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AUTORADIOGRAPHIC STUDIES ON THE UPTAKE AND DISTRIBUTION OF ^{14}C-GLUCOSE BY *PARAGONIMUS WESTERMANI*

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The carbohydrate matabolism of parasites is one of the best known phases of parasitic physiology, but little is known about the metabolism of trematodes. Especially, on the carbohydrate metabolism of lung fluke, *Paragonimus westermani*, only a few informations have been reported.

A series of experiments dealing with the carbohydrate metabolism of this fluke have been studied by several authors (Tada et al., 1961; Hamajima, 1964, 1965, 1966 & 1967). The glycolytic enzymes and Krebs cycle enzymes have been known to present in extracts of *Paragonimus westermani* and these were proved lactic dehydrogenase, phosphatases and malic dehydrogenase (Lee, 1967; Park & Seo, 1967; Lee & Seo, 1967).

Some informations on the distribution of carbohydrate were available in this fluke. Yokogawa et al. (1957) made histochemical studies on the adult worm of *P.ohirai* and Yoshimura et al. (1958) demonstrated the localization of glycogen in *P.westermani*. Ro et al. (1963) confirmed the result of Yoshimura et al. (1958) afterwards. Burton(1962) reported the experimental result of the in vitro uptake of radioglucose correlation with the histochemistry by a frog lung fluke, *Haematoloechus medioplexus*.

In recent years, the techniques of autoradiography have been applied for localization of exogenous radioactive tracers in tissue of certain helminths. Hahn et al. (1962) reported the distribution of radioactive glucose by Chinese liver fluke, *Clonorchis sinensis*, and Lee & Seo(1971) demonstrated more detailed distribution by applying the microautoradiography. In addition, distribution and fate of ^{14}C-glucose has been elucidated by liver fluke, *Fasciola hepatica*(Thorsell et al., 1968) and by *C.sinensis*(Kang et al., 1969).

However, little information is so far known concerning the uptake and distribution of exogenous radioglucose in lung fluke, *P.westermani*. Therefore, the present study was carried out to demonstrate the uptake and distribution of exogenous ^{14}C-glucose in lung fluke, *P.westermani*.

The distribution of the radioactive substances in this fluke was investigated in detail by microautoradiography.

MATERIALS AND METHODS

Collection of worms: Adult worms of *P.westermani* were obtained alive from the lungs of freshly killed dogs which had been sacrificed 6 months after infection with metacercariae. Intact and healthy worms selected and immediately immersed in sterilized Tyrode solution, at 37°C.

Isotope and incubating fluid used: D-glucose-^{14}C(U), specific activity 3.9 mCi/mM,

was obtained from Nuclear Chicago Corp oration, U.S.A. This labeled glucose was dissolved in Tyrode solution as to contain the concentration of 10 μCi/ml. This solution was applied as incubation medium.

Incubation procedure: The procedure of incubation was essentially the same as that of Lee et al. (1971). Ten worms of actively motile fluke were incubated in 25 ml Erlenmeyer flask with 10 ml of above mentioned incubation medium. This incubation flask was maintained in Dubnoff metabolic shaking incubator for 1 hour at 37° C. After incubation, the flukes were immediately removed from the incubation medium, and freed of external radioactivity by a series of washing in chilled Tyrode solution.

Microautoradiography: The flukes were fixed in AFA(alcohol-formal-in-acetic acid) fixative and were passed through a graded series of alcohol to xylene. They were embedded iu paraffine, sectioned at 5μ, and then mounted on subbed slides which had been prepared according to Kodak Data Sheet.

The stripping film technique(Fitzgerald et al., 1953; Lotz et al., 1953) was applied for microautoradiography. The slides were covered with Kodak AR-10 fine grain stripping film under the safelight fitted with a Wratten series I filter, and exposed for 30 days. The autoradiographs were developed for 10 minutes in Kodak D-19 developer, and fixed for 5 minutes in Kodak acid fixer.

The autoradiographs, after development and fixation, were stained with Delafield's hematoxylin.

EXPERIMENTAL RESULTS

Microautoradiographs of lung fluke, *P.westermani*, showed that there were apparent densities in regard to various anatomical structures of the parasite.

The highest density of black grains derived from ^{14}C-glucose were monitored in the parenchymal tissues (Fig. 1). High power magnification showed that relatively fine grains, in comparison with *Clonorchis*(Lee & Seo, 1971), were accumulated in subcuticular musculature and reticular tissues. In general, the parenchyma lying just beneath the integument was much more radioactive than the deeper parenchyma.

The muscular tissues of this fluke, such as oral sucker, pharynx and ventral sucker, showed fine discrete distribution of grains(Fig. 2, 3 & 4). As shown in Fig. 2 & 3, the oral sucker revealed less radioactivity than the pharynx. The slight radioactivity was also detected in ventral sucker, especially in outer surface and in musculo-reticular junction(Fig.4). Some black grains were observed in the intestinal ceca, along the intestinal lining cells(Fig. 5).

Figure 6 presented that the conspicuous particles of labeled carbon were distributed over the eggs in uterus, but there was negligible density of grains in uterine tubule. Reproductive systems of this fluke, i.e., ovary, testes and vitelline follicles showed slight radioactivity (Fig. 7, 9 & 11). High magnification of these regions showed that low density of grains were distributed correspondly to their structure (Fig. 8, 10 & 11).

Excretory bladder of this fluke also showed the occurrence of black spots according to its lining(Fig. 12).

DISCUSSION

Detection of sugars in lung fluke, *P.westermani*, is important in biochemical studies on carbohydrate metabolism of this parasite. Previous authors have reported on the presence of sugars and metabolic products of sugar in the lung fluke.

By applying histochemical techniques, Yoshimura et al. (1958) reported that a lot of glycogen was observed in reticulum, vitelline glanps, yolk cells of the egg in the uterus and

subcuticular cells of *P.westermani*. They also noted that other polysaccharids except glycogen were distributed in the muscle cells under the cuticle, vitelline glands, ovary, testes and the eggs in uterus. Similar results were obtained by this fluke from the experiment of Ro et al. (1963).

In addition, presence of substances associated glycolytic pathway and Krebs cycle was declared by Tada et al. (1961) and Hamajima(1964, 1965, 1966 & 1967); they claimed that carbohydrate degradation occurred by these scheme, in the lung fluke. By the experiment of Hamajima(1966), the sugars, hexose phosphate, and glycogen were detected in the uterine eggs, larvae and adults of *P.westermani* in chromatograms.

In comparison with the reports of above authors, the experimental result of present microautoradiography closely corresponded to their conclusions.

As shown in autoradiographs of *P.westermani*, the black grains from radioactive carbon were distributed in the organs or tissues where a lot of glycogen had been known to present by histochemical study (von Brand, 1966; Halton, 1967).

Thorsell et al. (1968) studied the fate of ^{14}C-glucose in liver fluke, *F.hepatica*, and observed the pronounced accumulation of radioactive carbon in the parenchyme. In present experiment, high radioactivity appeared in the parenchyme of *P.westermani* and this result might reflect that a high energy turn over took place by the degradation and transformation of glucose with in mitochondria of this site. Lee et al. (1971) proved that ^{14}C-glucose congregated into larger particles in the parenchyme of *C.sinensis*, but in this fluke, it was hardly observed. The density of radioactivity in the parenchyme of this fluke was usually lower than that of *C.sinensis* in autoradiographs. The muscular tissues like pharynx, oral and ventral sucker also revealed detectable radioactivity in compatible with the result of Thorsell et al. (1968), Lee & Seo (1971) and Burton(1962).

It may be considered that the radioactivity in the worm body represents glucose and or substances formed by anabolic processes, or by catabolic processes of glucose. Thus it could be explained that the distribution of radioctivity in the tissues or organs was not always compatible with the glycogen or polysaccharide content. On the other hand, as in *C.sinensis*, the male reproductive system is usually glycogen-poor, although the sperm cells not rarely give distinct polysaccharide reactions(von Brand, 1966). It is well known that the vitellaria of *Schistosoma* were glycogen free and consequently the uterine eggs contained but little polysaccharide.

It was suggested that absorbed exogenous ^{14}C-glucose was rapidly converted, and radioactive carbon appeared in the forms of degradation or transformation products in the worm body. Thus it can be explained that the organs or tissues which were known to contain glycogen revealed slight radioactivity, and *vice versa*. But, generally speaking, the result of present experiment closely corresponded to the histochemical studies of Yoshimura et al. (1958) and Ro et al. (1963).

SUMMARY

Autoradiographic study was performed in order to know the distribution of exogenous ^{14}C-glucose by lung fluke, *Paragonimus westermani*, incubated in Tyrode medium containing 10 μCi/ml of labeled substance.

After 1 hour incubation at 37°C, microautoradiographs of this fluke showed that black grains derived from radioactive carbon were accumulated mainly in the parenchyme and subcuticular musculature. The muscular tissues such as oral sucker, pharynx and ventral sucker revealed considerable density of fine grains. Slight radioactivity was also observed in the regions of ovary, testes, vitelline follicles, eggs in uterus, intestinal ceca, and even in excretor

bladder. Structures showing the least activity included the cuticle and uterine tubules of this fluke.

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=抄　錄=

^{14}C-glucose를 利用한 肺吸虫의 Autoradiography

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肺吸虫의 炭水化物代謝에 關해서는 解糖過程 및 Krebs cycle의 存在가 알려졌고 또 이에 關與하는 酵素系 및 中間代謝產物等이 檢出되었으며 組織化學的方法에 依해서 glycogen 및 多糖類의 分布가 알려졌으나 外部에서 投與한 ^{14}C-glucose의 虫體內 吸收 및 分布에 關해서는 究明된바가 없다.

이에 著者等은 肺吸虫에서 ^{14}C-glucose 의 吸收 및 虫體內 分布를 알기 위하여 microautoradiography를 實施하였다.

肺吸虫의 成虫은 人工感染시킨 개의 肺에서 採取하였고, 37°C의 滅菌 Tyrode 液으로 數回 洗滌한 다음 ^{14}C-glucose가 10μCi/ml 含有된 Tyrode培地에 옮겨 Dubnoff metabolic shaking incubator에서 37°C, 1 時間 保存하였다. 이때 培地는 容器當 10ml 씩 分注하여 使用하였고 虫體는 한 容器에 10마리씩 넣어서 實驗하였다.

Incubation이 끝난 虫體는 곧 AFA 固定液으로 固定하고 paraffine 包埋하여 5μ 두께의 切片을 만든 다음 slide에 附着시키고 이어 Kodak AR—10 film을 被覆하여 4°C의 暗箱子에서 30日間 露出시켰다. 露出이 끝난 標本은 10分間 Kodak D—19 現像液에서 現像하고 Kodak acid fixer로 定着한 다음 Delafield's hematoxylin 染色을 하여 鏡檢하였다.

그 結果 虫體內 吸收된 ^{14}C-glucose의 放射能에 依한 黑化顆粒은 虫體의 網狀組織에 가장 密集되어 있었는데 深層에서 보다는 表層에 가까울수록 더 濃厚하였다. 角皮에서는 顆粒이 보이지 않았으나 角皮下 筋肉組織, 口吸盤, 咽頭頭, 腹吸盤에서는 相當한 黑化의 分布가 認定되었다.

子宮管內 卵子에는 뚜렷한 黑化顆粒이 보였으나 子宮管壁에서는 보이지 않았고 卵巢, 睾丸, 卵黃腺에서는 낮은 密度의 黑化를 觀察할수 있었다.

腸壁 그리고 排泄囊壁에서도 또한 어느 程度의 黑化 顆粒을 認定할수 있었다.

Explanation of Figures

Fig. 1. Microautoradiograph of *P.westermani* showing high concentration of radioactivity in parenchymal tissue (x 100).

Fig. 2. Low density of fine grains were observed in oral sucker, mostly lined near the surface area (x 100).

Fig. 3. The radioactivity was also detected in pharynx, higher than that of oral sucker (x 430).

Fig. 4. Black grains were visible in the ventral sucker (x 100). Relatively fine grains of them also accumulated near the outer surface.

Fig. 5. High power magnification of intestinal wall revealed that grains were lined along the basement of the intestinal epithelium (x 430).

Fig. 6. Conspicuous black grains were observed inside of eggs, in uterus (x 430).

Fig. 7. In the region of ovary, slight radioactivity was monitored (x 100).

Fig. 8. High power magnification of Fig. 7 (x 430).

Fig. 9. Microautoradiograph of testes in which low density of grains were observed (x 100).

Fig. 10. High power magnification of the testes (x 430).

Fig. 11. High power magnification of vitelline follicles showed slight density of grains (x 430).

Fig. 12. Some black grains lined along the wall of excretory bladder in high power magnification (x 430).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

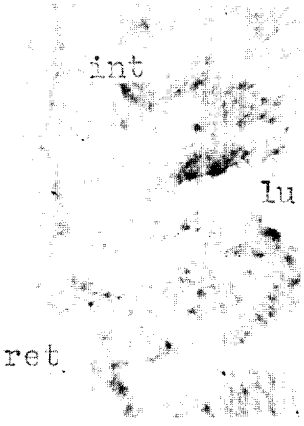


Fig. 5.

Fig. 6.

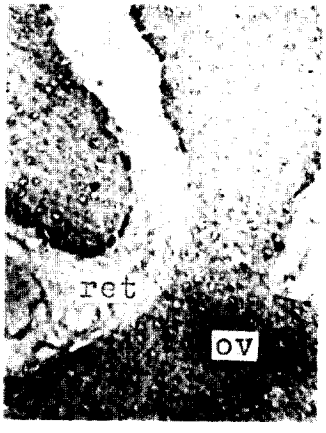
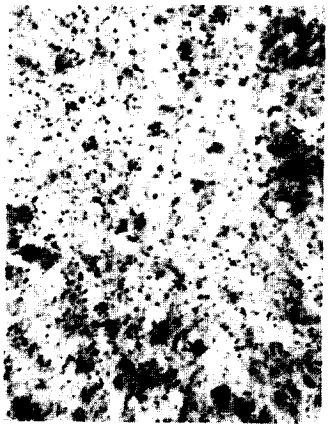


Fig. 7.



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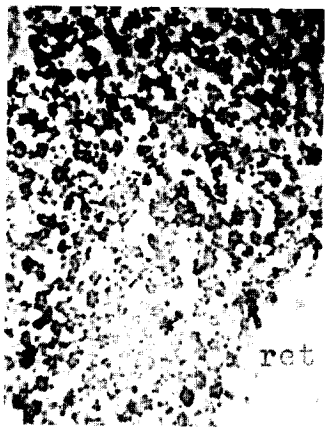

Fig. 9.

Fig. 10.

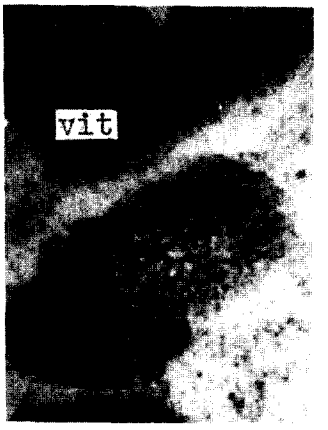

Fig. 11.

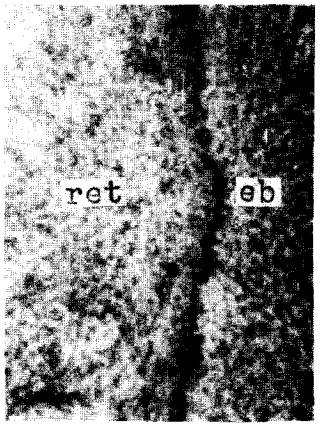

Fig. 12.