to the techniques developed by Epstein et al.[13] for carbonate and by Longinelli[14–16] for phosphate. The standard reproducibility of the measurement was ± 0.1 per mil (1σ) for carbonate (both oxygen and carbon) and ± 0.2 per mil (1σ) for phosphate (oxygen). Isotopic analyses were carried out on single stones and analyses of phosphate were made on groups of 10–20 stones because of the relatively low phosphate content. The isotopic compositions are reported in the δ-notation:

$$\delta = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000.$$

R is the isotopic ratio (O^{18}/O^{16} or C^{13}/C^{12}). The isotopic values are reported versus SMOW-standard as defined by Craig[17] for the oxygen and versus PDB-1 Chicago standard for the carbon.

Results and discussion. The results obtained exhibit 2 main features (table). First, $\delta_{C_{13}}$ of the carbonate is essentially identical for all the samples. It is rather difficult to interpret this. We do not know the C^{13}/C^{12}-ratios of the food and consequently cannot advance hypotheses concerning biological fractionation effects.

Second, oxygen isotopic composition of carbonate is also fairly constant among stones from one single case but varies significantly from area to area, along with the phosphate isotopic composition. Moreover, these differences between areas exhibit a striking latitudinal pattern. 2 conclusions can be drawn from the $\delta_{O_{18}}$-values. First, in each case physical chemical conditions probably remained constant during the precipitation of the stones, because there is a lack of variability, within the experimental error, for the carbonate isotopic ratios even when stones of different size were measured (table). Second, variability between areas seems to be closely related to the $\delta_{O_{18}}$ of average rain water in the same areas. The oxygen isotopic composition of pancreatic stones reflects that of the water of the animal's environment, which probably is not far from the value of average rain water. A similar relationship was previously found in the case of the oxygen isotopic composition of some mammal bones[18]. Recent unpublished measurements on bones confirm the previous findings and those reported here. This seems reasonable since temperature and oxygen isotopic composition of the water in a solution are the only variables which can affect the final isotopic composition of calcium carbonate and calcium phosphate precipitated under isotopic equilibrium conditions or when biological fractionation factors remain constant. In our case, the temperature of precipitation was likely to be constant through time. The isotopic composition of the water in the body fluids is not known, but it should reflect the isotopic composition of the environmental water taken in by the organism, even if relative humidity and environmental temperature may be important as they affect vapor loss during breathing. Differences caused by isotopic fractionations during vital processes should cause constant or quasi-constant isotopic effects through time. It follows that the minor differences between average $\delta_{O_{18}}$ of the 3 cases from Denmark could reflect similar differences in the average isotopic composition of local water.

Since it is well-known that the average isotopic composition of rain water depends mainly on local climatic conditions[19], and since the oxygen isotopic composition of some mammalian hard tissues seems to reflect that of rain water, it may be possible to use isotopic measurements of mammalian hard tissues for studying short-term climatic variations. For instance, isotopic study of fossil bones of known age could provide a $\delta_{O_{18}}$ versus time curve, whose gradients could reflect variations of average rain water $\delta_{O_{18}}$ and, consequently, climatic variations in their area of origin. These isotopic measurements could then represent a completely independent tool to compare paleoclimatic curves obtained in continental areas (e.g. studying bones of Quaternary age from food refuse in caves) with sections of 'paleotemperature' curves obtained from oceanic sediment cores.

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On the in vitro behaviour of mouse submaxillary gland cells. II. Metabolic differences between male and female C3H mice[1]

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Summary. Submaxillary gland cells from female C3H mice were isolated, cultivated in vitro and their metabolic properties compared with those of male derived cells. From the results it can be concluded that these cells retain their metabolic differences when grown in vitro.

It is now well established that the sexual dimorphism of murine submaxillary glands is related to differences in the cytological differentiation of the tubular portion of the gland[2]. In addition, biochemical sex-linked differences, which are due mainly to specific properties of regulatory enzymes of the glycolytic pathway, have been found[3–6]. Moreover cells from male C3H submaxillary glands have been isolated and cultivated in vitro and their biochemical and morphological characteristic have been described[7]. Because these cells retain some biochemical properties related to their enzymatic content and their responsiveness to dibutyrylcyclic-AMP, it is conceivable that sex-related biochemical differences may be genetically determined and so independent of environmental conditions. The aim of this study was to verify this hypothesis. The cells from submaxillary glands of female mice grown in vitro and their metabolic properties compared with those of male derived cells.

Materials and methods. Submaxillary glands were randomly collected from 3-month-old male and female C3H/He mice (I.R.E. colony), removed aseptically, minced and resuspended in Hank's balanced salt solution. The cells were isolated and cultivated in vitro as previously described[7]. Cells were harvested from the growth medium

by centrifugation at 600–900 × g, washed twice with Krebs-Ringer bicarbonate at pH 7.4 and resuspended in the same medium at a concentration of 1×10^7/ml. Respiration was measured with an Oxygraph (mod. KM, Gilson Medical Electronics) equipped with an oxygen electrode polarized at −0.8. Oxygen consumption was determined by adding 0.2 ml of cellular suspension to 1.8 ml of air-satured medium. The temperature was 37 °C and dissolved oxygen was calculated according to Chance and Williams[8]. Measurements of aerobic and anaerobic glycolysis were carried out according to Gregg et al.[9]. The oxidation of labelled glucose was evaluated as previously reported[4].

Results and discussion. Comparative values for respiration, aerobic and anaerobic glycolysis of female and male mice submaxillary glands cells in vitro are reported in table 1. Striking differences in oxidative metabolism can be observed. The oxygen consumption of female cells is 38% higher than that of male cells.

Table 1. Oxygen consumption (QO_2), aerobic $\left(Q_{CO_2}^{O_2}\right)$ and anaerobic $\left(Q_{CO_2}^{N_2}\right)$ glycolysis of male and female C3H submaxillary gland cells in vitro

	Female cells	Male cells	Percent
QO_2	17.3 ± 1.20	10.8 ± 0.80	+ 38
$Q_{CO_2}^{O_2}$	0.95 ± 0.02	0.44 ± 0.03	+ 54
$Q_{CO_2}^{N_2}$	1.66 ± 0.09	1.75 ± 0.10	
PE	43	75	

The results are expressed as µl O_2/mg dry wt/h for oxygen consumption and as µmoles lactate/mg dry wt/h for glycolysis. The Pasteur effect (PE) was calculated in the usual manner. Each figure (± SD) was averaged from 4 different determinations performed in duplicate.

Table 2. Glucose-1-^{14}C, glucose-6-^{14}C and glucose-U-^{14}C oxidation by in vitro cells derived from submaxillary glands of female and male C3H mice

	Female cells	Male cells	Percent
Glucose-1-^{14}C	33004 ± 427	26244 ± 1320	+ 20
Glucose-6-^{14}C	10365 ± 853	6009 ± 585	+ 41
Glucose-U-^{14}C	32241 ± 900	25580 ± 758	+ 21

The results are given as cpm/mg dry wt. Each figure (± SD) was averaged from 4 different determinations performed in duplicate.

As far as aerobic glycolysis is concerned, the production of lactic acid is considerably greater for female cells (Δ % = 54), while no statistically significant differences have been found for anaerobic glycolysis. (As result of different oxygen consumption and higher aerobic glycolysis). As result of different oxygen consumption and higher aerobic glycolysis, the Pasteur effect, i.e. the inhibition of the glycolysis by oxygen, is 75% for male and 43% for female derived cells. In order to establish whether the differences in oxygen consumption might be due to different capacity for glucose oxidation, experiments with labelled glucose were carried out and the results are shown in table 2. Female derived cells oxidize every type of labelled glucose to a greater extent than those derived from males. It is noteworthy that a greater difference has been found with glucose-6-^{14}C, which is metabolized only by the glycolytic pathway and is inert in the pentose cycle. Table 3 summarizes the results of estimation of ATP production potential rate by the cultured submaxillary glands cells, when glycolysis and respiration are proceeding at the rates given in table 1. Because P/O ratio of both cells populations, in the presence of pyruvate as substrate, is 2.8 (Floridi, unpublished data), it was possible to calculate that cells from females could synthesize 4.31 µmoles ATP/mg dry wt/h, whereas in male-derived cells ATP production is much lower (2.69 µmoles ATP/mg dry wt/h). These values correspond to the complete oxidation of 0.31 and 0.19 µmoles of pyruvate by female and male cells respectively. Under these conditions, a total of 5.57 and 3.32 µmoles of ATP/mg dry wt/h would be produced by cells from females and males, with remaining 1.26 and 0.63 µmoles of ATP coming from glycolysis.

The data reported above clearly show that female and male cells in vitro still retain some sex-linked metabolic features. Higher oxygen consumption of female derived cells is probably due to a greater glucose uptake. The experiments carried out with glucose-6-^{14}C have demonstrated that female derived cells are able to oxidize glucose more readily than male derived cells.

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Table 3. Contributions to ATP production from glycolysis and respiration of male and female C3H mice submaxillary gland cells in vitro

Gas phase	Cells	ATP from respiration	Pyruvate oxidized	ATP from glycolysis	Pyruvate reduced	ATP total	Δ percent
Aerobic	Female	4.31	0.31	1.26	1.26	5.57	+ 39
Aerobic	Male	2.69	0.19	0.63	0.63	3.32	
Anaerobic	Female	0	0	1.66	1.66	1 66	
Anaerobic	Male	0	0	1.75	1.75	1.75	

The results are expressed as µmoles/mg dry wt/h. These calculations are based on the respiratory and glycolytic rates summarized in table 1. The assumptions are that 1 µmole of ATP is formed via glycolysis per µmole of pyruvate formed and 14.5 µmoles of ATP are formed via terminal oxidation of 1 µmole of pyruvate to CO_2 and H_2O (i.e. P/O ratio of 2.8).

The increments, either in oxygen consumption or in glucose oxidation are of the same order of magnitude. The submayillary gland cells, when cultivated in vitro are able to carry out aerobic glycolysis, whereas tissue slices do not show this capacity[3]. The appearance of aerobic glycolysis is a general biochemical characteristic of cultured cells, but it must be emphasized that the female derived cells show an aerobic production of lactic acid twice that of male cells. Nevertheless, in spite of higher oxygen consumption and higher ATP production (tables 1 and 3), the Pasteur effect in female cells is lower. To explain this phenomenon, it is necessary to keep in mind that we are dealing with highly structured system composed of cells in which glycolysis and respiration are strictly correlated and each pathway has a profound effect on the other. In the presence of mitochondria, regulatory factors of glycolysis are changed because they may stimulate the glycolysis by means of all reactions that produce ADP and inorganic phosphate and this auxiliary enzyme system may be referred to as 'ATP-ase in the broadest sense'[10]. Moreover, it has been demonstrated that the Pasteur effect depends essentially on the allosteric properties of phosphofructokinase[11-13], i.e. it is increased by compounds which inhibit this enzyme and decreased by those which enhance its activity. The phosphofructokinase of female cells has a greater affinity for the substrate than that of male cells[14]. It is, therefore, conceivable that in submaxillary gland cells in vitro both mechanisms are working and that the lower Pasteur effect of female derived cells is attribuable mainly to different phosphofructokinase sensitivity.

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Alterations in the phospholipid composition of *Nocardia polychromogenes* during growth[1]

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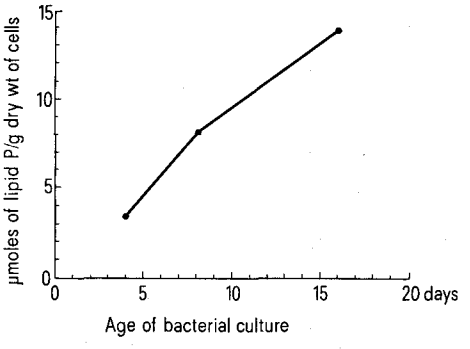
Summary. The major phospholipid classes of *Nocardia polychromogenes* were quantitated at different stages of the growth cycle. Significant differences were observed both in the total lipid phosphorus per g (dry weight) of cells, and in the relative percentages of individual phospholipids. The total amount of lipid-phosphorus increased throughout the growth cycle. Cardiolipin and phosphoinositides contents increased with significant decrease in phosphatidyl ethanolamine and unknown phospholipids.

Phospholipids in bacteria are almost exclusively localized in their membranes and these structures are the site of a number of enzymes involved in phospholipid biosynthesis[2]. Cardiolipin, phosphatidyl ethanolamine and phosphatidyl inositomannosides are the major phospholipids of *Nocardia*[3-5]. The effects of culture age are important, because cells in exponential phase of growth are physiologically more active as compared with the stationary phase when the greatest mass is obtained. As a preliminary to a better understanding of phospholipid biosynthesis, metabolism and its regulation in *Nocardia*, we have undertaken a study to investigate the effects of culture age on the phospholipids of *Nocardia polychromogenes*. To our knowledge, alterations of phospholipids during growth have not been investigated in *Nocardia*, although studies with other microorganisms have been reported[6].

Materials and methods. Large quantities of *Nocardia polychromogenes* were grown at 27 °C as described earlier[5] for varying intervals of time. Extraction and purification of lipids were as described elsewhere[7]. The separation, isolation, characterization and quantitation of phospholipids were as detailed in previous publications[5,8].

Results and discussion. The total lipid-phosphorus per g (dry weight) of *Nocardia polychromogenes* during growth is shown in the figure. The changes in the relative percentages of each of the major phospholopid fractions of *Nocardia polychromogenes* with respect to age of the cul-



Quantitation of total lipid phosphorus during growth of *Nocardia polychromogenes*.

Distribution of phospholipids of *Nocarda polychromogenes* during growth

Phospholipid	Age of the bacterial culture (days) 4(4)	8(4)	16(4)
Phosphoinositides	35.7 ± 2.9	45.0 ± 3.3	44.0 ± 3.7
Phosphatidyl ethanolamine	20.7 ± 1.4	12.8 ± 0.7	10.7 ± 1.8
Cardiolipin	33.5 ± 2.2	38.7 ± 2.3	40.2 ± 2.5
Unknown phospholipids	13.5 ± 0.5	3.3 ± 0.8	4.9 ± 3.8

Distribution of total lipid phosphorus (%, mean ± SD).
The number in parentheses represent the number of different batches analyzed.