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The Usefulness of Lung Surfactant Phospholipids (LSPs) in the Diagnosis of Drowning

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ABSTRACT: The authors have studied the usefulness of some lung surfactant phospholipids (LSPs) isolated from lung tissues as markers of drowning. Two different groups of rabbits were sacrificed by drowning in fresh and salt water, and their phospholipid compositions were compared with those of a non-drowned control series.

For the phospholipids studied in lung lavages (phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl glycerol) the proportions differed between the control group and the drowned group, and between the fresh-water and salt-water drowned animals. According to these results, the lipids we have analyzed can be employed as markers in forensic autopsies, where it is necessary to differentiate between death by drowning and postmortem immersion and between fresh-water and salt-water drowning.

In lung tissue, only phosphatidyl choline and phosphatidyl inositol showed significative differences.

These results also confirm that LSPs are strongly affected in drowning.

KEYWORDS: pathology and biology, drowning, phospholipids, fresh water, salt water, surfactant

Drowning is one of the three leading causes of accidental death in children and causes over 8000 deaths per year in the United States [*1*]. The world total of death by drowning is estimated at about 150 000 per year (about 5 to 6 per 100 000 population [*2*]).

Because of its especially complex physiopathology—which varies greatly between fresh and salt water and can, in addition, be strongly influenced by many different factors, such as the age and state of consciousness of the victim; any kind of intoxication, especially with alcohol [*3*]; the ambient and fluid temperatures; and other factors—the postmortem diagnosis of drowning can be one of the most difficult problems in coroner's pathology, as the findings are often minimal, ambiguous, or even completely negative [*4*].

Most of the cases of death by drowning can be perfectly diagnosed with an appropriate autopsy: in these cases it is not necessary to employ the many complementary laboratory techniques that exist. However, there are also some cases that present so many difficulties

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that, even employing all of these techniques, it would not be possible to answer all the questions that could be raised. In such cases, it can be especially difficult to distinguish between death by drowning and postmortem immersion or between fresh-water and salt-water drowning. These especially complex situations are frequently related to criminal deaths, where an exact diagnosis is required (by the judge and police).

Nowadays, there are a number of complementary markers, and it is well known that all of them have disadvantages and can be useless in certain circumstances. In this experimental design, we have studied the usefulness of lung surfactant phospholipids (LSPs) as markers of drowning. Pulmonary surfactant is a highly surface-active material that maintains alveolar stability at low lung volumes. It is a complex material that consists mainly of phospholipids but also contains specific proteins [5]. Its phospholipid composition is characteristic and distinctly different from those of the whole lung, alveolar lavage effluent, and other tissues. Phosphatidyl choline (PC) accounts for about 80% of the total phospholipid in pulmonary surfactant, and phosphatidyl glycerol (PG) is the second most abundant phospholipid and accounts for up to 11% of the total [6]. As this substance is directly and strongly affected in most drownings, we have assumed that this alteration could be observed as a variation in the composition of the surfactant, and that this variation could depend on the tonicity of the drowning fluid.

Materials and Methods

We have employed 45 New-Zealand rabbits (body weight, 1.5 ± 0.150 kg), which were divided into the three following series:

1. *A control series (Series I)*—Fifteen animals were sacrificed using an overdose of sodium pentobarbital (150 mg/kg, given intraperitoneally).
2. *A fresh-water series (Series II)*—Fifteen animals were sacrificed by drowning in fresh (tap) water, according to a technique previously described [7].
3. *A salt-water series (Series III)*—Fifteen animals were sacrificed by drowning in salt water [7], which had been artificially prepared [8].

Once an animal was dead, an autopsy was immediately performed, and the lungs with 5 cm of trachea were removed.

The pulmonary surfactant phospholipids were determined using two different but parallel techniques: endobronchial lung lavage, which was used to quantify phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), and phosphatidyl glycerol (PG), and lung tissue analysis, which was used to determine the levels of phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), phosphatidyl serine (PS), and sphingomyelin (S).

The lungs were lavaged by means of an endotracheal tube [9] with 20 mL of fluid—isotonic saline in the control series, fresh water in the second series, and salt water in the third one—and the fluid was drained through the trachea; this lavage procedure was performed two times. The lavage effluents were combined and the phospholipids were extracted with chloroform/methanol (2:1 ratio) [10].

Subsequently, four pieces of 1 g each were excised from the upper and lower lobes of both right and left lungs, and the phospholipids were extracted with chloroform/methanol (2:1 ratio) [11]. The final extracts from the lavage effluents and tissues were evaporated under a nitrogen stream, divided into aliquots, and frozen at −30°C until used.

The surfactant phospholipids were separated into their component species by thin-layer chromatography (TLC) in silica-gel 60 plates [high-performance thin-layer chromatography (HPTLC) silica-gel plates, from E. Merck, Darmstadt, FRG] with chloroform/methanol/petroleum ether/acetic acid/boric acid (40-20-30-10-1.8, v/v/v/v/w), ac-

cording to a single-plate method widely used [*12*]. In this technique, the solvent system was freshly prepared and the chromatogram was developed to within 1 cm of the top of the plate in tanks lined with solvent-saturated filters. After being dried under a stream of nitrogen, the lipids were made visible by brief exposure to iodine vapor. Spots of the different phospholipids were identified using appropriate standards (purchased from Sigma, St. Louis, MO) and were removed. Their phosphorus contents were then quantified directly in the gel using a reaction based [*13*] on the acid digestion of the gel with 70% perchloric acid. Amidol and ammonium molybdate were added, and the resulting blue color was quantified at 830 nm by spectrophotometry.

To develop the calculations, phosphorus was assumed to be 1/25 of the total weight of any phospholipid [*14*]. The results for all the different phospholipids are expressed as a percentage of the lipid in the total phospholipid composition of a sample.

Statistical analysis was carried out using both an ANOVA-1 test, to compare the differences between the different series, and a linear regression test.

Results and Discussion

The percentages of phospholipids in the lung lavage effluents are expressed in Fig. 1, and their values (n = 15) and statistical significance are shown in Table 1.

The main problem we have in discussing our results is the lack, to our knowledge, of literature on the phospholipidic composition of the surfactant referred to in drowning.

Lung lavage phospholipids must be differentiated from lung tissues ones. Lung lavage phospholipids show spectacular results, with highly significant differences. The highest values of PC appear in the controls, Series I. This fact may be due to the process of endobronchial lavage of live animals—active washing—which assumes the passage of drowning fluid to the lungs. This lavage is potentiated by the vigorous respiratory movements that occur; in consequence, pulmonary surfactant is actively washed and expulsed

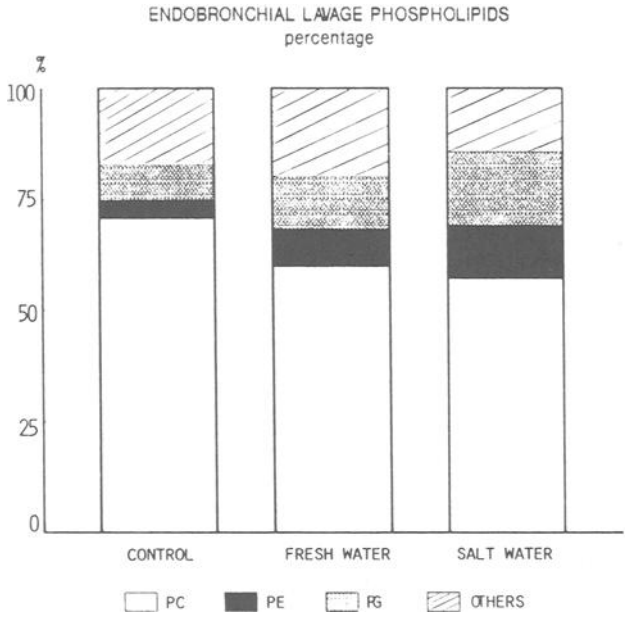

FIG. 1—*Percentage of endobronchial lavage phospholipids in the three different series.*

TABLE 1—*The values for and statistical significance of the percentages of endobronchial lavage phospholipids in the three series, given in Fig. 1.*

Phospholipid	Series	X, %	SD, %	Significance, P
Phosphatidyl choline	I	70.7	4.9	I–II <0.01
	II	65.9	2.9	I–III <0.001
	III	57.4	5.1	II–III <0.001
Phosphatidyl ethanolamine	I	3.91	0.67	I–II <0.001
	II	9.19	0.79	I–III <0.001
	III	11.67	1.67	II–III <0.001
Phosphatidyl glycerol	I	8.22	1.79	I–II <0.001
	II	12.95	1.54	I–III <0.001
	III	16.80	2.41	II–III <0.001

to the drowning fluid, where it appears as foam. We must also consider that lavage produces a process of pulmonary acute edema, which removes surfactant from the alveolar walls to the water or any other drowning fluid. In this way, when the lungs of drowned animals are washed, their phospholipid compositions are different from those of control—not drowned—animals, which receive only a passive washing. The difference in composition observed between the fresh-water and salt-water series—where Series II shows higher values than Series III—may be due to the greater insult produced by salt water, which can destroy the surfactant system more easily.

PE and PG results show an opposite behavior, because the minimum values appear in the control series, and the highest ones in Series III. This fact may be due to two different causes: first, the proportional decrease in the PC percentage may elevate the proportion of both PE and PG, and second, PE and PG could be less affected by destruction of the surfactant system than PC and, therefore, are not expulsed as much as PC in bronchoalveolar foam.

This differences in the values of PC, PE, and PG can be useful in establishing the difference between death by drowning and postmortem immersion. As the differences in the concentrations of PC, PE, and PG are also statistically significant between Series II and III, they are useful in differentiating between fresh-water and salt-water drowning.

These results are also useful in confirming the physiopathologic hypothesis widely accepted that pulmonary surfactant is affected by drowning fluid, and this destruction may be one of the causes of death by drowning—together with ionic alterations, especially increasing potassium levels [*15*]; the disappearance of pleural negative pressure [*16*]; and hypoxia, leading to fatal anoxia [*17*]. Alterations in the plural surfactant, which could be objectively quantified by knowing its phospholipidic composition, could be studied in all near-drowning victims as a way to evaluate pulmonary damage and to initiate the appropiate techniques for recuperation.

Lung tissue phospholipids show fewer differences than lung lavage phospholipids, as is shown in Fig. 2; the exact values and statistical significance of these data are shown in Table 2.

This minor variation is logical if we consider that pulmonary tissue is the place of synthesis, storage, and action of the different phospholipids. It is necessary for there to be a great decrease in the quantity of any phospholipid before one can observe statistically significant differences; therefore, a process like drowning, which usually takes no more than 3 or 4 min, cannot diminish the tissue storage in a significant way. Having made this assumption, note that only PI and PC show significant differences, and these differences are not highly significant. Nevertheless, we think that it would be necessary to employ larger series before really significant differences could be obtained.

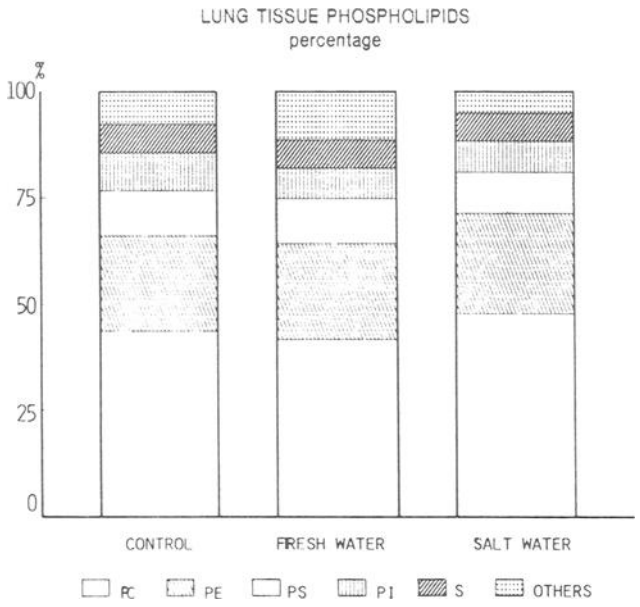

FIG. 2—*Percentage of lung tissue phospholipids in the three different series.*

TABLE 2—*The values for and statistical significance of the percentages of lung tissue phospholipids in the three series, given in Fig. 2.*

Phospholipid	Series	*X*, %	SD, %	Significance, *P*
Phosphatidyl choline	I	43.6	3.7	I–II NS[a]
	II	41.7	2.4	I–III <0.05
	III	47.8	2.7	II–III <0.001
Phosphatidyl ethanolamine	I	22.39	2.77	I–II NS
	II	22.59	2.75	I–III NS
	III	23.51	1.67	II–III NS
Phosphatidyl inositol	I	8.89	1.50	I–II <0.01
	II	7.22	1.03	I–III <0.05
	III	7.33	0.88	II–III NS
Phosphatidyl serine	I	10.68	1.25	I–II NS
	II	10.53	1.63	I–III NS
	III	9.77	1.41	II–III NS
Sphingomyelin	I	6.85	1.01	I–II NS
	II	6.71	0.83	I–III NS
	III	6.63	1.12	II–III NS

[a]NS = not significant.

Conclusions

To summarize, this first experimental approximation shows that, when using the lung lavage technique, the pulmonary surfactant PC, PE, and PG may be useful markers for drowning—first, to distinguish between drowning and postmortem immersion and second, to distinguish between fresh-water and salt-water drowning. In addition, alteration

of pulmonary surfactant phospholipids causes pulmonary instability and death by respiratory distress syndrome.

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