streaks and light spots are chemically identical although their appearance is identical. The occasional presence of similar clear, bright areas along the arms of the chromatids is also not explained by diffraction. Lastly, the appearance of the centromere area was unchanged when observed by monochromatic light. Taken together, however, these points constitute sufficient evidence to make diffraction of light an unlikely cause.

Differential thinning or tearing of the cytoplasm during fixation, with a resulting increase in transmission of light in the centromere area is a second possible explanation. The occurrence of this appearance under a wide variety of conditions and fixation makes this unlikely. These conditions included aceto-orcein squashes, air drying following fixation with methyl-acetic acid and Carnoy's, as well as the sedimentation technique recently described by GAILLARD et al.[11] which permitted use of a fixative not containing alcohol or acetic acid (formaldehyde).

It is likely that both the bright spots adjacent to the centromere and the streaks extending out from the centromere, as well as the material noted by GERMAN to distort the chromatids in areas of somatic crossing over, are due either to spindle fiber material which was unstained by the procedures employed or to the structures recently observed in this area by electron microscopy[4,5]. The small size of the areas in question undoubtedly contribute to the difficulty in histochemical identification of the area by light microscopy, and in the absence of positive histochemical identification a final conclusion cannot be reached.

The appearance of the configuration shown in Figure c, as well as the association of the acrocentric chromosomes with the centromere of No. 1 in man[12] suggest that the centromere, as well as the nucleolus and secondary constrictions, plays a role in chromosome association[13].

Zusammenfassung. Das dem Centromer der menschlichen Chromosomen (ebenfalls bei andern Arten) anliegende Gebiet erscheint hell, klar und rund. Dies wurde in einigen Zellen bei fast jedem Centromer, bei andern Zellen nur selten oder überhaupt nicht beobachtet. Eine ähnliche Erscheinung trat auch in Form von Streifen auf, die sich manchmal vom Centromer aus zu anliegenden Chromosomen erstreckten oder sich ins Cytoplasma ausbreiteten. Die mögliche Deutung dieser Beobachtung wurde besprochen.

H. A. LUBS and SYLVIA L. BLITMAN

Department of Medicine, Yale School of Medicine and VA Hospital, West Haven (Connecticut, USA), 19 June 1967.

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Regulation of Tryptophan Metabolism in the Parasitic Wasp, *Habrobracon juglandis*

The synthetic pathway from tryptophan to the dark eye-pigments (ommochromes) has been analyzed by paper chromatography, fluorometric methods, and enzymatic assay. For quantitative determination of intermediate products, extracts of wild type o^+ (stock 33) and of the mutants *o* (orange eyes) and o^3 (for survey see [1]) were centrifuged and the supernatants chromatographed in *n*-propanol (70%) and Na-citrate (4%). Kynurenine, kynurenic acid, 3-hydroxykynurenine, and xanthurenic acid were identified as fluorescent spots (365 nm). The concentration of these substances during postembryonic development is shown in Figure 1 (for abbreviations see this Figure). There are higher concentrations of kynurenine in *o* than in o^+ at all stages of development. No 3-hydroxykynurenine is synthesized in *o*. Even the eggs of the mutant *o* contain more kynurenine than do those of wild type. This is due to predetermination. The mutant o^3 accumulates kynurenine at the same rate as *o*, except in ♂♂ of 3-day-old pupae ($X \pm t_{(\alpha=2\%;\, f=8)} \times s_{\bar{x}} = 8.9 \pm 1.89$ fluorometric units/2 p_3 of the mutant *o* and 13.1 ± 2.1 fluorometric units/2 p_3 of the mutant o^3). There is still no explanation for this significant difference.

In *Habrobracon* tryptophan is degraded via kynurenine to kynurenic acid and via 3-hydroxykynurenine to xanthurenic acid[2]. Whereas the orange-eyed mutant lacks xanturenic acid, both substances are found in o^+. It has been proved that kynurenine is rapidly metabolized to 3-hydroxykynurenine in fl_3 and sl. Therefore the concentration of kynurenic acid is lower at these stages than the concentration of xanthurenic acid (Figure 1). A high level of kynurenine in the mutant *o* or in wild type which had been fed tryptophan (by injection into the Ephestia hosts, mutant *a*) yields the large amount of 1 γ kynurenic acid/5 sl of *o* respectively, 2 γ/5 sl of wild type. Nearly all kynurenic acid and xanthurenic acid is excreted during the moult to prepupa.

In spite of high concentrations of kynurenine and 3-hydroxykynurenine at the pupal stages, there are very low but constant amounts of kynurenic acid and xanthurenic acid at this time of development. It seems, therefore, that only in fl_3 and sl there are strong in vivo activities of kynurenine and 3-hydroxykynurenine transaminase. It is still unknown whether these reactions are performed by 2 specific transaminases or by 1 transaminase which is less specific for kynurenine than for 3-hydroxykynurenine.

Enzymatic assays (Figure 2, II and III) of crude extracts show, however, low transaminase activities in old feeding larvae and spinning larvae and high activities in 2-day-old pupae and in adults. This result is in contrast to the assumed high in vivo activity at fl_3 and sl and the very low activity at pupal and imaginal stages. Therefore, it is thought that substrate concentrations are less responsible for the contrasting results in vivo and in vitro

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than are stage-specific high or low concentrations of the transaminase cofactor pyridoxal-5-phosphate and α-ketoglutarate. It is also possible that the distribution of these 2 substances with respect to the enzymes in the tissues may influence transaminase activities.

Further results[3] confirm the hypothesis that enzyme activities in *Habrobracon* are regulated by the amount of essential cofactors and specific inhibitors rather than by the rate of enzyme formation.

Curve 1 in Figure 2 shows the activity of tryptophan pyrrolase tested after 1 h of incubation. Almost no activity is found in 2-day-old pupae. The mixture of i- and p_2-homogenates (Table I) shows that p_2-extract contains a pyrrolase inhibitor which is inactivated at 100 °C. Since freshly emerged flies have a high pyrrolase activity, it is assumed that the inhibitor is excreted with the meconium or is inactivated. Further amounts must disappear during the first days of imaginal life because pyrrolase activity increases by a factor of 2 in 3- to 4-day-old adults.

Neither the injected substrate nor the resulting high level of kynurenine cause an adaptive increase of trypto-

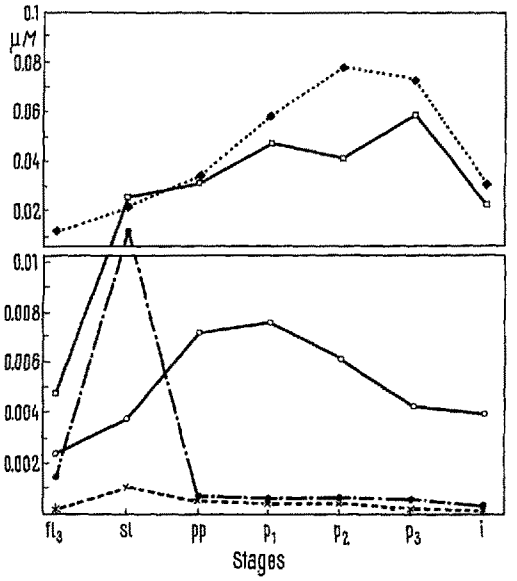

Fig. 1. Concentration of kynurenine (μM) in wild type o^+ (—o—) and in the orange-eyed mutant o (—□—). The other symbols indicate 3-hydroxykynurenine (—■—), kynurenic acid (—×—), and xanthurenic acid (—●—) in o^+. The number of animals used for each determination was 5 (kynurenic acid and xanthurenic acid) and 10 (kynurenine and 3-hydroxykynurenine), respectively. Each point represents an average obtained from 10–20 experiments. The abbreviations (age in days at 30 °C after egg laying) are: fl_3 (3-day-old feeding larva), sl, spinning larva (4), pp, prepupa (5), p_1, pupa at the first day after pupation (6), p_2, 2-day-old pupa (7), p_3, 3-day-old pupa (8), i, adults (9–10).

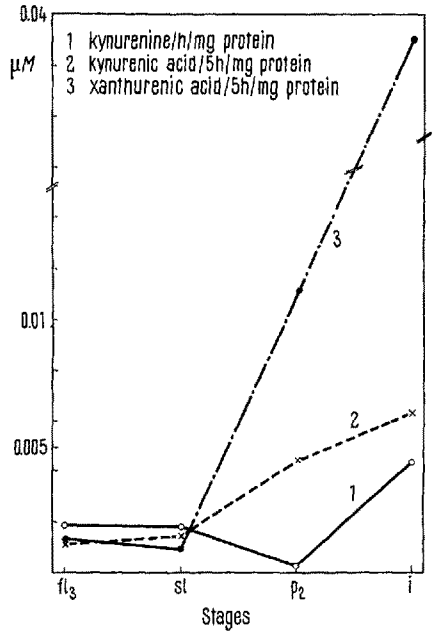

Fig. 2. Enzyme activities during postembryonic development of *Habrobracon* studied in crude extracts made by homogenizing the animals in $M/15$ phosphate buffer (pH 6.5) and by centrifuging the homogenate at 19×10^3 g (0 °C). To assay kynurenine transaminase (—×—) 0.1 ml of the supernatant, 0.1 ml of $10^{-2}M$ pyridoxal-5-phosphate, 0.1 ml of $10^{-2}M$ α-ketoglutarate and 0.1 ml of $10^{-2}M$ kynureninesulphate were mixed; in the case of 3-hydroxykynurenine transaminase (—●—) 0.1 ml of 0.2% 3-hydroxykynurenine was added. The tryptophan pyrrolase test (—o—) was performed in a 0.1% tryptophan solution ($M/15$ phosphate buffer pH 7.2) in which the animals were directly homogenized. Kynurenine, kynurenic acid, and xanthurenic acid were determined fluorometrically. Each point represents an average obtained from 2–4 assays.

Table I. Tryptophan pyrrolase activity in crude extracts of 1 day (EI_1) and 3–4 days (EI_3) old adults. Addition of extract of 2-day-old pupae (EP_2) has an inhibitory effect whereas heat inactivated extract of 2-day-old pupae and additional inert protein has no effect (1 U of pyrrolase activity = 0.1–0.19 γ kynurenine/h/mg protein). 3–4 experiments were performed for each combination

Addition of	EI_1	EI_3	EI_1	EI_1
buffer	13	24	9	11
EP_2	6	13		
50 γ			11	
200 γ			11	
bovine serum albumin				
EP_2 heat inactivated				10

Table II. Dependence of tryptophan pyrrolase activity on the substrate concentration. There is no real maximal velocity of enzymatic catalysis because inhibition by an excess of tryptophan takes place already at lower concentrations than 0.008 M tryptophan/l. The Michaelis-Menten-constant is estimated to be approximately 0.0026 M tryptophan/l for the enzyme of the adults and 0.0028 M tryptophan/l for the enzyme of 3-day-old feeding larvae

M tryptophan/l	0.001	0.002	0.004	0.006	0.008	0.01	0.06
$x \cdot 10^{-6}$ M kynurenine/h/mg protein	0.0023	0.0035	0.0053	0.0060	0.0047	0.0012	0.0012

phan pyrrolase activity[3] as they do in *Drosophila*[4,5], in *Rana*[6], and in rat liver[7–10]. However, high concentrations of tryptophan inhibit pyrrolase activity in vitro (Table II) in contrast to the enzyme of *Drosophila*[11,12]. There may exist ES_2-complexes instead of the normally functional enzyme-substrate-units (ES). If so ES_2 must be assumed to be nearly inactive. But the small activity of 0.0012 μM kynurenine/h/mg protein at substrate concentration of 0.01 M try/l remains constant until the tryptophan concentration is raised by a factor of 6. It is not known whether ES_2-complexes exist in vivo after tryptophan has been fed. However, the normal tryptophan concentration in vivo is not high enough to have a regulatory influence on pyrrolase activity.

Zusammenfassung. Die Metabolite und 3 Enzyme des Tryptophanstoffwechsels von *Habrobracon* weisen während der parasitären Phase sowie während der Puppenruhe und im Imaginalstadium signifikante Konzentrations- bzw. Aktivitätsveränderungen auf. Diese sind das Ergebnis mindestens zweier Regulationsmechanismen.

F. LEIBENGUTH[13]

Zoologisches Institut, Abteilung Entwicklungsphysiologie der Universität Tübingen (Germany), 12 May 1967.

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The Close Karyological Affinities between a *Ceratophrys* and *Pelobates* (*Amphibia Salientia*)

The South American Anuran, which are of very considerable taxonomic interest in view of the great number of families and species that exist in that area, are the object of karyological research at the present moment, being undertaken chiefly by the school of SAEZ. SAEZ and BRUM[1] have observed, among other things, that *Ceratophrys ornata* has a chromosome set that is numerically very high ($2n$ = 89, 92, 98 and 108).

I myself have collected karyological data regarding certain species of South American Anuran, and I here describe the results of a karyological study of specimens of *C. calcarata* originating from Columbia, which I consider to be interesting.

The chromosome set of this species consists of 26 chromosomes: 12 large and 14 smaller ones; one pair of these shows vast heterochromatic areas (Figure 1, arrows). In the male line there are 13 meiotic bivalents, each of which is usually provided with 2 terminal chiasmata.

As regards the number and morphology of the various pairs of homologues, the karyotype of *C. calcarata* (belonging to the family of the *Leptodactylidae*, which, however, various authors classify in a new family detached from the former: the *Ceratophrydae*) is very similar to that of *Pelobates cultripes* (Figure 2; MORESCALCHI[2]), belonging to the family of the *Pelobatidae*.

Figure 3 shows the genomes of the 2 species, reconstructed schematically by using a chromosome of each pair of homologues of a somatic metaphase of *Ceratophrys* (below) and of *Pelobates* (above). It may be seen that the various chromosomes correspond in order, as regards form and relative dimensions, in the 2 species (this is also the case for the chromosomes provided with heterochromatic areas, situated in the seventh place), except those in the sixth place, which in *Pelobates* have the centromere in a more distal position.

With regard to certain characteristics, the karyotype of *Ceratophrys* may also be compared with that of *Hyla* (MORESCALCHI[3]) which, however, appears more differentiated from this point of view, in accordance with the systematic data (REIG[4]; GRIFFITHS[5]; HECHT[6]).

I maintain that the considerable karyological affinity that exists between *Ceratophrys* and *Pelobates* may provide useful indications regarding the taxonomic relationships of the Leptodactylids, or of a part of them (the possible *Ceratophrydae*).

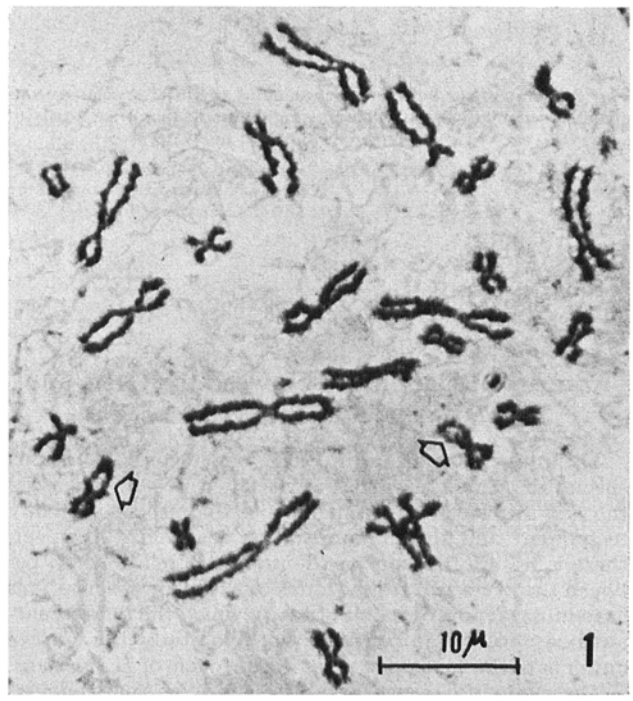

Fig. 1. Intestinal metaphase plate of a ♂ of *Ceratophrys calcarata*. The arrows indicate the chromosomes provided with heterochromatic areas. Mayer's acid hemalaun.