Mean levels of α_1-AT in the autumn and spring

Statistics season of the year	Boys				Girls				Boys and girls			
	n	$\bar{x} \pm$ SD	Range	95% c.i.	n	$\bar{x} \pm$ SD	Range	95% c.i.	n	$\bar{x} \pm$ SD	Range	95% c.i.
Autumn 1974	49	2.43 ± 0.32	1.73–3.5	2.62–2.33	35	2.4 ± 0.26	1.85–2.95	2.49–2.31	84	2.41 ± 0.3	1.73–2.5	2.48–2.35
Spring 1975	49	2.47 ± 0.46	1.5 –3.55	2.6 –2.34	35	2.49 ± 0.35	1.95–3.4	2.61–2.37	84	2.48 ± 0.41	1.5 –3.55	2.57–2.39
F-test		$p < 0.05$				Insignificant				$p < 0.01$		
t-test		Insignificant				Insignificant				Insignificant		

sexes is also different: $r = 0.8616$ between the autumn and spring values in the girls' subgroup is highly significant ($p < 0.001$), whereas in the boys' subgroup $r = 0.3568$ is only slightly significant ($p < 0.05$).

The Table shows the means of α_1-AT in boys and girls in the 2 seasons. Except for a significant difference between the value dispersions in boys (F-test $p < 0.05$) and in the whole group (F-test $p < 0.01$) in the 2 seasons, no significant difference of mean levels was found by t-test.

There is no correlation between the values of α_1-AT and proteinemia: in the autumn sampling, $r = -0.0123$, in the spring sampling, $r = -0.0193$.

Discussion. The levels of α_1-AT could be changed under certain physiological or pathological circumstances, as stated in the introduction. This paper shows the changes of the α_1-AT values in prepubertal children under seasonal influences. It is interesting that this influence is very strong in boys but not in girls. The α_1-AT levels in boys in spring tend to shift either to high or to low values. The variability is, therefore, great in both directions. Consequently, the mean values of α_1-AT in the boys' subpopulation are practically the same in both seasons. However, the differences in the distribution of values are highly significant in the χ^2-test, and this result is confirmed by the course of the regression line and by the correlation coefficients. The results suggest a more stable genetic control in girls' subpopulation. It is beyond the scope of this paper to explain why the α_1-AT levels are more labile in boys seasonally. One reason for this may be a different start of prepubertal hormonal changes in both sexes, while girls are actually physiologically older.

When the α_1-AT results observed in the autumn sampling are considered as initial values, then the changes in the α_1-AT levels assessed at the beginning of spring could with some probability be attributed to the influence of the previous winter season. The nutrition factor probably has no significant influence on the formation of α_1-AT levels, because there is no correlation of α_1-AT with proteinemia.

However, this season comprises many factors which should be taken into account: low temperature, a reduced vitamin content in fresh fruit and vegetables, increased incidence of infectious diseases and an increased amount of exhalation pollutants in the air. Some of the factors mentioned may be followed in a further study which is now being compiled.

The Mannophosphoinositides of *Nocardia asteroides*

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Summary. The phospholipid of *N. asteroides* has been investigated. It was found to contain phosphatidyl ethanolamine, cardiolipin, phosphatidyl inositol and a family of mannophosphoinositides. Dimannophosphoinositides with 3 and 4 moles of fatty acid per phosphate residue represented the major glycophospholipids besides small amounts of other more glycosylated mannophosphoinositides.

The close phylogenetic relationship of *Mycobacteria*, *Corynebacteria* and *Nocardia* is supported by morphological[1] and immunological[2] evidence. Lipids of *Mycobacteria*[3] and *Corynebacteria*[4–6] have been the subject of intensive studies in the recent years whereas little information is available concerning *Nocardia*. *Mycobacteria* contain unusual phosphatidyl myoinositol oligomannoside where the number of mannose units may vary from 1 to 5[7]. However, recent studies suggest that these lipids are more complex and differ in the number of fatty acyl groups[8, 9]. The phospholipids of *Nocardia* have been shown to contain cardiolipin, phosphatidyl ethanolamine and either mono or dimannophosphoinositide[10, 11]. This report pertains to the nature of mannophosphoinositides of *Nocardia asteroides*.

Materials and methods. Nocardia asteroides were grown for 4 weeks in a medium containing glucose, beef extract and peptone[11]. Extraction and purification of the lipids were described elsewhere[12]. Mannophosphoinositides were separated by thin layer chromatography (TLC) using silica gel H plates impregnated with 0.18% ammonium

[1] H. A. Lechevalier and M. P. Lechevalier, A. Rev. Microbiol. *21*, 71 (1967).
[2] A. R. Prevet, in *Traits de systematique, Bacterinne* (Dunod, Paris 1961), vol. 2, p. 771.
[3] M. B. Goren, Bacteriol Rev. *36*, 33 (1972).
[4] G. K. Khuller and P. J. Brennan, Biochem. J. *127*, 369 (1972).
[5] P. J. Brennan and D. P. Lehane, Lipids *6*, 401 (1971).
[6] J. A. Hackett and P. J. Brennan, Biochem. J. *148*, 253 (1975).
[7] Y. C. Lee and C. E. Ballou, Biochemistry *4*, 1395 (1965).
[8] M. C. Pangborn and J. A. McKinney, J. Lipid Res. *7*, 627 (1966).
[9] P. J. Brennan abd C. E. Ballou, J. biol. Chem. *242*, 3046 (1967).
[10] I. Yano, Y. Furukawa and M. Kusunose, J. Bact. *98*, 124 (1969).
[11] T. Kataoka and S. Nojima, Biochim. biophys. Acta *144*, 681 (1967).
[12] G. K. Khuller and D. Subrahmanyam, Experientia *24*, 851 (1968).

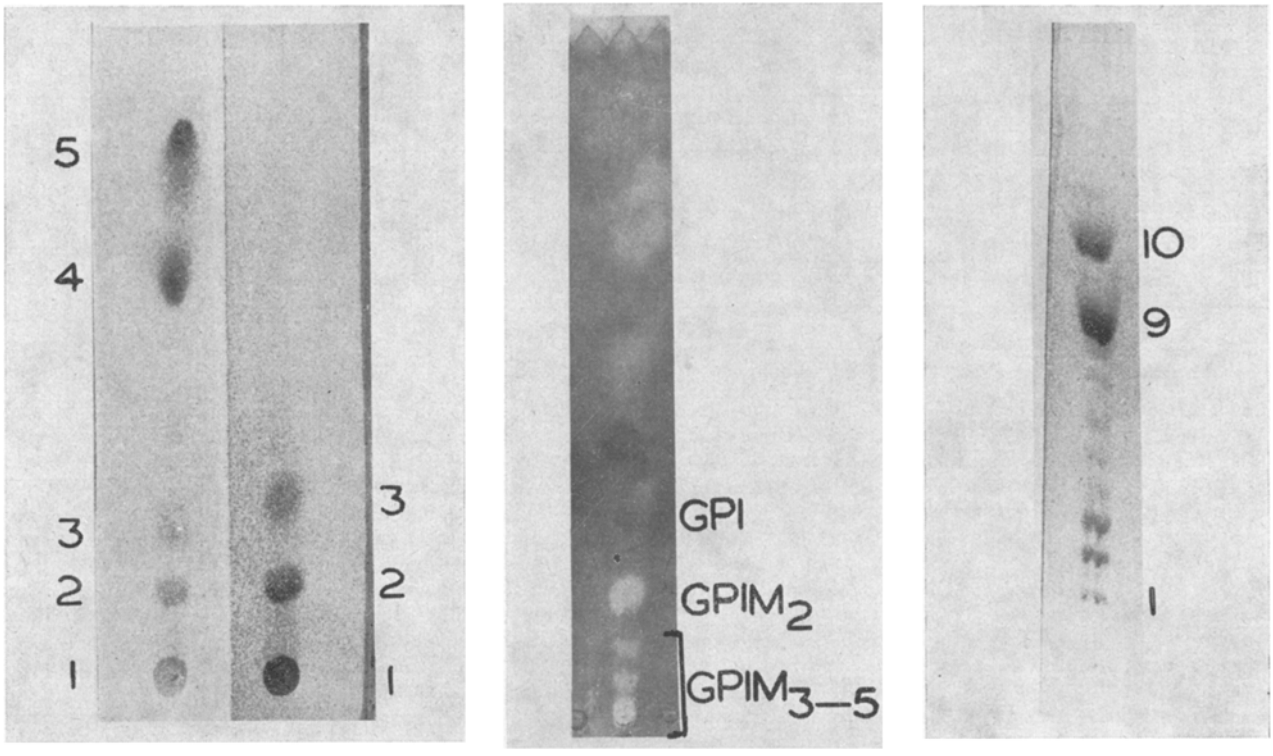

Fig. 1. Thin layer chromatograph of the phospholipids of *Nocardia asteroides* in chloroform-methanol-7 *N* ammonia, 65:25:4 as developing solvent. Spots were located with, Left: molybdenum blue reagent, Right: α-naphthol sulphuric spray.

Fig. 2. Paper chromatogram of deacylated phospholipids of *Nocardia asteroides* in isopropanol-ammonia, 2:1. The chromatogram was stained with benzidine dip reagent.

Fig. 3. Thin layer chromatograph of the phospholipids of *Nocardia asteroides* on silica gel H plates impregnated with ammonium sulphate in chloroform-methanol-water, 10:5:1. The plate was sprayed with α-naphthol sulphuric acid spray.

sulphate[13] with chloroform-methanol-water, 10:5:1, and chloroform-methanol-7 *N* ammonia, 65:25:4. Carbohydrate containing lipids were detected by spraying with α-naphthol sulphuric acid spray. All other chromatographic and analytical procedures used have been described before[4,14–16].

Results and disscussion. Phosholipids were found to be separated in 5 components on TLC with chloroform-methanol-7 *N* ammonia, 65:25:4 (Figure 1). Of these phospholipids, the first 3 from the origin were found to be glycophospholipids. Cardiolipin and phosphatidyl ethanolamine were identified from their chromatographic properties. Paper chromatography of the deacylated phospholipids in isopropanol ammonia, 2:1 showed 5 components (Figure 2) similar to that of previously examined *Mycobacteria*[16]. Compounds corresponding to glycerylphosphorylinositol (GPI), glycerylphosphorylinositol dimannoside ($GPIM_2$) and higher homologous were identified. When the material corresponding to GPI was eluted and rechromatographed in the same solvent or in the ethyl acetate-pyridine-water, 5:3:2, it was found to be identical with GPI from yeast. Identification of GPI suggests the presence of phosphatidylinositol. Components running below $GPIM_2$ had Rf values suggestive of glycerylphosphorylinositol trimannoside, tetramannoside and pentamannoside respectively.

10 glycophospholipids were identified by TLC in chloroform-methanol-water, 10:5:1 (Figure 3). The major phosphoinositides (spot 9,10, Figure 3) were isolated in adequate amounts for analysis and their purity was checked in different TLC solvent systems. Paper chromatography of the acid hydrolysate showed glycerol, inositol and mannose. Deacylation and paper chromatography of these components gave a single component corresponding to $GPIM_2$. Quantitative analysis of spot 10 for phosphorous, mannose, acyl groups and inositol demonstrated their presence in the molar ratio of 1:2.0:3.8:0.9 and for spot 9, 1:1.9:2.9:1 respectively. These results, along with the chromatographic properties of these components, strongly suggested the presence of tetra acylated dimannophosphoinositide (spot 10) and triacyl dimannophosphoinositide (spot 9) in this organism. Dimannophosphoinositides with different number of fatty acyl groups have been identified in *Mycobacteria*[8,9] and *Corynebacteria*[5]. The mannose containing lipids accounted for 30% of the total phospholipids of *N. asteriodes* of which the dimannophosphoinositides were the major glycophospholipid as in *N. coeliaca*[15] and *Mycobacteria*[17].

The higher mannophosphoinositides (with more than 2 mannose residues) constitute less than 15% and the identification of them is in progress. These results suggest that *N. asteroides* contain a family of mannophosphoinositides similar to *Mycobacteria*, thus further indicating close similarities in *Mycobacteria*, *Corynebacteria* and *Nocardia*.

[13] B. BANERJEE, S. K. JAIN and D. SUBRAHMANYAM, J. Chromat. 94, 342 (1974).
[14] G. K. KHULLER and D. SUBRAHMANYAM, J. Bact. *101*, 654 (1970).
[15] G. K. KHULLER and P. J. BRENNAN, J. gen. Microbiol. *73*, 409 (1972).
[16] G. K. KHULLER and P. J. BRENNAN, Am. Rev. resp. Dis. 106, 892 (1972).
[17] G. K. KHULLER, B. BANERJEE, B. V. S. SHARMA and D. SUBRAHMANAYAM, Indian. J. Biochem. Biophys. *9*, 274 (1972).