

group to extract into hexane when this solvent was used instead of chloroform indicates it is not a carotenoid. The extraction into chloroform after protein denaturation with acidic methanol is similar to that of chromoproteins having bilin prosthetic groups^{4,7,16}. However, attempts to isolate the prosthetic group from the chloroform solution were not successful. The solution gradually changed from blue-green to yellow and finally the color disappeared completely.

Two temperate-water fishes, *Clinocottus analis* (wooly sculpin) collected in tidal pools along the Southern California coast and *Scorpaenichthys marmoratus* (cabezon), which was collected¹⁷ off the coast of Southern California on the ocean bottom at a depth of about 20 m, were also found to have blue or blue-green blood plasma. Preliminary experiments indicate that a blue-green protein, with properties similar to those of the chromoprotein found in the blood plasma and skin of the Arctic sculpin, could be isolated from the plasma of both species¹⁸. The blue-green protein therefore appears to occur in the blood plasma of 3 different species of fish. The 3 species belong to the family *Cottidae* and further studies of the blood plasma and skin of fishes in this family may reveal other examples of the occurrence of the chromoprotein. The biosynthetic pathway and the physiological function, if any, of the blue-green protein are not known¹⁹.

Zusammenfassung. Aus dem Blutplasma und der Haut des arktischen Spinnenfisches *Myoxocephalus scorpioides* wurde ein blaugrünes Chromoprotein isoliert.

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¹³ W. RÜDIGER, W. KLOSE, M. VUILLAUME and M. BARBIER, *Experientia* 24, 1000 (1968).

¹⁷ These specimens were provided by Mr. D. W. WILKIE.

¹⁸ Besides the blue-green pigment, there also appears to be a reddish pigment present in the plasma of *Scorpaenichthys marmoratus*. Except for some ammonium sulfate fractionations, no attempt was made to purify this pigment.

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Hydrolysis of Amino Acid β -Naphthylamides by Aminopeptidases in the Parotid Gland

In a previous paper¹, we reported the enzymic hydrolysis of amino-acid β -naphthylamides by aminopeptidases in human parotid saliva. The hydrolysis of glycyl-L-prolyl β -naphthylamide in parotid saliva was relatively higher than those of other amino-acid β -naphthylamides, indicating the presence of a newly described kidney enzyme by HOPSU-HAVU and GLENNER² in parotid salivary fluid.

We have recently found that an aminopeptidase which hydrolyzes glycyl-L-prolyl β -naphthylamide was predominantly present in bovine parotid gland³. This communication describes the comparison of the substrate specificities with aminopeptidases in bovine parotid gland and the intracellular distribution of the enzymes.

Bovine parotid gland was obtained fresh, packed in ice, from the slaughterhouse. The tissue was homogenized by the use of an Ultra-Turrax homogenizer with 9 vol. of 0.25 M sucrose. After removing cell debris and nucleus by low-speed centrifugation, mitochondrial and microsomal fractions and soluble supernatant were separated by differential centrifugation. The substrate amino-acid β -naphthylamides, which were synthesized as described by GLENNER et al.⁴, were kindly supplied from Dr. G. G. GLENNER. Glycyl-L-prolyl β -naphthylamide hydrobromide was kindly synthesized by Drs. S. SAKAKIBARA and K. TAKADA by the method of GLENNER et al.². The incubation mixture contained 90 μ moles Tris-maleate buffer, pH 7.0, 0.45 μ mole amino-acid β -naphthylamide and water to 0.90 ml. The activity for the hydrolysis of α -L-glutamyl β -naphthylamide and α -L-aspartyl β -naphthylamide was measured in the presence of 1 mM of Ca^{2+} ⁵. Incubation was carried out at 37°C for 60 min. Increase of fluorescence intensity of 410 nm of β -naphthylamine released by enzymic hydrolysis of amino-acid β -naphthylamide was measured with the excitation light at 335 nm using an Aminco-Bowman spectrophotofluorometer⁶.

Results are shown in the Table. Aminopeptidases hydrolyzing amino-acid β -naphthylamides were distributed mainly in the microsomal fraction, as well as in the soluble fraction. The enzyme activities in mitochondrial and nuclear fractions were low and probably due to contamination of the microsomal fraction. Among 21 amino-acid β -naphthylamides, naphthylamides of glycyl-proline, alanine, leucine, methionine, arginine, norleucine, and norvaline were good substrates for aminopeptidases in the parotid gland. These amino-acid β -naphthylamides were also good substrates for salivary aminopeptidases¹.

The enzyme which hydrolyzes glycyl-prolyl β -naphthylamide was the most active aminopeptidase in the parotid gland, as well as in parotid saliva¹. This enzyme was mainly localized in the microsomal fraction as shown in the Table. Analysis of the reaction product in the hydrolysis of glycyl-prolyl β -naphthylamide by paper chromatography demonstrated that N-terminal glycyl-proline was liberated from the substrate. This result indicated that the aminopeptidase in the salivary gland is similar to the enzyme in the kidney newly described by

¹ I. NAGATSU, T. NAGATSU and T. YAMAMOTO, *Experientia* 24, 347 (1968).

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³ H. OYA, M. HARADA and T. NAGATSU, *Aichi-Gakuin J. dent. Sci.* 6, 362 (1969).

⁴ G. G. GLENNER, L. A. COHEN and J. E. FOLK, *J. Histochem. Cytochem.* 13, 57 (1965).

⁵ G. G. GLENNER, P. J. McMILLAN and J. E. FOLK, *Nature* 194, 867 (1962).

⁶ The Aminco-Bowman spectrophotofluorometer was purchased by United States Public Health Service Research Grant No. 7R05 TW-00219-01A1 for T. NAGATSU, which is gratefully acknowledged.

HOPSU-HAVU and GLENNER². This enzyme seems to be excreted into parotid saliva from the salivary gland in a higher concentration than the other aminopeptidases, since the hydrolysis of the substrate was found to be relatively higher in parotid salivary fluid¹. This may indicate a selective mechanism for the enzyme secretion from the salivary gland. Physiological significance of the presence of this enzyme in the salivary gland and in saliva remains unknown. Since the amino-acid sequence

glycyl-proline is predominant in collagen, the enzyme may act on collagen metabolism. The possibility of implication of this enzyme for collagen metabolism has been suggested by HOPSU-HAVU, RINTOLA and GLENNER^{7,8}. Since collagen is a main protein in the oral tissues including dentine, physiological and pathological role of this enzyme remains to be elucidated.

Zusammenfassung. Die Aktivität der Aminopeptidasen in Ohrspeicheldrüsen wurde gemessen. Glycyl-Prolin β -naphthylamid, Alanin β -naphthylamid, Leucin β -naphthylamid, Methionin β -naphthylamid, und Arginin β -naphthylamid wurden von der Mikrosomenfraktion und der löslichen Fraktion schnell gespalten. Das Glycyl-Prolin β -naphthylamid spaltende Enzym war in Ohrspeicheldrüsen in relativ grösserer Menge vorhanden. Die Aufspaltung von Glycyl-Prolin β -naphthylamid in Glycyl-Prolin und β -Naphthylamin wurde papierchromatographisch nachgewiesen.

Hydrolysis of amino-acid β -naphthylamides by aminopeptidases in bovine parotid gland

Amino-acid β -naphthylamide	Aminopeptidase activity	
	Microsomal fraction nmoles/min/mg protein	Soluble fraction nmoles/min/mg protein
Ala	22	7.9
Arg	4.1	7.0
Asn	1.1	1.2
α -L-Asp	0.0	0.0
Gln	1.2	2.2
α -L-Glu	0.1	0.5
D-Glu	0.0	0.0
γ -Glu	0.0	0.0
Gly	3.6	0.8
Gly-Phe	1.3	0.4
Gly-Pro	31	4.3
Ile	0.5	0.0
Leu	14	3.7
Lys	—	3.7
Met	11	4.8
Norleu	6.7	3.5
Norval	6.3	3.2
Phe	—	5.4
Pro	0.6	0.8
Ser	0.7	0.7
Val	0.6	0.0

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⁷ V. K. HOPSU-HAVU, P. RINTOLA and G. G. GLENNER, *Acta chem. scand.* 22, 299 (1968).

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Effect of Adrenalectomy on the Response of Rat Liver Polyribosomes to Whole-Body Gamma Radiation

Polyribosomes from regenerating rat livers^{1,2} and from intact livers of both rats and guinea-pigs exposed to whole-body X-irradiation are characterized by a marked shift in their pattern of distribution^{3,4}. After either of the above treatments, the liver C-ribosome profile on a linear sucrose density gradient shows a marked decrease in monomers and smaller oligomers and a concomitant increase in heavier aggregates. In contrast, whole-body exposure of rats with regenerating livers to either gamma or neutron radiation within a few hours after surgery initially prevents the characteristic shift in the polyribosome pattern normally observed 24 h after partial hepatectomy. However, when a longer time interval is allowed between surgery and killing (36 h), a heavy aggregate profile, characteristic of 36 h regenerating liver from unirradiated animals, is obtained^{2,5}.

In this communication we report experiments with adrenalectomized rats which were performed in an attempt the better to understand the varying response to ionizing radiation obtained with intact and regenerating rat liver. Unfortunately the data presented deal only with the effect of adrenalectomy and radiation on rats with intact liver because the combined stress of adrenalectomy,

partial hepatectomy and radiation employed in these studies was incompatible with survival.

Material and methods. Male Badger rats (Badger Research Corp., Madison, Wisconsin, USA) weighing 240–260 g were used. The rats were bilaterally adrenalectomized and given ad libitum access to a 1% NaCl drinking solution. 24 and 48 h after surgery, some rats were injected s.c. with one adrenal gland, which had been prepared for injection by homogenizing in 0.5 ml of 0.85% NaCl.

One hour after the second adrenal gland injection the rats were subjected to whole-body radiation. Irradiation at 60 R/min was delivered by a 2000 Ci ¹³⁷Cesium source emitting 662 KeV gamma rays. All rats were fasted for 20 h prior to killing (by decapitation) and their liver polyribosomes prepared and analyzed as previously reported².

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