OBSERVATIONS
Enzymatic analysis

Activities of the enzymes of glycolysis, Kreb's cycle, phosphatases and transaminases were detected in *T. globulosa*. Glucose-6-phosphatase, fructose-1, 6-diphosphatase, alanine and aspartate oxoglutarate transaminase, glycogen phosphorylase, glucose-6-phosphate isomerase, lactate dehydrogenase, adenosine triphosphatase (Mg$^{++}$-dependent), alkaline phosphatase and acid phosphatase activities were found to be more as compared to the specific activity of succinate dehydrogenase, hexokinase, glucose-6-phosphate dehydrogenase and malate dehydrogenase in *T. globulosa*. All these enzymes followed typical Michaelis-Menten substrate saturation kinetics (Figs. 3-16).

Thiabendazole and fenbendazole were shown to affect the activities of these enzymes when added *in vitro* to the standard enzyme assay system at the concentration of 100 µmoles of each one dissolved in 0.1% dimethyl sulphoxide (DMSO) (Table 1).

Glycogen phosphorylase and hexokinase activity were inhibited by both the drugs. The $V_{\text{max}}$ of the enzymes was lowered, while the $K_{m}$ was not altered (Fig. 3-4, Table 1), indicating the non-competitive nature of inhibition.
Activity of glucose-6-phosphatase was elevated by the presence of thiabendazole and fenbendazole. $V_{\text{max}}$ of the enzyme was unaffected by thiabendazole while fenbendazole lowered the same. $K_m$ was increased by both the drugs (Fig. 5, Table 1).

Thiabendazole elevated the activity of fructose-1, 6-diphosphatase and glucose-6-phosphate isomerase while fenbendazole enhanced the activity of fructose-1, 6-diphosphatase and reduced the activity of glucose-6-phosphate isomerase. $V_{\text{max}}$ of fructose-1, 6-diphosphatase did not change but that of glucose-6-phosphate isomerase was lowered in the presence of drugs. Both the drugs increased the $K_m$ value of fructose-1, 6-diphosphatase but lowered it in case of glucose-6-phosphate isomerase (Figs. 6 & 7, Table 1). Both the drugs also inhibited the activity of lactate dehydrogenase by lowering the $V_{\text{max}}$ but $K_m$ remained unaltered (Fig. 8, Table 1). Activity of succinate dehydrogenase was lowered by thiabendazole but elevated by fenbendazole. Both the drugs changed the $V_{\text{max}}$ while $K_m$ was not changed (Fig. 9, Table 1).

Malate dehydrogenase activity was increased in the presence of the two drugs. Fenbendazole increased the $V_{\text{max}}$ whereas it was not changed by thiabendazole. $K_m$ was also unaltered (Fig. 10, Table 1). Fenbendazole increased the activity of glucose-6-phosphate dehydrogenase whereas thiaben-
bendazole decreased it. Thiabendazole increased the $K_m$ as well as $V_{\text{max}}$ but fenbendazole lowered the $K_m$ and enhanced the $V_{\text{max}}$ (Fig. 11, Table 1).

Both drugs inhibited the activity of adenosine triphosphatase. $K_m$ was increased in the presence of drugs. Thiabendazole lowered the $V_{\text{max}}$ but fenbendazole increased it (Fig. 12, Table 1).

Activity of acid phosphatase was decreased by both the drugs. $V_{\text{max}}$ was unchanged, whereas $K_m$ was lowered by thiabendazole but fenbendazole did not change it (Fig. 13, Table 1).

These drugs also elevated the activity of alkaline phosphatase. $V_{\text{max}}$ was not affected but $K_m$ value was increased by fenbendazole and decreased by thiabendazole (Fig. 14, Table 1).

Activity of alanine oxoglutarate was inhibited by both the drugs. $K_m$ was not changed. Fenbendazole lowered the $V_{\text{max}}$ while it remained unchanged with thiabendazole (Fig. 15, Table 1).

Thiabendazole elevated the activity of aspartate oxoglutarate. Fenbendazole lowered it. $K_m$ of the enzyme was not changed in the presence of the drugs but $V_{\text{max}}$ was lowered by both (Fig. 16, Table 1).
Fig. 1. Digramatic sketch of female *T. globulosa*. 
Fig. 1
Fig. 2. Digramatic sketch of male *T. globulosa*. 
Fig. 3 (a) Effect of TBZ and FBZ on glycogen phosphorylase (+AMP) in T. globulosa. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of glycogen phosphorylase in the presence of TBZ and FBZ (1/v vs 1/s Lineweaver-Burk plot)
Glycogen-phosphorylase

(a)  

(b)  

\[ \frac{\mu \text{moles/mg protein/hr}}{1/\text{mM Glucose-1-phosphate}} \]

Fig. 3
Fig. 4. (a) Effect of TBZ and FBZ on Hexokinase in T. globulosa. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_{\text{m}}$ of Hexokinase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).
Hexokinase

(a)

(b)

Fig. 4
Fig. 5. (a) Effect of TBZ and FBZ on glucose-6-phosphatase in *T. globulosa*. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of glucose-6-phosphatase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).
Glucose-6-phosphatase

---

**Fig. 5**

(a) 

- **Absence of drugs**
- **Presence of thiabendazole**
- **Presence of fenbendazole**

(b) 

1/μmoles/mg protein/hr vs. 1/mM Glucose-6-P0₄
Fig. 6. (a) Effect of TBZ and FBZ on fructose-1, 6-diphosphatase in *T. globulosa*. Longitudinal bars indicate S.D.

(b) $V_{max}$ and $K_m$ of fructose-1, 6-diphosphatase in the presence of TBZ and FBZ ($1/V$ vs. $1/S$ Lineweaver-Burk plot).
Fructose-1,6-diphosphatase

Fig. 6

(a)

(b)

$\frac{1}{\mu\text{moles/mg protein/hr}}$

$\frac{1}{\text{mM Fructose-1,6-diphosphate}}$

$\mu\text{moles/mg protein/hr}$

- O - Absence of drugs
- △ - Presence of thiabendazole
- × - Presence of fenbendazole

Fig. 6
Fig. 7. (a) Effect of TBZ and FBZ on glucose-6-phosphate isomerase in T. globulosa. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of glucose-6-phosphate isomerase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver - Burk plot).
Glucose-6-phosphate isomerase

**Fig. 7**

(a) 

- **Absence of drugs**
- **Presence of thiabendazole**
- **Presence of fenbendazole**

(b) 

- Presence of thiabendazole
- Presence of fenbendazole

mM Glucose-6-phosphate

μ moles/mg protein/hr

1/μ moles/mg protein/hr

1/ mM Glucose-6-phosphate
Effect of TBZ and FBZ on lactate dehydrogenase in *T. globulosa*. Longitudinal bars indicate S.D. of *V* and *K* of lactate dehydrogenase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).

**Fig. 8.**

(a) Effect of TBZ and FBZ on lactate dehydrogenase in *T. globulosa*. Longitudinal bars indicate S.D.

(b) *V*\textsubscript{max} and *K*\textsubscript{m} of lactate dehydrogenase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).
Lactate dehydrogenase

(b)

1/μmoles/mg protein/hr vs 1/mM Sodium lactate

(a)

μmoles/mg protein/hr vs mM Sodium lactate

- ○ Absence of drugs
- △ Presence of thiabendazole
- × Presence of fenbendazole

Fig. 8
Fig. 9. (a) Effect of TBZ and FBZ on succinate dehydrogenase in T. globulosa. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of succinate dehydrogenase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).
Succinate dehydrogenase

**Fig. 9**

- **(a)**
  - Graph showing the relationship between sodium succinate concentration and enzyme activity.
  - The graph includes data points and error bars, indicating variability.
  - Legend: 
    - ○○ Absence of drugs
    - △△ Presence of thiabendazole
    - ×× Presence of fenbendazole

- **(b)**
  - Graph showing the reciprocal of enzyme activity against the reciprocal of sodium succinate concentration.
  - The graph includes data points and lines indicating the relationship.
Fig. 10. (a) Effect of TBZ and FBZ on malate dehydrogenase in *T. globulosa*. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of malate dehydrogenase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).
(a) 

µ moles/mg protein/ hr

(b) 

1/µ moles/mg protein/ hr

1/mM Oxaloacetic acid

Malate dehydrogenase

Absence of drugs
Presence of thiaminose
Presence of fenbendazole

Fig. 10
Fig. 11. (a) Effect of TBZ and FBZ on glucoso-6-phosphate dehydrogenase in T. globulosa. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_{\text{m}}$ of glucose-6-phosphate dehydrogenase in the presence of TBZ and FBZ ($1/V$ vs. $1/S$ Lineweaver-Burk plot).
Glucose-6-P dehydrogenase

Fig. 11

(a) 

(b) 

1/μmoles/mg protein/hr

1/μmoles/mg protein/hr

0 1 2

μmoles/mg protein/hr

0 0.023 0.046 0.069 0.092 0.115 0.138 0.161

mM Glucose-6-phosphate

Absence of drugs

Presence of thiabendazole

Presence of fenbendazole

Fig. 11
Fig. 12. (a) Effect of TBZ and FBZ on ATPase (Mg$^{2+}$-dependent) in T. globulosa. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of ATPase (Mg$^{2+}$-dependent) in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).
**ATPase (Mg$^{2+}$ dependent)**

![Graph](image)

**Fig. 12**
Fig. 13. (a) Effect of TBZ and FBZ on acid phosphatase in *T. globulosa*. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of acid phosphatase in the presence of *T. globulosa* (1/V vs. 1/S Lineweaver-Burk plot).
Acid phosphatase

Fig. 13

(a)

(b)

\[ \frac{1}{\mu \text{ moles/mg protein/hr}} \]

\[ \frac{1}{\text{mM Sodium } \beta\text{-glycrophosphate}} \]

\( \mu \text{ moles/mg protein/hr} \)

\( \text{mM Sodium } \beta\text{-glycrophosphate} \)

- ○ ○ Absence of drugs
- △ △ Presence of thiabendazole
- × × Presence of tenbendazole

Fig. 13
Fig. 14. (a) Effect of TBZ and FBZ on alkaline phosphatase in *T. globulosa*. Longitudinal bars indicate S.D.

(b) $V_{max}$ and $K_m$ of alkaline phosphatase in the presence of TBZ and FBZ (1/V vs. 1/S Lineweaver-Burk plot).
Alkaline phosphatase

![Graphs showing the effect of sodium \( \beta \)-glycrophosphate on alkaline phosphatase activity with and without thiabendazole and fenbendazole.](image-url)
Fig. 15. (a) Effect of TBZ on alanine oxoglutarate transaminase in *T. globulosa*. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of alanine oxoglutarate transaminase in the presence of TBZ and FBZ ($1/V$ vs. $1/S$ Lineweaver-Burk plot).
Alanine oxoglutarate transaminase

Fig. 15

(b)

$\frac{1}{\text{mmoles/mg protein/hr}}$

$\frac{1}{\text{mM Alanine}}$

(a)

$\mu$ moles/mg protein/hr

$0$ $1.3$ $2.6$ $3.9$ $5.2$ $6.5$ $7.8$ $9.1$

mM Alanine

- ○ Absence of drugs
- △ Presence of thiabendazole
- × Presence of fenbendazole

Fig. 15
Fig. 16. (a) Effect of TBZ and FBZ on aspartate oxoglutarate in T. globulosa. Longitudinal bars indicate S.D.

(b) $V_{\text{max}}$ and $K_m$ of aspartate oxoglutarate in the presence of TBZ and FBZ ($1/V$ vs. $1/S$ Lineweaver-Burk plot).
Aspartate oxoglutarate transaminase

(b)

\[
\frac{1}{\text{mM Aspartic acid}}
\]

\[
\frac{1}{\mu \text{moles/mg protein/hour}}
\]

Fig. 16

(a)

\[
\mu \text{moles/mg protein/hour}
\]

- Absence of drugs
- Presence of thiabendazole
- Presence of fenbendazole

Fig. 16
Table 1: In vitro effect of thiabendazole and fenbendazole on the enzyme of carbohydrate metabolism in T. globulosa.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ ($\mu$ mol mg$^{-1}$ protein h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen phosphorylase (+AMP)</td>
<td>1.56</td>
<td>100.00</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>7.14</td>
<td>666.60</td>
</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>0.02</td>
<td>100.00</td>
</tr>
<tr>
<td>Fructose-1, 6-diphosphatase</td>
<td>0.76</td>
<td>4.54</td>
</tr>
<tr>
<td>Glucose-6-phosphate isomerase</td>
<td>1.42</td>
<td>25.00</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>0.40</td>
<td>31.25</td>
</tr>
<tr>
<td>Krebs Cycle and other NAD- and NADP-dependent enzymes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>6.25</td>
<td>3.33</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>0.37</td>
<td>0.31</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Phosphatases:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATPase (Mg$^{2+}$-dependent)</td>
<td>0.03</td>
<td>29.41</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>0.90</td>
<td>35.71</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>1.49</td>
<td>22.72</td>
</tr>
<tr>
<td>Transaminase:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine oxoglutarate</td>
<td>0.02</td>
<td>50.00</td>
</tr>
<tr>
<td>Aspartate oxoglutarate</td>
<td>0.04</td>
<td>71.42</td>
</tr>
</tbody>
</table>

Thiabendazole and fenbendazole have been added to standard enzyme assay medium at the concentration of 100 $\mu$ mole in incubation medium dissolved in 0.1 to dimethyl sulfoxide (DMSO). Control incubations received equal amounts of DMSO. The enzyme activities were determined in triplicate which agreed within 5% limit. The mean values were plotted according to Lineweaver-Burk (Siegel, 1976), and $K_m$ and $V_{max}$ were directly determined from the plot.
CARBOHYDRATE METABOLISM

Glucose uptake

The uptake of $\textsuperscript{14}$C-glucose was found to be a non-linear two component process as a function of time and also showed substrate saturation kinetics. An initial rapid uptake for the first 2 min was followed by a second lower rate of uptake to the next 15 min, then achieving steady state (Fig. 17). From this study, experimental incubations of 2 min were chosen as optimum for further studies which showed the maximal initial velocity. During prolonged incubations from 1 to 5 hrs, although the parasites were visibly alive and showed movements, they did not accumulate further radioactivity; instead the uptake rate was inhibited. This inhibition was greater in the presence of thiabendazole and fenbendazole (inset of Fig. 17). Glucose absorption was also found to be a non-linear function of limiting substrate concentration (Fig. 18). However, no clear indication of sigmoidal curve was evident (Fig. 18a). Linear transformation of the data of different external substrate concentration and uptake velocity gave a straight line as in Eadie plot (Fig. 18b). Thiabendazole and fenbendazole inhibited the uptake as higher $K_t$ (lower substrate affinity constant) resulted in the presence of these drugs compared to the control while $J_{\text{max}}$ (maximum of apparent uptake velocity) was not altered.

$\textsuperscript{14}$C-glucose uptake was also found to be specifically dependent on Na$^+$ ions as replacing NaCl in assay system with
KCl and LiCl resulted in reduced uptake. Uptake in the presence of choline chloride represented the non-mediated portion of the process (passive influx) (Fig. 19). Effect of pH on $^{14}$C-glucose absorption was studied using three buffer systems ranging from pH 5 to 9 (Fig. 20). The uptake was found to be dependent on the changes in pH of incubation medium, the maximum absorption being at pH 7.4. The glucose absorption was also found to be temperature dependent (Fig. 21). Maximum uptake occurred around 37°C.

Fifteen different compounds including monosaccharides and other sugars, sugar derivatives and metabolic inhibitors were tested as potential inhibitors of $^{14}$C-glucose absorption (Table 2). Phlorizin, ouabain and D-glucose were found to be the most potent inhibitors of $^{14}$C-glucose uptake, accounting for 56.8%, 50% and 70.0% inhibition, respectively. AMP (27.3%), ATP (21.8%), Maltose (26.2%), and iodoacetate (19.9%) also caused considerable inhibition of $^{14}$C-glucose uptake. Strong inhibition of $^{14}$C-glucose uptake by phlorizin and ouabain further suggested that the uptake process was an active process. The inhibitory effect of maltose and other compounds might be indirect while the inhibition by glucose-6-phosphate could be caused by the hydrolysis product of the compound, resulting in a competitive inhibition by the glucose itself.
The interaction of \(^{14}\)C-glucose absorption with phlorizin, ouabain and D-glucose was further examined in greater detail. Dixon plots (1/V versus [I], where V is \(\mu\) moles of \(^{14}\)C-glucose uptake/mg ethanol extracted dry weight/min and [I] is concentration of the inhibitor) of the data were linear (Fig. 22-24). Hence each of these compounds appeared to inhibit the \(^{14}\)C-glucose absorption in a competitive manner. The inhibition constant (\(K_i\)) for D-glucose, phlorizin and ouabain were found to be 8, 5 and 7 mM respectively. Further, the replot of the slope versus 1/[I] of these inhibitors showed that inhibition by D-glucose was solely competitive because the Y intercept passed through zero (Inset of Fig. 22). The inhibition by phlorizin and ouabain was interpreted as partially competitive or mixed because the intercepts were found to be greater than zero (Insets of Figs. 23 & 24) (Siegel, 1976).

Absorbed glucose was readily incorporated into different fractions as shown by the following experimental evidences. Maximum of \(^{14}\)C-carbon from glucose got incorporated into glycogen and lipids. 35% of the total recovered radioactivity derived from uniformly labelled glucose was in the form of TCA soluble fraction (presumably unidentified glycolytic intermediates, unpolymerised pyrimidine and purine, nucleotides and amino acids) 25% in glycogen, 15% in lipids, 3% in proteins and 7.5% in RNA and only 1.3% in DNA. The amount of radiolabelled
Fig. 17. Time course studies of uptake of $^{14}$C-glucose by *T. globulosa* in the absence (0-0-0) and presence of 100 $\mu$ mole of thiabendazole (Δ-Δ-Δ) or fenbendazole (x-x-x), the results are mean ± S.E. of 3-4 observations.

Inset: Effect of long time intervals on uptake of $^{14}$C-glucose by *T. globulosa* in the absence (0-0-0) and presence of thiabendazole (Δ-Δ-Δ) and fenbendazole (x-x-x).
[V] μmoles/mg dry wt of T. globulosa

Fig. 17
Fig. 18. The velocity of $^{14}$C-glucose uptake by *T. globulosa* in the absence (0-0-0) and presence of 100 $\mu$ mole of thiabendazole (Δ-Δ-Δ) or fenbendazole (x-x-x), as a function of substrate concentration. The results are mean ± S.E. of 3-4 observations.

(a) $V (\mu$ mole of $^{14}$C-glucose uptake mg dry weight of *T. globulosa* vs. $S (\mu$ M glucose concentrations) plot.

(b) $V$ vs. $V/S$ (Eadie-Hofstee) plot.
Fig. 18
Fig. 19. Time course kinetics of uptake of $^{14}$C-glucose in $T$. globulosa in the presence of $\text{Na}^+$, $\text{K}^+$, $\text{Li}^+$ and choline$^+$. Each point is the mean of 4 determination. Longitudinal bars indicate S.D. of the mean.
μmoles glucose uptake/mg ethanol extracted dry wt. T. globulosa

Fig. 19
Fig. 20. Effect of pH on the influx of $^{14}$C-glucose by T. globulosa. Each point is the mean ± S.D. of 4 observations. Longitudinal bars represent S.D.
Fig. 20

µ moles glucose uptake/mg ethanol extracted dry wt. of T. globulosa/2 min

pH

The graph shows the relationship between pH and the uptake of glucose by T. globulosa, as well as the extracted dry weight over a period of 2 minutes.
Fig. 21. Effect of temperature on the influx of $^{14}$C-glucose by *T. globulosa*. Each point is the mean ± S.D. of 4 replicates. Longitudinal bars represent S.D.
Fig. 21
Fig. 22. Dixon plots (Velocity/Inhibitor concentration) of unlabelled glucose inhibition of $^{14}$C-glucose uptake in T. globulosa. Substrate concentrations were 5 mM, 10 mM, 25 mM and 50 mM. Each point is the average of 3 replicates.
Inset: Replot of the slope versus $1/(I)$ where $(I)$ is the concentration of the inhibition.
Inhibitor [I] = Unlabelled glucose [mM]

Inhibitor [I] = Unlabelled glucose [mM]

Fig. 22
Fig. 23. Dixon plots (Velocity/Inhibitor concentration) of phlorizin inhibition of $^{14}\text{C}$-glucose uptake in T. globulosa. Substrate concentrations were 5 mM, 10 mM, 25 mM and 50 mM. Each point is the average of 3 replicates.
Inset: Replot of the slope versus $1/(I)$ where (I) is the concentration of the inhibition.
Fig 23
Fig. 24. Dixon plot (Velocity/Inhibitor concentration) of ouabain inhibition of $^{14}$C-glucose uptake in *T. globulosa*. Substrate concentrations were 5 mM, 10 mM, 25 mM and 50 mM. Each point is the average of 3 replicates. Inset: Replot of the slope versus $1/(I)$ where $(I)$ is the concentration of the inhibition.
Table 2: Uptake of $^{14}C$-glucose in *T. globulosa* in the presence of certain compounds.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>$\mu$ moles of $^{14}C$-glucose uptaken/mg dry wt. of <em>T. globulosa</em>/2 min</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.63±0.5</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.48±0.5***</td>
<td>70.0</td>
</tr>
<tr>
<td>Fructose</td>
<td>1.44±0.5*</td>
<td>4.0</td>
</tr>
<tr>
<td>Maltose</td>
<td>1.20±0.3*</td>
<td>26.20</td>
</tr>
<tr>
<td>Lactose</td>
<td>1.39±0.2*</td>
<td>14.70</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.40±0.2*</td>
<td>13.90</td>
</tr>
<tr>
<td>Glucose-6-Phosphate</td>
<td>1.57±0.5</td>
<td>3.30</td>
</tr>
<tr>
<td>Glucose-1-Phosphate</td>
<td>1.49±0.1</td>
<td>8.50</td>
</tr>
<tr>
<td>Fructose-1, 6-diphosphate</td>
<td>1.45±0.5*</td>
<td>10.90</td>
</tr>
<tr>
<td>AMP</td>
<td>1.18±0.1**</td>
<td>27.30</td>
</tr>
<tr>
<td>ATP</td>
<td>1.27±0.2**</td>
<td>21.80</td>
</tr>
<tr>
<td>Phlorizin</td>
<td>0.70±0.5**</td>
<td>56.80</td>
</tr>
<tr>
<td>Ouabain</td>
<td>0.81±0.5***</td>
<td>50.10</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>1.30±0.5*</td>
<td>19.90</td>
</tr>
<tr>
<td>P-nitrophenyl phosphate</td>
<td>1.40±0.5*</td>
<td>14.4</td>
</tr>
</tbody>
</table>

Uptake values are mean ± S.D. of three independent observations. * p < 0.05; ** p < 0.01 and *** p < 0.001 indicate the levels of statistical significance compared to control as determined by student's 't' test.
Table 3: Incorporation of radioactivity of $^{14}$C-glucose into various tissue fractions of *T. globulosa*.

<table>
<thead>
<tr>
<th>Isolated fraction</th>
<th>%age of incorporated $^{14}$C-glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>TCA-soluble</td>
<td>50±2</td>
</tr>
<tr>
<td>Proteins</td>
<td>3±0.2</td>
</tr>
<tr>
<td>RNA</td>
<td>7.50</td>
</tr>
<tr>
<td>DNA</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Lipids</td>
<td>15±1</td>
</tr>
<tr>
<td>Glycogen</td>
<td>35±2</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>40±2</td>
</tr>
</tbody>
</table>

Note: %age of the particular fraction is calculated by counts per minute for that particular fraction and by taking total incorporation of $^{14}$C-glucose/min as 100 counts. Each value is mean ± S.D. of 3 determinations.
Table 4: $\text{U}^{14}\text{C-D- glucose uptake in ligated and non-ligated } Trichuris \text{ globulosa}$

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control (non-ligated)</th>
<th>Experimental (ligated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>121644±1033</td>
<td>65900±2193</td>
</tr>
<tr>
<td>10</td>
<td>151245±1142</td>
<td>80334±3344</td>
</tr>
<tr>
<td>30</td>
<td>205600±7725</td>
<td>122466±6712</td>
</tr>
</tbody>
</table>

Data is expressed as CPM/100 mg of ethanol extracted Trichuris globulosa. mean ± S.D. of 3-4 observations.
Table 5: U-¹⁴C-L-alanine uptake in ligated and non-ligated *Trichuris globulosa*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (non-ligated)</th>
<th>Experimental (ligated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5278±100</td>
<td>1056±100</td>
</tr>
<tr>
<td>10</td>
<td>9345±246</td>
<td>3689±150</td>
</tr>
<tr>
<td>30</td>
<td>12478±270</td>
<td>6839±275</td>
</tr>
</tbody>
</table>

The data have been expressed as CPM 100 mg of ethanol extracted *T. globulosa*, mean ± S.D. of 3-4 observations.
Table 6: $^{14}$C-L-leucine uptake in ligated and non-ligated *Trichuris globulosa*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (non-ligated)</th>
<th>Experimental (ligated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3250±50</td>
<td>1000±102</td>
</tr>
<tr>
<td>10</td>
<td>8490±150</td>
<td>3080±140</td>
</tr>
<tr>
<td>30</td>
<td>11340±150</td>
<td>6830±200</td>
</tr>
</tbody>
</table>

The data have been expressed as CPM/100 mg of ethanol extracted *T. globulosa*, mean ± S.D. of 3-4 observations.
Table 7: U-\(^{14}\)C-L-aspartic acid uptake in ligated and non-ligated *Trichuris globulossa*.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (non-ligated)</th>
<th>Experimental (ligated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5123±200</td>
<td>2531±100</td>
</tr>
<tr>
<td>10</td>
<td>8236±222</td>
<td>2247±110</td>
</tr>
<tr>
<td>30</td>
<td>10343±324</td>
<td>4234±200</td>
</tr>
</tbody>
</table>

The data have been expressed as CPM/100 mg of ethanol extracted *Trichuris globulosa*, mean ± S.D. of 3-4 observation.
carbon incorporated into CO$_2$ was also measured separately. Approximately, 35% of the total recovered radioactivity was in the form of CO$_2$.

The incorporation of $^{14}$C-glucose into different fractions was also inhibited by both the anthelmintics, thiabendazole and fenbendazole. It was seen that thiabendazole inhibited the incorporation to a greater level (Table 3).

Transcuticular uptake

The uptake of $^{14}$C-glucose took place as a function of time by transcuticular absorption (Table 4). The uptake observed in non-ligated parasites was higher as compared to the uptake by ligated parasites; some of the absorption also took place by ingestion.

Similar results were obtained with L-U-$^{14}$C-aspartic acic, L-U-$^{14}$C-leucine and L-U-$^{14}$C-alanine (Table 5, 6 and 7).

AMINO ACID METABOLISM

Uptake of Amino Acids

$^{14}$C-L-aspartic acid, $^{14}$C-L-alanine and $^{14}$C-L-leucine were rapidly absorbed initially but the rate of uptake declined with time and achieved a steady state (Figs. 25-27), possibly owing to a saturation in the transport loci. The uptake of all the
three amino acids by *T. globulosa* was found to be inhibited in the presence of thiabendazole and fenbendazole (Fig. 25-27). The absorption of amino acids were also found to be non-linear function of limiting substrate concentration (Fig. 28-30). Linear transformations of the data gave straight line in Lineweaver-Burk and Eadie plot (inset of Fig. 28-30). Thiabendazole and fenbendazole inhibited the uptake of all the three amino acids as a higher $K_t$ (lower substrate affinity constant) resulted in the presence of these drugs compared to the control, while the $J_{\text{max}}$ was unaltered.

Amino acid absorption was found to be dependent on pH and temperature. Uptake of $^{14}$C-L-aspartic acid $^{14}$C-L-alanine and $^{14}$C-L-leucine was optimum at pH 7.6, 8.4 and 9.2 respectively (Fig. 31). The transport was also affected by changing the temperature. The rate of uptake was increased with temperature up to 50°C, followed by steady state or even decreased thereafter when studied upto 60°C (Fig. 32).

The uptake of all the three $^{14}$C-amino acids by *T. globulosa* was found to be specifically dependent on Na$^+$ ions as replacing NaCl in assay system isosmotically with KCl and LiCl resulted in less uptake. The uptake in the presence of choline chloride represented the passive influx (non-mediated absorption) (Figs. 33-35).
Fig. 25. Time course study of uptake of $^{14}$C-L-aspartic acid by *T. globulosa* in the absence (0-0-0) and presence of thiabendazole (Δ-Δ-Δ) or fenbendazole (x-x-x). $\nu$ is the uptake velocity. The results are mean ± S.D. of 4 observations. Longitudinal bars represent S.D.
Fig. 25

V (µmoles/mg ethanol extracted dry wt.)

5 15 30 60
min
Fig. 26. Time course study of uptake of $^{14}$C-L-alanine by *T. globulosa* in the absence (0-0-0) and presence of thiabendazole (Δ-Δ-Δ) or fenbendazole (x-x-x). $V$ is the uptake velocity. The results are means ± S.D. of 4 observations. Longitudinal bars represent S.D.
Fig. 26

$V$ (µ moles/mg ethanol extracted dry wt.)
Fig. 27. Time course study of uptake of $^{14}$C-L-leucine by *T. globulosa* in the absence (0-0-0) and presence of thiabendazole (Δ-Δ-Δ) or fenbendazole (x-x-x). $V$ is the uptake velocity. The results are means ± S.D. of 4 observations. Longitudinal bars represent S.D.
Fig. 27

V (μ moles/mg ethanol extracted dry wt.)

min

5 15 30 60

0.75
0.5
0.25
1

Fig. 27
Fig. 28. The velocity of uptake (V) of $^{14}$C-L-aspartic acid by *Trichuris globulosa* in the absence (0-0-0) and presence of thiabendazole (Δ-Δ-Δ) or fenbendazole (x-x-x), as the function of substrate concentration(s). The results are mean ± S.D. of 4 replicates.

Longitudinal bars indicate S.D.

Inset: Eadie-Hofstee plot of uptake of $^{14}$C-L-aspartic acid.
Fig. 28

V (umoles/mg ethanol extracted dry wt./min)

V/S

S (Aspartic acid, mM)

Fig. 28
Fig. 29. The velocity of uptake (V) of $^{14}$C-L-alanine by 
*Trichuris globulosa* in the absence (0-0-0) and 
presence of thiabendazole (Δ-Δ-Δ) or fenbendazole 
(x-x-x), as the function of substrate 
concentration(S). The results are mean ± S.D. of 
4 replicates. Longitudinal bars indicate S.D. 
Inset: Radio-Hofstee plot of uptake of $^{14}$C-L-alanine.
Fig. 29

\[ V \sim \frac{1}{S} \]

\[ V(S) = \frac{V_0}{1 + \frac{S}{K}} \]

V: velocity of the reaction
S: substrate concentration
V₀: maximum velocity
K: Michaelis constant
Fig. 30. The velocity of uptake (V) of $^{14}$C-L-leucine by *Trichuris globulosa* in the absence (0-0-0) and presence of thiabendazole (Δ-Δ-Δ) or fenbendazole (x-x-x), as the function of substrate concentration(S). The results are mean ± S.D. of 4 replicates. Longitudinal bars indicate S.D. Inset: Eadie - Hofstee plot of uptake of $^{14}$C-L-leucine.
Fig. 31. Effect of pH on the influx of $^{14}$C-L-aspartic acid (---), $^{14}$C-L-alanine (-----), and leucine (----...----) by T. globulosa. Each point is the mean ± S.D. of 4 replicates. V denotes uptake velocity. Longitudinal bars represent S.D.
Fig. 31

[Graph showing pH vs. V (μmoles/mg dry wt./min)]
Fig. 32. Effect of temperature on the absorption of $^{14}$C-L-aspartic acid (——), $^{14}$C-L-alanine (-----), and $^{14}$C-L-leucine (---). Each point is mean ± S.D. of 4 replication. V denotes uptake velocity. Longitudinal bars represent S.D.
Fig. 32
Fig. 33. Time course kinetics of uptake of $^{14}$C-L-aspartic acid in *T. globulosa* in the presence of Na$^+$, K$^+$, Li$^+$ and Choline. $V$ is the uptake velocity ($\mu$ moles/mg dry weight). Each point is mean of 4 determinations. Longitudinal bars indicate S.D. of the mean.
Aspartic acid

Fig. 33
Fig. 34. Time course kinetics of uptake of $^{14}$C-L-alanine by $T. globulosa$ in the presence of Na$^+$, K$^+$, Li$^+$ and Choline$^+$. $V$ is the uptake velocity (µ moles/mg dry weight). Each point is mean of 4 determinations. Longitudinal bars indicate S.D. of the mean.
Alanine
5 10 30 90 min

Fig. 34
Fig. 35. Time course kinetics of uptake of $^{14}$C-L-leucine in T. globulosa in the presence of Na$^+$, K$^+$, Li$^+$ and Choline$^+$. $V$ is the uptake velocity ($\mu$ moles/mg dry weight. Each point is mean of 4 determinations. Longitudinal bars indicate S.D. of the mean.
Fig. 35

Leucine

$V$ (μmoles/mg dry wt.)

min

Na$^+$
K$^+$
Li$^+$
Choline$^+$

Fig. 35
Fig. 36. Effect of 1.0 mM DL-aspartic acid, L-alanine, iodoacetate, ATP, AMP, L-leucine, isoleucine, ouabain and methionine on the uptake of $^{14}$C-L-aspartic acid in T. globulosa.

Incubation time: 30 min; $S$ = mM substrate concentration, $V$ = uptake velocity (μ moles/mg ethanol extracted dry weight/min).
Fig. 36
Effect of 1.0 mM DL-aspartic acid, glucose, L-alanine, iodoacetate, ATP, AMP, L-leucine, isoleucine, ouabain and methionine on the uptake of $^{14}$C-L-alanine in T. globulosa.

Incubation time: 30 min; $S$ = mM substrate concentration, $V$ = uptake velocity ($\mu$ moles/mg ethanol extracted dry weight/min).
Methionine
Ouabain
Iodoacetate
Leucine
Aspartic acid
Isoleucine
Normal

$\frac{1}{V_0}$ (umoles/mg dry wt./min)

$\frac{1}{S}$ (Alanine, mM)

Fig. 37
Fig. 38

Leucine

$V_v$ (μmoles/mg dry wt./min)

$V_S$ (Leucine, mM)

Methionine
Ouabain
Iodoacetate
ATP
AMP
Alanine
Isoleucine
Glucose
Aspartic acid
Normal
Fig. 38. Effect of 1.0 mM DL-aspartic acid, glucose, L-alanine, iodoacetate, ATP, AMP, L-leucine, isoleucine, ouabain and methionine on the uptake of $^{14}$C-L-leucine in *T. globulosa*. Incubation time : 30 min, $S$ = mM substrate concentration.
$V$ = uptake velocity (μ moles/mg ethanol extracted dry weight/min).
Table 8. Effect of different compounds on uptake of amino acids by Trichuris globulosa.

<table>
<thead>
<tr>
<th>Compounds tested</th>
<th>( K_t )</th>
<th>( J_{\text{max}} )</th>
<th>( K_{i1} )</th>
<th>( J_{\text{max}} )</th>
<th>( K_{i2} )</th>
<th>( J_{\text{max}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.30</td>
<td>2.5</td>
<td>100</td>
<td>0.50</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>DL-aspartic acid</td>
<td>0.37</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>0.75</td>
<td>10</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.57</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>0.54</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>L-alanine</td>
<td>0.58</td>
<td>1.2</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>ATP</td>
<td>0.58</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>L-leucine</td>
<td>0.54</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>0.58</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>ATP</td>
<td>0.58</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
<tr>
<td>L-leucine</td>
<td>0.54</td>
<td>2.1</td>
<td>50</td>
<td>0.59</td>
<td>3.3</td>
<td>10</td>
</tr>
</tbody>
</table>

\( K_t \) is mM substrate affinity constant. \( J_{\text{max}} \) is maximum velocity in \( \mu \) moles/mg dry weight/min and \( K_{i} \) is mM reciprocal inhibition constant. Each compound has been added at the concentration of 1.0 mM in the incubation medium.
Table 9. Incorporation of radioactivity of amino acids into various tissue fractions of *Trichuris globulosa.*

<table>
<thead>
<tr>
<th>Isolated fraction</th>
<th>%age of incorporation of $^{14}$C-L-aspartic acid</th>
<th>%age of incorporation of $^{14}$C-L-alanine</th>
<th>%age of incorporation of $^{14}$C-L-leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal/Fenbendazole treated/Thiabendazole treated/Fenbendazole treated</td>
<td>Normal/Fenbendazole treated/Thiabendazole treated/Fenbendazole treated</td>
<td>Normal/Fenbendazole treated/Thiabendazole treated/Fenbendazole treated</td>
</tr>
<tr>
<td>RNA</td>
<td>10±1/7±1.5/8±1</td>
<td>7±2/4±0.3/5.5±0.9</td>
<td>9±1/5±1/7±1.5</td>
</tr>
<tr>
<td>DNA</td>
<td>3±0.2/1.5±0.3/2.5±0.5</td>
<td>2±0.5/1±0.2/1.5±0.4</td>
<td>3±0.3/1±0.2/1.8±0.5</td>
</tr>
<tr>
<td>Lipids</td>
<td>5±0.8/2.5±0.7/3±0.8</td>
<td>6±0.8/3±0.2/4±0.9</td>
<td>4±0.9/2±0.3/2.3±0.5</td>
</tr>
<tr>
<td>Glycogen</td>
<td>1.5±0.8/0.5±0.2/0.9±0.2</td>
<td>1.9±0.2/0.8±0.2/1±0.4</td>
<td>1.7±0.5/0.8±0.1/1.2±0.4</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>10±1/8±1/8±2</td>
<td>12±1/8±0.8/9±2</td>
<td>15±2/10±0.8/11±0.9</td>
</tr>
</tbody>
</table>

Note: %age of the particular fraction is calculated by counts per min for that particular fraction and by taking total incorporation of $^{14}$C-L-amino acid/min as 100 counts.

Each value is mean ± S.D. of 3 determinations.
In the presence of the drugs, thiabendazole and fenbendazole, the incorporation of amino acids into different macromolecular fractions was inhibited. The inhibition was significant in case of TCA soluble fraction, protein fraction and also CO₂ evolved in an in vitro incubation (Table 9).

HISTOLOGICAL OBSERVATIONS

Trichuris globulosa measures 30 to 50 mm in length in which the thread like oesophageal region (whip) occupies almost two-third of the total length and the thick posterior part of the body contains the intestine and reproductive organs (Fig. 1). Worms were abundantly found during the rainy season and the females, predominantly found in infection, constituted approximately 70-90% of total Trichuris population. The reproductive organs are simple tubular structures which are continuous with their ducts so that neither sperm nor eggs can be shed into the body cavity. The males are slightly smaller than females and can be distinguished by the curled tail end of the body (Fig. 2).

Body Wall

The outermost layer of body wall is cuticle. It consists of an outer cortical layer, a middle median or matrix layer and inner basal or fiber layer. The cortical layer is further divided into two layers, the external cortical layer or
epicuticle and internal cortical layer. The cortical, median and fibre layers are non-cellular (Pmg. 1).

The hypodermis is thin and is situated directly beneath the cuticle. Hypodermis in oesophageal region becomes relatively large to give rise to a number of columnar projections termed the bacillary band (Pmg. 1). The latter forms a specialized zone of the cuticle while the hypodermis is confined to the lateral region. The structure of the bacillary band shows that it is a modified hypodermal chord, the hypodermal projections penetrating the cuticular layers and opening the exterior through cuticular pores (Pmg. 2). The bacillary band is one-celled thick and contains distinct gland cells termed the bacillary cells.

A single layer of somatic muscles is present beneath the hypodermis. The musculature in *T. globulosa* is polymyarian and coelomyarian type viz. the muscle cells are numerous and muscle fibres are next and perpendicularly to the hypodermis. The muscles show two distinct sub-regions, the proximal fibrillar zone or the contractile part that lies towards the hypodermis, an inner distal nucleated zone or the non-contractile part (sarcoplasmic part) (Pmg. 1 & 2). The body cavity or pseudocoel encloses various visceral organs such as alimentary canal and reproductive tract.
Alimentary Canal

Oesophagus

The pseudocoel of this region is almost entirely occupied by a large cytoplasmic body known as the stichosome or cell body (Pmg. 2 & 8). This structure encloses a narrow capillary like oesophagus. The stichosome possesses numerous fine canaliculi and has a segmented appearance. The segmentation appears to have been caused by invaginations at several points. The stichosome is a collection of gland cells or stichocytes each of which possesses a large nearly spherical nucleus (Pmg. 2).

Intestine

It is a straight tube extending through the thick posterior portion of the worm. It consists of tall columnar epithelial cells resting on basement membrane. It lacks musculature. The luminal surface of the intestine is furnished with a brush border composed of microvilli (Pmg. 12 & 13). The shape of the lumen varies depending on the presence and absence of food in it. In the absence of any food material the lumen gets closed due to the exerted by turgor pressure the pseudocoelomic fluid.

Female reproductive system

The female reproductive system is monodelphic and tubular. Female reproductive system consists of a thread like ovary, long and narrow u-shaped oviduct, tubular seminal receptacle
or spermatheca, a broad uterus, a short muscular vagina and vulva which is distinct and in the form of transverse slit. The latter is situated anteriorly near the junction of the oesophagus and the intestine.

Ovary

Ovary is hologonic i.e. the germ cell formation is not confined to one end of the ovary but occurs throughout its length. It is surrounded by a thin layer of squamous epithelium. These epithelial cells are elongated, flattened and have a distinct nucleus. Rachis is lacking. The oogonia and developing oocytes completely fill the ovary (Pmg. 17).

Oviduct

Oviduct is a long, narrow tube with epithelial walls formed by cuboidal cells and is a continuation of the epithelial covering of the ovary. Oviduct contains the mature oocytes (Pmg. 9). The oogonia and oocytes contain the cytoplasmic inclusions i.e. the refringent granules and hyaline spherules (Pmg. 3). The mature oocyte have a spherical nucleus with a prominent nucleolus.

Seminal receptacle

Distally, the oviduct gradually becomes narrow to change into tubular seminal receptacle which is known to store thousands
of spermatozoa and fertilization of oocytes taken place here.
The wall of seminal receptacle is composed of single layer of
cuboidal epithelium enclosing a narrow lumen (Pmg. 4). These
epithelial cells have prominent nucleus and nucleolus. The inner
surface of epithelial cells is not smooth but produces irregular
outgrowths of various sizes and shapes projecting into the lumen
of the seminal receptacle.

**Uterus**

The seminal vesicle leads into uterus. The wall of the
uterus consists of epithelium made up of flat cells covered with
a well developed muscular layer with circular and oblique fibers
(Pmg. 11 & 12). These muscles are responsible for moulding
the shape of egg before the shell hardens.

**Vagina**

The uterus continues distally as a highly muscular short
tube, the vagina (Pmg. 3 & 4) which is lined internally with
cuticle. Vagina opens to the outside through the female genital
pore or vulva. The vulval opening is surrounded by a ring of
cuticle.

**Egg**

The morphological differentiation of layers of the egg
shell has been made on the basis of observations on stained
sections and teased preparations of the unfixed material.
shaped eggs are observed in the lumen of middle and distal part of the uterus in different stages of shell formation. The proximal part of uterus contains the fertilized oocytes. Early eggs are covered by two distinct layers separated by a gap. In the distal region, uterus is full of eggs having fully formed egg shells. At this stage the egg shell comprises three distinct layers (Figs. 7 & 11). The egg cytoplasm is immediately surrounded by a thin lipid layer followed by a thick chitinous layer. These two layers develop internally to original oocyte oolemma which is known as vitelline layer. The diameter of the egg did not increase during egg shell formation. An opercular plug is present at both the ends of the egg.

**Male reproductive system**

The male reproductive system is monarchic. The testis is the thinner most part of the reproductive system. It leads into a tubular seminal vesicle which opens into the vas deferens, which in its turn joins the ejaculatory duct posteriorly. The latter joins the intestine a long way from the anus, forming a cloacal tube. The cloacal tube joins the spicular tube containing the spicule and the spicular sheath (Fig. 2).

**Testis**

Testis is hologonic and is covered by a thin layer of epithelium which is continuous with the epithelium of the
gonoduct (Pmg. 13). The epithelial cells have prominent nucleus.

**Seminal vesicle**

The epithelial cells lining this region are flat and elongated with prominent nucleus.

**Vas deferens**

Vas deferens is comparatively a wider glandular duct, which is subdivided into three regions, viz. anterior, middle and posterior. Vas deferens is surrounded by weak muscle layer and tall epithelial cells lining the lumen. These show a decrease in height from anterior to posterior direction. The cells of anterior vas deferens are somewhat loosely packed as compared to the other two regions. At certain places the cells lining the anterior vas deferens have pseudopodia-like projections in the lumen (Pmg. 13). The cells appear to contain massive inclusions which are presumably the engulfed residue left after the formation of the spermatid.

**Ejaculatory duct**

The ejaculatory duct has a thicker and more defined layer of muscle fibres. The lumen is much narrower as compared to vas deferens and is lined with epithelial cells which produce folds or villi into the lumen (Pmg. 15).
Table 10: Distribution and localization of carbohydrates and proteins in *Trichuris globulosa*.

<table>
<thead>
<tr>
<th>Anatomical features of the worm</th>
<th>Best carmine</th>
<th>PAS</th>
<th>Murcuric bromophenol blue</th>
<th>Ninhydrin Schiff</th>
<th>Toludine blue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body wall</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuticle</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Hypodermis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Musculature</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Oesophagus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stichosome</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Intestine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Ovary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Oogonia</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Oviduct</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Seminal receptacle</strong></td>
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Pmg. 1. Section showing different layers of body wall (Haematoxylin/eosin x 1000).

Pmg. 2. Section showing stichosome (Haematoxylin - eosin x 1000).
Ping. 3. Section showing vaginal wall (Haematoxylin -
eosin x 1000).

Ping. 4. Section showing seminal receptacle, oviduct and
vagina (Haematoxylin - eosiin x 125).
Pmg. 5. Section showing oviduct containing female germ cells (Haematoxylin - eosin x 1000).

Pmg. 6. Section showing proximal part of uterus containing oocytes (Haematoxylin - eosin x 1000).
Pmg. 7. Section showing detailed structure of egg (Haematoxylin - eosin x 1000).

Pmg. 8. Section showing glycogen deposits in body wall, (Bouin’s/Best carmine x 125).
Pmg. 9. Section showing glycogen deposits in the body wall, intestinal epithelium and egg cytoplasm (Bouin's/Best Cremine x 125).

Pmg. 10. Section showing intestinal epithelium having mucopolysaccharides with β-metachromasia (Bouin's/Toluidine blue x 125).
Pmg. 11. Section showing uterine epithelium, cuticle of egg shell and body wall musculature, bacillary band and hypodermis having mucopolysaccharides with ε-metachromasia (Bouin's/Toluidin blue x 125).

Pmg. 12. Section showing intestinal epithelium, cytoplasm of the eggs, having good amount of carbohydrates (Zenker/Periodic acid-Schiff x 125).
Ping. 13. Section showing vas deferens spermatogonia, spermatids and seminal vesicle containing good amount of carbohydrates (Zenker/Periodic acid-Schiff x 160).

Ping. 14. Section showing bacillary band of body wall and female germ cells rich in proteins (Zenker/Mercuric bromophenol blue x 750).
Cloacal tube

Cloacal tube is a long narrow tubular structure, lined internally by a layer of cuticle and an outer muscular layer (Fig. 2).

Spicule

The cloacal tube joins the spicular tube containing the spicule and its sheath or cirrus (Fig. 2). A single spicule is present. It is very long and covered with cuticle which is continuous with the cuticular lining of spicular tube. Spicular sheath is large, somewhat cylindrical and is beset with numerous recurved spines.

Spermatogenesis comprises the production of spermatogonia and ultimate development to spermatozoa. The spermatogonia comprises closely packed cells with large nuclei and indistinct cell boundaries. The spermatogonia become clearly defined and then change into spermatocytes which in turn give rise to spermatids (Fig. 13). The spermatids are said to develop into spermatozoa in the female.

HISTOCHEMICAL OBSERVATIONS

Carbohydrate

Distribution and localization studies of different carbohydrates were made on the basis of colouration and reactions during
test for carbohydrates i.e. periodic acid Schiff-test (PAS) for total carbohydrates and Best's carmine test for glycogen (Table 10). Presence of good amount of carbohydrates were observed in the intestinal epithelium, polar plugs and cytoplasm of the eggs which showed intense reaction with PAS (Pmg. 12). Carbohydrates in moderate amount were found to be present in almost all the organs of the nematode, i.e. body wall, oesophagus, intestine, female reproductive system, female germ cells (oogonia and oocytes), eggs and male reproductive system (Pmg. 12 and 13). Cuticle of body wall and vagina, and lipid layer of egg shell were found to be without carbohydrates (Table 10).

Muscles of body wall, intestinal epithelium, and egg cytoplasm were shown to have glycogen deposits as these structures were deeply stained with Best's carmine. Except cuticle of body wall, vaginal cuticle and lipid layer of egg shell, all the structures were found to have glycogen deposits in moderate amount (Pmg. 8 & 9).

Toluidine blue method for metachromasia stained mucopolysaccharides with \(\gamma\)-metachromasia in pink, mucopolysaccharides with \(\beta\)-metachromasia in purple and mucopolysaccharides with \(\alpha\)-metachromasia in blue colour. Cuticle of body wall, vaginal cuticle and lipid layer of egg was not found to show any
metachromasia. Uterine epithelium, cuticle of egg shell and body wall (Musculature, bacillary band and hypodermis) were found to show the presence of mucopolysaccharides with α-metachromasia, whereas vitelline layer of egg shell, cytoplasm of egg and intestinal epithelium were shown to have mucopolysaccharides with γ- and β-metachromasia, respectively (Pmg. 10 & 11 and Table 10).

Proteins

Deep blue colour of mercuric bromophenol blue (MBPB) was marked intensly in bacillary band of body wall, female germ cells and epithelial layer of seminal receptacle. Except the lipid layer of egg shell, all the organs were shown to have moderate amount of proteins by showing positive reaction with MBPB (Pmg. 14 & 15, Table 10).

Presence of ω-amino acids was intense in body wall, intestinal epithelium, female germ cells, wall of uterus, egg cytoplasm and germ cells reacted intensely with ninhydrin Schiff reaction test showing the presence of ω-amino acids. Rest of the organs reacted feebly (Pmg. 16 & 17, Table 10).

Histoenzymology

Alkaline and acid phosphatase enzymes activities were shown to be intense in intestinal epithelium and egg shell. The
cuticle of body wall was found to be devoid of activity of these enzymes whereas the rest of the structures showed moderate activity (Table 11, Pmg. 18-20).

Bacillary band and uterine epithelium were found to give intense reaction to SDH activity while the activity was found to be moderate in most of the other structures. Feeble activity was found in male reproductive system and oesophagus. The SDH activity was completely absent in cuticle of body wall, vaginal cuticle and egg shell (Pmg. 21, 23, Table 11).

Intestinal epithelium strongly reacted with the ATPase activity test. Otherwise moderate activity was reported in all the organs except hypodermis of body wall, seminal vesicle and ejaculatory duct, vaginal and uterine musculature which were shown to be feebly reactive toward the test of this enzymatic activity (Pmg. 22, 24 & 25, Table 11).

Glucose-6-phosphatase activity was found to be intense in intestinal epithelium only. Body wall musculature, stichosome, ovary, eggs, male germ cells (spermatogonia) and epithelial layer of vas deferens were shown to have moderate activity of glucose-6-phosphatase. Activity was feeble in hypodermis of body wall, musculature, uterine epithelium, vagina, testicular epithelium, seminal receptacle and ejaculatory duct while it was absent in cuticle of body wall (Table 11, Pmg. 26 & 27).
Pmg. 15. Section showing outer layers of body wall containing very little proteins as compared to bacillary band. (Zenker/Mercuric bromophenol blue x 750).

Pmg. 16. Section showing Ejeculatory duct, having little of α-amino acids (Zenker/Ninhydrin-Schiff x 125).
Ping. 17. Section showing presence of $\alpha$-amino acids in intestinal epithelium, female germ cells, uterine wall and egg cytoplasm (Zenker/Ninhydrin-Schiff x 125).

Ping. 18. Section showing intense activity of alkaline phosphatase in intestinal epithelium (unfixed cryostat section x 125).
Pmg. 19. Section showing cuticle of body wall devoid of alkaline phosphatase activity whereas the activity is enough in egg-shells. (unfixed cryostat section x 100).

Pmg. 20. Section showing intense activity of acid phosphatase in intestinal epithelium and egg shells (unfixed cryostat section x 125).
Ping. 21. Section showing intense activity of succinate dehydrogenase (SDH) in bacillary band and uterine epithelium (unfixed cryostat section x 125).

Ping. 22. Section showing feeble activity of Adenosine triphosphatase (ATPase) in egg shells and egg cytoplasm (unfixed cryostat section x 125).
Pmg. 23. Section showing no activity of SDH in cuticle of body wall (unfixed cryostat section x 100).

Pmg. 24. Section showing strong activity of Adenosine triphosphatase (ATPase) in intestinal epithelium (unfixed cryostat section x 125).
Pmg. 25. Section showing feeble activity of ATPase in uterine wall and egg cytoplasm (unfixed cryostat section x 125).

Pmg. 26. Section showing intense activity of glucose-6-phosphatase in intestinal epithelium (unfixed cryostat section x 125).
Fig. 27. Section showing weak activity of glucose-6-phosphatase in uterine wall, eggs and almost no activity in the cuticle of body wall (unfixed cryostat section x 125).