in non-polar solutions. But the correlation with the effect upon catalysis of luminol decomposition is only fair.

Examination of the catalytic ability of a larger series of derivatives with changes in other than the 2 and 4 positions should be illuminating. We have found heme *a* to be an extremely poor catalyst.[12]

[12] Acknowledgments. We are indebted to Mr. R. D. TOWNER for some of the chemiluminescence assays. We are grateful to Dr. J. R. TOTTER for helpful discussions.

[13] Reprint requests to be addressed to U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick (Maryland 21701, USA), 23 August 1973.

Zusammenfassung. Untersuchungen über den Wirkungsmechanismus der Chemolumineszenz von Luminol.

T. P. VASILEFF, G. SVARNAS, H. A. NEUFELD[13] and L. SPERO[13]

Biological Sciences Laboratories, Fort Detrick, Frederick (Maryland 21701, USA), 23 August 1973.

Activities of Decarboxylases of Histidine and Ornithine in Young Mice after Injection of Epidermal Growth Factor

Epidermal cell proliferation, initiated by surgically inflicted skin wounds, has been found associated with a striking increase in histamine formation, i.e. histidine decarboxylase activity, in the growing wound tissues of rats and man. On artificially lowering or elevating the histamine formation of rat skin, the rate of healing was retarded or accelerated, respectively. It thus appears that a causal relationship between growth processes in the skin wound and histidine decarboxylase activity exists. Some rapidly growing tissues generate ornithine decarboxylase activity (for references see KAHLSON and ROSENGREN[1,2]). An 'epidermal growth factor' (EGF), occurring in the submaxillary gland of adult mice[3], stimulates epidermal growth in several species[4]. These observations led us to investigate EGF for possible effects on activities of histidine and ornithine decarboxylases of skin and some other tissues.

Material and methods. EGF was obtained by procedures designed to simplify existing published methods and to increase the overall yield and reliability of the extraction from male mouse submaxillary glands. Complete details of the methodology will be described in a forthcoming publication and therefore only a summarized account will be presented here. The method consists of a first stage extraction similar to that described by COHEN[3] and also TAYLOR, MITCHELL and COHEN[5], up to and including the precipitation with ammonium sulphate. The resolubilised material is then subjected to acidic conditions at pH 3.0, followed by dialysis at pH 5.5. The precipitate is removed at each stage. The liquor is then applied to a column of DEAE cellulose at pH 5.5. After batch elution with 0.09 *M* salt, the eluate is re-applied to an identical column and eluted with a salt gradient. The active peak is dialysed, concentrated and subjected to molecular filtration on a column of Sephadex G-75 at pH 5.5.

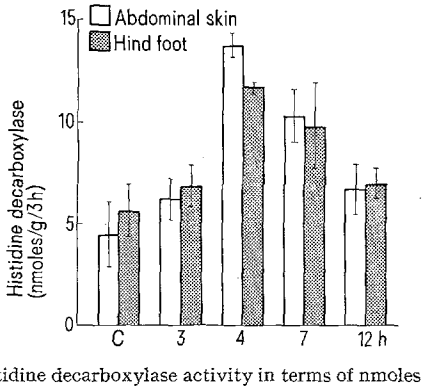

Fig. 1. Histidine decarboxylase activity in terms of nmoles histamine formed in abdominal skin and hind foot after injection of EGF (6 µg/g). C, controls; h, time after injection. The EGF-induced elevation is significant for the time course studied, $p < 0.001$ by Student's *t*-test.

The EGF obtained by the above procedure is found to be as active as that prepared according to the method of COHEN[3], when tested in vivo on the eye opening response of neonatal mice. However, the product differs in its molecular weight (5,750 by ultracentrifugation) and in its absorbance at 280 nm, a value for $E^{1\%}_{1\,cm}$ of 8.4 being obtained, compared to a reported value of 25.9 or 30.9 for COHEN's preparation[3,6]. The lower value is reflected in the presence of only trace amounts of tryptophan in the product. The amino acid, phenylalanine, is also absent in agreement with COHEN's findings but the presence of 2 other amino acids, lysine and alanine, is consistently shown. These amino acids have been claimed to be absent in the EGF molecule[3,5,6]. During the preparation of this manuscript, the extraction of EGF by a comparable technique involving low pH treatment of crude homogenates was reported[7,8]. At pH values in the range 3.2 to 3.5 a product designated EGF-2 was obtained in which the COOH-terminal leucine-arginine residue was absent, whereas the product described in this publication must be lacking a minimum of five COOH-terminal residues in order to account for the absence of tryptophan residues located at positions 49 and 50 on the native EGF molecule[8]. Therefore it is probable that the preparation presently employed represents the derivative EGF_{1-48} or an even lower molecular weight form.

The experiments were done on tissues of 6–9-days-old female mice, strain NMRI. EGF was injected s.c. in the back of the neck and the animals were killed at different times thereafter. Controls were injected with the solvent 0.9% NaCl solution. The appropriate tissue from 2 animals was pooled, finely cut and a sample removed for assay of enzymic activity.

Determination of histidine decarboxylase activity was made by incubating excised tissues with radioactive histidine and measuring the amount of histamine formed[9].

The ornithine decarboxylase activity of minced tissue samples was determined by measurement of the release of $^{14}CO_2$ from DL-1-^{14}C ornithine[10].

[1] G. KAHLSON and E. ROSENGREN, Physiol. Rev. *48*, 155 (1968).
[2] G. KAHLSON and E. ROSENGREN, in *Biogenesis and Physiology of Histamine* (Arnold, London 1971).
[3] S. COHEN, J. biol. Chem. *237*, 1555 (1962).
[4] S. COHEN, Devel. Biol. *12*, 394 (1965).
[5] J. M. TAYLOR, W. M. MITCHELL and S. COHEN, J. biol. Chem. *247*, 5928 (1972).
[6] J. M. TAYLOR, S. COHEN and W. M. MITCHELL, Proc. natn. Acad. Sci. USA *67*, 164 (1970).
[7] C. R. SAVAGE and S. COHEN, J. biol. Chem. *247*, 7609 (1972).
[8] C. R. SAVAGE, T. INAGAMI and S. COHEN, J. biol. Chem. *247*, 7612 (1972).

Results. The tissues examined for histamine formation were abdominal skin, hind foot (containing a high proportion of skin), kidney, lung, heart, liver and spleen. EGF was given in a dosage of 6 μg/g, and histamine formation was determined 3, 4, 7 and 12 h after the injection. Injection of EGF accelerated the rate of histamine formation in the skin, with a peak at 4 h when the rate of histamine formation was about 3 times the controls (Figure 1). Histamine formation of the hind foot, which contains tissue other than skin, was also elevated although to a lesser extent. The tissue specificity of the action of EGF was shown by the fact that it produced no increase in histamine formation in the other tissues examined, among which only the kidney was studied in detail.

In 4 mice, EGF 6 μg/g was injected daily from the 1st day of birth to the 6th day and the mice were killed 3 h after the last injection. In these experiments, no increase in histamine formation occurred. The observation that a single 6 μg/g injection of EGF increased histamine formation whereas repeated injections did not might be accounted for by the known inhibitory effect on growth of high doses of EGF.

Ornithine decarboxylase activity was measured in the same tissue pool in which histamine formation was determined. The activity of this enzyme was not significantly altered in the mice given EGF with one exception: At 4 h there was a slight elevation in the skin (Figure 2).

Discussion. Injecting EGF in newborn mice and rats has been reported to produce hyperplasia of the epidermis[11]. In newborn rats, injection of this factor gave rise to an increase in the protein and nucleic acid content per unit of skin[12]. A relationship between rate of protein synthesis in certain tissues and histidine decarboxylase activity has been proposed[2]. It would now appear that in the skin induction of histidine decarboxylase activity somehow is part of the mechanism of action of EGF. The kidney, under the growth stimulating influence of testosterone administration, has recently been found to bring about high ornithine decarboxylase activity, forming putrescine at increased rate, whereas that of histidine decarboxylase was depressed[10].

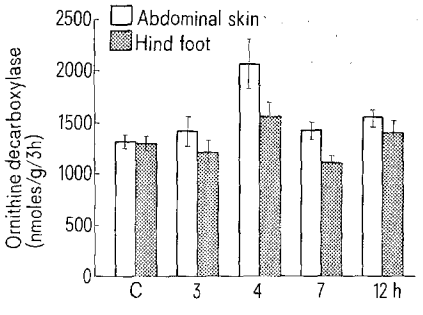

Fig. 2. Ornithine decarboxylase activity, in terms of nmoles $^{14}CO_2$ released, in abdominal skin and hind foot after injection of EGF. C and h as in Figure 1. Unlike the situation in Figure 1, the appropriate elevation is not significant.

Elevated ornithine decarboxylase activity has been reported to occur on stimulating skin and kidney with EGF: on injecting this factor subcutaneously in 6–9-day-old mice, the ornithine decarboxylase activity of the skin rose about 4-fold, reached a maximum in 4 h and then rapidly declined[13,14]. In the present experiments, no striking change in ornithine decarboxylase activity on injecting EGF in newborn mice could be demonstrated. This discrepancy between the results could be explained by the different nature of the EGF preparations used. As indicated earlier, it is probable that the active material is a derivative of EGF, lacking at least 5 COOH-terminal amino acid residues. Although this derivative appears to be as active as EGF prepared by standard methods[3] when measured by eye-opening response in neonatal mice, it is possible that the differences in the associated biological effects such as those described herein, may be attributed to the lower molecular weight derivatives.

The epidermis is a specific target organ for EGF in the sense that this tissue concentrates injected EGF whereas in the kidney for example the uptake is much less. This has been demonstrated by injecting ^{131}I-labeled EGF intraperitoneally in rats and determining the tissue content of the labeled product[15]. In the present study, EGF induced high histidine decarboxylase activity in the skin alone, thus supporting its specificity of action on epidermal tissue.

In conclusion, EGF brings about increased histamine formation, a phenomenon known to occur in some tissues in the process of normal growth[16].

Zusammenfassung. Nachweis, dass der epidermale Wachstumsfaktor EGF, in 6–9 Tage alte Mäuse s.c. injiziert, eine 3fache Steigerung der Histidin-Dacarboxylase-Aktivität in der Haut, nicht aber in anderen Geweben hervorruft, während EGF die Ornithin-Decarboxylase gar nicht beeinflusst.

P.T. Blosse, E.L. Fenton, S. Henningsson, G. Kahlson and Elsa Rosengren

Wellcome Research Laboratories, Beckenham (England); and Institute of Physiology, University of Lund, S-223 62 Lund (Sweden), 6 August 1973.

[9] G. Kahlson, E. Rosengren and R. Thunberg, J. Physiol. Lond. *169*, 467 (1963).
[10] B. Grahn, S. Henningsson, G. Kahlson and E. Rosengren, Br. J. Pharmac. *48*, 113 (1973).
[11] S. Cohen and G. A. Elliott, J. invest. Derm. *40*, 1 (1963).
[12] P. U. Angeletti, M. L. Salvi, R. L. Chesanow and S. Cohen, Experientia *20*, 146 (1964).
[13] M. Stastny and S. Cohen, Biochim. biophys. Acta *204*, 578 (1970).
[14] M. Stastny and S. Cohen, Biochim. biophys. Acta *261*, 177 (1972).
[15] I. Covelli, R. Rossi, R. Mozzi and L. Frati, Eur. J. Biochem. *27*, 225 (1972).
[16] This study has been supported by grant from the Swedish Medical Research Council (to E.R.) and from the Medical Faculty, University of Lund (to S.H.).

Amylases of the Lentil Roots

The starch degradation in higher plants is due mainly to the phosphorylases and the amylases. The latter were particularly studied in the seeds, where they are responsible for the mobilization of polysaccharide reserves[1,2]. Although in much smaller amount, the amylases were also found in the other organs, but few detailed papers have been published on this subject[3–5]. The lentil roots, in which several enzymatic systems – peroxidases and polyphenoloxidases[6], RNases[7], transaminases[8] – have already been analysed, show a significant amylolytic