Bull. Inst. Zool., Academia Sinica 21(1): 93-102 (1982)

THE UPTAKE *IN VITRO* OF CARBOHYDRATES, AMINO ACIDS AND NUCLEIC ACID PRECURSORS BY *ANGIOSTRONGYLUS CANTONENSIS*

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(Received December 1, 1981)

S. N. Chen, T. Tang, H. H. Shih and K. M. Lee (1982). The uptake *in vitro* of carbohydrates, amino acids and nucleic acid precursors by *Angiostrongylus cantonensis*. *Bull. Inst. Zool. Academia Sinica* 21(1): 93-102. The uptake and incorporation *in vitro* of various substances by adult male and female of *Angiostrongylus cantonensis* were investigated using scintillation counting and autoradiographic techniques. A significant uptake and incorporation of *D*-glucose, galactose, fructose, histidine, leucine, glycine, cytidine, guanosine and adenosine were demonstrated in the present study. However, no evidence was obtained for the incorporation of *L*-glucose, sucrose and thymidine by adult *Angiostrongylus cantonensis*.

In a previous study (Chen *et al.*, 1981), larval stages of *Angiostrongylus cantonensis* were cultivated in various culture media and cell cultures. The result suggested that the culture systems used in our experiment did not support a complete growth of *A. cantonensis* from the 1st stage or 3rd stage larvae to adult worms. It is probably related to the shortage of nutrient supply of this parasite in the culture systems. To improve the development of *A. cantonensis in vitro*, it is worthwhile to investigate the nutrient requirement for this parasite.

In the study of carbohydrate metabolism Yanagisawa and Von Brand (1965) and Shih and Chen (1981) reported that *A. cantonensis* can assimilate glucose for its major energy sources. Apart from this nutrient, no information is available on the utilization of other substrates by this parasite.

In the present study, the uptake of amino acids, monosaccharides, disaccharides and nucleic acid precursors by adult worms of *A. cantonensis* were investigated by using scintillation countxing and autoradiographic techniques.

MATERIALS AND METHODS

Male and female adults of *A. cantonensis* were obtained from pulmonary, heart and lung of infected albino rats as described previously (Chen *et al.*, 1981). Prior to experimental use, all worms were washed twice in Hanks' balanced salt solution (HBSS). Scintillation counting and autoradiographic techniques were employed to investigate the uptake of various substrates as described below.

Scintillation Counting Techniques

For scintillation counting experiments, four

adult worms for each sex were incubated in 10 ml of HBSS containing one of the following substances at a specific concentration of 1.5 μCi/ml, respectively:

D-[6-^{3}H]glucose (sp. act., 9.0 Ci/mmol);
L-[1-^{14}C]glucose (sp. act., 61.4 mCi/mmol);
D-[2-^{3}H]mannose (sp. act., 16 Ci/mmol);
D-[1-^{3}H]galactose (sp. act., 9.3 Ci/mmol);
D-[U-^{14}C]fructose (sp. act., 283 Ci/mmol);
[U-^{14}C]sucrose (sp. act., 434.6 mCi/mmol);
2-^{3}H glycine (sp. act., 12.3 Ci/mmol);
L-[2, 5-^{3}H]histidine (sp. act., 44 Ci/mmol);
L-[4, 5-^{3}H]leucine (sp. act., 165 Ci/mmol);
[5-^{3}H]cytidine (sp. act., 31 Ci/mmol);
[8-^{3}H]guanosine (sp. act., 8.1 Ci/mmol);
[2-^{3}H]adenosine (sp. act., 20 Ci/mmol) and
[methyl-^{3}H]thymidine (sp. act., 47 Ci/mmol).

All the radioactively labelled compounds were obtained from the Radiochemical Center, Amersham, England. The worms were incubated at 36 ± 1°C for 30 minutes and washed five times in 10-ml aliquots of unlabelled HBSS. Subsequently, the worms were blotted dry with filter paper and the body weight was determined. Single worm from each experiment was then dissolved in 0.5 ml of hyamine hydroxide (New England Nuclear, Boston, Mass., USA) at 36 ± 1°C for approximately 12 hours and mixed with 10 ml aquasol-2 (New England Nuclear, Boston, Mass., USA). The contol worms was killed at 50°C for 15 minutes and processed as those employed for live worms. Samples (approximately 0.1 ml) of the final washing solution for each experiment were counted using the scintillation counter to confirm the counts per minute (cpm) were similar to the background control. Radioactivity was measured in a Beckman LS-100 liquid scintillation counter.

Autoradiographic Techniques

For autographic study, adult males and females were exposed to each tested compound at a concentration of 10 μCi/ml in HBSS for 30 minutes. To investigate the incorporation of labelled amino acids, monosaccharides, disaccharides and nucleic acid precursors, the worms were fixed immediately after incubation in 6% glutaraldhyde in 0.2 M cacodylate buffer, pH 7.2, for 45 minutes at 4°C and post-fixed in 1% osmium tetraoxide in the same buffered solution. The worms were then dehydrated in ethanol and embedded in spurr epon. Two μm-thick sections were cut using a Sorvall MT 5000 ultra-microtome and mounted on 76×15 mm micro-slides. The sections were coated with Kodak nuclear track emulsion, exposed at 4°C for 2 weeks in dark, and developed with Kodak D-19 developer. Grain counts were made with the aid of a whipple eye-piece micrometer grid in conjunction with 12.5× eye piece and 100× oil-immersion objective. One-hundred μm^2 were counted for each given worm section and the background.

RESULTS

Liquid Scintillation Counting

Tables 1 and 2 present results obtained after incubation of adult *A. cantonensis* in HBSS containing radioactively labelled monosaccharides, disaccharides, amino acids and nucleic acid precursors at a concentration of 1.0 μCi/ml, respectively. These results show that there was a substantial uptake of D-[6-^{3}H]glucose, D-[1-^{3}H]galactose, D-[U-^{14}C]leucine, [2-^{3}H] glycine, [5-^{3}H]cytidine, [8-^{3}H]adenosine, [8-^{3}H] guanosine and significant differences were observed in the counting per minute (cpm) obtained for the test and control worms. On the contrary, the results demonstrated that there was no significant incorporation of L-[1-^{14}C]glucose, [U-^{14}C]sucrose or [methyl-^{3}H] thymidine by test worm as compared with that of heat-killed worms.

Autoradiography

The results obtained from light microscope autoradiographs of the adult male and female of *A. cantonensis* which had been incubated in HBSS containing the above mentioned substances are shown in Tables 3 and 4. Statistically, in the specimens obtained from the worms incubated in L-[1-^{14}C]glucose, [U-^{14}C]sucrose or [methyl-^{3}H]thymidine, the mean grain count over the worm tissue was not significantly ($p>0.5$) than that obtained over the background

(Figs. 1-2). In contrast to the results obtained with L-glucose, sucrose or thymidine, the autoradiographic experiments also demonstrated that [^{14}C] or [^{3}H-]labelled D-glucose, D-galactose, D-fructose, L-histidine, L-leucine, glycine, cytidine, guanosine and adenosine were readily incorporated into adult *A. cantonensis*. These results of autoradiography further confirmed the results of the liquid scintillation analysis. The distribution and density of D-[6-^{3}H]glucose in the structure of *A. cantonensis* revealed by autoradiography are presented in Figs. 3-4. This result shows that higher density of silver grains was found in somatic muscle, eggs and embryos. The autoradiograms from other specimens show that the incorporated compounds were distributed more evenly through the tissues of the worms than that of D-[6-^{3}H]glucose (Figs. 5-7).

DISCUSSION

The results obtained in the present study suggest that *A. cantonensis* is able to utilize exogenous glucose, galactose and fructose. The lack of uptake and incorporation of sucrose in either male or female worms with 30 minutes'

TABLE 1

The *In Vitro* Uptake of Radioactively Labelled Carbohydrates by Adult *Angiostrongylus cantonensis* After 30 minutes Incubation at 36±1°C.

Compound tested	Male		Female	
	Test (cpm/mg)	control (cpm/mg)	Test (cpm/mg)	control (cpm/mg)
D-[6-^{3}H]Glucose	19227±3160[a]	428±121	15051±1047[a]	379±149
L-[1-^{14}C]Glucose	317± 29	492±124	404± 42	399± 21
D-[1-^{3}H]Galactose	6797± 371[a]	425± 29	5938± 412[a]	224± 78
D-[U-^{14}C]fructose	5640± 748[a]	405± 49	3721± 125[a]	394± 77
[U-^{14}C]Sucrose	425± 13	462± 14	372± 14	385± 12

Incubation medium containing HBSS plus each compound at 1 μCi/ml.
Each reading was obtained at least from two worms and is expressed as mean±SD.
a: Test reading is significantly different ($p<0.01$) from control.

TABLE 2

The *In Vitro* uptake of Radioactively Labelled Amino Acids and Nucleic Acid precursors by Adult *Angiostrongylus cantonensis* After 30 Minutes Incubation at 36±1°C

Compound Tested	Male		Female	
	Test (cpm/mg)	Control (cpm/mg)	Test (cpm/mg)	Control (cpm/mg)
L-[2, 5-^{3}H]Histidine	42854±2034[b]	1642±479	32443±1579[b]	1462±317
L-[4, 5-^{3}H]Leucine	108338±5427[b]	1989±596	78532±3911[b]	1340±405
[2-^{3}H]Glycine	43518±5582[b]	3215±707	32081±1085[b]	3960±897
[5-^{3}H]Cytidine	5867± 802[b]	904±110	4292± 675[b]	797±154
[8-^{3}H]Guanosine	26681±1527[b]	1728±315	23454±1600[b]	1409±567
[methyl-^{3}H]Thymidine	74± 2[a]	81± 12	105± 9[a]	99± 15
[2-^{3}H]Adenosine	10325±2012[b]	2130±350	15720±1877[b]	3100±454

Incubation medium containing HBSS plus each compound at 1 μCi/ml.
Each reading was obtained at least from two worms and is expressed as mean±SD.
a: Test reading is not sigificantly different ($p>0.5$) from control.
b: Test reading is significantly different ($p<0.01$) from control.

TABLE 3

The *In Vitro* uptake and Incorporation of [^{14}C]- or [^{3}H] labeled carbohydrates by adult *Angiostrongylus cantonensis* after 30 minutes incubation at 36±1°C

[^{14}C]- or [^{3}H] labelled compounds	Mean grain counts for 100 μm^2 tissue in spurr epon section (2 μm thick)			
	Female		Male	
	Tissue	background	Tissue	background
D-[6-^{3}H]Glucose	68± 7 (Eggs or Embryos)	8±2	65±4 (Somatic muscle)	6±4
	47± 5[a]		38±7[a]	
	70±12 (Somatic muscle)			
L-[1-^{14}C]Glucose	9± 3	10±2	10±5	9±4
D-[1-^{3}H]Galactose	25± 6	12±2	27±3	10±4
D-[U-^{14}C]Fructose	30± 4	10±1	25±7	9±4
[U-^{14}C]Sucrose	8± 3	8±2	9±2	10±1

Worms were incubated in HBSS containing each compound at 10 μCi/ml.
Each reading was obtained from at least three worms and is expressed as mean±SD.
Background reading was obtained at least from five 100-μm^2 areas of the section not containing worm tissue.
a: Grains over the tissues not including eggs or embryos and somatic muscle areas.

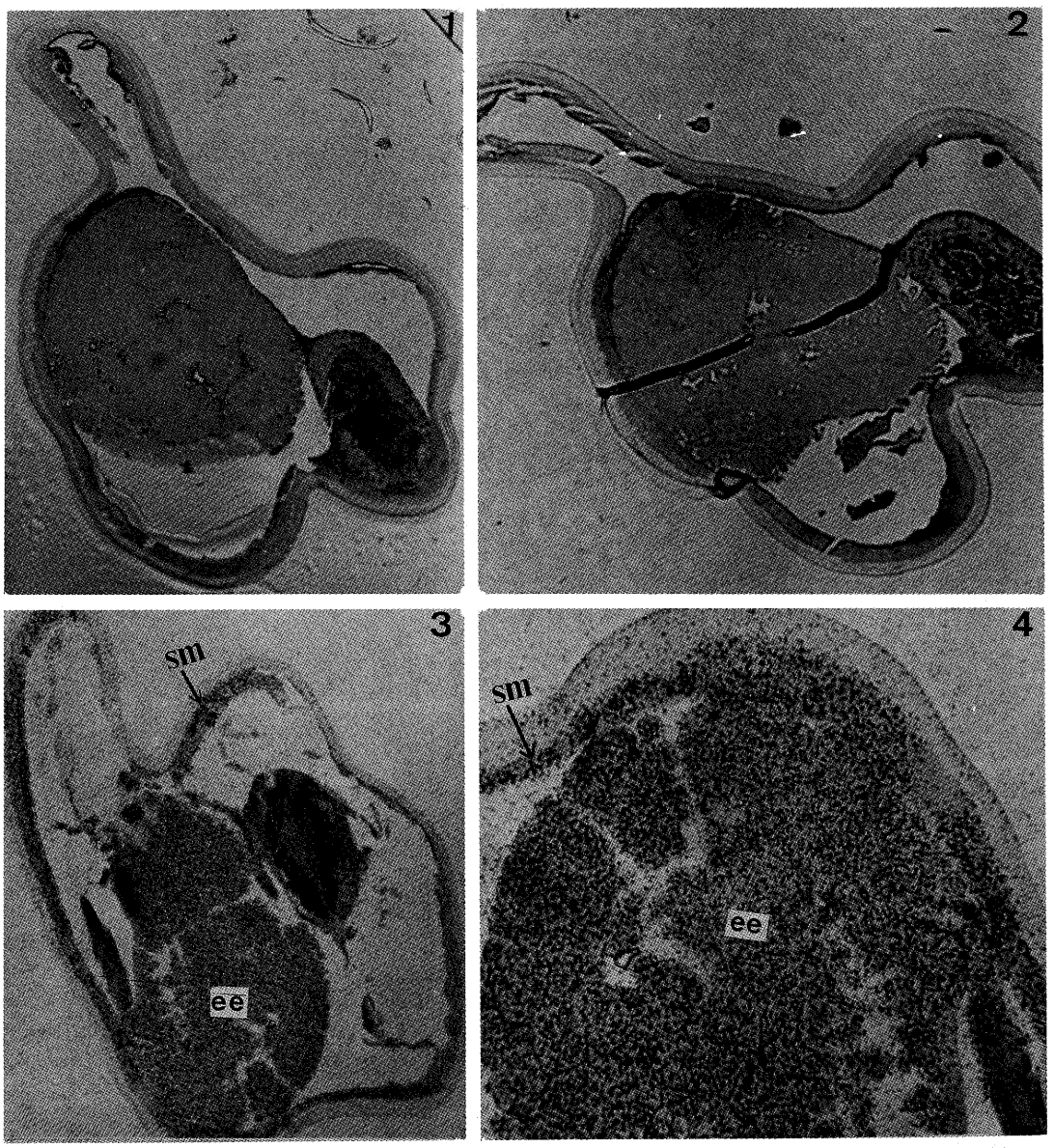
TABLE 4

The *In Vitro* Uptake and Incorporation of [^{14}C]- and [^{3}H] labeled Amino Acids and Nucleic Acid Precursors by Adult *Angiostrongylus cantonensis* After 30 Minutes Incubation at 36±1°C

[^{14}C]- or [^{3}H] labeled compounds	Mean grain counts for 100 μm^2 tissue in spurr epon section (2 μm thick)			
	Female		Male	
	Tissue	background	Tissue	background
L-[2, 5-^{3}H]Histidine	58± 9	7±1	49± 7	8±2
L-[4, 5-^{3}H]Leucine	62±10	9±2	57±12	8±4
[2-^{3}H]Glycine	65± 7	10±4	68±10	9±2
[5-^{3}H]Cytidine	69±12	12±1	65± 4	9±1
[8-^{3}H]Guanosine	+[a]	8±2	+[a]	7±1
[methyl-^{3}H]Thymidine	7± 1	8±3	11± 4	12±2
[2-^{3}H]Adenosine	69± 8	9±2	72±14	10±4

Worms were incubated in HBSS containing each compound at 10 μCi/ml.
Each reading was obtained at least from three worms and is expressed as mean±SD.
Background reading was obtained at least from five 100 μm^2 areas of the section not containing worm tissue.
a: The grain density is too great to count.

incubation indicated the *A. cantonensis* could not utilize this substrate for its metabolism. Similar results related to the favoured uptake of glucose and fructose by this parasite were also reported by Yanagisawa (1976). In a previous report (Shih and Chen, 1982) we demonstrated that *A. cantonensis* equipped with all the enzymes required to operate Embden-Meyerhof pathway for glucose degradation and may confirm the present results in the utilization of



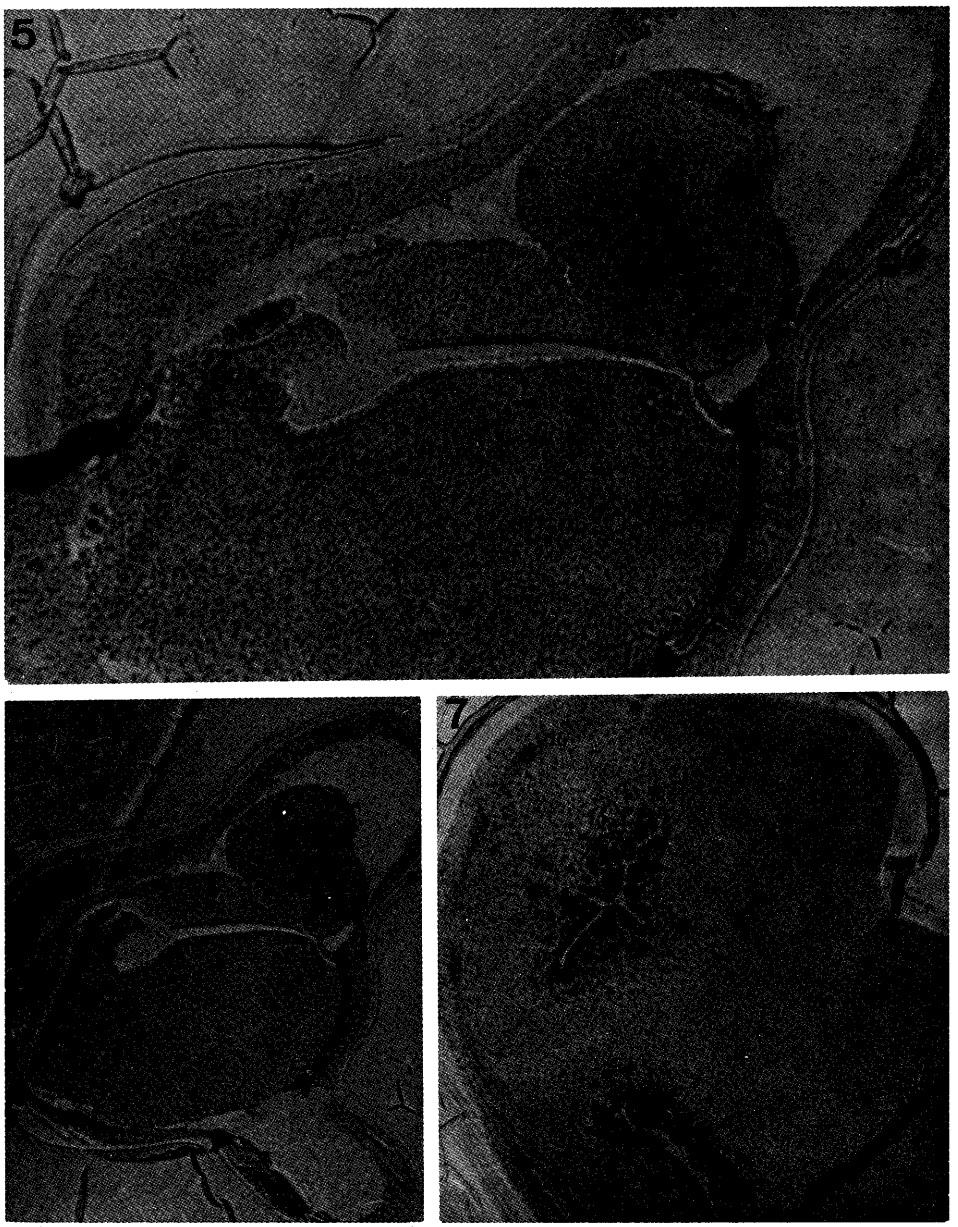
Explanation for the Figures

Figs. 1-7. Light microscope autoradiograms showing sections of adults of *Angiostrongylus cantonensis* exposed to radioactively labelled compound for a period of 30 minutes at 36±1°C.

Fig 1. Female *A. cantonensis* exposed to [U-^{14}C] sucrose. ×200.

Fig. 2. Female *A. cantonensis* exposed to [methyl-^{3}H] thymidine. ×200.

Figs. 3-4. Female *A. cantonensis* exposed to D-[6-^{3}H] glucose.
Fig. 3. ×200 Fig. 4. ×800. sm: somatic muscle. ee: eggs and embryos.



Explanation for the Figures

Figs. 5-6. Female *A. cantonensis* exposed to [2-^{3}H] adenosine.
Figs. 5. ×500. Fig. 6. ×200.
Fig. 7. Male *A. cantonensis* exposed to L-[2,5-^{3}H] histidine. ×300.

glucose by *A. cantonensis in vitro.*

Autoradiographically, greatest accumulation of incorporated, isotopically labelled D-glucose occurred in the apical region of somatic layer and in the eggs and embryos areas, suggesting that glyconeogenesis occurs in these structures. The similar results were also obtained in *B. pahangi* by Chen and Howells (1979).

The uptake and incorporation of various sugars were also demonstrated in other nematodes and Acanthocephala. Bueding (1949) and Anwar *et al.* (1977) reported that the filarial parasite of cotton rat (*Sigmodon hiapisua*), *Litomosoides carinii*, consumed glucose, galactose, fructose and mannose (Anward *et al.*, 1977). *Chandlerella hawkingi* (Srivastava *et al.*, 1968; Srivastava and Ghatak, 1974) and *Sataria cervi* (Anward *et al.*, 1975), the filarial nematodes of Indian jungle crow and water buffalo were also found to be able to utilize glucose and mannose to their energy requirement. According to the results of Cavier and Savel (1952) and Laurie (1959) *Ascaris* can synthesize glycogen from fructose, sorbose, maltose and saccharose and *Maniliformis dubius* from fructose, mannose and maltose. In contrast to the glucose assimilation, it is also demonstrated that there is no significant utilization of sucrose by larval or adult stage of *B. pahangi.*

Apart from carbohydrates, amino acids may probably provide alternative sources of metabolism for *A. cantonensis.* In the present study amino acids including histidine, leucine, glycine and cytidine were incorporated into the worms. Whether these amino acids provide significant amounts of energy needs further investigation. In an experiment using *Fasciola hepatica*, although large amount of alanine, arginine, glutamate, histidine, phenylalanine and serine were taken up, they were not metabolized *in vitro* to volatile fatty acids which may suggest that they are not major energy sources for *F. hepatica.*

Neither male nor female worms of *A. cantonensis* incorporate exogenous thymidine and this suggests that this parasite is unable to utilize this performed pyrimidine and may indicate an extremely limited ability of the worms to synthesize DNA. This inability to utilize preformed pyrimidines has been observed in *B. pahangi*, (Chen and Howells, 1979) and *Schistosoma mansoni* (Senft *et al.*, 1973; Levy and Read, 1975) and in a variety of protozoa including *plasmodia* (Bungener and Neilson, 1967; Walsh and Shelman, 1968; Van Dyke *et al.*, 1970) and piroplasms (Irvin *et al.*, 1978). However, the present study also demonstrated that the exogenous purine neucleotides, adenosine and guanosine was utilized by *A. cantonensis.* This may reflect a dependence by the worms *in vivo* on host-derived adenosine and guanosine. The dietary requirement for purine nucleotides are also present in other parasites. Bungener and Nielson (1967; 1968) Van Dyke *et al.* (1970), Gutteridge and Trigg (1970) and Trager (1971) reported that malarial parasites, *Plasmodium bengkei, P. vinckei* and *P. lophurae* incorporated adenosine and guanosine. The infective larvae, juvenile and adult worms of *B. pahangi* were demonstrated to assimilated adenosine *in vitro.*

In the light of the present results it suggests that there is no requirement of sucrose and thymidine for the *in vitro* cultivation of *A. cantonensis.* The other compounds including D-glucose, galactose, fructose, histidine, leucine, cytidine, guanosine and adenosine may provide the nutrient factors which may stimulate the development of *A. cantonensis in vitro.*

Acknowledgements: This study was supported by a grant [NSC-70-0201-B002-12] from National Science Council, Republic of China.

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廣東住血線蟲體外攝取碳水化合物，氨基酸及核酸先質之研究

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本實驗乃利用液態閃光計數法及自動放射照相術來探討廣東住血線蟲於試管內對各種營養物質之攝取及利用之可能性。實驗結果顯示，廣東住血線蟲可攝取 D-glucose, D-galactose, D-fructose, L-histidine, L-leucine, L-glycine, cytidine, guanosine 及 adenosine，但並不攝取 *L*-glucose, sucrose 及 thymidine。