$) in lung weight. Incorporation of glucose-U-^{14}C by the lung was significantly depressed by 30% in total lipids and by 32% in phospholipids (PL) in the ADX group. Hydrolysis of PL revealed that the depressed incorporation was proportionately greater in PL fatty acid than in PL glyceride glycerol moiety. The ability to oxidize labeled glucose was also significantly reduced (26%) in the ADX lungs. The C_1/C_6 ratio was significantly lower in the ADX group, suggesting relatively less carbon flow through the hexose monophosphate shunt. The data shows that adrenal deficiency has a profound influence on glucose metabolism and lipogenesis in the rat lung. Adrenalectomy did not affect lung surface tension properties. The suggestion is made that following adrenalectomy there is an increase in amount of growth hormone or an increase sensitivity to normal secretions of growth hormone which leads to higher lung weight and altered carbohydrate metabolism.

A sincere thanks is extended to Mary Lou Eskew for her technical assistance.

1. Stein, M., Kimbel, P., and Johnson, R. L., Jr., Clin. Invest. **40**, 438 (1960).

2. Massey, D. G., Becklake, M. R., McKenzie, J. M., and Bates, D. V., New Eng. J. Med. **276**, 1104 (1967).

3. Redding, R. A., Douglas, W. H. J., and Stein, M., Science **175**, 994 (1972).

4. Mason, R. J., Manganiello, V., and Vaughan, M., Amer. Rev. Resp. Dis. **106**, 767 (1972).

5. Brody, J. S., and Buhain, W. J., Amer. J. Physiol. **223**, 1444 (1972).

6. Jain, B. P., Brody, J. S., and Fisher, A. B., Amer. Rev. Resp. Dis. **108**, 49 (1973).

7. Kotas, R. V., and Avery, M. E., J. Appl. Physiol. **30**, 358 (1971).

8. Blackburn, W. R., Potter, D. M., Travers, H., Gassenheimer, L. L., and Rhoades, R. A., Proc. Soc. Exp. Biol. Med. **140**, 885 (1971).

9. Blackburn, W. R., Travers, H., and Potter, D. M., Lab. Invest. **26**, 306 (1972).

10. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., "Manometric and Biochemical Techniques," p. 146, Burrgess Publ. Co., Minneapolis (1972).

11. Buhler, D. P., Anal. Biochem. **4**, 413 (1962).

12. Frings, C. S., Ratliff, C. R., and Dunn, R. T., Clin. Chem. **16**, 282 (1970).

13. Zenker, N., and Benstein, D. E., J. Biol. Chem. **231**, 695 (1958).

14. Williams, R. A., Rhoades, R. A., and Adams, W. S., Med. Biol. Eng. **9**, 729 (1971).

15. Snedecor, G. W., "Statistical Methods," 5th ed., Iowa State University Press, Ames (1956).

16. Felts, J. J., Health Phys. **10**, 973 (1964).

17. Heineman, H. O., and Fishman, A. P., Physiol. Rev. **49**, 1 (1969).

18. Scholz, R. W., and Rhoades, R. A., Biochem. J. **124**, 257 (1971).

19. Salisbury-Murphy, S., Rubenstein, D., and Beck, J. C., Amer. J. Physiol. **211**, 988 (1966).

20. Long, C. N. H., Smith, O. K., and Fry, E. G., *in* "Metabolic effects of adrenal hormones," (G. E. W. Wolstenholme and M. O'Conner, eds.), CIBA Foundation, Study Group 6 pp 4-24, Little, Brown, and Co., Boston (1960).

21. Turner, D. C., and Bagnara, J. T., "General Endocrinology," pp. 348–383, W. B. Saunders, Philadelphia (1971).

22. Long, C. N. H., Katzin, B., and Fry, E. G., Endocrinology **26**, 309 (1940).

23. Winternitz, W. W., Dintzis, R., and Long, C. N. H., Endocrinology **61**, 724 (1957).

24. Harper, H. A., "Review of Physiological Chemistry," p. 495, Lang Med. Publications, Los Altos (1969).

25. Korner, A., *in* "Metabolic effects of adrenal hormones." (G. E. W. Wolstenholme and M. O'Conner, eds.). CIBA Foundation, Study Group 6, pp. 38–59, Little, Brown and Company, Boston (1960).

Received Oct. 23, 1973. P.S.E.B.M., 1974, Vol. 145.