Results and discussion. As in theraphosids[6] the three main leg nerves running through the leg of *Cupiennius salei* join in the coxa. In cross sections 2 mm from the CNS, this large nerve shows a bean-like shape (fig. 2). In contrast to insects, in which small and large fibers are distributed across the nerve's area[3,7,8], in *Cupiennius* there is a characteristic pattern of axon distribution: the dorsal portion of the leg nerve contains large axons ca 10–60 µm in diameter; the ventral part is filled up with small axons from 1 to 10 µm in diameter. Ventrally at the anterior edge are some large axons ca 20–50 µm in diameter (fig. 2). The cross-sectional area of the leg nerve decreases from the 1st to the 4th leg by about 40% (fig. 3a). This may indicate that a smaller number of axons run through the posterior legs. From axon counts in the tarsi of legs of the web spider *Zygiella x-notata* it is known that the 1st and 2nd legs contain up to 1500 sensory fibers; in the 3rd and 4th legs this number is considerable lower (600–800)[9].

The ratio between the area filled by large axons and that of the entire nerve is, however, similar for all legs (fig. 3b). As in insects, amputation of specific segments always results in degeneration of distinct regions within the nerve's cross section (fig. 4). Ablation of proximal segments causes degeneration of larger areas. These degenerations always occur in the ventral part of the nerve. Since in arthropods the sensory nerve cell bodies are located peripherally, only the sensory nerve fibers degenerate after amputation of peripheral leg segments. Therefore, one can distinguish a dorsal part with motor axons from a ventral part with sensory axons (compare fig. 2 with fig. 4). Figure 6 shows the representation of the afferents from the various segments within this ventral part of the leg nerve. A prominent feature is that all segments exept the tarsus are represented in a single distinct area: The metatarsus and the tibia in the posterior pole, the patella in the middle, the femur and trochanter in the anterior pole. The afferents of the coxa are located in a small area at the ventral margin of the nerve. The tarsus, however, is represented in two arrays in the middle next to the afferents of the patella.

The areas of the femur and the trochanter were determined together from autotomized legs (point a_5 in fig. 1). The coxa afferents were identified as the ones which remained intact after autotomy.

In general the degeneration process in the spider is similar to that in insects[3,7] as far as morphological symptoms are concerned. Initially the axoplasm darkens, then it clumps together and lysosomes and residual bodies appear (fig. 5). The glial sheath thickens and glial cells branch out. The fibers then lose their round shape and flatten. Finally the fibers collapse and the glial cells grow into the empty space. Differences exist in the time course of degeneration: In insects, 1 day after operation several axons have collapsed, and all fibers have collapsed after 6 days[3]. In *Cupiennius,* however, the degeneration process is much slower; axoplasmatic darkening does not appear earlier than 24 h after sectioning, collapse of some fibers happens only after 6 days, and all axons collapse only after 20 days post operation.

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Chitin synthetase activity and inhibition in different insect microsomal preparations

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Summary. To facilitate massive screening and for structure-activity relationship studies of chitin synthesis inhibitors, methods to obtain the chitin synthetase (CS) containing microsomal fraction from the postmitochondrial supernatant were examined. Compared with fractionation by differential centrifugation, the $CaCl_2$ precipitate yielded the most active CS preparation. Acidification (pH 5.6) and polyethylene glycol 8000 (5%) treatments resulted in relatively low CS activity. Inhibitory effects were detected with polyoxin-D and 1-geranyl-2-methyl benzimidazole, a novel CS inhibitor, but not with benzoylphenyl ureas.
Key words. Chitin synthetase; microsomal fraction, insect; chitin synthesis inhibitors; flour beetle; *Tribolium castaneum.*

Chitin is an amino sugar biopolymer which serves as an essential structural component in insect cuticles[1,2] and in fungal cell walls[3]. Chitin has been considered as an attractive target for the action of selective pesticides since any interference with its normal pathway and timing of synthesis is detrimental. Recently several benzoylphenyl urea insecticides which act by blocking insect chitin synthesis have been developed. Based on the original discovery that DU-19111, a combination between dichlobenil and diuron, is insecticidal[4,5], several highly effective benzoylphenyl urea insecticides were prepared[6–8]. It has been established that these insecticides inhibit in vivo insect chitin synthesis[5,6]. However, chitin formation in fungi was insensitive to benzoylphenyl urea compounds[9,10]. Evidence from studies involving whole organisms and organ cultures suggests that chitin biosynthesis inhibition occurs at the polymerization step[6,9]. The precise biochemical lesion could be clarified by insect cell-free preparations capable of chitin polymerization. Such preparations have been recently obtained from *Tribolium castaneum* gut[11], integuments of *Trichoplusia ni* and *Hyalophora cecropia*[12] and from *Stomoxys calcitrans* whole pupae[13]. It appears that the insecticidal benzoylphenyl ureas do not affect directly the insect chitin synthetase (CS) (EC. 2.4.1.16)[10,13] and their action involves other possible mechanisms[14].

The *Tribolium* CS assay[11] is simple, reproducible and reliable. It can be useful for massive screening and evaluation of compounds acting as chitin synthetase inhibitors. For this assay, the microsomal fraction as the enzyme source is routinely prepared by differential centrifugation. In the present communication alternative methods in which the high-speed centrifugation step is avoided are explored. These methods are based

Scheme. Preparation of Microsomes

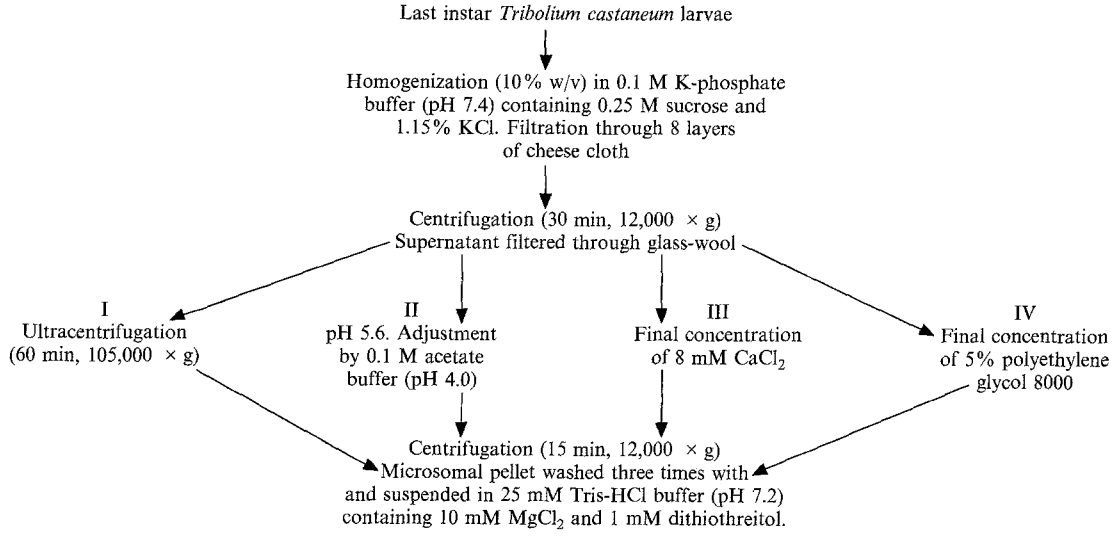

Table 1. Chitin synthetase activity in various microsomal fractions prepared from *Tribolium castaneum* lavrae

Microsomes	Chitin synthetase activity** (DPM/min/mg protein	%	Total*** radioactivity (DPM)
I Differential centrifugation	1130	100.0	1.0×10^6
II Acid precipitation pH 5.6	740	65.5	4.9×10^5
III Calcium chloride 8 mM*	1790	158.4	2.7×10^5
IV Polyethylene glycol 8000, 5%	510	45.1	3.8×10^5

* The quantity of microsomes obtained using this treatment is very small. ** The reaction mixture in 25 mM Tris-HCl buffer (pH 7.4) contained 0.5 mg microsomal protein, 10 mM $MgCl_2$, 1 mM dithiothreitol, 17 mM N-acetyl-D-glucosamine and 4.4×10^5 DPM of the tritiated substrate uridine 5'-diphospho N-acetyl-D-glucosamine (specific activity 6.6 Ci/mmol). The reaction was carried out at 22 °C for 30 min and stopped with 5% ice cold TCA. The precipitate was collected on glass-fiber filters (G/FC), washed four times with 5 ml portions of 5% TCA, dried and radioassayed. *** Calculated for 30 min of incubation and total amount of microsomal proteins obtained. The experiment was repeated three times with similar results.

Table 2. Effect of various chitin synthesis inhibitors on *Tribolium castaneum* chitin synthetase activity

Compound	Concentration (μM)	% Inhibition I	II	III	IV
Diflubenzuron	280	0	6.1	0.8	6.0
IKI-7899	280	0	0	2.4	0
Polyoxin-D	2.8	59.2	74.4	70.0	34.6
1-Geranyl-2-methyl-benzimidazole	280	85.4	77.5	97.6	87.6

I, differential centrifugation; II, acid precipitation, pH 5,6; III, precipitation by $CaCl_2$; IV, precipitation by polyethylene glycol 8000. The experiment was repeated three times with similar results.

on precipitation of microsomes from the postmitochondrial supernatant. The chitin synthetase activity in the various microsomal preparations and the effect of several chitin synthesis inhibitors are reported.

Materials and methods. The flour beetle *Tribolium castaneum* (Herbst) CTC-12 strain was kept at 30 °C and raised on wheat flour supplemented with 5% brewer's yeast. For experimental purposes last instar larvae were removed from cultures. The procedures for obtaining the various microsomal fractions are described in the scheme. The postmitochondrial supernatant was divided into 4 equal portions and microsomes were obtained as specified. The differential centrifugation (I) is the standard procedure for preparing the microsomes. The ultracentrifugation step is omitted in the alternative procedures. Instead the precipitate formed by various treatments of the postmitochondrial supernatant is collected by an additional 12,000 × g centrifugation. These treatments include lowering the pH to 5.6 with 0.1 M acetate buffer pH 4.0 (II) and addition of either solid $CaCl_2$ (III) or a 50% polyethylene glycol (PEG) 8000 (IV) solution to give final concentrations of 8 mM and 5%, respectively. The CS radioassay was carried out according to Cohen and Casida[11]. Except for polyoxin-D which is water soluble all other inhibitors were dissolved in dimethylsulfoxide and preincubated for 10 min with the enzyme system prior to addition of the substrate. The inhibitory compounds included two insecticidal benzoylphenyl ureas, diflubenzuron ([1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl)urea]; Thompson-Hayward, Co., USA) and IKI-7899 ([N-2,6-difluorobenzoyl-N'-4-(3-chloro-5-trifluoromethyl-pyridin-2-yloxy)-3,5-dichlorophenyl urea]; Ishihara Sangyo Kaisha Co., Japan), the nucleoside peptide antibiotic polyoxin-D (Kaken-Kagaku Co., Japan) and a terpenoyl benzimidazole 1-geranyl-2-methyl-benzimidazole (Dr. E. Kuwano, University of Kyushu, Japan) which represents a novel group of CS inhibitors (unpublished). All the reagents used were of analytical grade.

Results and discussion. Differential centrifugation is normally used to obtain the particulate CS system. To avoid the high speed centrifugation step which is expensive and time consuming, three other methods for preparing microsomes were examined. These alternative methods involve precipitation of microsomal membranes in the 12,000 × g supernatant, followed by an additional centrifugation (12,000 × g) for 15 min (scheme). The activity of the various CS preparations was compared using the *Tribolium* radioassay (table 1). The Ca^{2+} treatment facilitates formation of microsomal aggregates which are precipitable at low-speed centrifugation[15]. This procedure has been used successfully to isolate active microsomal enzymes from rat liver[16–18] and from the yeast *Candida tropicalis*[19]. The specific activity of the insect CS prepared by Ca^{2+} treatment (table 1, III) is the highest in comparison to the other preparations. Yet because of the very small amount of precipitable microsomes obtained by this procedure, the overall radioactivity is

reduced. It is noteworthy that Ca^{2+} sedimentation of microsomes from the insects *Musca domestica* and *Prodenia eridania* resulted in reduced activity of the mixed function oxidase enzymes[20]. PEG treatment facilitated the isolation of proteins, certain enzymes, nucleic acids, bacteria and viruses[21]. This polymer was also used to obtain active hepatic microsomal enzymes[22] and to precipitate ligand-receptor complexes[23]. It has been demonstrated that the acid precipitation procedure was useful in obtaining active rat liver microsomal enzymes[24,25]. The *Tribolium* CS enzyme complexes obtained by either acidification (II) or PEG precipitation (IV) were relatively less active, with specific activities of 65.5% and 45.1% of the control (I), respectively.

In the inhibition study (table 2), diflubenzuron and IKI-7899 did not affect the various CS enzymes at 280 µM. This result confirms previous reports which suggest that insecticidal benzoylphenyl ureas do not act directly on the chitin polymerization step[10,14]. Polyoxin-D which is an established competitive CS inhibitor in insect[10] and fungal[3] systems affects all the CS preparations. However, for unknown reasons the CS in PEG precipitated microsomes is less inhibited (34.6%) by polyoxin-D compared with the other enzyme systems (59.2–74.4%). The benzimidazole compound at 280 µM is inhibitory almost to the same degree in all the CS preparations examined. This novel compound blocks the molting process and was found to be insecticidal in silkworm larvae[26]. Extensive structure-activity relationship study on a series of benzimidazoles having a terpenoyl moiety has been recently conducted discovering compounds with considerable inhibitory effects on the insect CS (unpublished).

The available insect CS radioassay can serve as a useful tool for massive screening and evaluation of bioactive compounds which interfere with chitin polymerization. This assay coupled with in vivo studies might yield valuable information for developing potential pesticides. Although the activity of CS obtained by Ca^{2+} aggregation is high, the small yield of microsomal proteins is disadvantageous for large screening programs (table 1). It appears that despite a certain loss in specific activity (⅔ of the control), the acid precipitation alternative might be considered as the procedure of choice for massive screening and for quantitative structure-activity relationship studies of insect CS inhibitors.

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Hepatic acid hydrolases of albino rats, *Mastomys natalensis* and albino mice during *Plasmodium berghei* infection

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Summary. Changes in liver acid hydrolase activities during the infection of albino rats, *Mastomys* or mice with *Plasmodium berghei* are described. B-Glucosidase, B-galactosidase and N-acetyl-B-D-glucosaminidase exhibited widely different responses with acid phosphatase and cathepsin-B the least responsive and are likely to be causally related to immunity of animals.
Key words. Mouse, Swiss; *Mastomys natalensis;* rat, C.F. strain; acid hydrolases, liver; *Plasmodium berghei* infection.

Liver plays an important role in recovery from blood stage malaria infection and also in the modification of the immune system of the host[2,3]. The role of phagocytic cells of the liver (Kupffer cells) in host defense inflammatory processes, immune mechanisms and erythrocyte destruction has been amply documented[4–6]. Studies made with an electron microscope have also confirmed that Kupffer cells of the liver, during malaria infection, contained phagocytized hemozoin and parasitized erythrocytes[7,8]. The function of these cells, which contain a variety of hydrolases is considered to be intracellular breakdown and disposal of phagocytized materials[9]. Reports regarding increase in the levels of various tissue acid hydrolases during *Mycoplasma*