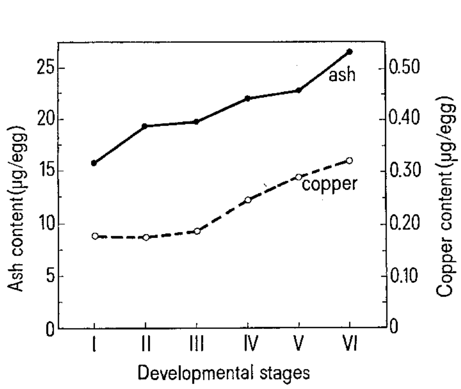
connections between the eggs removed. For ash and copper analyses, the following 6 stages of development were chosen: Stage I: Soon after spawning; eggs deep green in color, oval in shape (age: 0 to 24 h). Stage II: Transparent blastoderm visible at one end of the egg (age: ca. 48 h). Stage III: Streaks of pigmented eyes noticed. The egg slightly milky white. Occasional pulsations of the heart noticed (age: 6 to 7 days). Stage IV: Eyes and appendages fully developed (age: 10 to 12 days). Stage V: Jerky movements of the embryo noticed occasionally (age: 14 to 16 days, just prior to hatching). Stage VI: Freshly hatched zoeae (age: 16 to 17 days).

Ash content of the different stages of eggs was determined by incineration of the sample (50 mg) at 560 °C for 5 h[8]. Copper contents of developing eggs and zoeae were estimated colorimetrically following the procedure detailed by KOLMER et al.[9].



Intake of ash and copper in the eggs of *Palaemon lamarrei* as a function of incubation time.

Results and discussion. Marine crustacean eggs are known to be initially impermeable to water. However, PANDIAN[4,5] reported that they were permeable to salts throughout their development. In the eggs of *Palaemon lamarrei* also, salts were absorbed from the beginning of the incubation till hatching (Table). Ash (= salt) content increased during the developmental stages both as a percentage and quantitatively, indicating that the developing egg actively absorbed salts from the surrounding freshwater medium over a concentration gradient. It was therefore interesting to know whether this increase was due to the absorption of copper, a very important element necessary for the formation of haemocyanin. Copper content, which was about 175.3 µg/egg or 364.4 µg/g dry egg in stage I to III (age: 1 to 7 days) exhibited a marked increase in stage VI (freshly hatched zoea). Intake of copper in the eggs of *P. lamarrei* as a function of incubation time also exhibited a more or less similar trend to that of total salt intake (Figure). In a closely related species, *Palaemon malcomsonii*, RAJYALAKSHMI[10] observed that the development of heart took place in 7- to 11-day-old embryos. In *P. lamarrei* pulsation of the heart was observed from 7 to 9 days of age. Hence, a marked increase in the copper content from 0.182 µg/egg in stage III to 0.245 µg/egg in stage IV and further to 0.316 µg/zoea can be attributed to an increased synthesis of haemocyanin. On the whole, it was observed that during the entire embryonic development, lasting for 14 to 16 days, out of a total salt intake of 11 µg as much as 0.143 µg was due to the absorption of copper.

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Lipid and Phospholipid Content and Fatty Acid Composition of the Chick Lung During Embryonic Development

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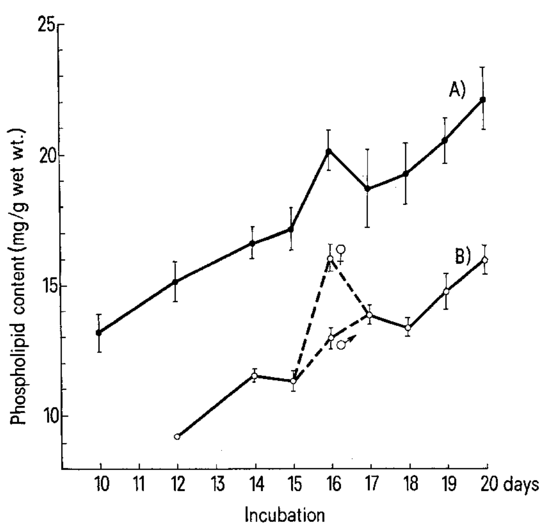
Summary. The evolution of total phospholipid, phosphatidylcholine and phosphatidylethanolamine content of chick lung during embryonic development is in good agreement with morphological data. Saturated fatty acids are predominant. A sex-linked difference is observed in the evolution of phosphatidylcholine.

Although the existence of a pulmonary surfactant in birds was first denied[1], it is now well established that the epithelium of the functional lung is lined by a surface active phospholipoproteic material, whose main features – high lecithin content, high percentage of saturated fatty acids - are similar to those of the mammalian surfactant[2]. Despite the particular structure of the respiratory parenchyma, which is a continuous network of air capillaries, the role of the surfactant in birds is comparable to that in mammals: it prevents transsudation of plasma fluids across the blood-air barrier, and it helps to keep the air capillaries open, to ensure sufficient ventilation[3]. Its physiological role is thus considerable, and its appearance in the course of foetal development is a very important step in functional differentiation.

In mammals, there is much information on normal evolution of lung lipids during foetal life, and recent results indicate that maturation of the pulmonary tissue, both morphological and biochemical, depends on the pituitary-adrenal axis[4–12]. In birds, the maturation of lung tissue has so far been studied only from the morphological point of view; as in mammals, the granular pneumocytes, which are responsible for surfactant synthesis, begin to differentiate at a late stage[13], and as in mammals this differentiation depends on humoral factors, secreted by the hypophysis or controlled by it[14–15]. From the biochemical standpoint, however, there are, so far as we know, no available data, either about normal development, or about the endocrine control of lung maturation.

The results reported here concern normal evolution, quantitative and qualitative, of lung lipids during the embryonic development of the chick. They represent the preliminary step in the study of hormonal control of lung differentiation.

The lungs were removed from white Leghorn chick embryos, between 10 and 20 days of incubation. Since NAYE et al.[16] have pointed out a difference in the rate of the morphological differentiation of human lung according to sex, lungs from females and males were pooled separately. Total lipids were extracted by chloroform-methanol, according to FOLCH et al.[17], and their amount was measured by gravimetry. The phospholipid content was evaluated on an aliquot part of the extracts, by measuring the amount of phosphorus[18], modified. The phosphatidylcholine (PC), i.e. lecithin, and phosphatidylethanolamine (PE) content were evaluated in the same way, following separation by thin layer chromatography, according to ROUSER et al.[19]. The fatty acid composition of PC and PE was determined by gas liquid chromatography, following sulphate methylation at room temperature[20].



Total lipid and phospholipid content in the lung of the developing chick embryo (mg/g of wet weight ± SE). A. Total lipid content. Each value is the mean of measurements of 6 to 20 separate extracts (2 to 12 pairs of lung for each extract, depending on stage). Since comparison of ♂ and ♀ values using Student's *t*-test did not show any significant difference, all the results are pooled. B. Total phospholipid content. Each value is the mean of measurements of 3 to 8 separate extracts. Comparison of the ♂ and ♀ phospholipid content on day 16 using Student's *t*-test gives $p < 0.01$. No significant difference at other stages.

Table I. Lecithin and phosphatidylethanolamine content of chick embryonic lung (mg/per g of wet weight ± SD)

Age in days of incubation	Number of extracts	Phosphatidylcholine	Phosphatidylethanolamine
14♀ + ♂	8	5.71 ± 0.79	2.69 ± 0.43
16♀	4	9.13 ± 1.37	5.27 ± 0.79
♂	3	5.54 ± 0.73	5.24 ± 0.63
18♀ + ♂	8	6.37 ± 0.98	5.31 ± 0.54
20♀ + ♂	8	7.16 ± 0.62	6.47 ± 0.66

Comparison between the ♂ and ♀ PC levels on day 16, using Student's *t*-test gives $p < 0.01$.

The Figure represents the evolution of the total lipid and phospholipid content in the course of development. As shown by curve A, the total lipid content increases during incubation, this increase being strongly enhanced between days 15 and 16. The variation of the total phospholipid content (curve B), is, on the whole, parallel to that of the total lipids: the amount of phospholipids, which represents about 70% of lung lipids whatever the stage considered, increases sharply between days 15 and 16; moreover, a difference is seen, according to the sex, the phospholipid level being higher in females than in males. This difference does not last, and is no longer observed on day 17.

Among individual phospholipids, PC and PE are quantitatively the most important: they represent 40 to 50% and 30 to 40% respectively of the total phospholipids. Their variation (Table I) is similar to that of total phospholipids. But only lecithin exhibits 'sexual dimorphism'.

Fatty acid composition of PC and PE at different stages is given in Table II. In both cases, saturated fatty acids are strongly predominant. In PC, the main acids are palmitic and stearic acids. Their percentage, already high at day 14, rises progressively until the end of the incubation, at which time they represent 80% of the total fatty acid content. The 'sexual dimorphism', which was observed in the quantitative evolution of PC, seems to be also present in the relative amount of individual fatty acids: first, the over-all degree of saturation is higher in females than in males; second, the mean ratio of palmitic acid to stearic acid is different (3,5 in females, and 2,5 inmales at the end of the incubation). In PE, a much larger variety of fatty acids is found: short-chain acids (14 carbons or less) and long chain acids are well represented. But, unlike that of PC, the fatty acid composition of PE is identical

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Table II. Percentage composition of PC and PE fatty acids of the embryonic chick lung

Age in days of incubation	⩽ 14	16:0	17:0	18:0	18:1	18:2	20:4	Total saturated	Total insaturated
Phosphatidylcholine (PC)									
14 – ♀	—	49.6	—	15.8	21.1	4.8	—	65.4	25.9
♂	—	39.5	—	19.6	30.2	4.1	—	59.1	34.3
16 – ♀	—	56.4	—	19.3	17.0	2.4	—	75.7	19.4
♂	—	42.9	—	28.7	20.8	4.6	—	71.6	25.4
18 – ♀	—	50.6	—	23.1	20.9	0.6	—	73.7	21.5
♂	—	51.0	—	25.3	17.9	2.3	—	76.3	20.2
20 – ♀	—	62.5	—	17.8	15.9	1.04	—	80.3	16.9
♂	—	54.7	—	21.5	19.5	2.0	—	76.2	21.5
Phosphatidylethanolamine (PE)									
14 – ♀	4.9	25.4	2.0	33.0	19.9	1.1	13.3	65.3	34.3
♂	7.6	23.3	1.4	30.1	17.4	5.1	10.6	62.4	33.1
16 – ♀	1.8	19.1	4.6	29.7	20.5	5.0	17.7	54.2	43.2
♂	3.3	16.5	4.1	31.4	23.8	6.7	6.8	55.3	37.3
18 – ♀	3.4	12.7	5.0	30.6	19.7	6.8	17.6	51.7	41.1
♂	2.0	11.9	5.8	27.2	18.6	8.4	18.7	46.9	45.7
20 – ♀	3.8	20.2	6.1	33.2	21.0	3.6	8.9	63.3	33.5
♂	4.2	13.3	7.6	32.5	21.8	6.1	12.7	57.6	40.6

Each value is a mean of results obtained on 3 separate extracts.

in males and females, and does not change in the course of development.

In conclusion, these results allow us to extend to the chick some of the data concerning mammalian foetal lung; first, as in mammals, the total phospholipid level, and, more precisely the PC and PE levels, rise significantly in the course of development[21]; then, the rapid increase in the total and individual phospholipids amounts observed between days 15 and 16, coincides with the time when granular pneumocytes start to differentiate. Finally, as has been described for rabbit and monkey[22,23], the percentage of saturated fatty acids of lecithin increases during maturation.

Our results show also, and this for the first time, a distinct sexual dimorphism in the evolution of the lecithin level. This dimorphism, reflected in the evolution of the phospholipid content, is no longer perceptible in the level of total lipids, where it is probably masked by the presence of cholesterol, triglycerides, free fatty acids, etc, which we did not determine. At the present time, one can only speculate about the factors which cause this earlier differentiation of lungs in females, already observed in man by NAYE et al.[16] on a morphological basis. Since it is widely accepted that pulmonary maturation is influenced by corticosteroids[5–9], it would be of great interest to check whether this phenomenon can be related to a sex-linked difference in the onset of adrenocortical activity.

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Abnormal Mitochondria in Retinoblastoma

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Summary. In examination of six retinoblastoma tumor specimens, bizzare mitochondria were often found. Some are irregular forms with focal expansion and constrictions. Occasionally, a portion of the mitochondria forms rings. Branching mitochondria are also seen. Other striking features of the mitochondria from tumor cells are the alteration of cristae. Dense bodies are also occasionally observed within the mitochondria. Morphological modifications of the mitochondria may be as results of pathological conditions of the tumor cells.

Although alterations of mitochondria have been observed in tumor cells such as: renal cell carcinoma[1,2], oncocytoma[3,4], and Warthin's tumor[5,6], changes of mitochondria in the tumor cells of retinoblastoma have not previously been reported[7].

In examination of 6 retinoblastoma tumor specimens, bizzare mitochondria were often found. The tumor tissues were taken from a family of 3 children, 2 of which were fraternal twins. The ages of the children were from 12 to 21 months. Pieces of tumor tissue about 1 mm^3 were immediately placed in White's saline (pH 7) which contained 1% osmium tetroxide or Dalton's chrome-osmic fixative (pH 7.6) for 1 h. The sections were stained with uranyl acetate and lead citrate.

Mitochondria within the tumor cells varied considerably in size, ranging from 0.3 to 4 μm in diameter. The