crease in order of tropoelastin can be expected to be compensated for by a decrease in free electrostatic energy of the system. The biological significance of the present observation rests in that proteoglycans are present in the ground substance in which elastin fibres are deposited. This suggests that the type of interaction described above can take place also during elastogenesis in vivo.

Depending on reaction conditions, 2 different types of structure have been observed: a) fibrils, b) segments. In this a certain analogy can be seen to the other connective tissue protein, collagen, which can be prepared either in fibrillar form or in the form of segments. The detailed investigation of the respective reaction conditions is now under way.

Some Metabolic Disorders Affecting the Carotenoid-Linked Haemolymph Proteins in *Rhynchosciara americana* (Diptera, Sciaridae)

W. R. TERRA[1], A. G. DE BIANCHI[1], M. PAES DE MELLO and R. BASILE[2]

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 20780, São Paulo (Brasil); and Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo C.P. 11461, São Paulo (Brasil),
25 September 1975.

Summary. Disorders in the carotenoid metabolism are proposed to explain the absence of the yellow and violet or only the violet carotenoid-linked proteins from the haemolymph of some *R. americana* larvae. The lack of only the yellow chromoprotein is considered to be due to a failure in the biosynthesis of its apoprotein.

The study of metabolic disorders affecting carotenoid-linked proteins can provide useful data on the metabolism of carotenoids by the animal under study, as well as on the assembly of these proteins. On the other hand, following the physiological and/or morphological changes associated with those metabolic disorders, one may have an insight into the function of the carotenoid-linked proteins. Findings in this field are desirable, since the only firmly established function of those proteins in the animal is to provide protection against photodynamic action, although they certainly have other functions[3].

Rhynchosciara americana have 3 pigments in the haemolymph[4] from which one has an unknown nature and the two other are carotenoid-linked proteins[5]. BASILE et al.[4] found in *R. angelae* (*R. americana*)[6] larvae with only one pigment in the haemolymph, and they were able to show that it was a consequence of a sex-linked mutation. In this paper we describe some other metabolic errors affecting the haemolymph pigments of *R. americana*. Although the animals did not survive for a genetic analysis be accomplished, sufficient data were collected for the establishment of a tentative explanatory model of the metabolic disorders from a biochemical point of view.

Figure 1 shows electrophoretograms of the pigments found in normal larvae and in larvae showing metabolic disorders which we will call 'mutants'. LI-mutant corresponds to that one previously described[4] and has only the lemon-coloured pigment which has an unknown nature[5]. This mutant, therefore, does not have any protein-bound carotenoid but, except for its color, it is similar to the wildtype. The LII-mutant does not have the violet chromoprotein in the haemolymph. No changes

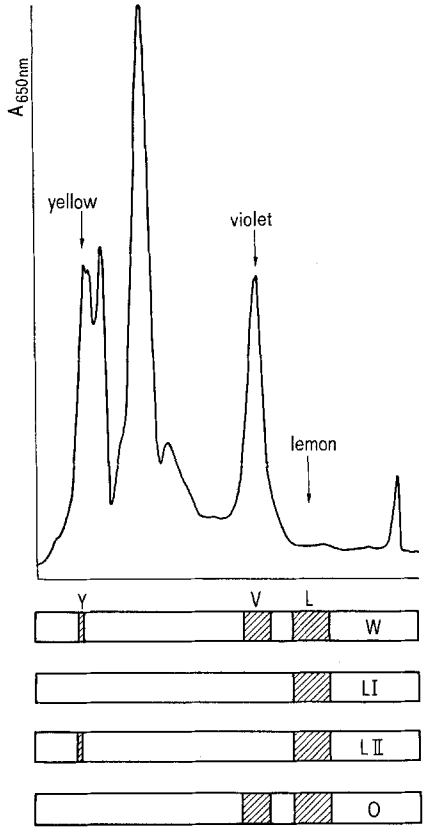

Fig. 1. Densitometric scan of a 7% acrylamide gel electrophoretogram of protein[7] from the heamolymph of wild-type larvae. The diagram below the densitogram shows the migration of the lemon (L), violet (V) and yellow (Y) pigments displayed by the haemolymph of the wild-type larvae (w) and of the mutants lemon I (LI), lemon II (LII) and orange (O).

[1] Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, C.P. 20780, São Paulo, Brasil.

[2] Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisas (CNPq). W.R.T. and A.G. de B. are indebted to Prof. F. J. S. LARA for advice, encouragement and for laboratory facilities. We thank Mr. A. MALAVASI for collecting larvae in nature, Mrs. R. M. ZANELATO for larvae culture and Miss I. L. JORGE for typing.

[3] N. KRINSKY, in *Carotenoids* (Eds. O. ISLER, Birkhäuser Verlag, Basel 1971), p. 669.

[4] R. BASILE, S. A. TOLEDO FILHO, A. B. DA CUNHA, J. S. MORGANTE and J. MARQUES, Revta bras. Biol. *30*, 471 (1970).

[5] W. R. TERRA, A. G. DE BIANCHI and F. J. S. LARA, Comp. Biochem. Physiol. *47B*, 117 (1974).

[6] M. E. BREUER, Archos Zool. Est. S. Paulo, *17*, 167 (1969).

[7] B. J. DAVIS, Ann. N.Y. Acad. Sci. *121*, 404 (1964).

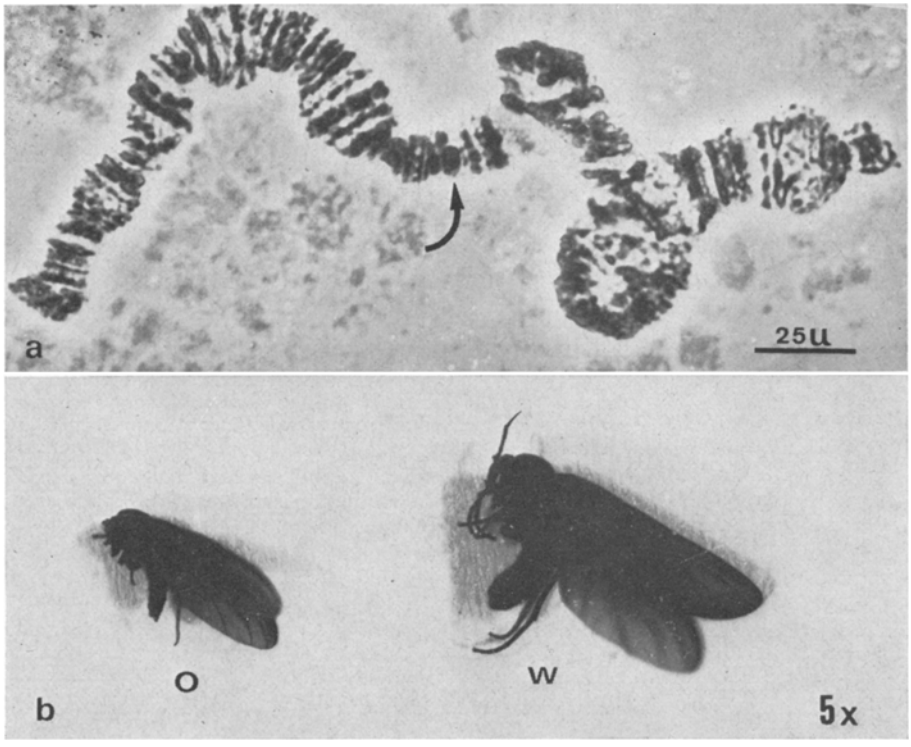

Fig. 2. a) Chromosome A from salivary gland of the LII-mutant. Arrow shows an heterozygote band in section 10[8]. Phase contrast; b) General aspect of a female wild adult fly (w) and O-mutant (O).

in morphological or physiological aspects had been detected in the larvae or adults, except for the salivary gland chromosomes. As is shown in Figure 2, the LII-mutant shows a heterozygote band in chromosome A. Heterozygote bands in *Rhynchosciara americana* have never been described before. Nevertheless, the implication of this chromosome change with the metabolic disorder observed is only speculative, since a genetic analysis was not carried out. A densitometric scan of the LI- and LII-mutants haemolymph proteins electrophoretogram is similar to that shown in Figure 1. These results suggest that the metabolic block resulting in the absence of the pigment must affect the carotenoid metabolism and not the apoprotein biosynthesis.

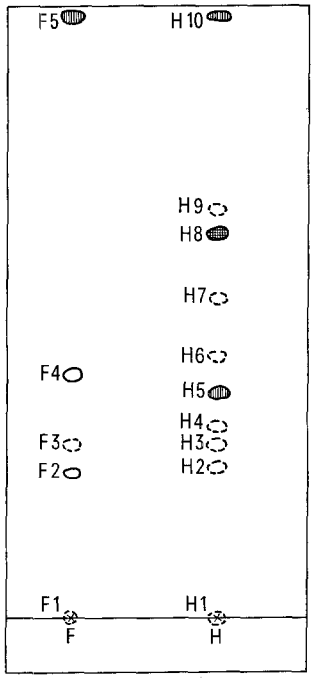

Fig. 3. Thin-layer chromatography of the carotenoids from the haemolymph (H) and food (F) of *R. americana* larvae. The samples were run in Silica gel G (Merck) plates employing 25% acetone in hexane. The shading of the spots is proportional to the amount of color.

The O-mutant does not show the yellow chromoprotein in the haemolymph. The mutant larvae have the same length but are significantly narrower than the wild type larvae from the same laying group. The difference between wild and mutant animals are the most remarkable in the imagoes. Mutant adult flies weighed 2.6 $\pm$ 1.0 mg/fly (50 determinations) while wild adult flies weighed 5.2 $\pm$ 1.0 mg/fly (50 determinations). Their sizes were also considerably different (Figure 2). Unfortunately we have no data on the presence or absence of the apoprotein of the yellow chromoprotein in those mutants.

In order to identify which carotenoids are synthesized by *R. americana*, we extracted samples of *Rhynchosciara* food[9] and of wild haemolymph with chloroform-methanol (2:1, v/v). The extracts were filtered, evaporated, saponified according to HARASHIMA[10] and finally spotted in Silica gel G plates. From the three main carotenoids present in *Rhynchosciara americana* haemolymph (H5, H8 and H10; see Figure 3), only H10 is also present in the food (F5). Since H10 is β-carotene[11], it is possible that this carotenoid absorbed from the diet is oxidized by the larvae resulting in H8 and H5, as occurs in other insects studied[12,13]. These results, and the findings of TERRA and DE BIANCHI[11] that the chromophore of the yellow

[8] M. E. BREUER, Revta bras. Biol. *27*, 105 (1967).
[9] F. J. S. LARA, H. TAMAKI and C. PAVAN, Am. Nat. *99*, 189 (1965).
[10] K. HARASHIMA, Int. J. Biochem. *1*, 523 (1970).
[11] W. R. TERRA and A. G. DE BIANCHI, in preparation.
[12] F. LEUENBERGER and H. THOMMEN, J. Insect Physiol. *16*, 1855 (1970).
[13] A. VEERMAN, Comp. Biochem. Physiol. *36*, 749 (1970).

chromoprotein is mainly β-carotene and echinenone, while that of the violet chromoprotein is chiefly echinenone and another ketocarotenoid, suggest a scheme of the metabolism of the carotenoids in *R. americana* as represented in Figure 4.

Basing on Figure 4, it is possible to propose a molecular interpretation of the metabolic disorders found in *R. americana*. The LI-mutant must be a consequence of failure in step I, which obviously results in a complete absence of β-carotene and its derivatives in the larvae.

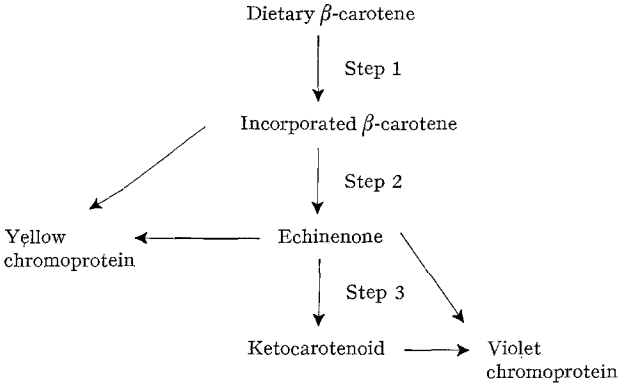

Fig. 4. Possible pathways in the metabolism of carotenoids in *R. americana*.

The absence of the violet pigment in the LII-mutant should be a consequence of failure in the step 2 or in the step 3. The fact that the yellow chromoprotein seems to be normal in the LII-mutant does not discount the possibility of the block being in step 2. It is possible that the yellow chromoprotein carries only β-carotene in the LII-mutant, since that protein is a lipoprotein[14] and the specificity of binding of carotenoids to lipoproteins is not absolute in some known cases[15].

The O-mutant is hardly a consequence of a disorder in the metabolism of carotenoids since the violet chromoprotein is present in the haemolymph fo those animals. The disorder must be due to some failure in the synthesis of the apoprotein. The absence of the yellow chromoprotein, which is the most important lipoprotein in *R. americana* haemolymph[14], must affect the lipid metabolism of the larvae in which it is lacking. The involvement of haemolymph yellow lipoprotein in the lipid transport has been demonstrated in some insects[16,17]. A serious impairment in the metabolism of lipids could cause the O-mutant larvae to be narrower and the O-mutant imagoes to be smaller than the wild type ones.

[14] A. G. DE BIANCHI and W. R. TERRA, J. Insect Physiol., in press.
[15] D. F. CHEESMAN, W. L. LEE and P. F. ZAGALSKY, Biol. Rev. *42*, 131 (1967).
[16] H. CHINO, S. MURAKAMI and K. HARASHIMA, Biochim. biophys. Acta *176*, 1 (1969).
[17] Y. PELED and A. TIETZ, Insect Biochem. *5*, 61 (1975).

Caractères différentiels de la 4-aminobutyrate-2-cétoglutarate transaminase ($GABA_T$) intra- et extra-synaptosomale de cerveau de porc

Differential Properties of Two Molecular Forms of 4-Aminobutyrate-2-Ketoglutarate Transaminase ($GABA_T$) from Pig Brain

M. TARDY, B. ROLLAND, C. FAGES et P. GONNARD

Département de Biochimie, C. H. U. Henri Mondor, 51, Avenue de Lattre de Tassigny, F-94010 Créteil Cedex (France),
20 octobre 1975.

Summary. The two forms isolated exhibit some differences concerning their physicochemical and functional properties. They are identical with the previously purified molecular forms, $GABA_T$ I and $GABA_T$ II, separated by DEAE cellulose chromatography.

La destinée de l'acide γ-aminobutyrique (GABA) dans le système nerveux central, après sa libération dans le compartiment intersynaptique est encore mal connue. Sa dégradation par transamination avec l'acide α cétoglutarique sous l'influence de la GABA transaminase (E.C. 2.6.1.19) ou $GABA_T$ conduit à la formation de semialdéhyde succinique ultérieurement oxydé en acide succinique, constituant du cycle de Krebs.

Nous avons purifié la $GABA_T$ à partir de cerveau de porc et mis en évidence l'existence de deux formes moléculaires par chromatographie sur DEAE cellulose et étudié les caractères différentiels de ces deux formes[1], ($GABA_T$ I et II). La deuxième forme: $GABA_T$ II est étroitement associée à une activité aspartate aminotransférasique soluble.

Des études récentes on considéré certains aspects du rôle de la $GABA_T$[2,3–6], mais l'importance de son rôle dans le mécanisme d'inactivation du GABA, in vivo, n'est pas exactement précisé. L'existence d'une forme intrasynaptosomale et extrasynaptosomale de l'enzyme nous a amené à envisager une relation entre ces deux formes et celles que nous avons isolées au cours de notre purification.

Matériel et méthodes. a) Préparation des synaptosomes et des mitochondries. Les cerveaux de porc sont prélevés aux abattoirs immédiatement après la décapitation de l'animal, débarrassés des méninges et de la majeure partie de la matière blanche, homogénéisés en solution saccharose 0,35 *M* à l'aide d'un appareil de Potter et Elvejhem verre-teflon (clearance 0,25 mm).

[1] M. BLOCH-TARDY, B. ROLLAND, P. GONNARD, Biochimie *56*, 823 (1974).
[2] S. C. CHENG, *Handbook of Neurochemistry* (Plenum Press, New York 1971), vol. 5, p. 283.
[3] J. C. HYDE and N. ROBINSON, Brain Res. *82*, 109 (1974).
[4] L. SALGANICOFF, E. DE ROBERTIS, J. Neurochem. *12*, 287 (1965).
[5] A. WAKSMAN and M. BLOCH, J. Neurochem. *15*, 99 (1968).
[6] A. WAKSMAN, M. K. RUBINSTEIN, K. KURIYAMA and E. ROBERTS, J. Neurochem. *15*, 351 (1968).