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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tyrosine from Z-Glu-Tyr per second at pH 3.7 and 30 °C. Release of the carboxy-terminal amino acid resdues from bradykinin (from Protein Research Foundation, Osaka, Japan) by the acid caroxypeptidase was investigated using amino acid analyzer according to the previous paper[17].

Anti-inflammatory activity of the acid carboxypeptidase was assessed by determining its ability to inhibit rat hind-paw carrageenin edema according to Winter et al.[18], and α-chymotrypsin (Sigma) and bromelain (Sankyo, Tokyo, Japan) were used as the standard.

Results and discussion. By the incubation with 1.32 μg acid carboxypeptidase, carboxy-terminal arginine of the nonapeptide bradykinin was rapidly released, followed by slow liberation of phenylalanine (Table I). On the other hand, 13.2 μg acid carboxypeptidase hydrolyzed sequentially carboxy-terminal arginine, phenylalanine, proline, and serine (Table I).

Table I. Release of carboxy-terminal amino acid residues from 0.49 μmole bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) at 30 °C by acid carboxypeptidase from *Penicillium janthinellum*

Enzyme (μg)	Incubation		Amino acid released (μmole)				
	(h)	(pH)	Gly	Ser	Pro	Phe	Arg
1.32	1	4.15	—	—	—	—	0.385
1.32	5	4.15	trace	trace	trace	trace	0.410
1.32	10	4.15	trace	trace	trace	0.065	0.443
13.2	1	4.8	trace	trace	trace	0.043	0.444
13.2	5	4.8	trace	0.097	0.097	0.146	0.484
13.2	13	4.8	trace	0.163	0.171	0.317	0.444

Incubation: bradykinin (0.49 μmole, 0.5 mg) was dissolved in 15 ml distilled water, and the acid carboxypeptidase in minimum amount of sodium acetate buffer was added to the solution. 1.32 μg of the acid carboxypeptidase exhibits 0.831 nkatal activity for Z-Glu-Tyr hydrolysis at pH 3.7 and 30 °C. The mixture was incubated at 30 °C for the time indicated. An equal volume of 10% trichloroacetic acid was added, to inactivate the enzyme. After extraction with ether to remove trichloroacetic acid, the aqueous layer was evaporated to dryness in vacuo. Samples of the hydrolyzate were investigated on the column of a Hitachi model KLA-3B automatic amino acid analyzer.

We previously reported the production and purification, and some properties of a new type of acid carboxypeptidase from *P. janthinellum*[13–16]. Ethylene diamine tretra-acetate and *o*-phenanthroline have no effect on the enzymatic activities, suggesting that there is no requirement for metal ions[14]. The inhibitory effects of monoiodoacetic acid, *p*-chloromercuribenzoate, hydrocinnamic acid, and di-isopropylfluorophosphate have been studied[14]. The enzyme can remove acidic, neutral and basic amino acides as well as proline from the carboxy-terminal postion of synthetic peptides or peptide hormone in the acidic pH range (2.5–5.5)[13,14,17]. Synthetic N-substituted peptides Z-Gly-Pro-Leu-Gly, Z-Gly-Pro-Leu-Gly-Pro, decapeptide angiotensin I, native lysozyme, and reduced S-carboxymethyl-lysozyme have been degraded at pH 5.2[17] demonstrating the combined activity of pancreatic carboxypeptidase A (EC 3.4.12.2) and B (EC 3.4.12.3), but showing important difference in the release of carboxy-terminal proline from Z-Gly-Pro-Leu-Gly-Pro. Furthermore, it was suggested that the penultimate position from carboxy-terminal of substrates is very important on the binding site in the enzyme reaction of the *Penicillium* acid carboxypeptidase[17]. The specificity of acid carboxypeptidase in particular is similar to the *Aspergillus* acid carboxypeptidase[19–21]. In this experiment of Table I, the acid carboxypeptidase showed the same specificity as that obtained from the previous experiments using several peptides and proteins[17]. Removal of the carboxy-terminal arginine from bradykinin was reported quite early to yield an inactive peptide[22]. The acid carboxypeptidase was confirmed to degrade bradykinin, producing inactive peptides and amino acids in vitro. Furthermore, it is widely known that the pH of inflammating cells falls to between 5 and 6. These results stimulated us to investigate the effects of this enzyme in vivo experiments.

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Table II. Anti-inflammatory activity of acid carboxypeptidase from *Penicillium janthinellum*

Sample	Dose[a] (mg/kg)	Time (h) after carrageenin							
		1		2		3		4	
		Swelling[b] (%)	Inhibition (%)	Swelling (%)	Inhibition (%)	Swelling (%)	Inhibition (%)	Swelling (%)	Inhibition (%)
Control	—	17.3 ± 2.4	—	51.5 ± 3.8	—	54.8 ± 3.8	—	59.2 ± 2.4	—
Acid	1.6	12.2 ± 2.0	29.5	14.1 ± 2.8	72.6	9.1 ± 3.6	83.4	11.3 ± 5.1	80.9
carboxypeptidase	0.8	11.7 ± 1.4	32.4	15.8 ± 2.5	69.3	12.6 ± 4.3	77.0	13.2 ± 3.8	77.7
	0.2	14.7 ± 1.3	15.0	24.8 ± 3.7	51.8	23.4 ± 5.1	57.3	23.8 ± 4.8	59.8
Bromelain	10	7.7 ± 1.3	55.5	15.2 ± 2.4	70.5	23.8 ± 3.5	56.6	38.1 ± 4.1	35.6
	1	15.5 ± 1.3	10.4	43.1 ± 3.1	16.3	61.1 ± 2.1	11.3	61.3 ± 2.5	3.5
α-chymotrypsin	20	14.7 ± 2.5	15.0	25.1 ± 2.6	51.3	35.8 ± 3.3	34.7	42.6 ± 3.0	28.0
	10	13.0 ± 1.2	24.9	37.0 ± 4.5	28.2	46.9 ± 3.8	14.4	50.7 ± 2.3	14.4

[a] Enzyme drugs in aqueous suspension were administered i.p. Controls received only distilled water. This treatment was given 1 h before injection of the carrageenin into the feet of male rats. A volume of 0.05 ml carrageenin prepared as 2% suspension in sterile 0.9% NaCl was injected through a 26-gauge needle into the plantar tissue of right hind paw. Swelling of the paw reached a peak in 3 to 5 h, then retained about the same degree of edema for several hours. For drug testing, increase in foot volume 1, 2, 3 and 4 h after carrageenin was adopted as a measure of effect. [b] Average of 8 rats.

Anti-inflammatory activity of the acid carboxypeptidase on carrageenin-induced paw edema is summarized in Table II. The acid carboxypeptidase showed a more potent anti-inflammatory activity than α-chymotrypsin and bromelain. In the analysis at 2, 3 and 4 h after injection of carrageenin (Table II), the log dose response curves for the acid carboxypeptidase were linear within the limit of experimental error. However, in the analysis at 1 h after injection of carrageenin, the log dose response curve was not linear. This phenomenon seems to indicate the slow absorption of the acid carboxypeptidase which has a molecular weight of 51,000. These findings seem to suggest that the acid carboxypeptidase hydrolyzed bradykinin in vivo. No appreciable adverse reactions, such as hemorrhage and ascites, were observed at the doses given.

Summary. The acid carboxypeptidase from *Penicillium janthinellum* catalyzed the rapid release of arginine, and the slow release of phenylalanine, proline, serine and glycine from the carboxy-terminal of bradykinin at pH 4.15 to 4.8. Anti-inflammatory activity of the acid carboxypeptidase seems to suggest that the enzyme hydrolyzed bradykinin in vivo.

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Interaction Between Dehydroepiandrosterone, Glucose-6-Phosphate Dehydrogenase, and Cyclic Adenosine-3',5'-Monophosphate in Neoplastic and Normal Human Mammary Tissue

Only recently, the apparent interaction between dehydroepiandrosterone (DHEA), 3β-hydroxy-5-androsten-17-one, glucose-6-phosphate-dehydrogenase (G-6-PDH, EC 1.1.1.49), and cyclic adenosine-3',5'-monophosphate (c-AMP) in normal and diseased subjects has been reported[1]. Although in vitro a distinct activation of c-AMP phosphodiesterase (EC 3.1.4.17) by DHEA and DHEA sulfatide could be demonstrated, this direct effect seems to be secondary to the indirect inhibition of said enzyme by DHEA and its sulfatide via the G-6-PDH system[2]. Here, the allosteric inhibition of G-6-PDH by DHEA and DHEA sulfatide[3] probably leads to the accumulation of G-6-P, which in turn is known as a competitive inhibitor of c-AMP phosphodiesterase[4]. Hence, the influence of DHEA, its endogenous or synthetic sulfoconjugates, as well as of other steroidal G-6-PDH inhibitors upon the growth of cell cultures from normal and neoplastic human tissue[5,6] eventually may also be ascribed to such regulatory effects. Furthermore, this hypothesis is supported by the observation that DHEA inhibits the mitosis of cultured human lymphocytes[7]. Based on these experiments, DHEA, G-6-PDH activity, and c-AMP were determined in normal and neoplastic human mammary tissue.

Material and methods. In the course of mastectomy, due to suspected mammary cancer, samples were removed from neoplastic and adjacent normal tissue and immediately submitted to histological inspection. For determination of total DHEA and G-6-PDH activity, one tissue aliquot was weighed and homogenized in 1.0 ml 0.9% sodium chloride/0.025% EDTA per 200 mg of wet tissue. After centrifugation for 10 min at 5000 *g*, the supernatant was decanted and assayed for total DHEA[8], G-6-PDH[9], and soluble proteins[10]. A second aliquot was homogenized in 1.0 ml 6% trichloroacetic acid per 50 mg of wet tissue and the homogenate centrifuged for 10 min at 10,000 *g*. Following decantation, the supernatant was diluted with 0.1 ml 0.1 *N* hydrochloric acid per ml and extracted with 4 volumes water-saturated ether. The ether extract was discarded and the aqueous phase subjected to the assay of c-AMP[11].

Results and discussion. Whereas the G-6-PDH activity in 7 samples of cancer tissue averaged 421 ± 241 mU/mg soluble proteins, the enzyme activity in all corresponding normal tissues did not even reach 25 mU/mg protein. Conversely, the concentration of total DHEA in cancer tissue, as compared to the normal tissue, was found to be decreased by 56.6 ± 13.6%. The individual concentrations, however, varied between 0.8 and 6.1 μg/mg soluble proteins in cancer tissue and between 1.8 and 12.3 μg/mg protein in normal tissue, thus preventing a direct statistical evaluation of tissue levels. From such findings it might be concluded that in cancer tissue the lack of DHEA or its endogenous sulfoconjugate, e.g. DHEA sulfatide, results in a considerable rise of intracellular G-6-PDH activity. Relatively low concentrations of DHEA in cancer patients have already been reported by SONKA et al.[12]. According to the aforementioned concept, the

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