intercept of the abscissa is $-1/K_d$. In the presence of a competitive ligand the plot has the same intercept on the ordinate axis, but the slope increases by a factor equal to $(1 + [I]/K_i)$ where [I] is the concentration and K_i the dissociation constant of the competitive ligand[12]. The results reported in the Figure indicate a molarity of binding sites of 43×10^{-9}. The K_d of the complex of methyl-γ-amanitin with RNA-polymerase II was $3.6 \times 10^{-9} M$. The presence of 100 nM α-amanitin inhibited in a competitive way the binding of ^{14}C-methyl-γ-amanitin and the K_i for α-amanitin, calculated from the ratio between the two slopes of the Figure, was $3.2 \times 10^{-9} M$. These results indicate that the affinities of amanitins for RNA-polymerase II are very high, the dissociation constants being of the same order as those reported for the strongest binding between haptens and their specific antibodies (see[13].) The Figure shows that the presence of 100 nM methyl-aldoamanitin does not interfere with the binding of ^{14}C-methyl-γ-amanitin. The non-toxic methyl-aldoamanitin lacks the γ-OH group present in the isoleucine side chain of both methyl-γ-amanitin and α-amanitin[14]. It does not inhibit RNA-polymerase[15] and our results indicate that it does not bind to the enzyme[16].

Riassunto. Sono state calcolate le costanti di dissociazione dei complessi fra la RNA-polimerasi II e la metil-γ-amanitina, la α-amanitina e la metil-aldoamanitina. La K_d è risultata essere $3.6 \times 10^{-9} M$ per la metil-γ-amanitina e $3.2 \times 10^{-9} M$ per la α-amanitina. La metil-aldoamanitina non si lega all'enzima.

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Free Amino-Acids Composition of Larvae and Pupae of *Euproctis fraterna* Moore (Lepidoptera: Lymantriidae) Reared on the Leaves of Different Host Plants

The *Euproctis fraterna* Moore is commonly known as castor hairy caterpillar. The larvae are polyphagous and attack a variety of host plants e.g., plum, apple, mango, apricot, peach, pear, grapevines, citrus, rose, castor and cotton[1]. Recently, some attention has been focussed on the nutritional studies of insects and insect-plant relationship which partly reflect the capacity of the insect to utilize the various constituents of hosts including amino acids and proteins[2-4]. Since very little is known about the physiology of *Euproctis fraterna*, the present investigations were undertaken to study the changes in free amino acids composition of larvae and pupae reared on the leaves of different host plants.

Material and methods. The larvae were reared on the leaves of 3 host plants viz., castor bean (*Ricinus communis*), ber (*Ziziphus mauritiana*) and crab-apple (*Malus hupehensis*) under the laboratory conditions. Before crushing the larvae in 80% alcohol for amino acid analysis, they were starved for 24 h. The larvae were analyzed at the 5th or 6th instar, whereas the pupae were taken at the full grown stage. The larvae and pupae were washed with distilled water, then dried on blotting paper and weighed. 20 insects were taken for analysis and there were three replicates in each treatment. The alcoholic extract of the insect was made and the free amino acids were analyzed by the procedure of PAL and LALORAYA[5] employing two dimensional ascending paper chromatography. Whatman No. 1 filter paper was used in the present investigation. Phenol (80% in double distilled water) saturated with 0.5% ammonia solution was used as the first running solvent, whereas *n*-butanol, acetic acid and water (4:1:5) was the second solvent. The dried chromatograms were sprayed with 0.1% ninhydrin and the various amino acid spots were developed by heating the chromatograms in an oven at 80 °C for 30 min. The amino acids were identified on the basis of their Rf values and colour reactions in different solvent systems. The quantitative estimation of amino acids was done by the colorimetric method. The data are expressed in terms of glycine.

Results. The Figure shows the growth rates of larvae of *Euproctis fraterna* reared on the leaves of different host plants. It is clear that larvae on the castor leaves comparatively develop faster than on ber and crab-apple. Furthermore, the weights of the larvae and pupae on castor leaves are more than on ber and crab-apple. This may be due to host preference of this pest to castor as compared to ber and crab-apple.

The alcoholic extracts of larvae and pupae of *Euproctis fraterna* contain about 18 amino acids (Table). These are leucines and phenylalanine, valine, γ-aminobutyric acid, tyrosine, proline, α-alanine, glutamic acid, threonine, arginine, aspartic acid, glycine and serine, asparagine, glutamine, histidine and lysine and 2 unidentified spots numbered 'x' and 'y'. It is clear from the Table that the amino acids composition of the larvae and pupae reared on the leaves of different host plants, shows marked differences in their relative concentration. It has been observed that larvae reared on castor bean leaves show, in general, more amino acids as compared to ber and crab-apple. Leucines and phenylalanine, valine, proline, glutamic acid, glycine and serine show highest concentration in larvae reared on castor bean leaves; whereas γ-aminobutyric acid and threonine show maximum concentration in larvae reared on crab-apple. However, tyrosine, α-alanine, arginine, aspartic acid, asparagine, glutamic acid, histidine and lysine

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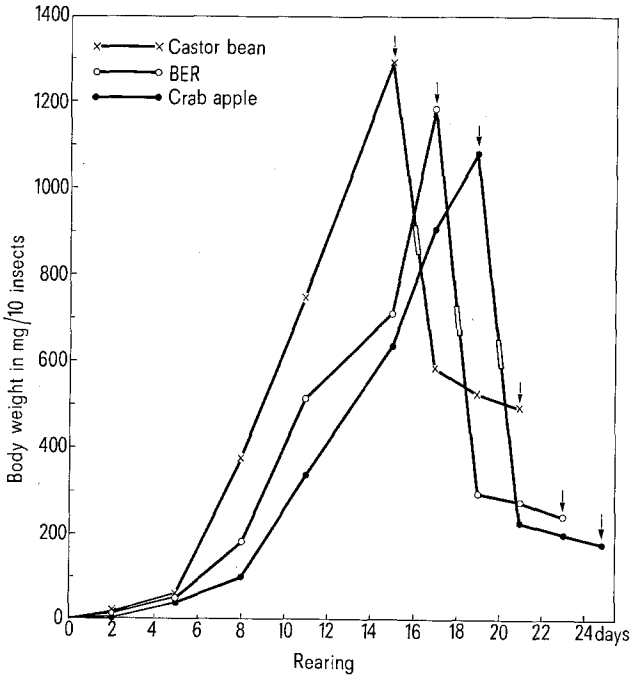
Free amino-acids composition of the larvae and pupae of *Euproctis fraterna* Moore reared on the leaves of different host plants

Sr. No.	Amino-acids	Castor		Crab-apple		Ber	
		Larvae	Pupae	Larvae	Pupae	Larvae	Pupae
1.	Leucines and phenylalanine	233	366	133	500	133	400
2.	Valine	233	400	Tr.	266	66	400
3.	γ-aminobutyric acid	66	—	200	—	66	—
4.	Tyrosine	66	233	Tr.	66	33	33
5.	Proline	700	433	266	500	266	566
6.	α-alanine	Tr.	100	Tr.	66	Tr.	33
7.	Glutamic acid	733	266	400	633	266	266
8.	Threonine	Tr.	366	166	100	—	66
9.	Arginine	66	233	66	300	133	400
10.	Aspartic acid	66	100	166	Tr.	66	66
11.	Glycine and serine	966	333	333	733	633	333
12.	Asparagine	233	233	233	200	233	200
13.	Glutamine	300	200	133	266	200	100
14.	Histidine and lysine	300	233	66	300	100	333
15.	Unidentified 'X'	Tr.	—	Tr.	—	33	—
16.	Unidentified 'Y'	Tr.	Tr.	Tr.	66	33	66
Total minus 'Tr.'		3962	3493	2162	3996	2261	3262

The values are based on the average of 6 chromatograms. The variations between different chromatograms of the same insect stage were insignificant. Data expressed as γg amino acid/g body weight of insect.

show little or no change among larvae reared on the leaves of different host plants.

In general, amino acids show an increase from larval to pupal stage, although some amino acids show decrease in their content. It is remarkable that γ-aminobutyric acid is entirely absent in the pupal stage, though present in larvae reared on the leaves of all 3 host plants. The concentration of leucine and phenylalanine, glutamic acid, glutamine, glycine and serine are highest in pupae reared on crab-apple leaves; whereas valine, tyrosine, α-alanine, aspartic acid and threonine show maximum concentration when they were reared on castor bean leaves. However, arginine, histidine and lysine show highest concentration in pupae reared on ber leaves.



Relative body weight changes in *Euproctis fraterna* reared on the leaves of different host plants. –□– in the curves indicate the transformation from the larval to pupal stage. ↓ shows the insect stage taken for amino acid analyses.

Discussion. The earlier studies on other lepidopterous larvae have shown the presence of α-alanine in the blood of *Prodenia eridania* and *Galleria mellonella*[6]; cysteic acid and taurine reported in *Agrotis ypsilon, Heliothis virescens* and *Estigmena acraea*[7]; cysteic acid in *Phlegethontius quinquemaculatus, Malacosoma americana* and *Arachips cerasivorana*[8]; homoserine, cyteine and methionine in *Eublemma amabilis*[9]; whereas these amino acids have not been detected in the body extract of larvae and pupae of *Euproctis fraterna* in the present investigation. DANG and DOHAREY[3] studied the variations in the amino acid compositions of the larvae and pupae of *Chilo zonellus* and have shown that free proline, tyrosine and aspartic acid were absent in the larval stage but these amino acids make their appearance in the pupal stage. It is interesting to mention that in our present study γ-aminobutyric acid, which is present in the larval stage, completely disappears in the pupal stage. This may mean that amino acid has been utilized in the transformation of larvae to pupae. It has also been shown that amino acids in general are more concentrated in the pupal stage, which may be due to increased breakdown of proteins[10].

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Zusammenfassung. Der Gehalt an freien Aminosäuren von auf Rizinus-, Zizyphus- und Holzapfelblättern ernährten Larven und deren Puppen von *Euproctis fraterna* wurde vergleichend untersucht. Zwischen den beiden Stadien werden massgebende Unterschiede festgestellt. Während die Gesamtmenge der Aminosäuren im Larven- und Puppenstadium der mit Rizinus ernährten Insekten ähnlich war, wurden in den Puppen anders ernährter Tiere bedeutend mehr Aminosäuren beobachtet als in Larven. γ-Aminobuttersäure kam nur im Larvenstadium vor.

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Étude de l'activité in vitro de la glycogène phosphorylase de la Carpe (*Cyprinus carpio* L.)

La dégradation enzymatique du glycogène a fait l'objet de très nombreux travaux mettant en évidence le processus d'activation de la phosphorylase hépatique ou musculaire et l'existence de deux formes a et b de cette enzyme, respectivement active et inactive mais activable par le 5'AMP[1-3].

Relativement peu d'études ont été faites sur ce sujet en dehors des Mammifères; citons toutefois des recherches récentes chez *Tetrahymena pyriformis*[4] et sur la phosphorylase musculaire de la Roussette[5] et du Brochet[6].

Dans le cadre d'une étude du métabolisme glucidique chez la Carpe, nous avons observé des modifications importantes des taux de glycogène tissulaire en fonction de facteurs saisonniers, nutritionnels ou endocriniens[7,8]. Nous nous sommes alors proposés d'évaluer l'activité des enzymes intervenant dans la mobilisation du glycogène hépatique, cardiaque et musculaire.

Matériel et méthodes. Nos essais ont porté sur un total de 52 Carpes, variété commune ou miroir, de 300 à 600 g et gardées dans des bassins à eau renouvelée au laboratoire. Nous avons également utilisé, à titre comparatif, 4 Rats albinos et 5 Tanches communes.

Le dosage de la glycogène phosphorylase (E.C.2.4.1.1.) se fait classiquement en mesurant in vitro la libération de phosphore inorganique en présence d'un excès de glucose-1-phosphate selon la réaction G-1-P $\rightleftarrows$ glycogène + Pi[9].

Nous avons utilisé un tampon maléate[10] qui nous a donné des résultats très reproductibles chez le Rat et la Carpe (acide maléique, 1,16 g; NaF, 86 mg; mercapto-2-éthanol, 320 mg; sérum-albumine, 100 mg; eau distillée QS 100 ml; amené à pH 6.5 par NaOH 10 *N*).

Le milieu d'incubation est constitué de: tampon maléate, 500 µl; glucose-1-phosphate (30 mg/ml), 200 µl; eau distillée ou 5'AMP (2 mg/ml), 200 µl; broyat de tissu, 200 µl; le glycogène présent dans le tissu servant de 'primer'.

200 à 500 mg de tissu (foie, myocarde, muscle) sont prélevés très rapidement dans 5 ml de tampon maléate glacé et broyés à 'l'ultra-Turrax'. 1 ml d'acide trichloracétique à 15% glacé est ajouté au temps 0 (blanc) et au bout de 15 min. Après centrifugation, le phosphore inorganique est dosé par la méthode de FISKE et SUBBAROW. Les résultats sont exprimés en µmoles d'élément phosphore libéré en 15 min/g de tissu.

Résultats et discussion. 1. Cas de la glycogène phosphorylase musculaire. Le Tableau I montre que l'activité enzymatique chez la Carpe est beaucoup plus importante dans le myocarde et le muscle rouge que dans le muscle blanc qui est généralement considéré comme peu actif du point de vue métabolique. Les activités sont assez proches quoique plus faibles de celles observées dans le myocarde de Rat dans les mêmes conditions expérimentales. On remarque également qu'en présence de 5'AMP la quantité de phosphore libéré est très augmentée: la valeur obtenue correspond alors à la phosphorylase totale (forme a et forme b) contenue dans le tissu.

Nous avons essayé de déterminer l'optimum thermique de la réaction en incubant dans une série d'expériences un un même broyat de tissu aux températures de 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C.

Le Tableau II montre que nous obtenons significativement le maximum d'activité pour les tissus de poissons à 25 °C au lieu de 30 °C pour les Mammifères[1].

2. Cas de la glycogène phosphorylase hépatique. Sur les 52 Carpes utilisées, il n'a pas été possible d'obtenir une libération de phosphore inorganique au cours de l'incubation de broyat hépatique de Carpe. Au contraire, dans tous les essais, la quantité de phosphore libre existant dans le tissu au départ de la réaction (temps 0) était diminuée sensiblement, quoique de façon variable après 15 min d'incubation. Nous avons observé que pour une

Tableau I. Activité de la glycogène phosphorylase (µ*M* Pi libéré/15 mn/g de tissu, à 25°C)

Tissus	Nombre d'animaux	Sans 5' AMP	Avec 5' AMP
Myocarde de Carpe	10	61,7 ± 7,9	101,5 ± 12,5
Muscle rouge de Carpe	10	68,6 ± 11,3	104,4 ± 16,1
Muscle blanc de Carpe	8	13,9 ± 7,4	22,6 ± 11,4
Foie de Carpe	52	Essais négatifs	
Myocarde de Tanche	5	82,1 ± 12,3	117,3 ± 20,4
Foie de Tanche	5	118,1 ± 24,5	169,2 ± 27,9
Myocarde de Rat	4	103,0 ± 20,8	135,7 ± 23,9
Foie de Rat	4	180,3 ± 29,6	216,2 ± 31,8

(valeur moyenne pour des animaux pris dans les mêmes conditions ± Sm. *t* 5%)

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