nation from the carotid artery 2 h after alcohol administration. A 0.15 ml sample of blood was added to 1.95 ml of ice-cold 1.06 M perchloric acid solution in an ice-cold test tube, which was tightly capped with a rubber stopper. Livers were removed and immediately frozen in liquid nitrogen. The frozen livers were pulverized in homogenizers cooled with liquid nitrogen. Ethanol and acetaldehyde concentrations in the blood and liver were determined according to our previously reported methods[3,4]. Average recovery using this method was found to be 100.1% for acetaldehyde and 98.1% for ethanol. Plasma and liver amino acid contents were determined 1 h after taurine administration. Quantitative determinations of serum and liver amino acids were carried out with a Nihon-Denshi JCL-6AL amino acid analyzer as described previously[5]. Results were expressed as mean ± SD. Significance ($p < 0.05$) of deviation from the control was calculated by Student's t-test.

Results and discussion. Serum and liver taurine concentrations 1 h after the oral administration of taurine (0.5 g/kg b.wt) were much higher than in control rats: 11.2 ($p < 0.1$) and 4.0 ($p < 0.05$) times higher, respectively (table 1). Blood and liver acetaldehyde concentrations following ethanol loading were significantly reduced when rats were pretreated with taurine. Taurine, an amino acid which does not occur in proteins, exists free in many cells in large amounts. In chronic alcoholics, urinary taurine excretion increases and plasma taurine levels diminish[6]. The effect of taurine administration on ethanol-induced sleeping time in mice[7] and on alcohol withdrawal[8] have been studied. The former study revealed that the brain-depressant effect of ethanol (4 g/kg b.wt) was markedly reduced in mice by simultaneous i.p. injection of taurine (45 mg/kg b.wt). The latter study stated that an oral administration of taurine (3 g/day) for 7 days showed preventive and therapeutic effects on alcohol withdrawal. Blood acetaldehyde levels after drinking a taurine-containing alcoholic beverage were much lower than those after drinking alcohol (A. Watanabe et al., unpublished observations). Taurine as well as pantethine may be clinically useful for preventing alcoholic liver damage and alcohol addiction, since acetaldehyde has been implicated in the pathogenesis of these problems[9].

Table 1. Effect of intragastric taurine administration on serum and liver taurine concentrations

	Serum (μmoles/l)	Liver (μmoles/kg)
Control	253 ± 15	2030 ± 493
Taurine	2833 ± 1305*	7933 ± 1436**

Mean ± SD. No. of rats = 3. *$p < 0.1$ and **$p < 0.05$.

Table 2. Effect of intragastric taurine administration on ethanol and acetaldehyde concentrations in the blood and liver following ethanol loading

	Blood Ethanol (mM)	Acetaldehyde (μm)	Liver Ethanol (mmoles/kg)	Acetaldehyde (μmoles/kg)
Control	25.9 ± 2.2	3.9 ± 0.7	15.5 ± 1.5	13.5 ± 3.2
Taurine	24.1 ± 5.0	1.5 ± 0.6*	19.0 ± 1.5	4.4 ± 0.5*

Mean ± SD. No. of rats = 3. *$p < 0.05$.

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Synthesis of trypsin inhibitor CMTI III from squash seeds *(Cucurbita maxima)*[1]

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Summary. Using the solid-phase procedure, a 29-peptide cross-linked by three disulphide bridges was synthesized. The synthetic product was shown to be identical with the trypsin inhibitor CMTI III from squash seeds *(Cucurbita maxima)*.
Key words. 29-peptide; trypsin inhibitor; solid phase peptide synthesis; squash seeds.

In 1980 Polanowski et al.[2] isolated low molecular mass (M_r 3300) polypeptide trypsin inhibitors, coded CMTI I and CMTI III[3], from squash seeds *(Cucurbita maxima)*. The inhibitors were purified with immobilized trypsin[4]. In 1981 Nowak et al.[5] published one sequence of the CMTI III. The authors suggest that the inhibitor molecules consists of 28 amino acid residues cross-linked by two disulphide bridges.

In 1983 another sequence of CMTI III and a sequence of CMTI I were published by Wilusz et al.[6]. According to these authors, the molecule of CMTI III, as well as that of CMTI I consist of 29 amino acid residues cross-linked by three disulphide bridges. The sequences of the two inhibitors differ in only one position, Lys_9[7] in CMTI III being replaced by Glu_9 in CMTI I[6]. It was established that Arg_5-Ile_6 is the reactive site peptide bond in both inhibitors[5,6]. The interaction of CMTI III or CMTI I with trypsin is accompanied by cleavage of this peptide bond, resulting in the modified inhibitors CMTI III* and CMTI I*[2,4], the molecules of which consist of two peptide chains held together by one disulphide bridge[5].

In this paper we present the synthesis of two polypeptides: the 28-peptide with the sequence suggested for CMTI III by Nowak et al.[5]: Arg-Val-Cys-Pro-Arg-Ile-Leu-Met-Lys-Cys^{19}-Lys-Lys-Asp-Gln-Ser-Asp-Leu-Ala-Glu-Val^{20}-Cys-His-Leu-Glu-Cys-Gly-Gly-Tyr^{28}, and the 29-peptide with the sequence suggested

for CMTI III by Wilusz et al.[6]: Arg-Val-Cys-Pro-Arg-Ile-Leu-Met-Lys-Cys-Lys-Lys-Asp-Ser-Asp-Cys-Leu-Ala-Glu-Cys-Val-Cys-Leu-Glu-His-Gly-Tyr-Cys-Gly.

The peptides were synthesized by the solid-phase procedure[8] using, in the case of the 28-peptide Boc-Tyr(2,6-Cl-Bzl)-Pam-resin (capacity 0.15 meq/g) and in the case of the 29-peptide Boc-Gly-O-CH_2-resin (capacity 0.20 meq/g). All deblocking, rinsing and coupling steps were carried out using an automatic peptide synthesizer (Beckman, Model 990). N-Boc-protection was used throughout the synthesis with the following side chain protecting groups: Arg(NO_2), Asp(OBzl), Cys(Acm), Gln(Xan), Glu(OBzl), His(Tos), Lys(2-Cl-Z), Ser(Bzl) and Tyr(2,6-Cl-Bzl). Double couplings in CH_2Cl_2 were performed using DCC. Deblockings were achieved using 33% TFA/CH_2Cl_2 with 2% dimethylsulphide. For neutralization 10% TEA/CH_2Cl_2 was used. After completing the synthesis, the Acm-peptides were cleaved from the resins with liquid HF at 0 °C in the presence of anisole. The Acm-peptides were purified on a Sephadex G-25F column. Both peptides gave the required amino acid composition on amino acid analysis. Then the Acm-groups were removed with mercuric acetate according to a previously described procedure[9]. The reduced peptides were desalted on a Sephadex G-10 column and reacted with an equimolar mixture of oxidized and reduced glutathione in Tris-HCl buffer (pH 8.5) at 35 °C for 20 h[10].

The oxidized 28-peptide based on the sequence as suggested by Nowak et al.[5] did not show any antitrypsin activity in the assay according to Erlanger et al.[11].

Yields obtained in the preparation and purification of synthetic 28- and 29-peptides

Product	Yield (%)
Crude Acm-28-peptide	53
Purified Acm-28-peptide (Sephadex G-25F, twice)	20
Reduced 28-peptide (removal of Acm groups, Sephadex G-10)	70
Crude Acm-29-peptide	42
Purified Acm-29-peptide (Sephadex G-25F, twice)	23
Reduced 29-peptide (removal of Acm-groups, Sephadex G-10)	67
Synthetic CMTI III (oxidation, affinity chromatography)	10

The oxidized 29-peptide with the sequence suggested by Wilusz et al.[6] was purified on a column of immobilized anhydrotrypsin. The peptide had the same antitrypsin activity as native CMTI III and gave identical electrophoretic and immunological[12] patterns, as well as similar UV and CD spectra. The synthetic peptide was modified with a catalytic amount of trypsin to the CMTI III* in the same manner as the native CMTI III.

Our results indicate that Wilusz et al.[6] presented the correct sequence for trypsin inhibitor CMTI III.

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7 Abbreviations. The symbols of amino acids and peptides are in accordance with the IUPAC-IUB Commission on Biochemical Nomenclature. All amino acids except glycine are in the L-configuration. Pam: the phenylacetamidomethyl group, Xan: the xanthyl group, TFA: trifluoroacetic acid, TEA: triethylamine, DCC: dicyclohexylcarbodiimide.

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Unisexual murine schistosomiasis: portal hepatitis in subacute infections[1]

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Summary. Liver changes induced by unisexual male infection with *S. mansoni* were studied in mice during 2–20 weeks post-infection, in order to distinguish changes related to released worm substances from changes related to schistosome egg deposition. In subacute unisexual infection the venous wall appears as the target for inflammation which remains focal for a long time and affects limited segments of the main portal veins. Schistosomal pigment deposited in the lobule does not induce inflammatory or fibrogenic reactions.

Key words. Unisexual schistosomiasis; liver pathology; portal hepatitis.

Most tissue changes in schistosomiasis are related to the immunopathological response of the host to the parasite[2]. Although extensive studies have shown that the determining factor in development of hepatosplenic schistosomiasis is the host granulomatous response to schistosome eggs which are trapped in the liver[3,4], it is generally accepted that development of the disease results in the synergistic action of multiple antigens from both the worm and the egg[5].

In schistosomiasis, there have been numerous reports indicating permanent circulation of toxic or antigenic substances produced by schistosomula or adult worms[6–8], allergens and hormones[9,10]. It has been suggested that secretions, excretions and breakdown products from living worms enter the liver via the portal circulation and could be at least partly responsible for hepatic changes[11,12]. Surprisingly, little attempt has been made to differentiate these immunopathological changes in the liver into those exclusively related to schistosome egg deposition and those related to released worm substances.

In the present study, we report on the hepatic changes induced in mice by living worms following unisexual male infection with *S. mansoni*.

Material and methods. Unisexual infection: The first group (n = 130) of Swiss albino SWR mice, weighing 18–20 g, was infected by 300 cercariae of *Schistosoma mansoni* (PR strain)