deviation of the generated ATP from availability to the permeability changes by an accelerated decay process other than the ATPase activity. This last point could also explain, at least in part, the same effect of DNP on the swelling.

From the compounds which increased the rate and degree of reduction in the absorbance of suspensions of protoplasts from *S. faecalis* metabolizing glucose, the action of dicumarol seems now to be related to its capability for increasing energy production. If the suggested increase in the intracellular concentration of $K^+$ induced by gramicidin[2] proves true, it could account for the same kind of activity of this compound on the swelling. The swelling is known to be stimulated by $K^+$[3]. In the particular case of arsenate, the disorganization of the internal structure of the cell induced by this compound on *S. faecalis*[9] seems able to explain the more pronounced decrease of the absorbance in swelling protoplasts[10,11].

Influence of various compounds on the linear regression coefficient for Pi liberation from exogenous ATP by lysed protoplast suspensions from *S. faecalis*

| | Linear regression coefficient |
|---|---|
| 2,4-Dinitrophenol $1 \times 10^{-3} M$ | 1.13 |
| Sodium azide $10 \times 10^{-3} M$ | 0.53 |
| Dicumarol $50 \times 10^{-6} M$ | 1.06 |
| Gramicidin $22 \times 10^{-6} M$ | 0.58 |
| Oligomycin $15 \times 10^{-6} M$ | 0.81 |
| Valinomycin $0.35 \times 10^{-6} M$ | 0.78 |
| Rutamycin $100 \times 10^{-6} M$ | 0.92 |

The value for the control is taken as unity. The linear regression coefficient was determined by the least squares method. An addition of Na-arsenate was followed by a heavy floculation and interference with the phosphorus assay; no results for this compound were recorded.

*Résumé.* L'activité ATPasique après l'addition d'ATP et en présence de diverses substances est étudié chez *Streptococcus faecalis*. Les effets les plus évidents ont été les effects inhibiteurs de l'acide sodique et de la gramicidine.

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[9] D. Walker and W. Forrest, J. Bact. *87*, 256 (1964).

[10] We wish to thank J. C. Maia and M. M. Freitas for help and R. L. Mann, The Lilly Research Laboratories, U.S.A. and J. C. MacDonald, Prairie Regional Laboratory, Saskatoon, Canada, for their generous gifts respectively of rutamycin and valinomycin.

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## Effect of Ecdysone on Glutamic Decarboxylase in Rat Brain

The rapid advances in the understanding of mechanisms of protein synthesis and its genetic control has made the study of the regulation of specific enzyme formation of the greatest current interest. Control of metabolism through induction and repression of the synthesis of specific enzyme proteins is now well established in microbial systems. However, it is now generally agreed that the synthesis of enzyme proteins in animal systems is mediated by hormones. Of special interest in this connection is the insect moulting hormone, ecdysone which has been demonstrated to induce DOPA decarboxylase in *Calliphora*[1,2], a key enzyme in the process of sclerotization. Previous studies[3] indicate that ecdysone may have an effect on mammalian cells also. In the present study, we have investigated the possible effects of ecdysone on the activity of glutamic decarboxylase in rat brain.

*Material and methods.* Hooded male rats (Long Evans strain) weighing approximately 150 g were used. Ecdysone was solubilized in Tween 20 and doses ranging from 0.25–5 µg/ml were injected i.p. into different batches of rats. The animals were later sacrificed by decapitation at different time intervals of 4, 12 and 24 h. Parallel controls were run under identical experimental conditions (control animals were injected with a solution of Tween 20). For each time interval (including control) 8 animals were sacrificed. Brains were removed immediately and homogenized in $0.02 M$ phosphate buffer (pH 6.4). In the presence of 1-$C^{14}$-labelled glutamic acid, the homogenate was incubated at 37 °C for 30 min in Warburg manometric flasks. The released radioactive $CO_2$ was absorbed on hyamine saturated filter paper which was later deposited in scintillation vials containing PPO-POPOP. The radioactivity was counted by means of a liquid scintillation counter (Nuclear Chicago). Further details of the method are essentially the same as described by Lupien and Hinse[4].

*Results and discussion.* As shown in the Table, there is a significant increase ($t$-test) in the activity of glutamic decarboxylase in the brains of ecdysone-treated animals as compared to control animals. However, there does not appear to be a well-defined dose-effect relation over the entire range. 24 h after the injection, the increase in enzyme activity is slightly less pronounced compared with its activity at 4 and 12 h after the injection. This could perhaps be due to inactivation or excretion of the hormone. In earlier studies, Burdette and Coda[3] have demonstrated an enhanced rate of mammalian protein synthesis by ecdysone. In our present investigation, whether the increased activity of glutamic decarboxylase represents one of the more general effects of ecdysone

[1] P. Karlson and C. E. Sekeris, Acta Endocr. *53*, 505 (1966).

[2] C. E. Sekeris, in *Mechanism of Hormone Action* (Ed. P. Karlson; Academic Press, New York 1965), p. 149.

[3] W. J. Burdette and R. L. Coda, Proc. Soc. exp. Biol. Med. *112*, 216 (1963).

[4] P. J. Lupien, C. Hinse and L. Berlinguet, Analyt. Biochem. *24*, 1 (1968).

Effect of ecdysone on glutamic decarboxylase activity in rat brain

| Dose of ecdysone (μg/100 g body weight) | h of sacrifice | Enzyme activity dpm/mg protein $M^a \pm \sigma M$ | $P^b$ |
|---|---|---|---|
| Control | 4 | 123 ± 5 | |
| | 12 | 124 ± 6 | |
| | 24 | 122 ± 8 | |
| 0.025 | 4 | 168 ± 10 | 0.01 |
| | 12 | 160 ± 20 | 0.05 |
| | 24 | 158 ± 13 | 0.01 |
| 0.1 | 4 | 156 ± 12 | 0.01 |
| | 12 | 162 ± 8 | 0.01 |
| | 24 | 148 ± 19 | 0.05 |
| 1.0 | 4 | 169 ± 6 | 0.01 |
| | 12 | 174 ± 15 | 0.01 |
| | 24 | 160 ± 21 | 0.05 |
| 5.0 | 4 | 171 ± 11 | 0.01 |
| | 12 | 169 ± 9 | 0.01 |
| | 24 | 165 ± 5 | 0.01 |

[a] $M = \sqrt{(m-M)^2/(N(N-1))}$. N, total number of determinations; m, each determination; M, mean; $\sigma$ M, standard deviation. [b] Statistical analysis shows a significant difference between the treated and control animals for each corresponding time interval.

on protein synthesis or the increased enzyme activity is the result of an allosteric effect of ecdysone as a steroid, rather than as a hormone, is not known. In fact allosteric phenomena have been reported to be quite widespread in nature and several steroids have been implicated as capable of altering catalytic activities of enzymes through this mechanism[5]. In insects, ecdysone by inducing DOPA decarboxylase, has been shown to be implicated in processes leading to hardening and tanning of the cuticle. It is, therefore, of interest to compare the effect of ecdysone on glutamic decarboxylase activity in rat brain with that of its normal mode of action in insects[6].

*Résumé.* Nous avons étudié l'effet que produisait l'ecdysone, une hormone de la mue chez les insectes, sur l'activité de l'acide glutamique decarboxylase du cerveau de rat. Nous avons observé que l'activité de cet enzyme augmentait de façon significative chez les rats traités par cette hormone.

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[5] H. G. WILLIAMS-ASHMAN, Cancer Res. *25*, 1096 (1965).

[6] Acknowledgments. This work was supported by a grant from Medical Research Council of Canada. We acknowledge with thanks the technical assistance of Mr. J. G. BERTRAND. We are indebted to Hoffmann-La Roche for providing the free samples of ecdysone.

## A Non-Dialyzable Inhibitor of Proteolytic Activity in Soluble Extracts of *Escherichia coli*

In recent years, a number of polypeptide inhibitors of proteolytic activity have been isolated from mammalian pancreas[1,2], blood[3-5], and colostrum[6]. Inhibitors have also been observed in brain, ovary, breast, and bowel[7-10].

In view of the considerable quantities of trypsinogen and chymotrypsinogen released into the intestine from the pancreas[11], and in view of the survival of *Escherichia coli* in the intestinal tract in the presence of large quantities of such secreted proteinases, *E. coli* was selected and examined for its possible capacity to resist digestion by trypsin (T) and chymotrypsin (CT).

This communication reports the presence of a non-dialyzable, heat-stable inhibitor of tryptic and chymotryptic activity in the 90,000 *g* supernatant fraction of sonicated *E. coli* cells.

Five grams of *E. coli* strain ECB-6504, lyophilized (Worthington Biochemical Corporation, Freehold, New Jersey) were suspended in 120 ml of 0.1 *M* phosphate buffer, pH 7.0, and sonicated for 12 min. The suspension was then centrifuged at 90,000 *g* for 1 h to obtain a soluble 90,000 *g* supernatant fraction, which was stored frozen in aliquots until used. Assays for proteolytic and antiproteolytic activity were performed essentially according to the procedure of KUNITZ[12], using 0.5% casein as a substrate and twice crystallized T (Nutritional Biochemical Corporation, Cleveland, Ohio) and thrice crystallized CT (Worthington Biochemical Corporation, Freehold, New Jersey) as proteases. Protein nitrogens were performed according to the procedure of LOWRY et al.[13].

The inhibition of the tryptic and chymotryptic hydrolysis of casein by the 90,000 *g* supernatant fraction derived from sonicated *E. coli* cells is given in the Figure. Analogous curves have been obtained with extracts of mammalian normal and pathological breast, bowel, and with extracts of human glioma, adenocarcinoma of the ovary[10], and with astrocytoma extracts[8]. The soluble inhibitor is non-dialyzable (Table I) and is heat-stable (Table II). It can be sedimented with $(NH_4)_2SO_4$, indicating that the inhibitor is considerably larger than the pancreatic trypsin inhibitors isolated by NORTHROP and KUNITZ[1], and by KAZAL et al.[2] The bacterial inhibitor bears a

[1] M. KUNITZ and J. H. NORTHROP, J. gen. Physiol. *19*, 991 (1936).
[2] L. A. KAZAL, D. S. SPICER and R. A. BRAHINSKY, J. Am. chem. Soc. *70*, 3036 (1948).
[3] H. E. SCHULTZE, K. HEIDE and H. HAUPT, Klin. Wschr. *40*, 427 (1962).
[4] K. HEIDE, N. HEIMBURGER and H. HAUPT, Clinica chim. Acta *11*, 82 (1965).
[5] H. F. BUNDY and J. H. MEHL, J. biol. Chem. *234*, 1124 (1959).
[6] M. LASKOWSKI JR. and M. LASKOWSKI, J. biol. Chem. *190*, 563 (1951).
[7] A. S. BRECHER and N. M. QUINN, Biochem. J. *102*, 120 (1967).
[8] A. S. BRECHER and N. M. QUINN, J. Neurochem. *14*, 203 (1967).
[9] A. S. BRECHER, B. B. OLIPHANT and V. P. WASILAUSKAS JR., Archs int. Physiol. Biochim. *76*, 287 (1968).
[10] V. P. WASILAUSKAS JR. and A. S. BRECHER, submitted.
[11] A. CANTAROW and B. SCHEPARTZ, in *Biochemistry*, 4th edn (W. B. Saunders Company, Philadelphia 1967), p. 261.
[12] M. KUNITZ, J. gen. Physiol. *30*, 291 (1947).
[13] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).