can be adduced from the ratio of *erythro*- to *threo*-isoleucine. Preponderance of either L-Val[2]- or D-Val[4]-angolide must cause this ratio to depart from unity. Angolide fractions were isolated from *P. sacchari* grown in the presence of L-valine at 8 different concentrations in the range 2.5–20 µg/ml. In acid hydrolysates of these fractions the mean Ile:*a*Ile ratio was 1.03, with a range 0.97–1.18. Previous work with the 2 epimeric isoleucine derivatives produced by mild hydrazinolysis of angolide has shown that little if any epimerization of isoleucines occurs during acid hydrolysis[3]. We therefore conclude that the ratio of *erythro*- to *threo*-isoleucine residues in the angolide fractions is close to unity. It follows that exogenous L-valine promotes the biosynthesis of L-Val[2]- and D-Val[4]-angolide in equal amount.

This conclusion has interesting biochemical implications. Formation of the 2 stereoisomers in equal amount is plausibly explained only if both arise from a common precursor. This precursor must contain one residue of valine and one of isoleucine, with the added proviso that both residues are biochemically equivalent, so that which one undergoes inversion is entirely a matter of chance. This supposes a high degree of symmetry in the precursor molecule, and excludes from consideration any open-chain structure, which must necessarily contain 2 aminoacid residues in non-equivalent environments.

We therefore propose that biosynthesis of the 'monovaline angolides', and hence by implication of angolide itself, proceeds by way of an all-L-cyclotetradepsipeptide precursor, the single D-residue being introduced by random inversion of one or other of the two environmentally and stereochemically equivalent L-aminoacid residues. It seems to us likely that a similar mechanism may operate in the biosynthesis of other microbial peptides, such as sporidesmolide I, valinomycin, gramicidin S, and fungisporin, for which essentially symmetrical, all-L cyclic precursors may plausibly be formulated. These in turn may be special cases of a general mechanism, by which D-aminoacid residues in microbial peptides originate by inversion of previously incorporated L-residues. An enzyme-catalysed dehydrogenation/hydrogenation mechanism by which such inversion may occur has recently been proposed[6].

A detailed account of this work will be published elsewhere.

Zusammenfassung. Ein Gemisch von Angolid und seinen drei valinhaltigen Homologen aus L-valinhaltiger Kultur von *Pithomyces sacchari* enthielt gleiche Mengen zweier Isomere mit nur einem Valinrest, was wahrscheinlich macht, dass Angolid durch Umkehrung eines L-Rests aus einem zyklisch-symmetrischen Vorläufer entsteht.

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[6] B. N. BYCROFT, Nature, Lond. *224*, 595 (1969).

[7] This investigation was supported by a grant No. MA 2480 from the Medical Research Council of Canada. We are most grateful to Dr. J. A. VERPOORTE for the aminoacid analyses.

Xanthine Dehydrogenase in the Biosynthesis of the Eye Pterin Pigments of *Drosophila melanogaster*

It has been established in previous studies[1,2] that the wild type of *Drosophila melanogaster*, grown on the KELLER and GLASSMAN[3] culture medium, with 4-hydroxypyrazole (3,4-D) pyrimidine (or HPP) at 0.08 g/100 ml concentration, originates phenocopies of the 'maroon-like' (MAL) mutant. It is known that this mutant lacks the xantine deydrogenase enzyme (XDH), and therefore in the males there is no isoxanthopterin in any measurable amount, while there is an increase of its precursor 2-amino-4-hydroxypterin[3]. Furthermore, the MAL mutant shows a remarkable decrease of the amount of the eye pigment drosopterin.

Similar experiments performed with HPP on the 'sepia' (SE) mutant[4] have shown the disappearance of the isoxanthopterin in the males, an increase of the level of 2-amino-4-hydroxypterin and a decrease of sepiapterin which is the eye pigment of this mutant. This decrease reaches a certain level that cannot be lowered, even if the HPP concentration in the culture medium is increased. The effects of the addition of HPP to the wild type and to the SE mutant are obviously similar. While the wild type treated with HPP originates the phenocopy of the MAL mutant, the SE mutant treated does not have a corresponding biochemical phenotype in any *Drosophila melanogaster* single mutant.

We have therefore tried to isolate a mutant strain of which the SE mutant treated with HPP is the phenocopy. By crossing the SE with the MAL mutant, it has been possible to isolate a double mutant strain which, when analyzed by spectrophotometry and chromatography, shows the same biochemical phenotype of the SE treated with HPP. In fact, because of the SE mutation, sepiapterin is the eye pigment while, because of the MAL mutation, there is no XDH enzyme and accordingly no isoxanthopterin. Since it is impossible to distinguish by examination between the SE and the double mutant,

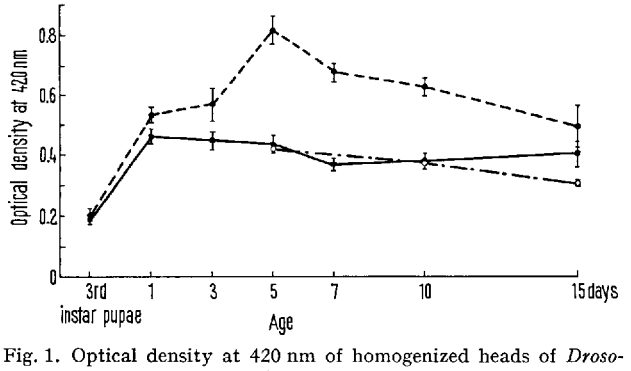

Fig. 1. Optical density at 420 nm of homogenized heads of *Drosophila melanogaster* males SE (----), MAL/SE (——) and SE treated with HPP (-·-·-). Means of 5 experiments for each different age. Vertical lines indicate S.E.

[1] P. BONI, B. DE LERMA and G. PARISI, Experientia *23*, 186 (1967).
[2] B. DE LERMA, P. BONI and G. PARISI, Boll. Soc. ital. Biol. Sper. *42*, 1764 (1966).
[3] E. C. KELLER and E. GLASSMAN, Nature, Lond. *208*, 202 (1965).
[4] P. BONI and G. PARISI, Experientia *23*, 1020 (1967).

a second *X* chromosome marker has been introduced, that is the 'crossveinless' mutation which modifies the alar phenotype only. Spectrophotometric measurements (Figures 1 and 2) have been carried out on both sexes at different stages of development to compare the SE mutant with the SE HPP[5] treated, and with the MAL/SE mutant strain. For each sex and for each of the 7 ages examined (3rd instar pupae and 1st, 3rd, 5th, 7th, 10th, 15th day from the emergence) 5 groups of 40 animals were weighed, boiled for 1 min in distilled water and beheaded. The heads were homogenized with 2.5 ml of EtOH/H_2O (30:70) brought to pH 2 with HCl, filtered through a millipore membrane (AP 200 1300) and examined by a Beckman DU 2 spectrophotometer in the range 200–500 nm. Among the absorption peaks we have chosen the one at 420 nm which is related to sepiapterin. The chromatographic analysis has been carried out with the n-propanol: NH_4OH 5% (2:1) system on Whatman No. 1 filter paper using a Mineral-light nm 365 as detecting lamp. Migration time was about 24 h. It was possible to observe the disappearance of the isoxanthopterin in the SE males treated with HPP, an increase of 2-amino-4-hydroxypterin, and a decrease of sepiapterin that reaches the same level as in the double mutant.

These results and the previous studies on the wild type suggest that the decrease of the red and the yellow pigments (drosopterin and sepiapterin respectively) is caused by the inhibition of the XDH enzyme. Since this enzyme is known to convert 2-amino-4-hydroxypterin into isoxanthopterin in the pterin metabolism, it seems clear that the disappearance of isoxanthopterin should be related to the decrease of the red and yellow pigments. In the normal males, the isoxanthopterin is partly stored in some organs (testicles, etc.) and partly converted into the eye pigment. The females, lacking the main receptor organs, can utilize only as much isoxanthopterin as is necessary to get the maximum amount of the eye pigment, the excess being eliminated. The inhibition of XDH obviously effects only the amount of the eye pigment which is synthesized through the isoxanthopterin, while the rest, coming from the normal pterin metabolism, cannot be inhibited.

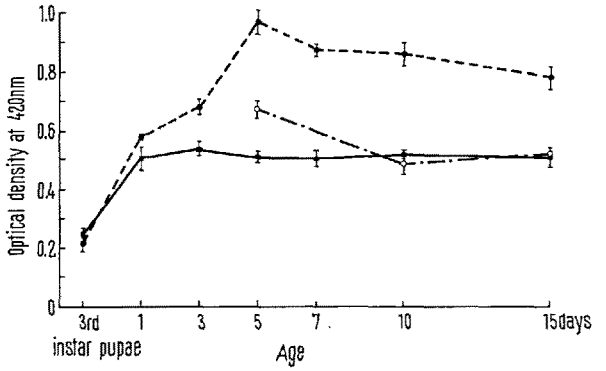

Fig. 2. Optical density at 420 nm of homogenized heads of *Drosophila melanogaster* females SE (---), MAL/SE (——) and SE treated with HPP (-·-·-). Means of 5 experiments for each different age. Vertical lines indicate the S.E.

Riassunto. Il trattamento con HPP di mutanti SE di *Drosophila melanogaster* ha dato la fenocopia biochimica del doppio mutante MAL/SE (diminuzione della sepiapterina, aumento delle biopterine e, nei maschi, assenza della isoxantopterina con accumulo del precursore 2-amino-4-idrossipterina). Si discute il ruolo dell'enzima XDH nel metabolismo in istudio.

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[5] We are indebted to 'Wellcome Italia S.p.A.', Rome, for a generous gift of HPP (Allopurinol®).

Mise en évidence d'une activité mannosyl-transferase dans les cellules embryonnaires de poulet et influence de la différenciation cellulaire

Dans l'étude de la biosynthèse des glycoprotéines, le problème actuel consiste à localiser, à isoler et à caractériser les systèmes enzymatiques responsables du transfert et de la fixation des molécules glucidiques sur la chaîne polysaccharidique. Parmi les systèmes cellulaires disponibles, les cellules embryonnaires de poulet, du fait de leur activité métabolique intense, sont un matériel de choix, d'autant plus qu'elles se prêtent remarquablement au fractionnement cellulaire[1,2].

Une étude préliminaire in vivo nous a montré que le mannose s'incorporait dans les macromolécules glycoprotéiniques des cellules d'embryon de poulet. Nous avons alors essayé d'élaborer un système acellulaire, susceptible d'incorporer in vitro ce précurseur glucidique à partir de sa forme coenzymatique active: le GDP-mannose.

Un fractionnement cellulaire limité, en saccharose 0,25*M*, conduit à 3 fractions successives. Après broyage de l'embryon à froid en tampon *Tris*-HCl $5 \times 10^{-2} M$, pH 7, 0,25*M* en saccharose, par 2 fois 10 passages successifs dans un homogénéiseur de type Potter-Elvehjem, une première centrifugation pendant 15 min à 15000*g* permet d'éliminer les cellules non broyées, les noyaux et les mitochondries. Les 4/5 du surnageant post-mitochondrial ainsi obtenu sont centrifugés pendant 1 h à 195000*g*; un culot de microsomes est ainsi séparé de la phase cytoplasmique non particulaire. Chacune de ces fractions subcellulaires est incubée avec le précurseur glucidique pendant 1 h à 37 °C. Les macromolécules sont précipitées par une concentration finale de 10% en acide trichloracétique sur filtre Whatman (Glass paper, type GF/B, diamètre 2,5 cm) et lavées sur filtre par un mélange méthylal-méthanol (4/1). Des témoins sont traités dans les mêmes conditions, au temps 0. La radioactivité, rapportée à la teneur en protéines dosées par la méthode

[1] R. Got, P. Louisot, J. Frot-Coutaz et L. Colobert, Biochim. biophys. Acta *157*, 599 (1968).

[2] J. Frot-Coutaz, P. Louisot, R. Got et L. Colobert, Experientia *24*, 1206 (1968).