Results and discussion. The effect of growth temperature on the phospholipid composition is given in the table. Compared with cells grown at 37 °C, significant differences were noted in the proportion of the different classes of phosphatides at 27 °C. It is known that changes occur in the fatty acid composition of complex lipids in microorganisms, plants and animals in response to growth temperature[10,11]. As a general rule, the composition of fatty acids is modified in the direction of lower average melting points as the growth temperature is lowered. We have now observed significant changes in the phospholipid compositions of cells grown at 27 °C as compared to cells grown at 37 °C. Similar to our observations, a decrease in phosphatidyl ethanolamine and an increase in cardiolipin has been observed in Vibrio cholerae with decreasing growth temperatures[12]. Ethanolamine phosphoglycerides have also been shown to decrease in Tetrahymena pyriformis with a drop in temperature[13]. In Clostridium butyricum, changes were seen in the phospholipid composition when cells were grown either at lower temperature[14] or in the presence of exogenous unsaturated fatty acids[15]. When the growth temperature was decreased from 37 °C to 25 °C, the proportion of glycerol phosphoglycerides increased at the expense of ethanolamine and N-methylethanolamine phosphoglycerides[14]. When the medium was supplemented with oleate at 37 °C, there was a decrease in the content of ethanolamine plus N-methylethanolamine plasmalogens with a corresponding increase in the glycerol acetals of these plasmalogens[15]. It was suggested that such changes may be involved in the maintenance of membrane fluidity. It is also known from model membrane studies that changes in the phospholipid head groups can have an effect on fluidity[16,17]. It therefore seems possible that the differences in phospholipid distribution found in Nocardia polychromogenes grown at high and low temperatures are a feature of adaptive processes to regulate membrane fluidity.

Composition of phosphatides of Nocardia polychromogenes grown at 37 °C and 27 °C

Phospholipid classes	Percent of total phospholipid (mean ± SD) 37 °C	27 °C
Inositol containing phospholipids	33.1(±2.1)	45.0(±3.3)
Phosphatidyl ethanolamine	25.9(±1.4)	12.8(±0.7)
Cardiolipin	32.7(±1.8)	38.7(±2.3)
Unknown phospholipids	8.4(±0.7)	3.3(±0.8)

At 37 °C and 27 °C, 4 different batches were analyzed. The differences observed are statistically significant.

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A study of broad bean α-amylase isoenzymes

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Summary. 2 α-amylase isoenzymes were isolated from cotyledons of germinated broad bean seeds. The examination of the 2 isoenzymes isolated by column chromatography on DEAE-cellulose and sephadex showed that these isoenzymes have the same behaviour towards soluble starch, amylose, amylopectin, heavymetal ions, and high temperature, and differ only in their mol.wt and their electrophoretic mobility.

The α-amylase (α-1,4 glucan 4-glucanohydrolase, E.C. 3.2.1.1.) is one of the enzymes involved in the starch hydrolysis of the living tissues, splitting the 1,4-bonds of the starch. For complete hydrolysis of the starch, the presence of R-enzyme (splitting the 1,6-bonds of the starch) is required in addition to-α-amylase. β-Amylase (E.C. 3.2.1.2.) splitting also the 1,4 bonds of the starch occur in the plants and sometimes in the same tissues where the α-amylase occurs. The physiological role of these 2 amylases (α and β) has not yet been fully understood. In addition the amylases occur in isoenzymes both in plants[1–7] and animals[8–11]. In the cotyledons of broad bean, the α-amylase occurs in 2 isoenzymes. In order to contribute to the further understanding of the significance of these isoenzymes, the 2 isoenzymes from the broad bean were isolated and examined separately.

Materials and methods. Seeds of broad beans (Vicia faba var. aquadulce) were grown and the cotyledons from 10-days-old plants were used as the source of the isoenzymes. For the enzyme assay, 1 ml of soluble starch solution (300 mg/100 ml acetate buffer 0.1 M, pH 6.0 + 10 mM $CaCl_2$) was incubated at 30 °C with 1 ml of the properly diluted enzymic solution. After 1–5 min, 1 ml of the iodine reagent[12] and 5 ml water were added and the A570 μm was read and transformed to mg of starch by proper standard curve. The hydrolyzed starch (mg) per min under the above conditions defines one enzymic unit. The specific activity is defined as the units/mg of protein measured by the method of Lowry et al.[13].

Electrophoresis. Polyacrylamide disc gel electrophoresis was used. For the preparation of gel, the following reagents were used: polyacrylamide (6.4% w/v) in a buffer consisting of Tris (6 g) and glycine (28.8 g)/l pH 8.6 and containing soluble starch (0.25% w/v). NNN'N'-tetramethylethylene diamine (0.15% v/v) and ammonium persulfate (0.05% w/v). After running the gels vertically at 4–5 mA/gel, they were incubated for 15 min at 30 °C in 0.1 M acetate buffer pH 5.6 containing 10 mM $CaCl_2$.

Isolation and purification of α-amylase isoenzymes from broad bean cotyledons

Step	Procedure	Volume (ml)	Protein (mg/ml)	Units/ ml	Total units	Specific activity	Purification	Yield
1	Extraction in 0.01 M Cal. acetate, filtration, centrifugation	2000	7.2	4	8000	0.55	–	100
2	Heating at 70 °C for 15 min cooling, centrifugation	1950	2.2	3.1	6045	1.41	2.5	75.5
3	Ammonium sulfate fraction 0.30–0.65	250	5.3	10	2500	1.88	3.4	31
4	Dialysis	300	4.15	8.3	2490	2.00	3.6	31
5	DEAE-cellulose chromatography (figure 1)							
	Peak α1	100	0.11	5	500	55	96	26
	Peak α2	200	0.15	8	1600	53	98	
6	Rechromatography on DEAE-cellulose							
	Peak α1	80	0.06	4	320	67	122	15
	Peak α2	150	0.08	5.7	855	71	129	
7	Rechromatography on sephadex G-75							
	Peak α1	40	0.045	3.4	136	75	136	4.8
	Peak α2	50	0.062	5.0	250	80	145	

After immersing in dilute iodine solution, amylase activity appeared as clear bands against a dark blue background. Coomassie brilliant blue was used as a protein stain. The subunit analysis was performed by Weber and Osborn's method[14] using bovine serum albumin, pepsin, trypsin and lysozyme with mol.wts of 68,000, 35,000, 23,300 and 14,300 respectively as mol.wt standards.

Results and discussion. The results of the procedure for the isolation of the α-amylase isoenzymes are summarized on the table. Samples for the 2 separated peaks of the last step showed one electrophoretic band of amylase activity per gel and also one band of protein per gel coincided with the correspondent enzymic band. Thus both isoenzymes are electrophoretically homogeneous.

The effect of pH. The 2 isoenzymes exhibited the same maximal activity in the pH 6.0. A-amylases from other sources[15] also presented the same maximal pH.

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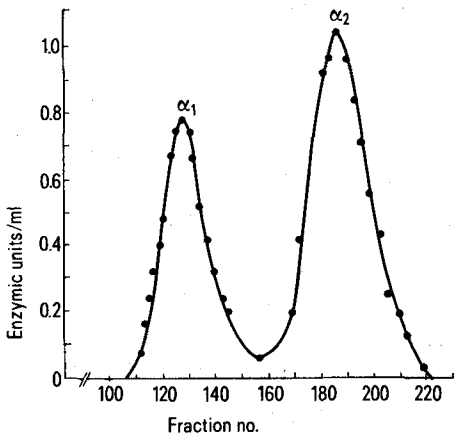

Fig. 1. Chromatography of broad bean cotyledon α-amylase isoenzymes (2.5 × 23 cm column). Gradient elution between 0.015 M and 0.06 M Tris-HCl buffer, pH 7.45 at constant concentration of 2 mM $CaCl_2$. Fraction per 4.3 ml.

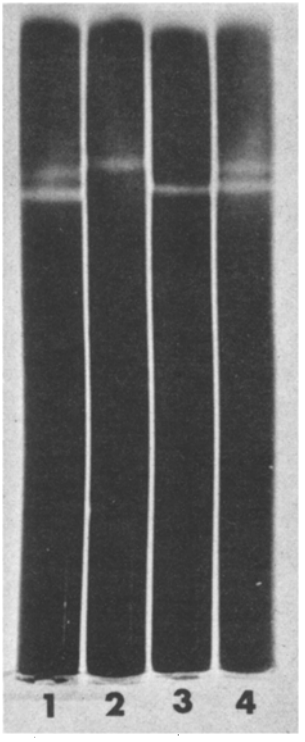

Fig. 2. Electrophoresis of α-amylases on polyacrylamide gel (staining with iodine). *1* α-Amylases from crude extract (step 1). *2* and *3*. The α_1 and α_2 peaks respectively (step 7). *4* The mixture of the 2 peaks.

Inhibitors and heavy metal ions. Incubating the 2 isoenzymes with 5×10^{-4} M EDTA Na_2 for 30 min at 25 °C lost all their activity which is not restored either with the addition of excess of $CaCl_2$ nor with dialysis against large volume of buffered solutions of $CaCl_2$. Thus Ca^{++} is necessary for the stability of both isoenzymes[1,3,4,15,16]. After preincubating the isoenzymes with 10^{-4} M $AgNO_3$, 10^{-4} M $CuSO_4$, 10^{-4} M $HgCl_2$ and 2×10^{-4} M p-chloromercuribenzoate-sodiumsalt (P-CMB) for 30 min at 25 °C, it was found that both amylases retained all their enzymic activity.

Substrate effect. After a long time of incubation of the 2 isoenzymes with soluble starch, the starch-iodine complex color disappeared. This property in combination with the other findings (calcium dependency, heat resistance, insensitivity to Ag^+ Cu^{++} and Hg^+) strongly indicates that both isoenzymes are of the α-type[1]. The 2 separated isoenzymes were also found to be inhibited by high substrate concentration (1%) in the same way, to exhibite practically the same K_m (0.8 mg/ml for the α1 and 0.7 mg/ml for the α2 isoenzyme) and to hydrolyze in the same way the amylose faster than amylopectin.

Subunit analysis. The α1 form was found to be composed of 2 different subunits of mol.wt 16,000 the one and 22,000 the other (mol.wt of α1 16,000 + 22,000 = 38,000 at least). Similarly the α2 form is composed of 2 different subunits of mol.wt 19,500 the one and 32,000 the other (mol.wt of α2 19,500 + 32,000 = 51,500 at least). The value for the α2 isoenzyme is apparently within the range (50,000–55,000) of the mol.wt found from other sources[3,7,11,17,18] while the value for the α1 isoenzyme seems low, although such isoenzymes have been reported in the literature[5,6,19]. Thus the 2 isoenzymes differ only in their mol.wt as well as in their electrophoretic mobility, and the occurrence in the living cell remains still without satisfactory explanation.

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Effect of aldosterone and methylprednisolone on cardiac NaK-ATPase

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Summary. Aldosterone (15 μg BID) and methylprednisolone (8 mg QD) administration to female guinea-pigs augmented both the total and the specific activity of NaK-ATPase but not the activity of adenylate cyclase in the cardiac sarcolemma. The rise in NaK-ATPase was due to increase in the number of enzyme molecules; catalytic activity and ouabain-sensitivity of individual molecules did not change.

In target tissues steroid hormones induce the synthesis of messenger and ribosomal RNA and subsequently that of specific proteins[2]. In the kidney aldosterone[3,4] enhances the synthesis of NaK-ATPase (EC 3.6.1.3), an enzymatic component of the Na^+-pump, and possibly of some Na^+-carrier also. In large doses methylprednisolone[5] and corticosterone[6] exert similar effects. In this paper we show that aldosterone and methylprednisolone preferentially increased the steady state level of NaK-ATPase also in the myocardium, without affecting the catalytic activity or ouabain-binding of the individual enzyme molecules.

Female Dunkin-Hartley guinea-pigs, weighing 337 ± 4 g initially, were randomly divided in 3 groups. Each group was injected daily as follows: 'controls' (24): 0.1 ml of 0.9% (w/v) NaCl i.p. twice daily for 14 or 24 days resp., 'aldosterone-treated' (16): 15 μg of aldosterone i.p. twice daily for 14 days; 'methylprednisolone-treated' (18): 8 mg of Depo-Medrol i.m. once a day for 14 or 24 days, resp. Previously similar experiments by other investigators were conducted on rats. We chose guinea-pigs because in this animal (unlike in the rat) the affinity of NaK-ATPase for ouabain is high and the number of the ouabain-binding sites can be measured directly and accurately by binding assay. After killing the animals, the heart ventricles were dissected free from pericardium and the atria, a piece of tissue was dried to constant weight at 120 °C, the rest of the ventricle was homogenized in ice-cold 0.33 M sucrose in 50 mM Tris-acetate (pH 7.2) containing 1 mM Tris-EGTA (ethyleneglycol-bis(α-aminoethylether)N,N-tetraacetic acid). Enzyme activities were monitored in this initial homogenate and in the sarcolemma, purified by extraction with 1 M KCl[7]. Protein was determined by the procedure of Miller[8]. NaK-ATPase was assayed as described earlier[9], with one modification: the reaction mixtures contained 1 mM Tris-EGTA and 2 mM NaN_3 also. The amount of phosphorylated NaK-ATPase in the sarcolemma was measured at 0.04 mM [γ^{32}P] ATP, ouabain-binding was measured in the presence of 0.05–1 μM [^{3}H] ouabain, dissociation constant of the enzyme-ouabain complex was calculated from Scatchard-plots (for details see Hegyvary[9]). Turnover number of NaK-ATPase was computed by dividing specific activity of the enzyme by the amount of phosphorylated NaK-ATPase at steady state. It was assumed that each enzyme molecule bound

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