between the levels of the metabolites obtained for the normal and the refed rats ($p > 0.05$). These differences were observed 24 h after chloroquine administration and 24 h after 7 days of chloroquine administration. The values in the table are considerably lower after 7 days of chloroquine administration than after 1 day; this is probably due to the fact that the amount of chloroquine and its metabolites excreted in urine decreases with time.

The fact that more chloroquine and less metabolites were excreted by the kwashiorkor rats as compared to the normal and the refed rats showed that chloroquine is probably slowly metabolised during protein-energy malnutrition. It is well known that the rate of metabolism of drugs and foreign compounds in mammals may be altered by changes in the quality and quantity of dietary protein[6-8]. It has been shown that chloroquine is metabolized in the rat via a dealkylation process such that 70% of the excreted quinolines was unchanged chloroquine and the rest consisted of the desethyl and bisdesethyl chloroquine as metabolites[9,10]. The reason for the difference observed in the urinary levels of chloroquine and its metabolites in both the normal and the kwashiorkor rats might be due to the effect of the disease. In the kwashiorkor rats, the effect of the disease might reduce the concentration or the activity of the drug-metabolizing enzymes; thus, when the rats were refed to normal from the malnourished state, the metabolism of chloroquine became normal. This finding is in agreement with a previous study by a group of workers[11] who studied th in vitro and in vivo effects of dietary protein intake on drug metabolism. They found very low drug metabolising activity in rats fed with a protein free diet and progressively higher activity when the protein content of the diet was raised to 50%.

The result from this study therefore shows that the metabolism of chloroquine was impaired in protein-energy malnourished rats, and refeeding corrected this abnormality. However, the measurement of chloroquine and its metabolites in urine might not be a direct evidence for altered metabolism of chloroquine since other changes such as altered binding to serum protein[12], or changes in excretory functions could play some part.

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Temporal storage of kynurenine and 3-OH-kynurenine in the fat body of metamorphosing *Ephestia kühniella*

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Summary. During the pharate pupal stage a massive accumulation of kynurenine and 3-OH-kynurenine is observed in the fat body of *Ephestia kühniella*. By injection it can be demonstrated that this organ is capable of sequestering at least 3-OH-kynurenine, the dominating tryptophan metabolite in *Ephestia*. It is suggested that the fat body reduces a possibly harmful excess of tryptophan metabolites at the beginning of metamorphosis. These sequestered metabolites provide a precursor depot for ommochrome synthesis in later development.

During metamorphosis, holometabolous insects can be regarded as closed systems. Chemical precursors required for the development of adult structures can be derived only from histolysis products or from reserves accumulated during the larval feeding period. Kynurenine and 3-OH-kynurenine are intermediates in the tryptophan → ommochrome pathway[1]. In the meal moth *Ephestia kühniella* Z. they are mainly utilized during the second half of the pupal stage for the synthesis of screening pigment inside the compound eyes[2]. But they are already present in relatively high concentrations at the beginning of the pharate pupal stage[3]. Kynurenine and 3-OH-kynurenine, however, are very reactive molecules; 3-OH-kynurenine, especially, causes abnormal development at higher concentrations[1,4,5]. Therefore the internal level of both tryptophan metabolites should be regulated. The aim of this study was to obtain some information about regulatory mechanisms by investigating the developmental patterns and tissue distribution of kynurenine and 3-OH-kynurenine.

Material and methods. Experiments were performed with last instar larvae of *Ephestia kühniella* Z. Feeding larvae 7 days after molt and pharate pupal stages A2 and A5 were analyzed. Timing and sexing have been described elsewhere[6]. Characteristics of the strains used (wild type and mutant *a*) and rearing conditions have been summarized by Caspari and Gottlieb[7]. Injections, ligations, hemolymph sampling, determination of hemolymph volume and the preparation of visceral fat bodies already have been described in detail[6]. Tryptophan, kynurenine and 3-OH-kynurenine were quantified by means of 2-dimensional TLC on cellulose[8,9]. Protein was determined after extraction of tryptophan metabolites with biuret reagent[10], with BSA serving as a standard. Statistical calculations were done using the tests of Mann and Whitney or Kruskal and Wallis as referred to by Campbell[11].

Results and discussion. During developmental phase analyzed here, no apparent sex-specific differences exist with respect to kynurenine and 3-OH-kynurenine. Other investi-

gations have shown[12], that males and females do not begin to diverge until the second half of the pupal stage. During this relatively late phase of metamorphosis, sex-specific differences are also observed in other lepidopterans[1]. In *Epestia* kynurenine remains at rather low concentrations, which is in accordance with Egelhaaf[3].

The total amount of kynurenine rises continuously from 0.43 ± 0.01 nmoles per animal at the end of feeding period (T7) to 1.49 ± 0.19 nmoles per animal at the end of the pharate pupal stage A5 (fig. 1). When the results are expressed on the basis of protein content, a similar and also statistically significant picture is obtained (0.24 ± 0.05 nmoles/mg protein in T7 and 0.58 ± 0.05 nmoles/mg protein in A5). The amount of 3-OH-kynurenine increases from 4.68 ± 0.10 nmoles per animal at T7 to 11.28 ± 1.29 nmoles per animal at the young pharate pupal stage A2 and then remains constant throughout further pharate pupal development. Since no statistically-significant difference is found in 3-OH-kynurenine content between the development stages studied, when results are expressed on the basis of protein, the increase from T7 to A2 only reflects animal growth. The average concentration is about 3.5 nmoles/mg protein (fig. 1). Nearly constant levels of tryptophan and its metabolites have also been found in the late larval development of *Protophormia*[13].

Profound changes occur in the visceral fat body (fig. 1). In T7 only traces of both tryptophan metabolites can be demonstrated in this organ. At stage A2 the fat body contains 0.10 ± 0.02 nmoles kynurenine and 0.22 ± 0.04 nmoles 3-OH-kynurenine per organ. In A5 kynurenine has increased to 1.34 ± 0.14 nmoles and 3-OH-kynurenine to 7.45 ± 0.45 nmoles per fat body. The accumulation can also be demonstrated with data normalized for protein content (fig. 1). Finally 90% of total kynurenine and 70% of total 3-OH-kynurenine are concentrated in the fat body. Data of Stratakis and Egelhaaf[14] indicating that most of 3-OH-kynurenine is localized in the integument of the middle to late pharate pupal stage, may be explained by heavy contaminations of their preparations by fat body[15]. Thus, in agreement with earlier qualitative studies[3] it can be concluded, that most of the kynurenine and 3-OH-kynurenine of the late pharate pupal stage is located in the fat body.

The increase of kynurenine and 3-OH-kynurenine in the fat body coincides with a decrease of both metabolites in the hemolymph (fig. 1). During development from A2 to A5, kynurenine is diminished from 0.24 ± 0.05 to 0.05 ± 0.005 nmoles and 3-OH-kynurenine is reduced from 1.34 to 0.18 ± 0.01 nmoles per total hemolymph volume. This is also apparent when data are normalized on the basis of protein content (fig. 1) or are expressed per ml hemolymph (hynurenine from 38 to 8 nmoles/ml and 3-OH-kynurenine from 216 to 36 nmoles/ml). Up to now it has not been clear whether there are binding proteins for kynurenine and 3-OH-kynurenine in the hemolymph, as it has been found for tryphtophan. About 70% of total hemolymph tryptophan (530 nmoles/ml) is specifically bound to protein[16].

As revealed by the study of normogenesis, the fat body accumulates large amounts of tryptophan metabolites, while no significant concentration changes occur in the whole animal. The storage coincides with a decrease of the titres in the hemolymph, which may be regarded as a transport organ. Taking into account results obtained with other lepidoptera, it can be stated that during the pharate pupal stage most organs[1] have very low activities of tryptophan metabolizing enzymes (studies on *Bombyx mori*). Further excretory activity of Malpighian tubules[17] nearly ceases during this stage (studies on *Calpodes ethlius*). The results described could therefore at least partially be explained by a transfer of kynurenine and 3-OH-kynurenine from other organs via haemolymph to the fat body. To test this hypothesis directly we injected 3-OH-kynurenine into mutant *a* animals, which are not capable of metabolizing tryptophan via the kynurenine pathway[3].

After injection of 11 nmoles 3-OH-kynurenine into A5 animals a rapid initial accumulation by the fat body is observed during the 1st 30 min, followed by a slow, nearly constant rate of uptake which continues for at least another 300 min. Finally about 60% of the injected 3-OH-kynurenine is concentrated in the fat body (fig. 2). Our results show that the fat body of late pharate pupal stage is able to sequester 3-OH-kynurenine from the hemolymph. The activity of tryptophan oxygenase, the first enzyme in tryptophan → ommochrome pathway, is however, known to be very low in the fat body of pharate pupal stage of wild type *Ephestia*. This speaks strongly in favor of uptake being the main source of accumulated kynurenine and 3-OH-kynurenine in the wild-type fat body.

Conclusions. During the pharate pupal stage of *Ephestia*, integument and Malpighian tubules are depleted of kynurenine and 3-OH-kynurenine[3]. The present study demonstrates that during the same period these tryptophan metabolites are concentrated in the fat body. 2 facts indicate that at least 3-OH-kynurenine is transferred via hemolymph from other organs to the fat body. First, there are no changes in total tissue content. Second, injection ex-

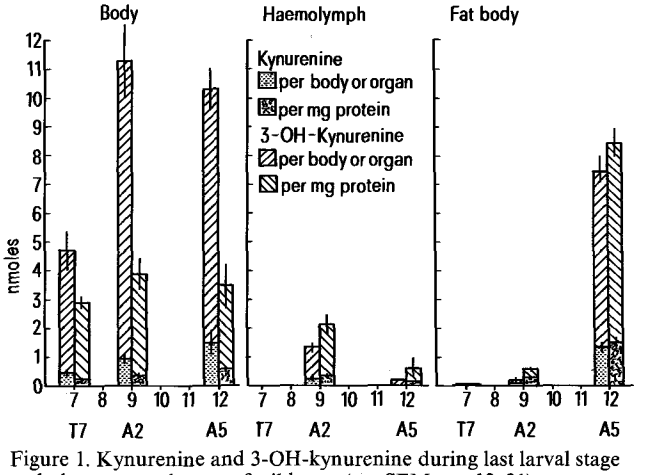

Figure 1. Kynurenine and 3-OH-kynurenine during last larval stage and pharate pupal stage of wild type ($\bar{x}$ + SEM, n = 12–21).

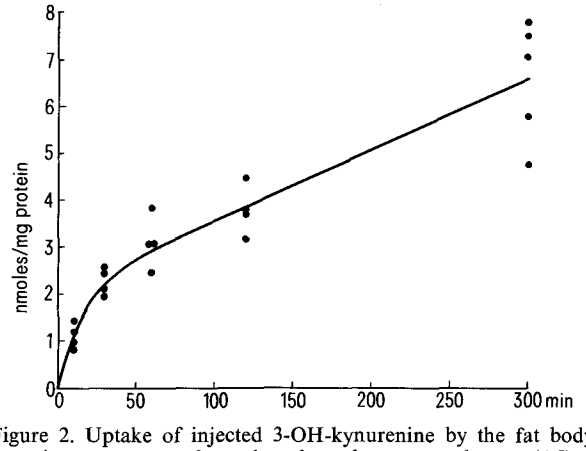

Figure 2. Uptake of injected 3-OH-kynurenine by the fat body. Experiments were performed on late pharate pupal stage (A5) of the mutant *a*.

periments have demonstrated that 3-OH-kynurenine is transferred into the fat body from hemolymph.

In young pharate pupal stage A2 the hemolymph concentration of 3-OH-kynurenine is about 220 nm/ml. This concentration would lead to chromatid breakages[4] and a decrease in thymidine incorporation[5] in human cell lines. Thus at least the biological significance of accumulating 3-OH-kynurenine in the fat body might be a protection of the developing adult against an excess of this compound. Possibly the same is true for kynurenine. In agreement with a role as a temporary storage site for precursor material needed later on, the fat body histolyses most intensively during the pupal phase just prior to the main onset of ommochrome synthesis in the developing compound eyes[2,6]. The total amount of tryptophan metabolites found in the fat body of the late pharate pupal stage would be sufficient to synthesize about 60% of the ommochromes found in the heads of adults 2 days after eclosion[9]. There are first indications that the accumulation is under direct or indirect control[18].

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Vasopressin does not stimulate fatty acid synthesis in mouse mammary explants[1]

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Summary. Fatty acid synthesis in mammary explants from pregnant mice increased after incubation with insulin, cortisol, and prolactin. Replacement of prolactin with vasopressin did not stimulate this activity. Prolactin treated with an anti-vasopressin antiserum had activity similar to untreated prolactin. Thus, stimulation of this lactogenic function by prolactin is not the result of its contamination with vasopressin.

Prolactin regulates a large number of functions in different tissues[2]. One well-studied function of this hormone is its stimulation of epithelial cell differentiation in mammary tissue. Treatment with prolactin either in vivo or in vitro can increase the synthesis of several biochemical markers of mammary differentiation[3]. In mouse mammary tissue, one marker of differentiation is the synthesis of medium chain fatty acids (MCFA)[4]. These fatty acids are not produced by mammary epithelial cells in virgin animals. In lactating animals, however, mammary epithelial cells synthesize a high proportion of MCFA, which are unique to this tissue and its secretions (milk fat from mice contains about 35% MCFA). The synthesis of these milk-specific fatty acids has been used as an index for assessing the in vitro response of mammary tissue from several species to lactogenic hormones[5-8]. These studies indicate that prolactin regulates the synthesis of milk fats in a manner similar to that of other milk-specific products.

The evaluation of this and other prolactin effects is complicated by the contamination of prolactin preparations with other pituitary hormones. For example, the reported antidiuretic effect of prolactin[9,10] may be the result of its contamination with small but physiologically active amounts of vasopressin[11-13]. Likewise, the stimulation of fatty acid synthesis in WRK-1 cells by prolactin is also the result of vasopressin contamination[14,15]. Since the WRK-1 cell line was established from a rat mammary tumor induced by DMBA, this observation led to the suggestion that fatty acid synthesis and possibly other lactogenic responses of normal mammary tissue might be stimulated by this neurohypophyseal hormone[14]. Therefore, the effect of vasopressin on milk fat synthesis needs clarification.

Methods. Rat prolactin (NIH B-1; 7 IU/mg) and ovine prolactin (NIH oPRL-10, 25 IU/mg; NIH oPRL-14, 30 IU/mg) preparations were kindly supplied by the National Pituitary Agency (NIAMDD). Bovine insulin (25 U/mg), cortisol, and ovalbumin were purchased from Calbiochem (LaJolla, CA). Synthetic arginine vasopressin (160 U/mg), oxytocin (16 U/mg), and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. (St. Louis, MO).

Vasopressin contamination of pituitary prolactin samples was measured by radioimmunoassay (RIA) using the method of Skowsky et al.[16]. Initial studies showed that extraction of these samples was not necessary, probably because of their relative purity. For the RIA, rabbit anti-vasopressin antiserum (Calbiochem, lot 142,124) at a final dilution of 1:80,000 in 0.08% normal rabbit serum was added to various dilutions of vasopressin or unknown samples in 200 µl of buffer (20 mM Na_2HPO_4, 0.15 M NaCl, 0.1% bovine serum albumin, and 10 mM EDTA, pH 7.4). After incubation for 24 h at 4 °C, approximately 10,000 dpm of [^{125}I]vasopressin (2200 Ci/mmole; New England Nuclear, Boston, MA) were added in 200 µl buffer. After a 2nd 24-h incubation, 600 µl of goat anti-rabbit antiserum (Sigma) in buffer was added; the 2nd antibody was used at a dilution that was previously shown to give maximum precipitation. After 2 h, all samples were centrifuged at 2000 rpm for 5 min, and the antibody-bound [^{125}I]vasopressin measured with a gamma counter. In the absence of added vasopressin approximately 28% of the labeled vasopressin was precipitated; in the presence of a large excess (100 pg/tube) of vasopressin, about 3% of the labeled material was precipitated.