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].

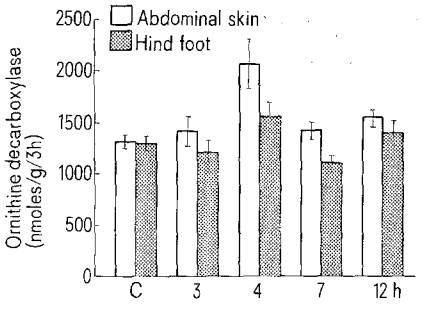

Fig. 2. Ornithine decarboxylase activity, in terms of nmoles $^{14}CO_2$ released, in abdominal skin and hind foot after injection of EGF. C and h as in Figure 1. Unlike the situation in Figure 1, the appropriate elevation is not significant.

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.

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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

activity. This is of particular interest in view of its possible relation to the changes in starch content of the amyloplasts following a gibberellin treatment[9]. This paper is precisely concerned with the study of amylase activity in lentil roots.

A hundred 3-day-old seedling roots of *Lens culinaris* were extracted in *Tris* buffer 0.001 *M*, pH 7.5, containing $CaCl_2$ 0.04 *M*. After centrifugation, the supernatant is used as the source of enzymes; 5 ml are subjected to gel chromatography on Sephadex G-50 and G-100, and eluted with the extraction buffer. The optical density (OD) at 254 nm is measured and the enzyme activity analysed by 2 ways. First, the colorimetric measurement of the reducing power of sugars[10] liberated from a solution of soluble starch 1% in acetate buffer 0.025 *M* pH 5.4 was performed. The activity, expressed as 'maltose equivalent' by comparison with a standard curve of maltose, can be considered as the total amylase activity. Second, the degradation of a solution of β-limit dextrin[11] by the α-amylase of the eluate was measured; the activity is expressed in IDC units[12] per ml eluate.

With Sephadex G-50 (Figure 1A), it can be seen that the peak of total activity coincides with that of α-amylase activity; both are eluted with the completely excluded proteins of the extract, consequently their molecular weight is greater than 30,000. On G-100 (Figure 1B), the volume of elution is larger, but only 1 peak includes both the total and the α-amylase activity. The molecular weight, estimated by the formula previously discussed by DETERMANN[13], is 81,000. Such value is greater than the molecular weight of malt α-amylase[14], and smaller than that of the sweet potato β-amylase[15], which probably means that the peak corresponds to a mixture of the two forms, not chromatographically separated. Such non-separation has already been mentioned in malt extract[16].

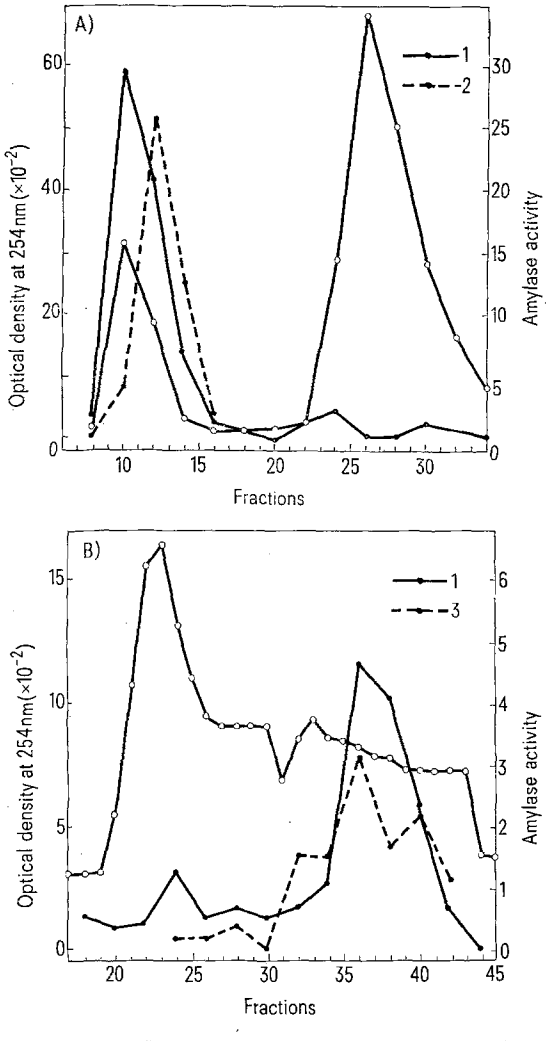

Fig. 1. Elution on Sephadex column (30 × 2.54 cm): optical density (○) and amylase activity (●). A) Sephadex G-50. 1. Total amylase activity in µg maltose equivalent/min/ml eluate; 2. α-amylase activity in IDC units × 5.10^{-2}/ml eluate. B) Sephadex G-100. 3. α-amylase activity in IDC units × 10/ml eluate.

According to these results, the root extract was subjected to polyacrylamide gel electrophoresis. A vertical gel slab apparatus[17], allowing the simultaneous electrophoresis of 7 samples, was used. The gel system was that of DAVIS[18], with a few modifications: suppression of the sample gel, buffer for spacer diluted 1:16, buffer for reservoirs diluted 1:2. With a syringe, 150 µl of supernatant were laid down in the holes prepared at the upper surface of the spacer gel; the whole apparatus was placed in a refrigerator to prevent heating. A current of 5 mA was applied for 2 h, allowing the proteins to enter the spacer gel; then the migration was effected with a current of 35 mA for 90 min. The gel slab was cut to separate the migration ways, and the slices were soaked for 4 h at 37 °C in solutions containing the substrate for amylases (starch soluble 1% in acetate buffer pH 5,4) and specific enzyme inhibitors. The enzymatically active bands were revealed by immersing the slices in a I_2 0.05%–KI 0.5% aqueous solution. As shown in Figure 2A, 3 bands with amylase activity (I, II, III) appear in the total extract, without inhibitor. If the extract was heated to 70 °C for 10 min before electrophoresis, the band I disappears (Figure 2B). Similar results are seen with phenylmercury chloride (PMC) in the incubation medium (Figure 2C). On the other hand, the addition of EDTA at 5×10^{-3} *M* involves the total inhibition of bands II and III (Figure 2D). Other assays show that only the bands II and III can hydrolyse the β-limit dextrin, and that none of the 3 bands were found to be sensitive to $CuSO_4$ 0.01 *M*.

The enzymes of bands II and III – heat-resistant, Ca-dependent, non-inhibited by PMC, hydrolysing the β-limit dextrin – are surely α-amylase isozymes. With regard to the enzymes of the band I, its properties – heat-labile, inhibited by PMC, requiring no Ca, not hydrolysing the β-limit dextrin – are those of a β-amylase, with the exception of its insensitiveness to Cu^{++}. Its migration

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speed is also quite different from that observed in extracts of barley seed[19] and pea cotyledon[20], where it is always slower than the α-amylase. But it is possible that the root β-amylase is rather different from that of the seeds, which is formed of several subunits and sometimes bound to protein-residues[19].

Present results indicate that *Lens* roots contain at least 3 amylases. In *Pisum*, the electrophoresis on polyacrylamide gel revealed 2 bands with amylase activity, which are not only located in the roots, but are found also in the other parts of the plant[3]. The analysis of the products of the enzymatic degradation seems to indicate that only the β-amylase is present in the axis of pea, whereas the cotyledons contain both α- and β-amylase[21]. But it must be noted that such plant contains also amylopectin 1,6-glucosidase[11], which was not found in the extracts of lentil roots.

If the physiological role of the amylases is now well established in seeds, it is not the case in other organs like roots. For instance, the ultrastructural observations on the destarching of amyloplasts in lentil root statenchyma[9] following a treatment with gibberellic acid, and in wheat coleoptile treated with GA_3 or kinetin – this latter correlated with an increase of amylase activity[22] – allow the hypothesis of the intervention of one or more amylases in the hormonal control of starch metabolism.

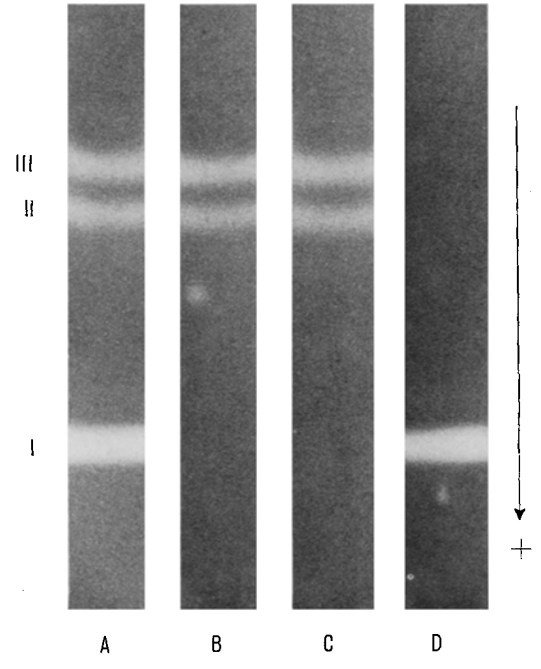

Fig. 2. Zymogram of the electrophoresis on 7% polyacrylamide gel; central parts of gel slices. A) total extract; B) 70°C heated extract; C) gel incubated with PMC; D) gel incubated with EDTA.

Résumé. Les amylases de la racine de *Lens culinaris* sont éluées en un seul pic par chromatographie sur gel de Sephadex G-50 et G-100. Par contre, l'électrophorèse en plaque de gel de polyacrylamide révèle 3 zones d'activité amylasique. Deux d'entre elles sont constituées d'isozymes d'α-amylase; la troisième pourrait être une β-amylase, bien qu'elle ne possède pas toutes les propriétés généralement attribuées à cette enzyme.

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Clearance of Concentrated Thymidine and Deoxycytidine from the Plasma of CBA Mice

Tritiated thymidine (^{3}HTdR) is commonly used as a labelled precursor of DNA both in vivo and in vitro and data is available on clearance from plasma at the concentrations used (usually $\sim 10^{-7}M$)[1,2]. At much higher concentrations (above $10^{-3}M$), TdR is used in vitro to induce synchrony of mitosis[3–5]. Phosphorylation of TdR produces large amounts of thymidine triphosphate which inhibits the cytosine diphosphate to deoxycytosine diphosphate pathway so that removal of TdR releases cells from a block of DNA synthesis[6,7]. Before attempting similar studies in vivo clearance rates of concentrated TdR were needed and results are presented here. As deoxycytidine (CdR) can bypass a TdR block in vitro[8–10] some data is also included on its clearance.

Materials and methods. Animals: CBA mice were used (F36-41), originally from Carshalton M.R.C. Laboratory Animal Centre. Animals were weighed to ± 0.1 g and in Experiment 1 the age recorded.

Inocula. TdR and CdR (Sigma) in Hank's BSS were membrane sterilised before i.p. injection. ^{3}HTdR (S.A. 5 Ci/mM) and ^{3}HCdR (S.A. 25 Ci/mM) were from Amersham and used at 5μCi/ml of inoculum.

Procedure. Blood was obtained in heparinized microhaematocrit tubes from the retroorbital sinus (ROS) under ether anaesthesia, or from cardiac puncture following cervical dislocation. The cells were spun down and two aliquots of either 0.1 ml cardiac plasma or 10 μl (Shandon microcaps) ROS plasma were taken, one for estimation of total ^{3}H activity and the other for tritiated water (THO) determination. As water is the most common ^{3}H labelled end product of ^{3}HTdR degradation, it was assumed that the difference would represent TdR, with only small quantities of intermediate products.

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