rate the TR, no difference in absorbance at 290 nm would be expected between mixtures containing only phenylalanine or phenylalanine and α-KG.

In radish, the TR activity is relatively high in cotyledons and very low in roots; therefore one must expect a higher degree of interference with the PAL assay in the case of cotyledons. This is in agreement with the finding that the coefficient of variation for the PAL activity (determined in crude extracts and borate) is higher for cotyledons than for roots[16]. In hypocotyls, the TR activity is quite low and independent from light. This fact, and the relative constance of the α-KG content in darkness and in light, suggests that the data of BELLINI and HILLMAN[5], obtained with hypocotyls from 48-h-old seedlings, can be interpreted as the sum of two parts, the first one stable and inherent to TR, the second one variable in the different experimental conditions and due to the true PAL activity. Moreover, the confirmation of the non-efficiency of one single red light pulse – this is the crucial point in the paper cited[5] – has subsequently been reported using determinations made on purified extracts[6].

Conclusions. Although our data do not represent a critical kinetic analysis of the development of PAL and TR activities, they are sufficient to indicate a completely different distribution pattern of TR and PAL in the various parts of the radish seedling. This may be connected with the different rôle of the 2 enzymes: PAL is a key enzyme in the lignification process and this may explain its higher level in roots, while TR activity seems involved in amino acid synthesis, which is very active in the cotyledons of germinating seeds[3]. The effect of light is clearly more evident on PAL than on TR level, and this agrees with other data suggesting a higher sensitivity of the development of PAL activity to light, as compared to other enzymes, also of the transcinnamic acid pathway. The present investigation also confirms that the presence of TR activity in plant extracts interferes with the determination of PAL by spectrophotometric methods. This interference can be eliminated by removal of α-keto acids present, for example by passing crude extracts through Sephadex.

Summary. In dark-grown *Raphanus sativus* seedlings the level of phenylalanine transaminase is higher in cotyledons than in root and hypocotyl. The maximum activity of phenylalanine ammonia-lyase (PAL) is found in the root. Only PAL is significantly increased by light.

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In vitro Lipid Synthesis by Lathyrogen-Treated L-929 Fibroblasts

A previous in vitro study has shown that the lathyrogen, B-aminopropionitrile fumarate (BAPN), in a concentration of 5 m*M* increased the secretion of collagen[1]. It has been suggested that certain lipids are present in close proximity to collagen fibres[2,3] and that the lipids play an important role in the calcification process[2]. The purpose of this study was to determine whether the rate of lipid synthesis paralleled the increased rate of collagen synthesis seen in 5 m*M* BAPN-treated cell cultures.

Materials and methods. Strain L-929 fibroblasts were grown in Eagle's minimum essential medium containing 10% calf serum. 5-day-old cultures were trypsinized and resuspended into a common suspension having approximately 100,000 cells per ml. The suspension was divided equally: one designated as the experimental to which was added 5 m*M* of BAPN, and the other as control to which was added an equal amount of sodium fumarate. Replicate cultures were prepared and grown for a 5-day period. On the 5th day, the medium was decanted and replaced with fresh control and experimental medium containing 1.25 μCi/ml ^{14}C-acetate. After 4 h incubation, the cells were washed and harvested.

For lipid analysis, the cells were extracted with chloroform/methanol (2/1); the lipid extracts were purified by washing with 0.9% sodium chloride[4]. Lipid classes were separated on silicic acid papers according to the chromatographic procedures of MARINETTI[5] and WUTHIER[6]. Radioactive lipid spots were located by autoradiography, cut from the papers, and counted in a Packard Tri-Carb Scintillation Spectrometer, model 3375, using BRAY's solution[7]. Lipids were identified on chroma-

Effect of 5 m*M* BAPN on lipid synthesis by L-929 fibroblasts from ^{14}C-acetate

Lipid class	Control		BAPN	
	CPM[a]	SD[a]	CPM[a]	SD[a]
Triglycerides	17,297	921	4,384	314
Lecithin	26.687	927	6,960	189
Mono- and diglycerides	5,335	644	2,089	338
Cholesterol	5,578	749	4,258	397
Free fatty acids	13,951	183	3,665	388
Cholesterol esters	877	558	582	249
Unknown	1,943	413	822	356
Phosphatidyl ethanolamine	2,996	168	1,187	10
Phosphatidyl inositol	4,032	229	1,266	83
Sphingomyelin	2,775	286	1,240	133
Lyso-lecithin	741	280	842	314
Phosphatidyl serine	1,464	59	707	55

[a] Mean and standard deviation of three samples; $p < 0.01$ in all cases. The samples analyzed were made by pooling 3 plates of cells. Each value represents the mean of 3 samples. The 3 control samples contained 15.49, 13.84 and 14.25 mg of phospholipid phosphorus, while the BAPN group contained 11.68 ,9.72 and 9.31 mg phospholipid phosphorus, respectively.

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tograms by various chemical and enzymatic tests[8,5]. Phospholipid phosphorus was determined on aliquots of lipid extracts by the micro-Bartlett method[5]; control and experimental values were compared on this basis[8].

Results. The Table shows the results obtained with L-929 fibroblasts grown for 5 days with 5 m*M* BAPN. The cells were incubated for 4 h with ^{14}C-acetate before harvest. Under the conditions of the study, BAPN had a pronounced effect on lipid synthesis by these cells. Triglycerides, lecithin, mono- and diglycerides, free fatty acids, an unknown lipid, phosphatidyl ethanolamine, phosphatidyl inositol, sphingomyelin, and phosphatidyl serine showed reduced radioactivity in BAPN cultures ($p < 0.01$), while cholesterol esters and lysolecithin values were not significantly different. All values were compared on a phospholipid phosphorus basis.

Discussion. In the present study, 5 m*M* BAPN was found to depress the de novo synthesis of lipids from ^{14}C-acetate in cultures of L-929 fibroblasts. Previous workers[9] studying the effect of BAPN on in vitro bone lipid synthesis had variable findings depending upon incubation time and BAPN concentration. At concentrations up to 10 m*M* BAPN and 20 h pre-incubation, they had enhanced synthesis while at 40 m*M* and above synthesis was retarded; concentrations of 20 m*M* showed no effective difference. NIAKARI et al.[10] observed that the non-collagen fraction from skin of lathyritic rats contained less neutral lipids than control animals, while SCHWARTZ[11] observed that when 50 mg BAPN was administered to cholesterol-fed rabbits, the severity of atheroma and of foam cell lipidosis was enhanced.

Although previous studies[1,12] using the model system of this investigation have shown increases in both collagen and mucopolysaccharide synthesis, it is obvious that the increase in macromolecular synthesis is not a general one. Aside from cholesterol, cholesterol esters and lysolecithin, the de novo lipid synthetic mechanisms which operate in cells grown in the presence of 5 m*M* BAPN are largely depressed and suggest that there may be in operation specific metabolic control mechanisms for regulation of cellular lipid composition.

Summary. Aside from cholesterol, cholesterol esters and lyso-lecithin, the de novo lipid synthesic mechanisms which operate in cells grown in the presence of β-aminopropionitrile are largely depressed and suggest that there may be in operation specific metabolic control mechanisms for regulation of cellular lipid composition.

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Kininase and Anti-Inflammatory Activities of Acid Carboxypeptidase from *Penicillium janthinellum*

Bradykinin is released from its plasma protein precursor(s) by the conjugated action of kallikrein and aminopeptidase. Its pharmacological actions include induction of acute arterial hypotension, vasodilation, increased capillary permeability, leucocyte migration and accumulation and pain, suggesting that bradykinin may be a mediator of conditions ranging from functional vasodilation to acute inflammation[1]. Anti-inflammatory activity of several proteolytic enzymes (trypsin, α-chymotrypsin, etc.[2–5]), in both the laboratory and clinic, has been reported, and absorption of these enzymes from intestinal tract has also been concisely investigated[6–12]. In the present study, we report that the acid carboxypeptidase (ACPase) from *Penicillium janthinellum*[13–17] shows kininase and anti-inflammatory activities in vitro and in vivo, respectively.

Materials and methods. P. janthinellum acid carboxypeptidase with a molecular weight of 51,000 was purified from kōji culture and submerged culture to yield a crystalline protein which was disc electrophoretically homogeneous at pH 9.4[15,16]. The crystals of the acid carboxypeptidase suspended in 0.05 *M* sodium acetate buffer (pH 3.7) were completely stable in 12 months at 5 °C[15,16]. 1 μg crystalline acid carboxypeptidase exhibits 0.63 nkatal activity at pH 3.7 and 30 °C for hydrolysis of benzyloxycarbonyl-L-glutamyl-L-tyrosine (Z-Glu-Tyr)[16].

One unit (katal) of acid carboxypeptidase was defined as the amount of enzyme required to liberate 1 mole of

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