JCI The Journal of Clinical Investigation

Radiation pneumonitis in mice. Some effects of corticosteroids on mortality and pulmonary physiology.

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J Clin Invest. 1980;66(3):504-510. https://doi.org/10.1172/JCI109881.

Research Article

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Radiation Pneumonitis in Mice

SOME EFFECTS OF CORTICOSTEROIDS ON MORTALITY AND PULMONARY PHYSIOLOGY

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ABSTRACT The fall in pulmonary compliance in mice with radiation pneumonitis is associated with increased microvascular leakage of plasma proteins into the alveolar spaces and increased surfactant phospholipids in the lung and alveolar fluid. In the present experiments we examined the effect of corticosteroid administration on these two effects and on pulmonary mechanics 16 wk after x irradiation of the thorax. Survival in irradiated animals that received corticosteroids was markedly better during the period of corticosteroid administration than that of irradiated animals that received no corticosteroids. The development of abnormalities in pulmonary mechanics and alveolar fluid surface tension appeared to be inhibited in the irradiated animals receiving corticosteroids as compared with irradiated animals not receiving corticosteroids. The increased microvascular protein leakage seen in the lungs of irradiated mice was not significantly different in the corticosteroid-treated group. However, corticosteroid administration was associated with a marked increase in the amount of phosphatidyl choline that could be recovered from the alveolar spaces by lavage, over and above the increase resulting from irradiation, and a significant increase in the incorporation of [^{14}C]palmitate into phosphatidyl choline by lung slices. The beneficial effects of steroids in this variety of adult respiratory distress syndrome may be the result of augmented surfactant production which may contribute to the maintenance of relatively normal pulmonary mechanics despite substantial leakage of plasma proteins into the alveolar space.

INTRODUCTION

In radiation pneumonitis the major mechanical abnormality in the lungs of humans and experimental animals is a fall in lung compliance (1). In previous experiments on mice I showed that this was due to abnormalities of the alveolar surface layer rather than to stiffness of the lung tissue (2). The abnormality of the alveolar surface layer appeared to be due to the presence of large amounts of plasma protein in the alveolar spaces, furthermore the rate of albumin leakage from the circulation into the alveolar space was strikingly increased (3).

In other experiments an increase in the content and synthesis rate of surfactant phospholipids occurred when mice developed radiation pneumonitis (4). It was not clear whether this was a direct effect of irradiation, or whether it was a secondary response to lung damage.

Corticosteroids have been shown by Phillips et al. (5) to be prophylactic against death in mice that received radiation to the lungs. We therefore examined the effects of corticosteroids on lung mechanics, leakage of protein into the alveolar lining layer, and surfactant phospholipid metabolism in the lung in radiation pneumonitis. Phospholipid synthesis and secretion were further increased in the lungs of mice that received corticosteroids, suggesting that this is a protective mechanism against the fall in lung compliance that occurs when there is excessive microvascular leakage of protein.

METHODS

All experiments were performed on CF_1 female mice aged 10–12 wk at the time of x irradiation. They received a total of 2,800 rad in two fractions to the thorax. The anesthetized mice were attached to a revolving surface. With the head, abdomen, and tail shielded by 3-mm lead shields, the thorax was exposed to x-rays generated by a Maxitron 250 unit (General Electric Co., Milwaukee, Wisc.) operated at 250 kVp and 30 mA, using a filter of 0.25 mm Cu and 1 mm Al (6). Mice of the same age and delivered in the same batch served as controls; these were treated identically except that they did not receive x irradiation.

Corticosteroid administration. The control and irradiated groups were each randomly subdivided into corticosteroid

Received for publication 17 December 1979 and in revised form 12 May 1980.

J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.* · *0021-9738/80/09/0504/07 $1.00*
Volume 66 September 1980 504–510

and no corticosteroid groups, giving four final groups of mice. Corticosteroid administration commenced 80 d after the completion of x irradiation. Animals in the two steroid groups received 250 μg methyl prednisolone in saline (SoluMedrol, Upjohn Co., Kalamazoo, Mich.) by intraperitoneal injection on alternate days for 20 d and daily thereafter until the time of study, another 7–12 d. The two nonsteroid groups received an equal volume of isotonic saline by intraperitoneal injection according to the same schedule.

Physiologic studies. The protocol for measuring mechanical properties of the lungs, obtaining blood, lung, and alveolar lavage samples and wet and dry weight lung measurements was similar to that in a previous study (3). Animals were anesthetized by intraperitoneal injection of 2 mg pentobarbital and 50 U of sodium heparin, weighed, and attached supine to a small animal board. The abdomen was opened and the inferior vena cava was cannulated. In rapid succession the aorta was transected, arterial blood was collected, and the vena cava perfused with isotonic saline at a hydrostatic pressure of 10 cm to exsanguinate the lungs. The perfusion was stopped after ~5 min at which time the fluid leaving the aorta appeared clear. The trachea was then cannulated (Angiocath 18, Deseret Pharmaceutical Co., Sandy, Utah) and closed with a three-way stopcock with the lungs at functional residual capacity. The thorax was opened and its ventral half resected. Static pressure-volume studies were performed by injecting 0.1-ml increments of air into the lungs via the tracheal cannula while transpulmonary pressure was measured under static conditions at each incremental inflation and deflation with a U tube water manometer through the sidearm of the three-way stopcock. The lungs were inflated to the first increment above transpulmonary pressure of 25 cm H_2O and deflated by increments of 0.1 or 0.05 ml air to transpulmonary pressure of zero or below.

Collection of lung and alveolar lavage samples. After physiologic studies, the left lung was resected, weighed, dried at 95°C to constant weight, and reweighed. Meanwhile, the animal was placed in a desicating chamber with its head inclined down at ~20° and with the tracheal cannula under degassed isotonic saline. Vacuum was applied (Welch Duo-Seal, Chicago, Ill.) for 5 min and then released. The right lung was then reinflated with 1.2 ml isotonic saline at room temperature; this was flushed into and out of the lung three times. Four additional lavages, 0.8 ml each, were obtained in the same manner and all five lavages were pooled. The combined alveolar lavage fluid was centrifuged at 250 *g* for 10 min and the supernate decanted, measured, divided into aliquots, and stored at −15°C. The cellular pellet was resuspended in 0.5 ml isotonic saline and cell counts were performed using a hemocytometer chamber to exclude obvious pulmonary infection. The presence of polymorphonuclear leukocytes amounting to >5% of the suspended cells is indicative of such infection. None of the mice in the present experiments had evidence of infection. The lavaged right lung was removed, chopped with scissors, homogenized in a final volume of 4.0 ml of distilled water in an all glass homogenizer, divided into aliquots, and stored at −15°C.

Protein studies. Microvascular leakage of protein was studied as previously described using radioiodinated serum albumin (3). Mouse albumin (50 μg, Sigma Chemical Co., St. Louis, Mo.) was labeled with ^{125}I by the chloramine-T method (7). The iodinated protein was purified in a column of Sephadex G-200 (0.9 × 30 cm) equilibrated with 0.05 molar sodium phosphate buffer, pH 7.0, and eluted with the same buffer. A sharp peak of acid precipitable radioactivity was eluted immediately after the 4-ml void volume. The three peak fractions (1 ml each) were combined. A small portion was assayed by cochromatography with 5 mg of authentic unlabeled mouse albumin in the same system. The elution profile of radioactivity corresponded very closely with that of protein as determined by optical density at 280 nm; the radioactivity was taken to represent radioiodinated serum albumin.

6 h before assay each mouse was anesthetized with ether and received 2 μCi radioiodinated serum albumin in 0.1 ml isotonic saline by tail vein injection. Aliquots of arterial blood, exsanguinated lung and alveolar lavage fluid, obtained and stored as described above, were thawed, and treated with trichloroacetic acid, final concentration 8%, at 0–4°C for 30 min. The sample was centrifuged, 5,000 *g* for 10 min, the supernate discarded, and the pellet assayed for radioactivity in a Nuclear-Chicago gamma counter (Nuclear-Chicago, Des Plains, Ill.). Results are expressed as the ratio of counts per minute in the lung homogenate or alveolar lavage sample per counts per minute in 10 μl of the simultaneously obtained blood sample. Correction for residual blood in the lungs was made by the cyanmethemoglobin method (3). Total protein in aliquots of the alveolar lavage fluid was measured by the method of Lowry et al. (8) using bovine serum albumin standards (fraction V, Sigma Chemical Co.).

Phospholipid studies. Phospholipid content was measured in aliquots of the lungs and alveolar lavage fluid as previously described (4). At the start of the purification procedure 2,200 dpm of [^{14}C]phosphatidyl choline (methyl labeled, 23.3 mCi/mM, ICN Pharmaceuticals Inc. Irvine, Calif.) was added to each sample to correct for losses. Phospholipids were purified by the method of Folch et al. (9) and separated by thin layer chromatography on silica gel HR plates (Analtech, Inc., Newark, Del.) in one dimension for alveolar fluid or two dimensions for lung using the solvent systems of Getz et al. (10). Spots corresponding to phosphatidyl choline (PC)[1] were eluted and assayed for phosphorus by the method of Bartlett (11). A portion of each sample was assayed for its disaturated PC content according to the method of Mason et al. (12) by osmium tetroxide oxidation and alumina column elution.

Phospholipid synthesis was assayed in a separate experiment on four groups of mice irradiated and treated with steroid or saline exactly as described above for the remainder of the experiment. Following the methods of Abe and Tierney (13), we resected the exsanguinated lung and chopped it into 0.5-mm slices (McIlwain tissue chopper, Brinkmann Instruments, Inc., Westbury, N. Y.). Weighed portions of lung slices (close to 40 mg each) were incubated in Krebs-Ringer bicarbonate medium containing 5% bovine serum albumin, 5 μCi of [^{3}H]glycerol (New England Nuclear, Boston, Mass., adjusted to sp act 6.25 μCi/μM) and 4 μCi [^{14}C]palmitate (ICN Pharmaceuticals, Inc., adjusted to sp act 5.6 μCi/μM). Incubation mixtures were gassed with 5% CO_2 in air and incubated at 37°C for 60 min with agitation. Triplicate assays and one control (boiled for 2 min before incubation) were performed on each lung sample. The reactions were stopped by addition of 1 ml methanol and immersion in ice. The sample was centrifuged at 10,000 *g* for 10 min and the pellet was homogenized, frozen, and stored. It was subsequently extracted for PC and disaturated PC as described above.

Surface tension studies. Surface tension was measured in duplicate 25% aliquots of the total of each pooled alveolar fluid as previously described (2). A Kimray Greenfield surfactometer (Kimray, Inc., Oklahoma City, Okla.) was adjusted for optimal sensitivity and linearity of output and calibrated with weights made from aluminum foil. The hypophase was 14 ml of Ringer's solution at room temperature, cleaned by suction of the surface. The aliquot was applied dropwise to the surface

[1] *Abbreviations used in this paper*: DPC, disaturated PC; PC, phosphatidyl choline.

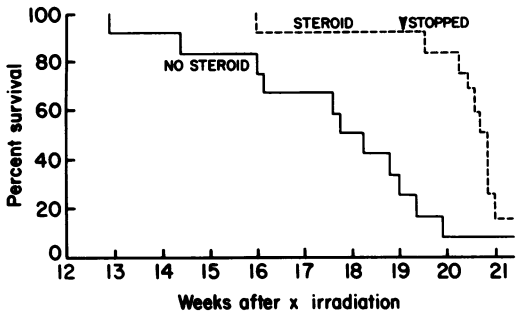

FIGURE 1 Survival of mice after 2,800 rad to the thorax. (——), no steroid administered. (– – –), mice received 250 μg methyl prednisolone on alternate days from 80 to 100 d after x irradiation and then daily. Steroid administration was discontinued at the arrowhead, 19 wk.

of the hypophase at maximal surface area. The surface was then aged without change in surface area for 2 h, this time being in excess of that required to reach equilibrium surface tension. The surface area was then cycled to 15% of maximum area and back to maximum area once, while the surface tension was recorded. Minimum surface tension was obtained from the chart. Hysteresis was determined by cutting out the area of the chart enclosed by the surface area-surface tension loop and weighing it.

RESULTS

Survival. The effect of corticosteroids on survival was tested in two groups of 24 mice that had received x irradiation to the thorax. The result is shown in Fig. 1. Animals that did not receive corticosteroids died over a period of many weeks, 50% survival occurring at ~18 wk, which is similar to previous reports (14, 15). Death at this time has been shown to be due to radiation pneumonitis (15). Survival at 19 wk was significantly enhanced in the group that received corticosteroids, $P < 0.02$. In this group deaths occurred at an accelerated rate shortly after steroid withdrawal; and by 21 wk there was no significant difference between the two groups.

Body and lung weights. Irradiated mice failed to gain weight at the same rate as controls, Table I; this change was not diminished in the steroid-treated group. The wet and dry weights of the left lungs were significantly increased in irradiated mice; this abnormality was not significantly reduced in the group that received corticosteroids. The ratios of wet to dry weights were similar in all four groups of mice.

Mechanical properties of lungs. The compliance of the lung was measured from the slope of the linear portion of the deflation limb of the pressure-volume curve. Table II shows that absolute lung compliance was significantly reduced in irradiated nonsteroid-treated mice, but not in irradiated steroid-treated mice. Compliance was made specific by dividing it by a lung volume, the volume of gas removed from the lung between transpulmonary pressures of 25 and 0 cm H_2O on the deflation limb of the pressure-volume curve. This volume was significantly reduced in both groups of irradiated mice, Table II. Specific lung compliance was low only in the irradiated nonsteroid-treated mice. Thus, although the lungs were smaller in both groups of irradiated mice, only the steroid-treated group had normal mechanical properties.

Surface tension of alveolar fluid. Results are shown in Table III. As was previously found (2), the alveolar fluid of irradiated nonsteroid-treated animals failed to achieve the minimum surface tension of that achieved by controls. However the alveolar fluid of irradiated steroid-treated animals achieved a minimum surface tension that was not significantly different from corresponding controls but significantly less than that of irradiated nonsteroid-treated animals ($P < 0.025$). Analysis of the area of hysteresis provided the same result.

Microvascular protein leak. This was studied in two ways: by quantitation of the total protein in the alveolar lavage fluid and by the flux of ^{125}I-albumin from the blood into the lungs and alveolar lavage fluid 6 h after tail vein injection. We have previously shown

TABLE I
Body Weight and Lung Weights 16 wk after Thoracic Irradiation

	Control	Control steroid	Irradiated	Irradiated steroid
Body weight, g	27.7±3.1	27.7±1.9	23.1±3.6*	23.9±2.9‡
Lung weights, mg§				
Wet weight	53.6±2.8	54.2±5.3	68.9±10.7*	68.4±8.8‡
Dry weight	9.8±1.2	10.7±2.0	13.6±3.9*	12.8±2.3*
Wet/dry ratio	5.5±0.5	5.2±0.5	5.2±0.5	5.3±0.6

Means±1 SD.
* Significantly different from corresponding nonirradiated control at $P = 0.05$.
‡ Significantly different from corresponding nonirradiated control at $P = 0.01$.
§ Left lung only.

TABLE II
Static Mechanical Properties of Lungs

	Control	Control steroid	Irradiated	Irradiated steroid
$C_{L\,abs}$	8.21±0.62	8.20±1.4	6.46±1.59*	7.11±0.89
Lung volume, ml	1.01±0.04	1.01±0.15	0.87±0.18‡	0.89±0.10‡
$C_{L\,sp}$	8.11±0.45	8.06±0.61	7.43±0.60*	8.00±0.52

* Significantly different from corresponding control at $P = 0.025$.
‡ Significantly different from corresponding control at $P = 0.05$.
Means±1 SD, compliance units are deciliter·centimeter H_2O^{-1}.
$C_{L\,abs}$, absolute compliance; $C_{L\,sp}$, specific compliance.
Differences were not significant unless indicated.

in this model that protein recovered by this lavage technique represents protein present in the alveoli rather than protein eluted from lung tissues (3). Total protein lavaged from the right lung is shown in Table IV. Both groups of irradiated mice had considerably more protein in the lavage fluid than did controls; the amount was not reduced in mice receiving steroids.

Data for the flux of ^{125}I-albumin from the blood into lung tissue and alveolar fluid are shown in Table V. In both groups of irradiated mice about twice as much ^{125}I-albumin appeared in lung tissue and alveolar fluid as in control mice which is similar to what was previously reported (3). Steroid treatment was not associated with a significant reduction in the flux of labeled albumin into lung tissue or alveolar fluid in either controls or irradiated mice.

Phospholipid content of lungs and alveolar fluid. Table VI shows the content of total phospholipids, PC, and disaturated PC (DPC) in the lungs of the four groups of mice. In both groups of irradiated mice PC and DPC were increased, as was previously found (4). However, there were no significant differences in the phospholipid content between steroid-treated and nonsteroid-treated groups. The proportion of PC that was disaturated (DPC/PC) was the same in all groups, 42–43%.

The same data for alveolar fluid is shown in Table VII. Irradiated nonsteroid mice had significantly more total phospholipid, PC, and DPC than corresponding controls. An even greater increase occurred in irradiated steroid-treated mice, where we found an increase of nearly threefold above steroid-treated controls, and of nearly twofold above irradiated nonsteroid-treated mice. The proportion of PC that was disaturated (not shown in Table VII) was between 68 and 73% in all groups and not significantly altered by either irradiation or steroid administration.

Incorporation of [^{3}H]glycerol and [^{14}C]palmitate into PC by lung slices in vitro is shown in Table VIII. Incorporation was increased in both groups of irradiated mice as compared with nonirradiated controls and was also increased in both steroid-treated groups as compared with the corresponding untreated groups. In the case of [^{3}H]glycerol, incorporation by steroid-treated irradiated lungs was not significantly increased above that by untreated irradiated lungs, but the same trend was present.

DISCUSSION

In previous experiments we have found two abnormalities associated with the reduction in lung compliance in radiation pneumonitis, an increase in surfactant phospholipids and an increase in leakage of protein from the circulation that occurs between 2 and 4 mo after irradiation (2–4). The relationship between the

TABLE III
Surface Tension Properties of Alveolar Lavage Fluid

	Control	Control steroid	Irradiated	Irradiated steroid
Minimum surface tension (dyn·cm^{-1})	6.4±2.2	6.0±3.8	16.5±6.7*	9.5±5.5
Area of hysteresis (arbitrary units)	24.9±5.5	23.8±6.2	13.7±5.1*	21.4±6.8

* Significantly different from corresponding control at $P = 0.001$.
Differences were not significant unless indicated.
Means±1 SD.

TABLE IV
Total Protein in Alveolar Lavage Fluid

	No steroid	Steroid	P*
	μg		
Control	487±170	400±177	NS
Irradiated	1,100±548	1,336±800	NS
P‡	<0.025	<0.01	

* P value of difference between values on the same line.
‡ P value of difference between values in the same column.
Means±1 SD.

mechanical changes and the biochemical changes was unclear. Because corticosteroids have been reported to have a protective effect in radiation pneumonitis (5), we used them to study their effects on each of the above two abnormalities associated with radiation pneumonitis. Although survival was enhanced and pulmonary mechanics were significantly better in the steroid-treated group, neither the amount of protein recovered from the alveolar spaces by lavage (Table IV), nor the flux of ^{125}I-albumin from the circulation into the alveolar fluid (Table V) was significantly lower in the steroid-treated mice. On the other hand significant effects on phospholipid biochemistry were found.

The most striking effects were the very large increases in total phospholipid, PC, and DPC in the alveolar fluid of irradiated steroid-treated mice as compared with irradiated but untreated mice (Table VII). Alternative mechanisms that could account for the increases are increased synthesis, decreased breakdown, or both. Increased synthesis is suggested by the increased rate of incorporation of precursors into PC by lung slices in vitro (Table VIII). This interpretation is consistent with previous studies of the effect of corticosteroids on the surfactant system of adult lungs, which show an increase in the size and number of normal rat type II cells and their lamellar bodies (16), increased incorporation of glycerol and palmitate into PC by rat lung slices in vitro (13), increased amount, synthesis, and possibly secretion of PC by lung in pregnant rabbits (17), and increased incorporation of choline into PC by type II cells in culture (18). In none

TABLE V
Flux of ^{125}I-albumin from Blood into Lung Tissue and Alveolar Fluid

	No steroid	Steroid	P*
Lung tissue			
Control	2.17±1.67	2.33±1.37	NS
Irradiated	4.94±2.69	3.39±1.00	NS
P*	<0.025	NS	
Alveolar fluid			
Control	1.64±0.30	1.46±0.74	NS
Irradiated	2.82±1.18	2.20±0.78	NS
P*	<0.025	<0.05	

2 μCi ^{125}I-albumin was injected into the tail vein 6 h before assay.
Results expressed as the ratio of acid-precipitable counts per minute in lung tissue or alveolar fluid to acid precipitable counts per minute in 10 μl blood, mean±1 SD.
* P, significance of the difference between values on the same line or in the same column.

TABLE VI
Phospholipid Content of Lungs

	No steroid	Steroid	P*
	μg phosphorus		
Total phospholipids			
Control	105.5±8.1	116.6±22.8	NS
Irradiated	142.3±30.5	135.9±27.2	NS
P*	<0.01	NS	
PC			
Control	53.0±7.4	57.2±10.2	NS
Irradiated	83.3±24.2	79.9±9.5	NS
P*	<0.01	<0.001	
DPC			
Control	22.5±1.9	24.0±2.7	NS
Irradiated	34.6±7.9	34.4±4.3	NS
P*	<0.002	<0.001	

All values for right lungs, means±1 SD.
* P, significance of difference between values on the same line or in the same column.

TABLE VII
Phospholipid Content of Alveolar Fluid

	No steroid	Steroid	P*
	μg phosphorus		
Total phospholipids			
Control	14.0±2.9	15.1±6.1	NS
Irradiated	20.4±6.6	40.3±18.4	<0.02
P*	<0.025	<0.01	
PC			
Control	12.3±2.6	14.1±4.7	NS
Irradiated	21.3±8.0	34.8±14.7	<0.025
P*	<0.02	<0.01	
DPC			
Control	8.4±2.1	9.4±3.1	NS
Irradiated	15.5±6.6	24.1±9.9	<0.05
P*	<0.02	<0.002	

All values for right lungs, means±1 SD.
* P, significance of the difference between values on the same line or in the same column.

TABLE VIII
Incorporation of Precursors into Lung PC In Vitro

	No steroid	Steroid	P*
	[^3H]*glycerol, nM/g wet tissue per h*		
Control	13.6±2.5	15.2±2.9	<0.05
Irradiated	16.6±4.1	17.5±2.3	NS
P*	<0.02	<0.02	
	[^{14}C]*palmitate, nM/g wet tissue per h*		
Control	55.4±10.0	67.9±8.7	<0.02
Irradiated	79.6±11.7	91.8±14.8	<0.025
P*	<0.001	<0.001	

Means±1 SD.
* *P*, significance of difference between values on the same line or in the same column.

of these studies was alveolar fluid PC separated from lung PC. If phospholipid synthesis is enhanced in the lungs of corticosteroid-treated animals, then the present finding that phospholipid content of the lung is unchanged (Table VI) requires explanation. The phospholipids that remain in the lung after lavage are the constituents of a heterogeneous population of cells, only some 6% of which are type II cells concerned with surfactant synthesis (19). Even in the case of the relatively surfactant-specific phospholipid DPC there is evidence for a substantial pool or pools in the lung cells that do not enter the alveolar spaces (20). Therefore, a significant change in the surfactant-related phospholipids in the lavaged lung could be obscured by the bulk of all nonsurfactant-related phospholipids. Another possibility is that secretion was enhanced as well as synthesis, in which case no increase in lung phospholipid content would be found, but an increase in alveolar fluid phospholipid might be expected. The data in Tables VI and VII are consistent with this interpretation.

An alternative mechanism of increased alveolar fluid phospholipid content in corticosteroid-treated animals is that degradation was decreased. Steroids have been shown to inhibit the activity of certain lung phospholipases (21). Although there is no data in the present study against decreased degradation, the likelihood is that increased synthesis is at least one of the mechanisms by which the amount of phospholipid in the alveolar fluid was increased in corticosteroid-treated animals.

The relationship, if any, of increased surfactant phospholipid to enhanced survival is unclear. As mortality in this model is attributable to the fall in compliance of the fluid lining layer (2), one can speculate that enhanced survival in the corticosteroid-treated group is due to the improvement in lung compliance and alveolar fluid surface tension properties by means of increased surfactant phospholipid production. Possibly increased production permits repletion of the surfactant layer in conditions such as radiation pneumonitis, where leakage of circulatory proteins accelerates the inactivation or desorption of surfactant from the air-fluid interface (22, 23). Alternatively, enhanced survival in the corticosteroid-treated mice may occur by mechanisms that are unrelated to phospholipid metabolism, of which many have been postulated (24–28).

Elucidation of the role of corticosteroids in this condition may have wider clinical implications. Radiation pneumonitis can be regarded as one of the group of conditions loosely known as adult respiratory distress syndrome. The role of steroids in respiratory distress syndrome is controversial and their use at present has little rational basis (29).

ACKNOWLEDGMENT

I thank Duane Smith for his excellent technical assistance.

REFERENCES

1. Gross, N. J. 1977. Pulmonary effects of radiation therapy. *Ann. Intern. Med.* **86**: 81–92.
2. Gross, N. J. 1978. Experimental radiation pneumonitis; changes in physiology of the alveolar surface. *J. Lab. Clin. Med.* **92**: 991–1001.
3. Gross, N. J. 1980. Experimental radiation pneumonitis IV. Leakage of circulatory proteins onto the alveolar surface. *J. Lab. Clin. Med.* **95**: 19–31.
4. Gross, N. J. 1979. Experimental radiation pneumonitis. III. Phospholipid studies in the lungs. *J. Lab. Clin. Med.* **93**: 627–637.
5. Phillips, T. L., M. D. Wharam, and L. W. Margolis. 1975. Modification of radiation injury to normal tissues by chemotherapeutic agents. *Cancer (Phila.).* **35**: 1678–1684.
6. Gross, N. J. 1977. Alveolar macrophage number: an index of the effect of radiation on the lungs. *Radiat. Res.* **72**: 325–332.
7. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy Appl. Immunol.* **29**: 185–189.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
9. Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation of and purification of total lipides from animal tissues. *J. Biol. Chem.* **226**: 497–509.
10. Getz, G. S., S. Jakovcic, J. Heywood, and M. Rabinowitz. 1970. A two-dimensional thin-layer chromatographic system for phospholipid separation. *Biochim. Biophys. Acta.* **218**: 441–452.
11. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234**: 466–468.
12. Mason, R. J., J. Nellenbogen, and J. A. Clements. 1976. Isolation of disaturated phosphatidyl choline with osmium tetroxide. *J. Lipid Res.* **17**: 281–284.
13. Abe, M., and D. F. Tierney. 1977. Lung lipid metabolism after 7 days of hydrocortisone administration to adult rats. *J. Appl. Physiol.* **42**: 202–205.
14. Field, S. B., and S. Hornsey. 1974. Damage to mouse lung with neutrons and x-rays. *Eur. J. Cancer.* **10**: 621–627.

15. Wara, W. M., T. L. Phillips, L. W. Margolis, and V. Smith. 1973. Radiation pneumonitis: a new approach to the derivation of time dose factors. *Cancer (Phila.).* **32:** 547–552.
16. Picken, J., M. Lurie, and J. Kleinerman. 1974. Mechanical and morphologic effects of long-term corticosteroid administration on the rat lung. *Am. Rev. Respir. Dis.* **110:** 746–753.
17. Tsao, F. H., G. R. Gutcher, and R. D. Zachman. 1979. Effect of hydrocortisone on the metabolism of phosphatidylcholine in maternal and fetal rabbit lungs and livers. *Pediatr. Res.* **13:** 997–1001.
18. Anderson, G. G., J. A. Cidlowski, P. M. Absher, J. R. Hewitt, and W. H. J. Douglas. 1978. The effect of dexamethasone and prostaglandin $F_2\alpha$ on production and release of surfactant in type II alveolar cells. *Prostaglandins.* **16:** 923–929.
19. Fulmer, J. D., and R. G. Crystal. 1976. The biochemical basis of pulmonary function. *In* Biochemical Basis of Pulmonary Function. R. G. Crystal, editor. Marcel Dekker, Inc., New York. 419–466.
20. Young, S. L., and D. F. Tierney. 1972. Dipalmitoyl lecithin secretion and metabolism by the rat lung. *Am. J. Physiol.* **222:** 1539–1544.
21. Flower, R. 1978. Steroidal anti-inflammatory drugs as inhibitors of phospholipase A_2. *Adv. Prostaglandin Thromboxane Res.* **3:** 105–112.
22. Taylor, F. B., and M. E. Abrams. 1966. Effect of surface active lipoprotein on clotting and fibrinolysis, and of fibrinogen on surface tension of surface active lipoprotein, with a hypothesis on the pathogenesis of pulmonary atelectasis and hyaline membrane in respiratory distress syndrome of the newborn. *Am. J. Med.* **40:** 346–350.
23. Said, S. I., M. E. Avery, R. K. Davies, C. M. Banerjee, and M. El-Gohary. 1965. Pulmonary surface activity in induced pulmonary edema. *J. Clin. Invest.* **44:** 458–464.
24. Pingleton, W. W., J. J. Coalson, L. B. Hinshaw, and C. A. Guenter. 1972. Effects of steroid pretreatment on development of shock lung: hemodynamic respiratory, and morphologic studies. *Lab. Invest.* **27:** 445–456.
25. Lillehei, R. C., R. H. Dietzman, G. J. Motsay, C. D. Beckman, L. H. Romero, and C. H. Shatney. 1974. Growth of the concept of shock and review of present knowledge. *In* Steroids and Shock. T. M. Glenn, editor. University Park Press, Baltimore, Md. 377–413.
26. Wilson, J. W. 1972. Treatment or prevention of pulmonary cellular damage with pharmacologic doses of corticosteroids. *Surg. Gynecol. Obstet.* **134:** 675–681.
27. Hammerschmidt, D. E., J. G. White, P. R. Craddock, and H. S. Jacob. 1979. Corticosteroids inhibit complement-induced granulocyte aggregation. A possible mechanism for their efficacy in shock states. *J. Clin. Invest.* **63:** 798–803.
28. Balis, J. U., E. S. Rappaport, L. Gerber, J. Fareed, F. Budding, and H. L. Messmore. 1978. A primate model for prolonged endotoxin shock: blood vascular reactions and effects of glucocorticoid treatment. *Lab. Invest.* **38:** 511–523.
29. Hopewell, P. C., and J. F. Murray. 1977. The adult respiratory distress syndrome. *In* Respiratory Emergencies. E. M. Shibel and K. M. Moser, editors. The C. V. Mosby Company, St. Louis, Mo. 101–128.