*Cotugnia digonopora*, a coparasite of *Ascaridia galli* in fowl intestines, for which no information regarding nutritional or metabolic aspects is yet available, was found to serve as an excellent model for conducting biochemical studies on comparative basis in the parasites of the same niche. Since carbohydrates constitute the major, if not the exclusive, source of energy in helminth parasites, various aspects of sugar metabolism in this cestode were investigated. Effect of certain known and promising anticestodals as well as a few other compounds was simultaneously examined. The major findings are as follows:

**CARBOHYDRATE METABOLISM IN WHOLE WORM:**

*C. digonopora* exhibited a high rate of glucose consumption, corresponding to 40% fresh body weight in 24 hr. Radiocarbon from glucose was found to be maximally incorporated into ether soluble organic acids; CO₂, ketoacids and lactate, comparatively, were produced in very low amounts. Pyruvate was the main keto-acid formed. Among body constituents, the 'free pool component' accounted for approximately 23% radioactivity of the consumed sugar. This was followed by glycogen while lipids, nucleic acids and proteins were poorly labeled.
Excretion of semioxidized organic acids predominated over other metabolic end-products of G. digonopora. Though the nature of these acids was not identified, steam volatile acids accounted for more than 25% of this fraction. These evidences, therefore, suggested that G. digonopora, like many other parasites, was also characterized for possessing an anaerobic type of metabolism. Interestingly, when the worms did not receive exogenously supplied glucose, lactate and other organic acids were found to be excreted in the medium at the expense of endogenous glycogen. This indicated that under starvation the cestode utilized reserve polysaccharide for maintaining its physiological needs.

ENZYMES OF CARBOHYDRATE METABOLISM IN CYTOSOL:

G. digonopora was found actively equipped upto PEP-level, with most of the enzymes of Embden-Meyerhof pathway. All the enzymes, as usual, did not show equal activities. Thus phosphoglucoisomerase (PGI), glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and aldolase exhibited very high activities. In contrast, hexokinase (HK) and phosphofructokinase (PFK) showed very low activities and hence appeared to catalyze the rate limiting steps of glycolysis. Similarly, glucose-6-phosphatase (G6Pase) and fructose-1,6-diphosphatase
(PDPase), showing low activities could be the means of control for gluconeogenesis and glycogenesis. Extremely poor glucose-6-phosphate dehydrogenase (G6PDH), yielding a ratio of 0.004 with PG1, indicated that the metabolism of glucose-6-phosphate through pentose-phosphate shunt was negligible and channelized mainly through glycolysis. The activity of glycogen phosphorylase, catalysing the breakdown of glycogen was, however, quite significant. Specific activity and catalytic properties of the enzymes concerned with the metabolism of PEP further explained the fate of carbon source during its catabolism. Activities of pyruvate kinase (PK) and lactate dehydrogenase (LDH) in C. digenopora were significantly lower than those of PEP-carboxykinase (PEPCK) and malate dehydrogenase (MDH). Ratios of the activities of the two kinases (PK/PEPCK) and dehydrogenases (LDH/MDH) were found to be of the order of 0.077 and 0.008 respectively. This suggested that the metabolism of PEP should be favoured in the direction of malate formation. Such a conclusion was also supported by the observation made above regarding the predominance of organic other than lactic acid among the metabolic end-products.

PK and PEPCK, regarding their dependance on coenzymes differed with each other; PK used ADP while PEPCK required GDP. However, their requirement for
divalent ions, Mg$^{++}$ and Mn$^{++}$ appeared to be similar. It was unusual to notice that Mg$^{++}$ and Mn$^{++}$ activated both the enzymes to the same extent. MDH, as judged by its relative activity in forward and backward directions, was found to catalyze the reduction of oxaloacetate (OAA) at a rate 17 times higher to that of the oxidation of malate. Qualitative differences in the activities and the number of isozymes of LDH and MDH were also demonstrated on polyacrylamide gel electrophoresis (PAGE) employing enzyme specific staining. The latter enzyme exhibited seven bands and three of them, constituting about 80% of the total activity, were cathodal.

All the enzymes, mentioned above were predominantly cytosolic in origin except that some activity of HK and MDH was detected in mitochondrial fraction also.

**Enzymes of Carbohydrate Metabolism in Mitochondria:**

Presence of isocitrate dehydrogenase in amounts below the level of detection revealed that TCA-cycle plays a physiologically insignificant role in *G. digonopora*. However, the enzymes concerned with malate metabolism were quite prominent. Both, malic enzyme (ME) and fumarase, utilizing malate as the common substrate, were present in high amounts. The former enzyme required NADP but not NAD as the coenzyme and was equally activated by
either Mg$^{++}$ or Mn$^{++}$. ME was found to favour the
decarboxylation of malate at a rate three times higher
to that of malate formation from pyruvate. It, therefore,
appeared that malate in this cestode also undergoes
dismutation reaction catalyzed by ME and fumarase.
Furthermore, fumarate reductase (FR), required for the
conversion of fumarate to succinate, was also demonstrated
to be present in significant amount in the mitochondria
of *G. digonopora*.

**ENZYMES OF BRUSH BORDER MEMBRANE (BBM):**

Purified BBM of *G. digonopora* exhibited the presence
of a number of phosphohydrolases. Among these, alkaline
phosphatase was extremely active. However, other enzymes
like G6Pase, FDPase, cAMP-phosphodiesterase, 5'-nucleo-
tidase (5'-Ntase) and adenosine-triphosphatase (ATPase)
were also quite active. Interesting observations were
recorded regarding the activities of various ATPases;
whereas the enzyme was activated by Ca$^{++}$ and Mg$^{++}$ in an
additive manner, its sensitivity to ouabain was negligible.
Furthermore, in presence of EDTA the enzyme activity was
unexpectedly quite significant. These results could
infer upon either of the followings; i) presence of
tightly bound Ca$^{++}$ and Mg$^{++}$, ii) an absence of a sodium
pump, iii) presence of an ouabain insensitive Na$^+$, K$^+$-
ATPase, and iv) presence of a permeability barrier for
ouabain near the site of Na\(^+\), K\(^+\)-ATPase (even in the isolated BBM).

**PURIFICATION AND CHARACTERIZATION OF MALATE DEHYDROGENASE:**

MDH from *G. digonopora* was purified 63-fold employing a two-step procedure. In the first step, out of seven isozymes present in the crude preparation, the major three cathodal forms (representing 80% of the total activity) were separated from the rest by negative adsorption on DEAE-cellulose column. This fraction was further purified by affinity chromatography on Blue Sepharose. On PAGE, the purified preparation illustrated the presence of three bands representing for both protein as well as the activity. These bands were found to match well with the corresponding activity bands obtained with the crude extract. The purified enzyme on Sephadex G-200 displayed a molecular weight of 40,000 daltons. The enzyme did not exhibit a sharp pH optima rather it was equally active between the range of 7.4 to 8.2. Vmax for the reduction of OAA (316 μmoles/min/mg protein) was 27 times higher than that for the oxidation of malate. The enzyme showed equal affinity for the substrate OAA and the cofactor (NADH), as their Km values were found to be 4.76 x 10\(^{-5}\)M and 5.55 x 10\(^{-5}\)M, respectively. However, affinity of the enzyme for different cofactors was not the same. Thus, the activity in presence of
NADH was 35 times higher than in presence of NADPH. The purified enzyme could be stored effectively in presence of ammonium sulfate at $-10^\circ C$ for three months. The enzyme was quite susceptible to the temperature, as it lost most of the activity above $40^\circ C$ within 5 min. Various activators and inhibitors affected the purified enzyme; sulfhydral reagents, pCMNB in particular, inhibited the activity. Among metal ions Cu$^{++}$ and Co$^{++}$ were strong activators while Zn$^{++}$ proved a potent inhibitor.

**ENERGY METABOLISM IN ISOLATED MITOCHONDRIA:**

Biochemical functions of intact mitochondria from *C. digonopora* were studied by investigating its utilization, decarboxylation and phosphorylation properties. Malate from the medium was utilized in amounts higher than pyruvate. Though all the three acids examined namely, malate, pyruvate and succinate were decarboxylated at significant rates, maximum CO$_2$ was produced by malate. Similarly, the phosphorylation rate of ATP was found highest in presence of malate followed by fumarate and succinate. Ketoacids like pyruvate, oxaloacetate and $\alpha$-ketoglutarate behaved as poor substrates while citrate and isocitrate were totally inert in this respect. Two molecules of ATP were found to be produced for each molecule of CO$_2$ liberated from
malate or succinate. However, this relation did not hold true for pyruvate, as significant amount of \( \text{CO}_2 \) was produced without ATP generation. One of the explanations, which could be offered towards this behaviour, may be that the decarboxylation and the phosphorylation in mitochondria occurred at two different sites possessing a permeability barrier between them for pyruvate.

**ANTHELMINTICS AND THEIR MODE OF ACTION:**

Certain aspects of the metabolism in *S. digonopora* including enzyme systems were examined for their response to various chemotherapeutic agents. For this purpose an important member from each group of anthelminitics was selected. These were niclosamide (anti-cestodal), mebendazole (broad spectrum), DEC (anti-filarial), tetramisole (antiascaris) and praziquantel (novel antischistosomal). Atrazine, representing triazine group was also included because of the reason that these compounds find an easy route to the digestive tract of fowls when used as herbicides in countryside areas. In addition 75/93, 76/544 and 78/265, all antiestodals prepared at this institute, were also checked for such effects.

Niclosamide, representing salicylanilide group of compounds, showed effect on the intact worm as well as on its homogenate. Investigations on sugar metabolism,
studied by incubating *G. digonopora* in presence of the drug, showed a marked reduction in the uptake of glucose, reduced production of other soluble organic acids and CO₂, and reduced synthesis of glycogen and other macromolecular components. Surprisingly, lactate production in the treated parasites increased 2 to 4 fold, indicating that the drug caused a shift in the metabolism of *G. digonopora*.

Cytosolic fraction of niclosamide treated *G. digonopora* revealed that HK, G6PDH, PEPCK and MDH were inhibited, although the extent of inhibition varied from enzyme to enzyme. It was interesting to observe that glycogen phosphorylase and PK were slightly activated.

Levels of enzyme activities in the homogenate after exposure to niclosamide yielded certain additional information. While niclosamide proved inhibitory to G6PDH, HK, PEPCK and MDH, the maximum inhibition was observed with MDH. The purified MDH also responded to the drug in a similar manner. This inhibition was found to be competitive in nature yielding Ki values of 1.3 x 10⁻⁵M and 4.5 x 10⁻⁶M at the drug concentration of 10⁻⁵M and 10⁻⁶M, respectively.

Enzymes like PK, LDH, glycogen phosphorylase, PGI, G6Pase, FDPase and PFK in the drug treated homogenate were left unaffected. All these observations supported
the earlier findings with intact worms, and led to the conclusion that niclosamide inhibited PEP-succinate pathway at MDH (to a great extent) and PEPCK levels, which forces PEP to get metabolised by the route of lactate production.

Among mitochondrial enzymes, FR and ME were susceptible to the action of niclosamide; ME appeared to be the preferential target site. Phosphohydrolases of isolated brush border membrane like ATPase, alkaline phosphatase, 5'-NTase and cAMP-phosphodiesterase, on the other hand, were insensitive to this drug.

The energy metabolism of *C. digonopora* when exposed to niclosamide revealed a drastic inhibition of malate and fumarate dependant $^{32}$P-incorporation into ATP by the intact mitochondria.

It may be concluded that, though niclosamide showed a variation in its effect on different biochemical systems, the target site for this drug was not limited to a single locus. The primary site of attack for this drug under *in vitro* conditions may not be inferred by these results, yet the reduction of sugar uptake may be attributed to the reduced energy production and, the increased production of lactate to the inhibition of enzyme systems associated with the PEP-branch point.
Interestingly, niclosamide was almost equally inhibitory to mammalian (porcine heart) MDH. Thus, further studies on the uptake of drug by different tissues of the host and the parasite may be of utmost importance to localize the exact site of action.

Anticestodals, like 75/98, 76/544 and 78/265 were also inhibitory to various enzyme systems with more or less similar pattern as shown by niclosamide. 76/544 appeared a toxic compound since it did not spare even PFK, PK and LDH from its inhibitory effect. 78/265 proved to be a strong uncoupler of malate and fumarate dependant phosphorylation, and was very close in its effect to niclosamide. It was, in addition, effective on PFK also. 75/98 was inhibitory against various enzymes in a similar fashion as niclosamide except that FR showed better response to this compound.

Though mebendazole and praziquantel in the intact worms altered the metabolism in a way similar to niclosamide but equivalent changes could be achieved only at higher concentrations. These drugs could also affect different enzymes equally except that praziquantel appeared ineffective against MDH and ME.

DEC and tetramisole neither exerted any significant effect on the metabolic process nor did they
exhibit any noteworthy effects on the enzymes studied.

Atrazine also exhibited a change in the metabolism of C. digonopora towards increased lactate production. Though, majority of the enzymes were unaffected by this compound, yet FR, was clearly susceptible to the action of this compound.