Evidence for the metabolism of phospholipids and triacylglycerols in human *Ascaris lumbricoides*

P.K. Sasi and R.K. Raj[1,2]

Department of Biochemistry, University of Kerala, Trivandrum (India), 5 December 1977

Summary. Human *Ascaris lumbricoides* has the necessary mechanism for the biosynthesis and degradation of phospholipids and triacylglycerols, as in most other species. Piperazine decreases the level of triacylglycerols of this parasite by stimulating the activity of lipase and partially inhibiting the activity of phosphatidate phosphatase.

Although much information is available on the metabolism of carbohydrates, amino acids and certain lipids in *Ascaris lumbricoides*[3,4], detailed information is not available on the metabolism of phospholipids and triacylglycerols. In fact the information on phospholipids is lacking in nematode parasites in general. It was shown recently that cestodes and trematodes have the essential mechanism for the metabolism of phospholipids[5-7]. Piperazine, an anthelmintic drug widely used in the treatment of ascariasis[8], has been shown to decrease the phospholipid content of human *A. lumbricoides*[9]. It also decreases the level of triacylglycerols in this parasite. These reasons prompted a study of the activity of enzymes known to be involved in the metabolism of these 2 groups of lipids on other species, in order to find if a similar metabolism is operating in human *A. lumbricoides*. Another reason being the worms' inability to synthesize sterols[10,11], and the presence of high concentrations of phospholipids[12] and triacylglycerols.

Materials and methods. Live round worms were collected from local hospitals, brought to the laboratory and incubated for 24 h at 37 °C in a modified Tyrode solution containing NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.01%, $NaHCO_3$ 0.015%, Na_2HPO_4 0.05% and glucose 0.5% at 50 ml/worm[13]. After incubation, the worms were separated and used for the following studies. Some of these were homogenized in the different buffers recommended for each enzyme assay to give a 33% homogenate. Homogenates were centrifuged at 2000 rpm for 5 min and the supernatant was used for the assays. Protein was estimated by the method of Lowry et al.[14]. Standard methods were employed for the assay of enzyme activities and these are indicated in table 1[15-27]. The methods were found satisfactory for the *Ascaris* system.

The remaining worms were separated into groups of 3 each to weigh about 5 g. 1 set of groups (controls) was incubated for 6 h in modified Tyrode solution. A 2nd set of groups (experimental) was incubated for the same 6-h period in modified Tyrode solution containing piperazine at concentrations of 1, 2, 3, 4 and 5 mg/ml. After incubation, the worms were washed free of the external medium and frozen. Total lipids were extracted using ethanol-ether (3:1) followed by methanol-chloroform (1:1). The pooled lipid extracts were made up to a known volume and the total triacylglycerol was separated as follows. Aliquots of the lipid extracts were taken and the solvents removed under reduced pressure. The residue so obtained was taken up in chloroform and passed through a florisil column and the chloroform washings which contain all the triacylglycerols collected. Total triglyceride was estimated by the method of Van Handel and Zilversmidt[28].

A 3rd set of groups was used for the ^{14}C incorporation study. This was done by introducing 1 μCi of ^{14}C acetate into the perienteric fluid of each worm by injection using a tuberculin syringe. After injection, the worms were incubated as before in the presence and absence of piperazine (1 concentration of 2 mg/ml alone was used). Triacylglycerol was recovered as described above from the control and experimental worms, and a liquid scintillation counter was used for the determination of radio activity. The phospholipid fraction was separated from non-treated worms as described in an earlier paper[9] and fractionated by TLC using silica gel G coated plates. Spots were removed from plates and the radio activity measured.

Egg yolk phosphatidyl choline and lysophosphatidyl choline were purchased from VP Chest Institute, New Delhi. Phosphatidic acid was prepared from phosphatidyl choline by the method of Möllerung and Bergmeyer[27] using cabbage leaf enzyme and separated from excess reagent by silicic acid chromatography[29]. Diglyceride was prepared from phosphatidic acid, using rat kidney enzyme by the method of Smith et al.[20], and separated from excess reagent by silicic acid chromatography. Coenzyme A, ATP, CTP, CDP-choline and phosphoryl choline were from Sigma. Other materials were of chemically pure quality.

Results and discussion. The activities of the enzymes studied are presented in table 1. In most cases, the specific activities of the enzymes are comparable to the specific activities of similar enzymes reported for other species. Phospholipase D was not detected in *Ascaris*. In fact this enzyme is found to be absent in many animal species. The activities of the enzymes reported clearly indicate the formation of acyl-CoA derivatives, phosphatidic acid and diglyceride as inter-

Table 1. The activities of the synthetic and degradative enzymes of phospholipid and triacylglycerol metabolism in human *A. lumbricoides*

No.	Enzyme	Sp. act. $\times 10^{-6} \pm$ SEM	Temperature	Reference
1	Choline kinase (EC 2.7.1.32)	55.600 ± 0.50*	30	15
2	Cholinephosphate cytidyltransferase (EC 2.7.7.15)	1.706 ± 0.07*	37	16
3	Glycerol kinase (EC 2.7.1.30)	4.117 ± 0.06*	37	17
4	Acyl-CoA synthetase (EC 6.2.1.3)	6.384 ± 0.20**	37	18
5	Glycerolphosphate acyltransferase (EC 2.3.1.15)	6.078 ± 0.25**	37	19
6	Lysolecithin acyltransferase (EC 2.3.1.23)	19.910 ± 1.00**	37	19
7	Phosphatidate phosphatase (EC 3.1.3.4)	4.452 ± 0.12*	37	20
8	Diglyceride acyltransferase (EC 2.3.1.20)	3.750 ± 0.05**	37	21
9	Lipase (EC 3.1.1.3)	3.990 ± 0.08*	37	22
10	Cholinephosphotransferase (EC 2.7.8.2)	0.100 ± 0.003*	37	23
11	Phospholipase A (EC 3.1.1.4)	5.930 ± 0.03**	37	24
12	Lysophospholipase (EC 3.1.1.5)	6.040 ± 0.47**	37	25
13	Phospholipase C (EC 3.1.4.3)	6.220 ± 0.08*	37	26
14	Phospholipase D (EC 3.1.4.4)	NA	30	27

Each value is an average of 6 independent estimations. * mM/min/mg protein, ** mEq/min/mg protein. NA, no activity.

mediates in the formation of phospholipids and triacylglycerols.

In order to confirm the results suggested by the enzyme studies, ^{14}C acetate incorporation study was carried out using the cuticular puncture technique. Other studies have shown this to be the most efficient method of introducing labelled lipid precursors into *Ascaris*[10,11,30]. Incubation of the worms for 1 h following injection showed appreciable amounts of the label in the phospholipids and triacylglycerols. The results are shown in table 2. The results confirm that the worms have the ability to synthesize both phospholipids and triacylgylcerols.

The triacylglycerol level in the presence and absence of piperazine is given in table 3. The percentage decreases are directly proportional to the concentrations of the drug in the external medium. The activities of most of the enzymes of triacylglycerol metabolism are also affected in the presence of piperazine (1 mg/ml) in the assay system. A comparison with the normal values given in table 1 gave the following results. It considerably enhanced the activity of lipase ($7.03\pm0.09\times10^{-6}$, $p<0.01$), causing a 75% increase. Similarly acyl-CoA synthetase ($8.05\pm0.12\times10^{-6}$, $p<0.05$) and glycerolphosphate acyltransferase ($7.64\pm0.20\times10^{-6}$, $p<0.05$) are stimulated to 26% and 24% respectively. Partially inhibited were the activities of glycerol kinase ($3.73\pm0.09\times10^{-6}$, $p<0.05$) and phosphatidate phosphatase ($3.84\pm0.08\times10^{-6}$, $p<0.01$), the inhibition being 10% and 15% respectively. Though pronounced effects are observed on the activities of the different enzymes at a concentration of 1 mg/ml, in the intact worm appreciable decrease in the triacylglycerol level was observed only from a concentration of 2 mg/ml. All the worms incubated in piperazine media up to a concentration of 2 mg/ml were found paralyzed, while only partial paralysis was observed at a concentration lower than 1 mg/ml.

While pronounced incorporation was found in the triacylglycerol fraction after the injection of ^{14}C acetate, less incorporation occurred in the piperazine-treated worms. When the average activity of the control group was 10,600 cpm (table 3) that of the experimental was 8700. Thus there is a decrease of 18% and it clearly indicates that, though triacylglycerol synthesis is taking place in presence of piperazine, its level is found low due to enhanced degradation and decreased synthesis. This action of piperazine is comparable to its effect on the phospholipids of this parasite, which also decreases in level in the presence of piperazine[9].

It may hence be concluded that the human *A. lumbricoides* also has the ability to metabolize phospholipids and triacylglycerols. Piperazine decreases the level of major lipid constituents of this parasite by stimulating degradation and partially inhibiting synthesis, and this decrease is associated with the observed paralysis.

Table 2. Result of ^{14}C acetate incorporation study

Compound	cpm/g wet weight of human *A. lumbricoides**	% of total activity
Total triacylglycerol	10,600	-
Total phospholipid	18,300	-
Phosphatidylethanolamine	1128	7
Phosphatidylcholine	4853	26
Phosphatidylserine	936	5
Lysophosphatidylcholine	6149	33
Sphingomyelin	2963	14

* Average of 6 independent experiments.

Table 3. Effect of piperazine on the total triacylglycerol level of human *A. lumbricoides*

Experiment	Weight of piperazine (mg/ml)	Triacylglycerol level after 6-h incubation in the medium (mg glycerol ± SEM)	p-value	Effect % decrease
1	nil	♂ 77.32 ± 3.15	-	-
		♀ 112.64 ± 5.15	-	-
2	1.0	♂ 71.24 ± 1.35	>0.05	8
		♀ 104.65 ± 5.00	<0.05	7
3	2.0	♂ 64.00 ± 3.00	<0.05	17
		♀ 99.13 ± 4.00	<0.05	12
4	3.0	♂ 62.09 ± 2.65	<0.05	19
		♀ 85.80 ± 0.95	<0.05	24
5	4.0	♂ 58.76 ± 1.85	<0.01	24
		♀ 77.57 ± 2.30	<0.01	31
6	5.0	♂ 53.76 ± 2.10	<0.01	30
		♀ 72.47 ± 1.95	<0.01	36

Each value is an average of 6 independent estimations. $p<0.01$ is highly significant, $p<0.05$ is significant and $p>0.05$ is not significant.

1 Present address: Department of Zoology, University of Iowa, Iowa City, USA.

2 Acknowledgments. Thanks are due to the University of Kerala for facilities provided, Dr T. Ramasarma of the I.I. Sc., Bangalore for help in the labelling studies, Dr G.D. Cain of the University of Iowa for valuable comments and Dr P. Vijayagopal of ANU for the gift of CDP-choline.

3 T. Von Brand, *Biochemistry of Parasites*, 2nd edn. Ed. T. Von Brand. Academic Press, New York 1973.
4 A. Greichus and Y.A. Greichus, Int. J. Biochem. *6*, 1 (1975).
5 R.A. Webb and D.F. Mettrick, Can. J. Biochem. *49*, 1209 (1971).
6 G.H. Buteau and D. Fairbairn, Exp. Parasit. *25*, 265 (1969).
7 V. Oldenborg, F. Vanvugt and L.M.B. Van Golde, Biochim. biophys. Acta *398*, 101 (1975).
8 H.J. Saz and E. Bueding, Pharmac. Rev. *18*, 871 (1966).
9 P.K. Sasi and R. Kaleysa Raj, Experientia *31*, 1261 (1975).
10 J. Barrett, G.D. Cain and D. Fairbairn, J. Parasit. *56*, 1004 (1970).
11 R. Kaleysa Raj and S. Ranganathan, Nature, New Biol. *240*, 247 (1972).
12 D. Subramanyan and S. Venkatesan, Comp. Biochem. Physiol. *25*, 733 (1968).
13 L.G. Goodwin, Br. J. Pharmac. *13*, 197 (1958).
14 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
15 J.B. Wittenberg and A. Kornberg, J. biol. Chem. *202*, 431 (1953).
16 L.F. Borkenhagen and E.P. Kennedy, J. biol. Chem. *227*, 951 (1957).
17 C. Bublitz and E.P. Kennedy, J. biol. Chem. *211*, 951 (1954).
18 A. Kornberg and W.E. Pricer, J. biol. Chem. *204*, 345 (1953).
19 H. Okuyama and W.E.M. Lands, J. biol. Chem. *247*, 1616 (1972).
20 S.W. Smith, S.B. Weiss and E.P. Kennedy, J. biol. Chem. *228*, 915 (1957).
21 P. Goldman and Vagelos, J. biol. Chem. *236*, 2620 (1961).
22 E.D. Korn, in: *Methods in Enzymology*, vol. 5, p. 542. Ed. S.P. Colowick and N.O. Kaplan. Academic Press, New York 1962.
23 E.P. Kennedy and S.B. Weiss, J. biol. Chem. *222*, 193 (1956).
24 A. Rimon and B. Shapiro, Biochem. J. *71*, 620 (1950).
25 O. Hayashi and A. Kornberg, J. biol. Chem. *206*, 647 (1954).
26 J.H. Kleiman and W.E.M. Lands, Biochim. biophys. Acta *187*, 677 (1969).
27 H. Möllerung and H.U. Bergmeyer, in: *Methods of Enzymatic Analysis*, p. 415. Ed. H.U. Bergmeyer. Academic Press, New York 1963.
28 Van Händel and D.B. Zilversmidt, J. Lab. clin. Med. *50*, 152 (1957).
29 G. Hubscher and B. Clark, Biochim. biophys. Acta *41*, 45 (1960).
30 A. Greichus and Y.A. Greichus, Exp. Parasit. *28*, 210 (1970).