Tableau II. Analyse des stérols de *Calliactis parasitica*[17]

Stérols	Temps relatifs de rétention en chromatographie gaz-liquide	Spectrométrie[a] de masse[15] Stérols *m/e*	propionates *m/e*	Relatifs approximatifs (%)
Cholestérol	1	386	368	87
Cholestanol	1	388	444	3
Déhydrocholestérol[a]	0,89	384	366	2
Méthylène-24 cholestérol	1,28	398	380	4
Brassicastérol (ou picstérol)	1,12	398	380	2
C_{26}	0,68		352	1,5

[a] Rappelons que les stérols Δ_5 fournissent un ion moléculaire alors que leurs propionates montrent un ion à M-74; en absence d'insaturation en 5, les propionates donnent l'ion moléculaire. Les fragmentations ultérieures ont été utilisées pour la détermination des chaînes latérales. La seconde insaturation du déhydrocholestérol est située dans la chaîne latérale, mais par suite des faibles quantités desubstance, nous n'avons pu préciser.

dans les acides (environ 3%) et dans la partie non stéroles résultats obtenus sont résumés au Tableau II. La présence du cholestérol en quantité abondante avait été signalée par SALAQUE[10]. Nous avons pu mettre en évidence une quantité non négligeable de stérols à 26 atomes de carbone (environ 1,5% des stérols)[12–14] provenant vraisemblablement du phytoplancton par la chaîne alimentaire[13].

La partie de l'insaponifiable non précipitée par la digitonine a été chromatographiée sur colonne d'acide silicique et on a recherché le squalène par chromatographies sur couches minces et gaz-liquide; n'ayant pu le trouver par ces procèdés, nous avons repris l'isolement après addition de 20 mg de squalène authentique purifié. Le produit isolé par chromatographie préparative sur couche mince d'acide silicique (développement par l'heptane, Rf 0,35) possède une radioactivité totale de 600 dpm devenant nulle après préparation et cristallisation de l'hexachlorure de squalène[11].

Summary. Sodium acetate I-^{14}C has been injected to the sea anemone *Calliactis parasitica* (coelenterate, *anthozoa*). Sterols and squalene were found unlabelled in this experiment. An analysis of the sterol mixture is reported.

J. P. FEREZOU, M. DEVYS et M. BARBIER[15,16,17]

Institut de Chimie des Substances Naturelles, C.N.R.S., F-91 Gif-sur-Yvette (France), 16 septembre 1971.

[10] A. SALAQUE, Thèse de Doctorat d'Université, Paris 1967.
[11] I. M. HEILBRON, E. D. KAMM et W. M. OWEN, J. chem. Soc. *1926*, 1631.
[12] D. R. IDLER, P. M. WISEMAN et L. F. SAFE, Steroids *16*, 451 (1970).
[13] J. L. BOUTRY, A. ALCAIDE et M. BARBIER, C.r. Acad. Sci., Paris, *272*, série D, 1022 (1971).
[14] A. ALCAIDE, J. VIALA, F. PINTE, M. BARBIER, M. ITOH et T. NOMURA, sous presse.
[15] Nous remercions MM. COSSON, BARDEY et VARENNE qui ont effectué les mesures de spectrométrie de masse sur un appareil AEI MS9, sous la direction du Dr B. C. DAS.
[16] Nous remercions le Professeur E. LEDERER pour l'intérêt qu'il a porté à ce travail.
[17] Nous remercions la Station Biologique de Roscoff pour l'expédition de ces animaux.

Activity of a Dipeptidyl Carboxypeptidase (Angiotensin Converting Enzyme) in Lungs of Different Animal Species

VANE et al.[1] have shown that the conversion of circulating angiotensin I to angiotensin II (as measured by bioassay) in the dog occurs mainly in the lungs[2]. Using a chemical assay with a synthetic substrate, we recently determined the converting enzyme in different tissues of the rat and also found the highest concentrations to occur in the lungs[3]. The purpose of the present investigation was to compare the enzyme content in lungs of different animal species in order to find a convenient starting material for future preparative work on the enzyme.

Material and methods. The dipeptidyl carboxypeptidase was assayed with the method previously described[4], except that the apparatus was an Aminco Fluoro-microphotometer. The method is based on the fluorimetric determination of histidyl-leucine released from the substrate, Z-Phe-His-Leu.

Enzyme preparations from different animal species. Depending on the size of the animal, either a single lung or both lungs were used as the starting material. Rat lungs were pooled from 15 and mice lungs from 70 animals. Healthy fragments of human lungs were obtained from 2 individuals after autopsy and pooled.

The intact organs were kept at 4°C for a time which was uniformly set at 48 h, after which they were hashed with a kitchen machine or scissors. The minced tissue was mixed and 2 aliquots were taken to provide duplicate samples. These were homogenized with a high speed blade homogenizer after addition of 3 parts (w/w) of water; the homogenates were centrifuged for 30 min at 107,000 × *g*. The supernatant was carefully decanted and replaced by an equal volume of water in which the centrifuged material

[1] K. K. F. NG and J. R. VANE, Nature, Lond. *218*, 144 (1968).
[2] Y. S. BACKLE; Nature, Lond. *220*, 919 (1968).
[3] M. ROTH, A. F. WEITZMAN and Y. PIQUILLOUD, Experientia *25*, 1247 (1969).
[4] Y. PIQUILLOUD, A. REINHARZ and M. ROTH, Biochim. biophys. Acta *206*, 136 (1970).

was resuspended. The enzyme activity was measured a) in the supernatant and b) in the resuspended sediment. In the latter case, incubation was performed in a Dubnoff-shaker with constant agitation.

Results. The enzyme activities found in the 107,000 × *g* supernatants and sediments of lung homogenates from various species are shown in the Table. Much higher activities occur in the sediment, except for rat and mouse. In accordance with our previous studies on the specificity of the assay, the enzyme activities measured could be abolished by dialysis against water and restored by addition of chloride ions.

Discussion. From recent studies[5-7] it appears that the enzyme responsible of the conversion of angiotensin I to angiotensin II is also able to attack other substrates. Thus, it can inactivate bradykinin by removal of the C-terminal dipeptide. The name 'dipeptidyl-carboxypeptidase' is therefore more appropriate than 'converting enzyme' which has been used so far. Availability of purified preparations of the enzyme would certainly help much in improving our knowledge of its properties and function. The present investigation shows that rat lungs represent a particularly rich source of the enzyme. In practice, however, organs of slaughter-house animals are often preferred as a convenient source for large-scale preparations, and in this respect a material such as horse or hog lung may prove more adequate.

A puzzling question is how circulating angiotensin I can be so easily converted to angiotensin II by a non-circulating pulmonary enzyme. This probably implies that the enzyme is located in the very vicinity of the blood flowing through the capillaries. Our studies show that in dog, rabbit, hog, horse, sheep, ox, guinea-pig and man, dipeptidyl carboxypeptidase is not a cytoplasmic enzyme, since it is found in a sedimentable fraction. They confirm the findings of BACKLE[2] and of YANG et al.[7], who found most of the activity of lung to sediment in the microsomal fraction.

We found the enzyme to occur in all 10 mammalian species investigated. The amounts present in each particular case are certainly of importance for the kinetics of angiotensin activation and bradykinin breakdown[8].

Activity of dipeptidyl-carboxypeptidase in lungs from different species

Species	Enzyme activity (mU/g tissue)		
	Supernatant	Pellet	Total
Rat	98.2	46.4	144.6
Rabbit	3.2	106.0	109.2
Mouse	60.4	47.6	108.0
Horse	18.1	47.8	65.9
Dog	9.6	52.2	61.8
Hog	13.0	48.8	61.8
Sheep	2.2	58.8	61.0
Beef	1.6	29.8	31.4
Guinea-pig	3.6	26.4	30.0
Calf	1.2	16.0	17.2
Human	3.6	12.2	15.8

Résumé. On a dosé la dipeptidyl-carboxypeptidase (responsable de la conversion de l'angiotensine I en angiotensine II) dans des préparations de poumons de 10 espèces différentes. Les espèces se classent comme suit par ordre décroissant de teneur en enzyme: rat > lapin, souris > cheval, chien, porc, mouton > bœuf, cobaye > veau, homme. Chez la plupart des espèces, l'enzyme est fixé a une structure subcellulaire sédimentable.

D. DEPIERRE and M. ROTH

Laboratoire Central, Hôpital Cantonal, CH–1211 Genève 4 (Switzerland), 13 September 1971.

[5] Y. E. ELISSEEVA, V. N. OREKHOVICH, L. V. PAVLIKHINA and L. P. ALEXEENKO; Clin. chim. Acta *31*, 413 (1971).
[6] D. W. CUSHMAN and H. S. CHEUNG, Biochem. Pharmac. *20*, 1637 (1971).
[7] H. Y. T. YANG, E. G. ERDÖS and Y. LEVIN; J. Pharmac. *177*, 291 (1971).
[8] Supported by Grant No. 3.243.69 from the Swiss National Fund for Scientific Research.

Effect of Acriflavine on the Radiation Resistance Enhancement Induced by Cysteine Treatment in *Escherichia coli* B/r

Cysteine is a good radioprotector. It has been established that there are at least two kinds of mechanism by which it enhances the radiation resistance of *Escherichia coli*[1]. The physico-chemical mechanisms represent radical scavinging, hydrogen donation, etc[2]. The metabolic or biochemical mechanisms mean that cysteine alters the cell metabolism in several ways and some of these metabolic alterations result in enhanced survival after ionizing radiation[1]. The same is true also for cysteamine[3].

The mechanism of the biochemical radioprotective effect of cysteine has been studied in our laboratory using the following method: *E. coli* cells are treated with cysteine for certain periods, thereafter cysteine is removed (by centrifugation and washing) and the bacteria are irradiated in cysteine-free buffer. Analyzing the changes in radiosensitivity and, at the same time, in cell metabolism induced by cysteine treatment, allowed us to propose a model to explain the mechanism of the metabolic radioprotective effect of cysteine[4]. According to this model, the sharp increase in radio-resistance, obtained by 1 min cysteine treatment, can be explained by the sharp increase in the acid-soluble SH level of bacteria. The further increase in radio-resistance, up to the maximum obtained by 30 min cysteine treatment, can be correlated with the asynchronous synthesis of macromolecula

[1] ZS. NAGY, F. HERNÁDI, P. KOVÁCS and T. VÁLYI-NAGY, Radiat. Res. *35*, 652 (1968).
[2] H. DERTINGER and H. JUNG, *Molecular Radiation Biology* (Springer-Verlag, New York-Heidelberg-Berlin 1970).
[3] D. M. GINSBERG, Radiat. Res. *28*, 708 (1966).
[4] ZS. NAGY, P. KOVÁCS, Cs. KARI and F. HERNÁDI, Arch. Mikrobiol. *70*, 65 (1970).