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INTERACTION BETWEEN NON-HISTONE CHROMOSOMAL PROTEINS OF
SCHISTOSOMA MANSONI WITH A GENE EXPRESSED ONLY IN ADULT
FEMALES.

BI-1

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Nuclear proteins from different stages of *Schistosoma mansoni* were extracted and characterized by polyacrylamide gel electrophoresis. The pattern was consistent with the occurrence of High Mobility Group proteins, HMG1, HMG2, HMG14, and HMG17. The pattern for *Schistosomula* was significantly different from the other stages. Using a filter binding assay, it was found that the proteins from all preparations bound to a schistosome gene (F-10), expressed only in adult females. Protein binding to DNA was inhibited by a synthetic oligonucleotide having a sequence containing a hexanucleotide motif, TGTCCT, occurring in binding sites of genes responsive to steroids. Blotting of the adult male and female nuclear proteins onto nitrocellulose followed by hybridization with ³²P-F-10 DNA, revealed distinct binding patterns. Female proteins showed besides, a higher affinity for the probe. The female proteins binding the F-10 probe were tentatively identified as HMG14 and 17, whereas the male proteins were not identified.
Support: CNPq, CAPES, FINEP

ECDYSTEROIDS PRODUCTION BY SEXUALLY MATURE AND IMMATURE
SCHISTOSOMA MANSONI WORMS

BI-2

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This work aims at studying changes on ecdysteroid production by *S. mansoni* after pairing between male and female worms. It is also an attempt to establish a possible role for these hormones on the male stimulus responsible for female sexual maturation and hence ovoposition. Ecdysteroids were extracted in methanol: water (7:3), and sequentially fractionated on Sep-Pak Cartridges (silica bound to C18 hydrocarbons) with 10, 30, 60 and 95% methanol in water. Each fraction was then fractionated in HPLC (silic acid columns). It was observed that males, previously mated or not, produce B-ecdysone (insect molting hormone). It was possible to detect, in all fractions examined, qualitative differences between the samples. The data indicate that mating induces changes in the hormonal metabolism alternating not only its turnover, but also driving biochemical modifications. These modifications may be important to the new functions (i.e. egg laying) of ovarium and differentiated vitelline glands in mature female worms.
Support: CNPq, CAPES.

BI-3

SCHISTOSOMA MANSONI: INTERACTION BETWEEN REGULATORY PROTEINS AND A GENE EXPRESSED BY SEXUALLY MATURE FEMALES

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The gene F-10, isolated and characterized by A. Simpson's group is expressed only by egg-laying female *S. mansoni*. This work aims at establishing the pattern of DNA: protein association in different *S. mansoni* protein extracts, trying to identify particular proteins that regulate the expression of gene F-10. Using south-western blot techniques we observed that immature worms and mated mature male worms have similar F-10 protein binding pattern. Interestingly, mature females have fewer bands able to bind the probe. When 10 mM Zn²⁺ was added to the binding buffer, improved DNA: protein interaction was observed. This seems to be a specific Zn²⁺ effect, since other divalent ions as Cu²⁺ and Mn²⁺, did not produce the same result. The interaction was also seen in the presence of a poly dIdC synthetic polynucleotide. The results suggest (1) the occurrence of "Zincfinger" structures in *S. mansoni* proteins, (2) the occurrence of specific interaction between proteins and gene F-10, specially with native proteins of immature female and male worms, where the gene is not expressed. (CNPq, CAPES)

BI-4

PREDICTED STRUCTURE FOR THE MAIN MOTIF OF SCHISTOSOMA MANSONI EGG SHELL PROTEIN

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The predicted sequence of the *Schistosoma mansoni* eggshell protein possesses a signal peptide, cleavage of which would result in a processed polypeptide of 160 residues. The sequence is very glycine-rich (50%) and also rich in tyrosine (12%). Both these residue types show a distinct asymmetry in their distribution either side of a short interruption in the glycine-rich background. The most striking feature of the sequence is the recurrence of the sequence motif YGGG, present on both sides of the PAP interruption the predict structure for the main motif we propose is a small beta-sheet in which three or four strands lie in a simple meander with the glycine in the centre and the tyrosines and cysteines at the turns.

Supported by CNPq.

CHARACTERIZATION OF A Ca^{2+} - STIMULATED, Mg^{2+} - DEPENDENT ATPase IN
SUBCELLULAR FRACTIONS FROM *SCHISTOSOMA MANSONI*

BI-5

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The information available on Ca^{2+} - ATPase activity in *Schistosoma mansoni* is limited to a few descriptions of ATPases activated by high concentrations of Ca^{2+} or Mg^{2+} probably not related to a Ca^{2+} pump. The aim of present work was to look for a Ca^{2+} - stimulated, Mg^{2+} - dependent ATPase activity that could be responsible for the transport of Ca^{2+} in *S. mansoni* in vivo. Tegumental material was obtained by incubation of male adult worms in a membrane disruption fluid containing saponin and $CaCl_2$. Four subcellular fractions were obtained by differential centrifugation of an untreated homogenate. A Ca^{2+} -stimulated, Mg^{2+} - dependent ATPase activity was found in subcellular fractions, but not in tegumental material, from *S. mansoni*. Its specific and relative activity were higher in the heterogeneous cuticle fraction and in the microsomal fraction. The $K_{0,5}$ for ATPase activation by free Ca^{2+} was 0.2-0.5 μM in the presence of saturating MgATP. This activity was inhibited by micromolar concentrations of vanadate and by the calmodulin antagonists calmidazole, trifluoperazine, W7 and 48-80 compound. These results indicate that the (Ca^{2+} + Mg^{2+})-ATPase here described in *S. mansoni* is of the $E_1 - E_2$ type, and is activated by calmodulin, supporting its participation in a Ca^{2+} pumping in vivo. Supported by CNPq, CAPES.

SCHISTOSOMA MANSONI: IDENTIFICATION OF POLYPEPTIDES IN SEXUALLY
MATURE WORMS

BI-6

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This work aims at establishing protein differences related to female maturation, that could be used as possible targets to interfere in this process, avoiding oviposition and hence schistosomiasis pathology. An attempt to characterize and compare protein composition of sexually mature and immature *S. mansoni* worms was made using metabolic incorporation of C -Leucine for 12 and 24 hours. The protein extracts were fractionated by 2-D electrophoresis and the protein patterns revealed by the autoradiograms have confirmed those previously obtained with silver staining procedures. The qualitative differences observed suggest that sexual maturation in *S. mansoni* females is triggered as in other systems, by the induction and repression of different sets of genes. No qualitative modification was noticed in protein pattern of male worm after pairing, although there was an increase in the biosynthesis of some polypeptides in mated male extracts after 24 hours incubation. This might indicate that coupling also affects male protein composition, inducing quantitative changes in a distinct set of proteins.