

**SCANNING ELECTRON MICROSCOPY ON ADULTS  
OF *SCHISTOSOMA MANSONI* TREATED  
IN VIVO WITH PRAZIQUANTEL AND RO-15 (5458 )**

By

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تغيرات البنيان الدقيق بالمجهر الألكتروني الماسح لسطح الأهاب

في ديدان شيسيتوسوما مانسوني البالغة

بعد العلاج بعقاري برازيكوانتل و رو-15 ( ٥٤٥٨ )

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تتضمن الدراسة الحالية متابعة تأثير عقارين مضادين لديدان البلهارسيا شيسيتوسوما مانسوني باستخدام الميكروسكوب الاللكتروني الماسح . وهذان العقاران هما البرازيكوانتل وعقار جديد هو رو-15 ( ٥٤٥٨ ) .  
وقد وصفت التغيرات التي تظهر على السطح الخارجي للديدان الناتجة عن استخدام الجرعات الشافية والتحت شافية لكل من العقارين ، كما وصفت أيضاً تشوهات الديدان الناجمة عن استخدام جرعة مركبة من كلا العقارين .  
وقد أوضحت الدراسة كفاءة عقار رو-15 ( ٥٤٥٨ ) الجديد ، في تأثيره على ديدان البلهارسيا المعوية بصورة مشجعة على مواصلة دراسته وإمكانية استخدامه ، في حالات المرضى الذين يظهرون دلائل على مقاومة الطفيليات للعلاج بعقار برازيكوانتل .

**Key words :** *Schistosoma mansoni*, scanning electron microscopy, Praziquantel, Ro-15 (5458).

**ABSTRACT**

In view of the known relationship between changes in the tegumental surface of schistosomes and the potency of antischistosomal drugs, the present work is done to study the effect of two oral drugs, namely Praziquantel and Ro-15 (5458), on the tegument of *Schistosoma mansoni* by scanning electron microscopy. The damage produced in the tegument of both male and female worms after treatment with the curative and subcurative doses of **Praziquantel and Ro-15 (5458)** are described. A pronounced deformation of the whole worms was detected after the treatment with a combined reduced dose of the two oral antibilharzial drugs. The potency of the new compound Ro-15 (5458) is encouraging and deserves further studies to be recommended as an antischistosomal drug, particularly in those cases which may show evidence of drug resistance to Praziquantel.

## INTRODUCTION

Few chemotherapeutic agents are available currently for the treatment of schistosomiasis. The future of these drugs is threatened by the possible emergence of drug resistant strains of the parasite (1, 2). Resistance to Hycanthon and Oxamniquine is well documented (3, 4). Praziquantel is an oral antischistosomal drug most commonly used now in Egypt and other endemic areas. The resistance of *S. mansoni* to Praziquantel was recently recorded in the Senegal where the drug showed unexpected failure in an outbreak of schistosomiasis (5).

Recently, some compounds of the class 9-acridanone-hydrazones derivatives have been developed by Hoffmann La Roche (Basel-Switzerland) and were shown to have promising antischistosomal activities. One of these compounds, Ro-15 (5458), has been demonstrated to be superior to other known antischistosomal drugs used against the three principal species of the parasite that infect man (6).

A preliminary acute toxicity study on Ro-15 (5458) revealed death of all mice 14 days after treatment with a single oral dose of 50 mg/kg (7). On the other hand, a single oral dose of 15 mg/kg, completely cleared mice of schistosomes, indicating that Ro-15 (5458) has a high margin of safety between the effective dose and the lethal dose(8). *In vitro* mutagenicity tests were all negative (9). Amer (10) recommended a higher dose of Ro-15 (5458) for reaching the same therapeutic efficacy as that of Praziquantel. Burger (11) recommended the use of a combination of therapeutics to obtain a better response.

The present study aims at determining the related surface alterations observed by scanning electron microscopy induced by different doses of Praziquantel and Ro-15 (5458) on the tegument of male and female *S. mansoni* worms after treatment. It is known that tegumental alterations are directly proportional with the potency of antischistosomal drugs (12).

## Material and Methods

Sixty mice ( *CD1* strain ) were infected each with 100 cercariae of an Egyptian strain of *Schistosoma mansoni* by the tail immersion technique (13).

Six weeks post-infection, animals were divided into 6

groups with 10 animals in each group. Each of these groups were treated as follows:

Group 1: Served as non - treated controls.

Group 2: Animals were treated with the curative dose of Praziquantel given orally in a dose of 500 mg/kg body weight for 2 consecutive days.

Group 3: Received a subcurative dose of Praziquantel (1/3 of the curative dose).

Group 4: Animals were treated with the curative dose of Ro-15 (5458) given orally in a dose of 10 mg/kg body weight for 2 consecutive days.

Group 5: Animals received subcurative dose of Ro-15 (5458) (1/3 of the curative dose).

Group 6: Animals received a combined subcurative doses of both Praziquantel plus Ro-15 (5458).

Animals were sacrificed 14 days post-infection and worms were recovered by perfusion (14). The worms were fixed in buffered 3% glutaraldehyde and processed for Scanning Electron Microscopy (SEM) and examined with Joel JEM-1200 EXII electron microscope.

## RESULTS

The body surface of the untreated normal male *Schistosoma mansoni* varies considerably from one part to another as seen by scanning electron microscopy. Anterior to the ventral sucker, the surface is relatively smooth, having a tegument dotted with small holes interspersed with sensory bulbs (Fig. 1). The dorsal-lateral surface of the mid body is covered with tubercles of fairly uniform size and distribution, bearing apically directed spines (Fig. 2). The oral and ventral suckers are round to oval in shape but the ventral sucker may acquire a rosette -like shape. Both suckers are lined with long well developed spines (Figs. 3 & 4).The tegument of the ventral side of the male is characterized by several folds, with spines in between (Fig. 5).

The tegumental surface of female is generally smooth and bears conspicuous sensory bulbs anteriorly and throughout its dorsal surface (Fig. 6), while the posterior end is covered with spines interspersed with sensory bulbs (Fig. 7).

### **1- Effect of the Subcurative Doses of Praziquantel and Ro-15 (5458)**

Due to the subcurative dose of praziquantel treatment, the suckers seem to be affected in both male and female through the reduction in number of the lining spines (Fig. 8).

In the male, the most intensely affected parts were posterior to the ventral sucker. Some of the tubercles had lost their spines (Fig. 9). In some other areas, the tegument was wrinkled and the sensory organs were surrounded by a raised tegumental borders. Elsewhere, the tegument showed many small crater-like structures (Fig. 10). The ventral side lost some of its inter-wrinkler spines. Moreover, it had shown some hairy appearance (Fig. 11). In the female worms, edema occurred in different areas and markedly swollen portions of the tegument were also observed (Fig. 12). However, the effect of Ro-15 (5458) was more pronounced on the tubercles of the dorsal areas; these tubercles were heavily wrinkled and distorted with their spines being short and blunt (Fig. 13). The edema involved also the tubercles which appeared as much thickened and raised knobs (Fig. 14).

### **2- The effect of the Curative Doses of Praziquantel and Ro-15 (5458)**

On treatment with Praziquantel, the spines of the oral and ventral suckers were destroyed and the oral sucker had got several pores, possibly replacing the sensory spines (Figs. 15 & 16). The greatest damage on the surface of males was on the dorsal side. Some host cells appeared firmly attached to the tegument which was already damaged as seen from the denuded surface of the tubercles which had lost all their spines (Fig. 17). In females, the prominent damage was peeling of the surface in different areas and erosion (Fig. 18). The peeling resulted in total exposure of the spines which probably occurred after spotty erosion had taken place. Edema also occurred, with extreme contraction and disruption of the sensory structures.

On treatment with Ro-15 (5458), extreme deformation of worms examined was observed (Fig. 19). The most frequent damage was a pronounced deformation and contraction of the oral and ventral suckers of male worms (Figs. 20 & 21), while the ventral sucker of the female

worm showed extreme shrinkage and constriction (Fig. 22). Edema and damage of the tubercles appeared on the dorsal surface of males and most of the tubercles appeared torn at the tip where sensory bulbs were often situated. The spines on the tubercles seemed to be affected by the drug; most of the tubercles had lost their spines and showed different types of damage, including collapse of the blebs found on the surface (Fig. 23). The female tegument was full of wrinkles and appeared devoid of papillae, spicules or spines (Fig. 24).

### **3- Effect of combined subcurative doses of Praziquantel plus Ro-15 (5458)**

All specimens examined were so severely damaged that they were virtually unrecognizable as schistosomes (Fig. 25 & 26). The tegument had peeled, resulting in exposure of the underlying tissue and muscles. Some areas still had recognizable but denuded tubercles (Fig. 27). In other areas big cavities were seen (Fig. 28).

## **DISCUSSION**

Continuous search for alternative antischistosomal drugs is necessary because of the possible emergence of drug-resistant strains of the parasites (2).

It was noted that irrespective of their mode of action or method of application, a number of antischistosomal drugs have been reported to induce various forms of tegumental damage in *S. mansoni*: Lucanthone (15), Hycanthone (16), Miridazole (Amiblhar) (17), and Praziquantel (12, 18, 19, 20, 21, 22, 23, 24). However, Praziquantel has become without doubt the drug of choice for treatment of schistosomiasis, because of its high efficacy against the adult worms (25, 26).

In the present study, several types of surface damage were recognizable on the surface of *S. mansoni* as a result of treatment with Praziquantel with different regimen dosages. This damage can be summarized as follows: contraction of the whole worm, collapse of sensory structures, edema all over the surface particularly in females and erosion at different areas.

Recently, the antischistosomal activity of Ro-15 (5458) was reported to be superior to other standard antischistosomal agents (4). In the present study, several alterations were

found on the tegumental surface in male and female *S. mansoni* after treatment with Ro-15 (5458) with different regimen dosages such as: destruction of tubercles, blunting of the spines, wrinkling of the tegument, destruction of the sensory spines and alterations of the suckers.

In the present study, the alterations induced by the curative and subcuratives doses of Ro-15 (5458) and Praziquantel are fairly similar. These observations are in agreement with other investigations done using Praziquantel for treating *S. mansoni*. (18, 19, 20, 21, 22, 23, 24, 27).

A pronounced deformation of the whole worms was detected after treatment with a combination of subcurative doses of Praziquantel and Ro-15 (5458). This agrees with the suggestion of using a combination of low doses of therapeutics to obtain a better response with few side effects.

It appears that Ro-15 (5458) causes varying degrees of tegumental damage in this study. This augments the known potency of this new compound as an antischistosomal drug which deserves more studies so as to qualify it for human trials, particularly in cases which show possible resistance to treatment with Praziquantel.

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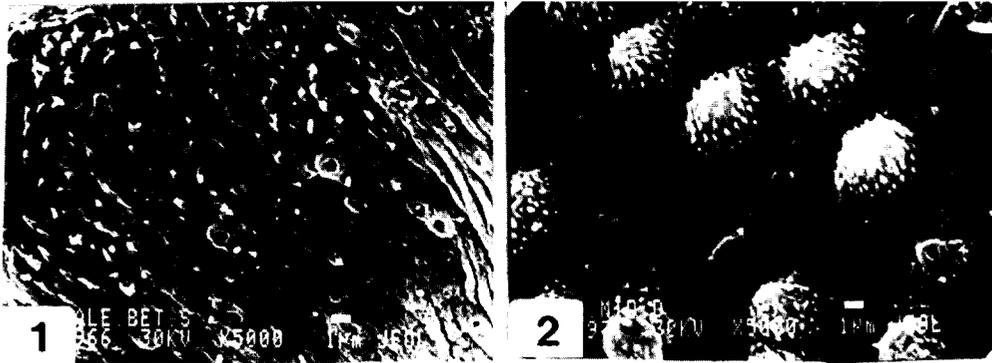


Fig. (1): Surface between suckers of normal *S. mansoni* male worm. (X 5000).

Fig. (2): Dorsal surface of normal male showing tubercles.  
(X 5000).

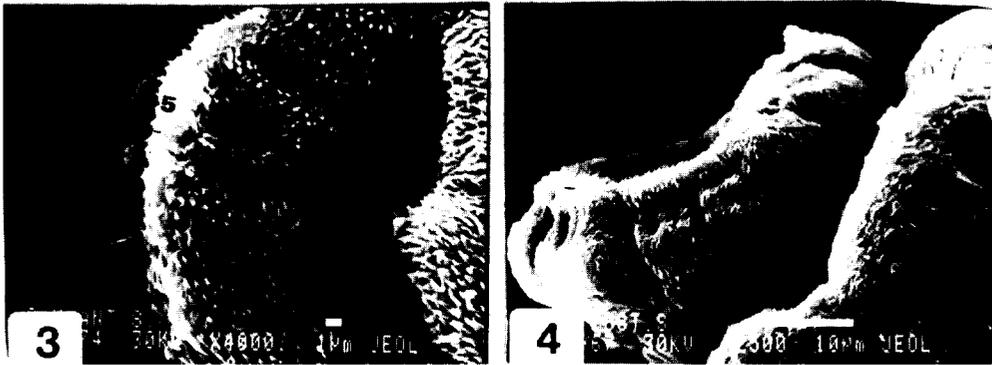


Fig. (3): Oral sucker of normal *S. mansoni* male. (X 4000).

Fig. (4): A rosette like ventral sucker. (X 2500).

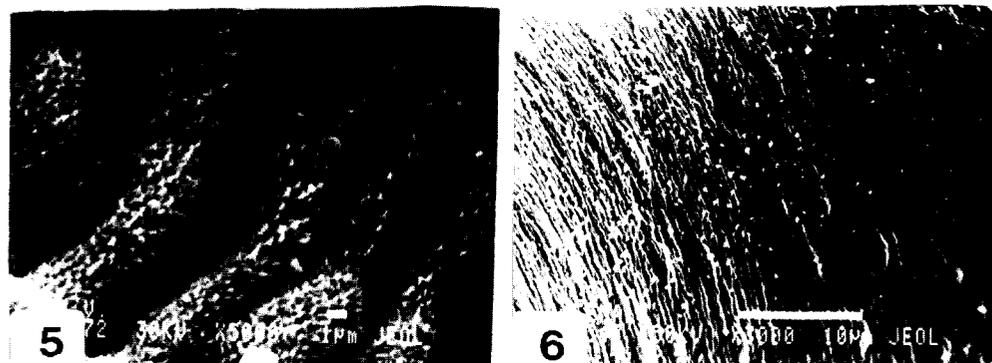


Fig. (5): Ventral surface of normal male. (X 5000).

Fig. (6): Tegumental surface of normal female. (X 3000).

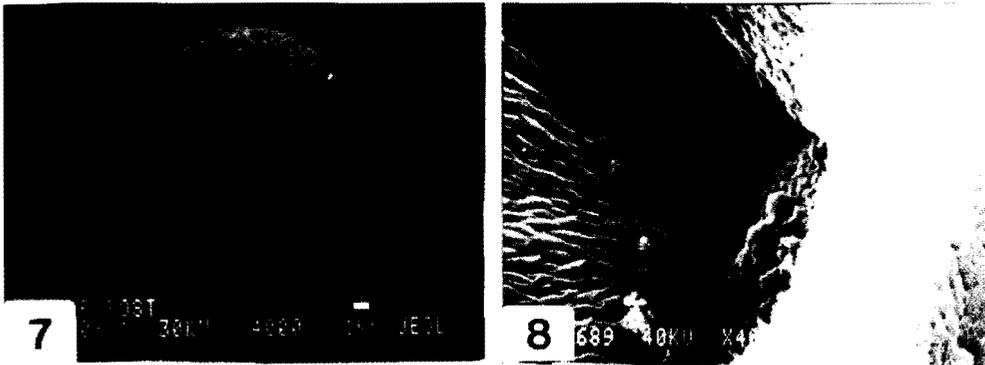


Fig. (7): Posterior end of normal female.(X 4000).

Fig. (8): Oral sucker of male *S. mansoni* treated with subcurative dose of Praziquantel (notice the absence of spines). (X 4000).

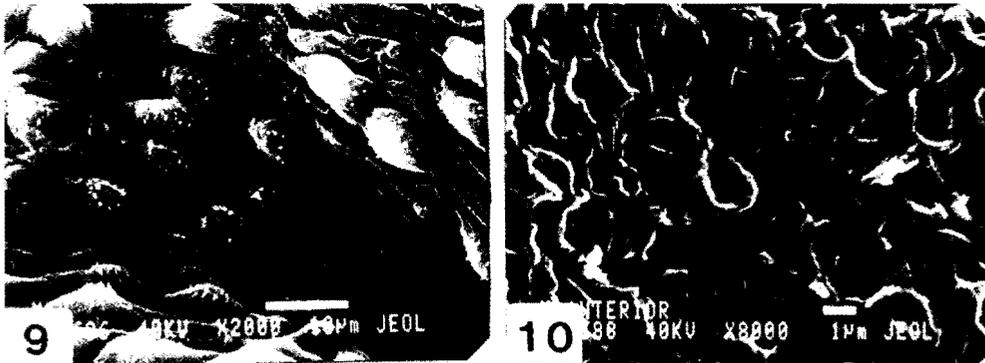


Fig. (9): Dorso-lateral surface of male *S. mansoni* treated with subcurative dose of Praziquantel showing that tubercles had lost their spines. (X 2000).

Fig. (10): Anterior area of male *S. mansoni* treated with subcurative dose of Praziquantel (notice the crater-like structure of the tegument). (X 8000).

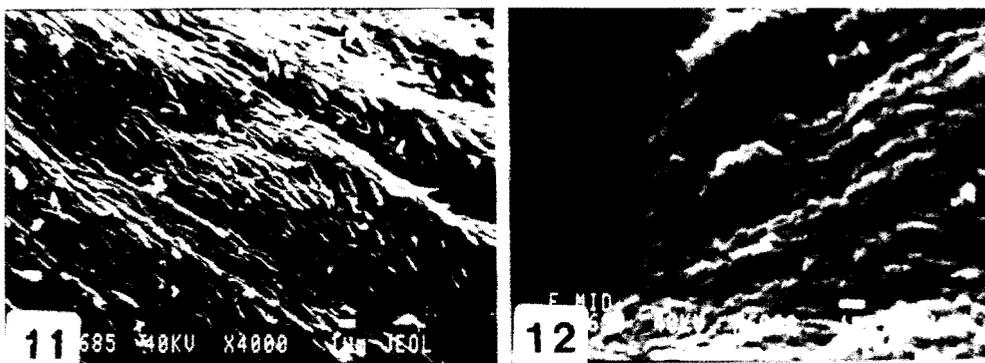


Fig. (11): Ventral surface of male *S. mansoni* treated with subcurative dose of Praziquantel (notice the hairy appearance of the surface). (X 4000).

Fig. (12): Edema and markedly swollen portions of the tegument of female *S. mansoni* treated with subcurative dose of Praziquantel. (X 6000).

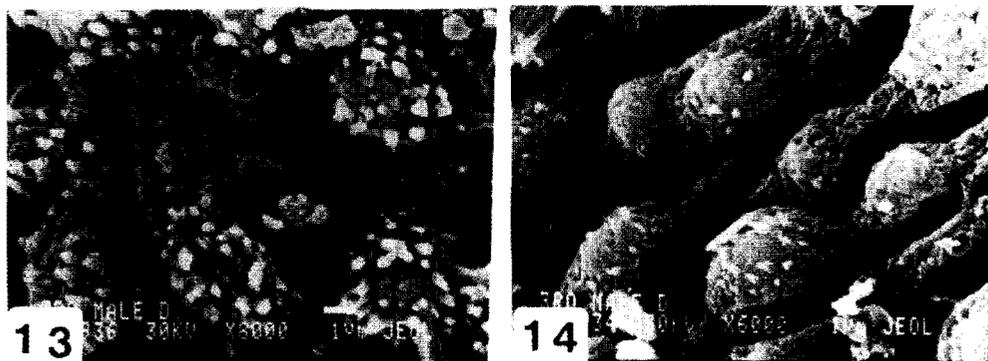


Fig. (13): Dorsal surface of male *S. mansoni* treated with subcurative dose of Ro-15 (5458) showing the distortion of the tubercles with short and blunt spines. (X 8000).

Fig. (14): Dorsal surface of male *S. mansoni* treated with subcurative dose of Ro-15 (5458) showing edema of the tubercles. (X 6000).

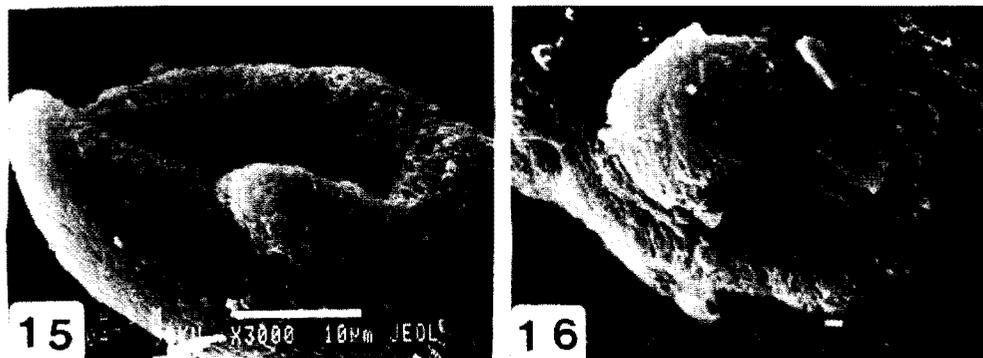


Fig. (15): Oral sucker of male *S. mansoni* treated with the curative dose of Praziquantel (notice the spines were destroyed).. (X 3000).

Fig. (16): Ventral sucker of male *S. mansoni* treated with the curative dose of Praziquantel (notice the presence of pores in place of sensory spines). (X 5000).

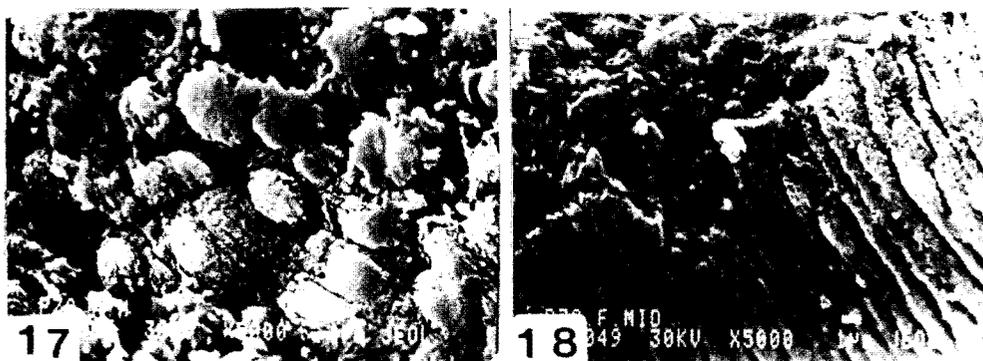


Fig. (17): Dorsal surface of male *S. mansoni* treated with the curative dose of Praziquantel showing attachment of the host cells to the tegument. (X 5000).

Fig. (18): Anterior half of female *S. mansoni* treated with the curative dose of Praziquantel showing extensive peeling of the tegument. (X 5000).



Fig. (19): *S. mansoni* worm treated with the curative dose of Ro-15 (5458) (notice the extensive deformation of the worm). (X 200).

Fig. (20): Oral and ventral sucker of male *S. mansoni* treated with the curative dose of Ro-15 (5458) showing the deformation of both suckers. (X 2500, 3500).



Fig. (21): Oral and ventral sucker of male *S. mansoni* treated with the curative dose of Ro-15 (5458) showing the deformation of both suckers. (X 2500, 3500).

Fig. (22): Extreme shrinkage of ventral sucker of female *S. mansoni* treated with the curative dose of Ro-15 (5458). (X 5000).



Fig. (23): Dorsal surface of male *S. mansoni* treated with the curative dose of Ro-15 (5458) (notice that most of the tubercles appeared torn at the tip where sensory bulbs often situated). (X 5000).

Fig. (24): *S. mansoni* female treated with the curative dose of Ro-15 (5458) showing that the tegument was heavily wrinkled and devoid of spines. (X 5000).

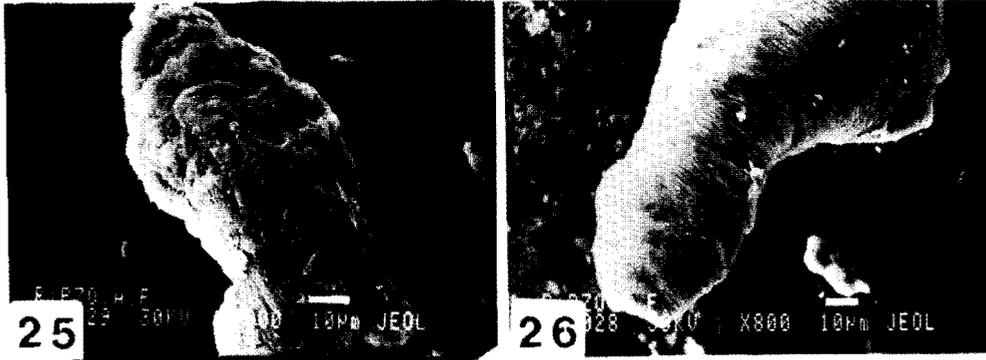


Fig. (25): Anterior end of male *S. mansoni* treated with a combined subcurative doses of Praziquantel plus Ro-15 (5458) [notice extreme deformation]. (X 800).

Fig. (26): Posterior end of female *S. mansoni* treated with a combined subcurative dose of Praziquantel plus Ro-15 (5458), notice extreme deformation. (X 800).

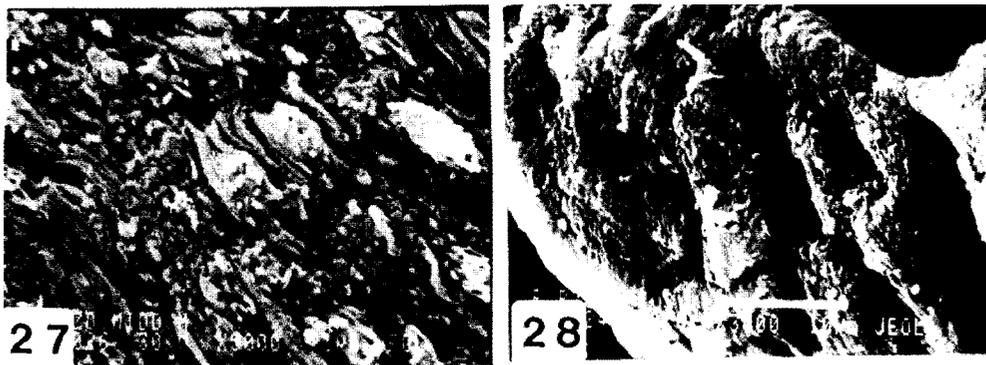


Fig. (27): Dorsal surface of male *S. mansoni* treated with a combined subcurative doses of Praziquantel plus Ro-15 (5458) showing denuded tubercles. (X 5000).

Fig. (28): Cavities appeared in the area between suckers of male *S. mansoni* treated with a combined subcurative doses of Praziquantel plus Ro-15 (5458). (X 3000).