Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 90, Number 5, September 1981, pp. 585-591.

Host-parasite relationship and carbohydrates of *Tanqua tiara* Linstow, 1879

H R BHONSLE, L NARASIMHA RAO and MYTHILI DHIMAHI
Department of Zoology, Kakatiya University, Warangal 506 009, India

MS received 13 February 1981; revised 9 May 1981

Abstract. The metabolic adaptations of the male and female species of *Tanqua tiara* Linstow, 1879, to the microenvironment in the stomach of its host *Tropidonotus piscator* and the adaptation of the organ to the presence of the parasite are studied. The parasites adapt by storing glycogen, by possessing free glucose, by diverting the pathway to PEP-malate-succinate type. The stomach adapts to the presence of parasites by increasing its glycogen, free glucose, Pyruvate and Phosphatases. The SDH, LDH and MDH activities are lowered suggesting the operation of Kreb's cycle.

Keywords. *Tanqua tiara* ; stomach: metabolic adaptation; *Tropidonotus piscator*.

1. Introduction

The carbohydrate metabolism in helminths received relatively much attention in recent years. Weinland (1901a, 1902 a, b) determined the glycogen content of various helminths and showed that they store enormous quantities of polysaccharide. He also observed its disappearance during starvation and the production of Co_2 and volatile acids. Since then a large number of observations were reported by a number of workers like Rogers (1948), van Grembergan (1949), Read (1956), Eckert (1967 a,b) and Eckert and Lehner (1971).

Similarly much information is available on post-helminth infection changes in the infected organs (Kuwamura 1958; Rubaj and Furmaga 1969; Lomukhin 1971; Ansari and Singh 1974; Zakhariv and Lemishkov 1975).

However, a study of the adaptations of parasites to its microenvironment in its infected organ and that of the infected organ to the presence of parasite is of much help in understanding the nature of the parasitism. As relatively less work is available on these lines, the authors made an attempt to elucidate the post-infection biochemical content changes of carbohydrate metabolism in the stomach of *Tropidonotus piscator* due to *Tanqua tiara* Linstow (1879) infection ; in relation to the parasites' biochemical requirements. The various biochemical parameters assayed were (I) Substrates— Glycogen free glucose, lactate, Pyruvate and inorganic phosphate and (II) Enzymes—LDH (L-Lactate, NAD Oxidoreductase EC 1·1·1·27) SDH (Succinate acceptor oxidoreductase EC 1.3.99.1); MDH (L-malate: NAD oxidoreductase EC 1·1·1·37) and acid and alkaline phosphatases.

2. Materials and methods

The hosts *T. piscator* were collected from the ponds, brought to the laboratory and sacrificed by decapitation. They were cut open and stomach screened for helminth infections. The infected and uninfected organs were separated, so also the male and female worms. After washing them in several changes of physiological saline the fresh weight was determined and the different biochemical parameters were assayed in male and female worms in normal and infected stomach by following procedures.

The glycogen content was determined by the modified anthrone method of Kliccpera *et al* (1957). The free glucose content was estimated by Nelson and Somogyi (1952) method. Pyruvic acid was estimated by the method of Friedman and Haugan (1943), Lactic acid by Barker and Summerson (1941) as modified by Huckbee (1961). The inorganic phosphate, acid and alkali phosphatases was assayed colorimetrically by the method of Fiske and Subbarow (1925). SDH, LDH and MDH activities were assayed following the mehod of Nachlas *et al* (1960).

3. Results

The results obtained in the present study on the substrate contents are shown in table 1. The table suggests that the female worms and infected tissue possess more glycogen and free glucose about 7·3%, 21% and 24%, 16% respectively. The pyruvate, lactate and inorganic phosphate contents are more in females by 36%, 25%, 25% respectively. In infected organs pyruvate is more by about 26% and lactate and inorganic phosphate less by about 38% and 11%.

The results obtained on enzyme activity levels are shown in table 2. This table suggests that the acid and alkali phosphatases are more in females and infected organ by about 11%, 16% and 21%, 16% respectively, whereas in the case of three dehydrogenases, viz, LDH, SDH and MDH the activity levels are more in females by about 11%, 37% and 32% and in infected organs these dehydrogenating enzyme levels are less by about 13%, 22% and 25% respectively.

4. Discussion

The parasite of the present study lives in a relatively less aerobic environment. Therefore, the energy yield mainly depends upon anaerobic type of metabolism. In this type the energy output is comparatively less, as most of the intermediates are excreted out instead of getting completely oxidized. For such parasites a steady supply of energy yielding molecules like glycogen, etc, must be readily available. Therefore, the storage of glycogen reserves is justifiable.

Table 1. Substrate contents in male, female worms of *T. tiara* and in normal and infected stomach tissue of *T. piscator*. The contents are mean of ten individual samples ± Standard deviation.

	Glycogen*	Free Glucose*	Pyruvate*	Lactate*	Inorganic* phosphate
Male	1774±164	8·5133±0·6841	8·2612±0·9629	25·4975±2·9295	30·842±1·509
Female	1914±124	11·3680±1·8575	12·9657±1·1041	34·2003±2·2051	40·672±4·519
Ratio M/F	0·9268	0·7488	0·6371	0·7455	0·7583
Percentage of difference	7·3145	25·1117	36·2911	25·4511	24·1689
'*P*' value	NS	< 0·01	< 0·001	< 0·001	< 0·002
Normal	1024± 27	6·2325±0·7855	5·8941±0·2626	26·0466±1·7034	37·382±2·524
Infected	1296± 81	7·4093±0·7265	7·8603±0·6587	16·1733±1·2504	33·166±3·787
Ratio N/I	0·7901	0·8411	0·7384	1·6104	1·1270
Percentage of difference	20·9876	15·8827	26·1593	37·9062	11·2734
'*P*' value	< 0·001	< 0·05	< 0·001	< 0·001	< 0·05

* μg/100 mg of wet tissue

Table 2. Enzyme activities levels in male, female *T. tiara* and in normal and infected stomach tissues of *T. piscator*. The activity levels are mean of ten individual samples ± Standard deviation.

	LDH*	SDH*	MDH*	Acid @ phosphatases*	Alkali @ phosphatases
Male	0·6045±0·0917	1·5447±0·1902	0·3144±0·0243	86·000±16·4544	101·865±13·1002
Female	0·6814±0·0309	2·4757±0·3401	0·4657±0·0193	96·945±14·3512	129·103±20·6272
Ratio M/F	0·8871	0·6240	0·6751	0·8871	6·7890
Percentage of difference	11·2855	37·5904	32·4887	11·2899	21·0981
P value	< 0·05	< 0·001	< 0·001	NS	< 0·001
Normal	0·6891±0·0655	1·7871±0·3038	0·4176±0·0449	68·158±11·9082	76·951±13·4006
Infected	0·5999±0·0178	1·3774±0·3295	0·3128±0·0141	81·482±9·1551	91·555±17·0542
Ratio N/I	1·1486	1·2973	1·335	0·8364	0·8404
Percentage of difference	12·9444	22·921	25·0957	16·3522	15·9504
P value	< 0·01	< 0·02	< 0·001	< 0·05	NS

* μmol of formazan/1 hr/100 mg of wet tissue @ μg of pyruvate liberate/1 hr/100 mg wet tissue.

The deposition of polysaccharides, therefore, to some extent also depends upon the availability of free glucose which contributes in the building up of polysaccharide reserves. The parasites do possess free glucose and its content is significantly more by about 25% in females. It may be said that females are generally metabolically more active as they also possess significantly higher quantities of lactate, pyruvate and inorganic phosphate. The enzyme activities are also more in females suggesting the higher rate of the glucose metabolism.

As the parasite is an anaerobe the glucose is metabolized to lactate. This is evident from the fact that male contains about 0·025 mg% of lactate and the female about 0·03 mg%. The lactate generally is converted to pyruvate depending upon NAD concentrations and thereby it should enter into the Kreb's cycle. But for this purpose the LDH activity must be slightly higher. The assay of this enzyme has suggested that its activity is less compared to SDH. Therefore, it may be presumed that most of the lactate is not oxidized but is stored and excreted out. May be because of this the pyruvate content is slightly less. The pyruvate/lactate ratios for males and females are 0·324 and 0·535 respectively, suggesting that the metabolism is more of anerobic than of aerobic type. If it is so this may be a shunt from PEP, as is evident from the activity of MDH suggesting the formation of malate. The malate thus formed ultimately has to be converted to succinate through fumarate. The SDH activity observed in the present worm is high. Though the activity of fumarase is not investigated in the present study, the termination of glycolysis with succinate as the end product is presumed.

Thus the parasites are presumed to adapt in the stomach *T. piscator* that contains very less oxygen, by storing glycogen as reserve polysaccharide, by possessing free glucose, by diverting the pathway to PEP, malate, succinate type, rather than through usual pathway of pyruvate and acetyl-CoA which needs high amount of oxygen.

The presence of parasite in the stomach brings about a variety of changes. There will be various types of biochemical changes also, but still the host survives indicating that the infected organ is biochemically adapting to the presence of the parasites. The glycogen content, in post-helminth period is increased by about 18% and it can be explained on the basis of the polysaccharide resistance property. As stated by Pal (1970) resistance to toxic agents and pathological process is more when the liver glycogen content is high. On par with the glycogen content the free glucose also increases which may not be surprising because the increased glycogen content demands excess of free glucose for its polymerization. The inorganic phosphate also occurs in large quantities presumably facilitating the rapid formation of ATP molecules or its breakdown.

The presence of the parasites in the stomach deprives the host of its nutrition. Hence, a demand on the part of the host for the rapid supply of nutrients for energy providing molecules arises. For the effective mobilization of free glucose molecules the rate of acid and alkaline phosphatases activities increase. As the excessive breakdown of glucose molecules takes place the lactate thus formed will either be transported to other organs to some extent or leaked into the lumen of the stomach. Hence the removal of this lactate accumulation occurs and it is beneficial for the organ in avoiding the toxicity. The lactate decrease observed is thus justifiable. Concomitantly the decrease in the LDH activity occurs. But the pyruvate content on the other hand goes up in infected stomach, suggesting the operation of Embden Mayerhof pathway. The MDH, SDH activities also corroborate that EMP is operating and succinate is not the end product as occurs in parasites.

Thus the stomach can be expected to adapt biochemically to the presence of the parasite by increasing its glycogen, free glucose and pyruvate contents and the activity of phosphatases ; in an attempt for the effective mobilization and the metabolism of energy producing molecules.

Acknowledgements

Authors express their thanks to the authorities of Kakatiya University for providing laboratory facilities, (HRB and MD) to the Council of Scientific and Industrial Reserach for financial assistance.

References

Ansari M J and Singh K S 1974 Histochemical studies of liver in opisthorchiasis; *Indian J. Anim. Sci.* **43** 438–446

Barker S H and Summerson W H 1941 Colorimetric determination of lactic acid in biological fluids: *J. Biol. Chem.* **138** 535–554

Eckert J 1967a Zur Physiologie invasion fashier laven der Trichostrongylodea; *Z. Parasitenkd.* **29** 209–741

Eckert J and Lebner B 1971 Oxygen consumption substrate absorption and lactate excretion of *Dicrocoelium dendriticum* ; *Z. Parasitenkd.* **37** 288–302

Fiske C A and Subbarow Y 1925 The colorimetric determination of phosphorus: *J. Biol. Chem.* **66** 375–400

Friedman T E and Haugen G E 1943 in *Hawk's physiological chemistry* 14th ed. (ed.) B L Oser (New Delhi: Tata McGraw-Hill) p. 1108.

Huckbee W E 1961 In *Hawk's physiological chemistry* 14th ed. (ed.) B L Oser (New Delhi: Tata McGraw-Hill) p. 1103

Klicpera M, Drahota Z and Zak R 1957 Notes on the determination of muscle glycogen; *Physiol. Bohemoslov.* **6** 569

Kuwamura T 1956 Studies on experimental clonarchiasis, especially on the histochemical changes in liver; *Shikoku. Acta Med.* **12** 28–57

Lomukhin V P 1971 Biochemical changes in the liver of cattle with chronic fasciola infection *Veternariya* **21** 127–132

Nachlas M M, Margeulius S P and Selligman A M 1960 A colorimetric method for the estimation , of succinate dehydrogenase; *J. Biol. Chem.* **235** 499–503

Nelson and Somogyi 1952 in *Hawk's physiological chemistry* 14th ed. (ed.) B L Oser (New Delhi: Tata McGraw-Hill) p. 1054

Pal R K 1970 in *A hand-book of modern physiology* (Calcutta: Oxford and I B H Publishing Company)

Read C P 1956 in *Biochemistry of parasites* by von Brand T (London, New York: Academic Press) 1973

Rogers W P 1948 in *Biochemistry of parasites* by von Brand T (New York, London: Academic Press) 1973

Rubaj B and Furmaga S 1969 Pathomorphological and histochemical studies of livers of sheep, experimentally infected with liver fluke; *Acta Parasitol. Pol.* **16** 77–81 van Grembergan 1949 Le metabolisme respiretoire du trematoda *Fasciola hepatica*; *Enzymologia* **13** 241–257

Weinland E 1901a Uber den glyokogen gehlat einigen parasitischer womer; *Z. Biol.* **41** 69–74

Weinland E 1902a Uber ausgepresste extrakte von *Ascaris lumbricoides*, and ihre wirkung; *Z. Biol.* **43** 86–111

Weinland E 1902b Uber antigermete; *Z. biol.* **44** 1–15

Zakhariv Y A and Lemiskov A M 1975 Histochemical investigatiots of lipids and lipases in the liver of sheep and cattle during acute fasciolasis; *Gel. mintolonov.* **27** 47–53