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Intermediary Metabolism in the Immature Liver Fluke, *Fasciola hepatica* L.

THE adult and juvenile forms of the liver fluke differ in their susceptibilities to a number of anthelmintics[1]. If it is assumed that anthelmintic drugs exert their effects by acting on enzymes or multiple enzyme systems in the parasite then the adult and immature fluke may not possess identical patterns of metabolism. Some of the pathways of intermediary metabolism in the adult fluke have recently been studied using substrates labelled with carbon-14 in conjunction with chromatographic and autoradiographic techniques for the separation of the labelled intermediates formed[2]. In the present work, a comparison has been made of the patterns of incorporation of radioactivity from carbon-14-labelled glucose, acetate and succinate into the soluble metabolic intermediates of adult and immature forms of *Fasciola hepatica*.

Adult flukes were removed from the livers of freshly killed sheep, stored in Hedon–Fleig saline[3], and used within 24 h. Viable cysts of *F. hepatica* were introduced with polythene cannulæ into the stomachs of mice. The mice were killed after 3 weeks and the immature flukes isolated from the liver parenchyma, washed with Hedon–Fleig saline and used immediately. The methods used for incubation of the parasites with ^{14}C-glucose, 2-^{14}C-acetate or 1 : 4-$^{14}C_2$-succinate and for separating, identifying and measuring the radioactivity in the soluble metabolic intermediates were those described previously[2].

Both forms of the fluke incorporated less than 1 per cent of the labelled acetate and succinate into the soluble intermediates and the distribution of the radiocarbon was very similar. The labelled glucose was metabolized to a much greater extent by the adult than the immature flukes and there were differences in the subsequent distribution of the isotope in the various fractions (Table 1).

Table 1. METABOLISM OF GLUCOSE LABELLED WITH CARBON-14 BY ADULT AND IMMATURE *Fasciola hepatica* L.

Radioactive intermediate	Adult fluke	Immature fluke
Glucose % utilized	80	35
Hexose phosphates	37	76
Alanine	12	2
Succinate	19	12
Fumarate	5	1
Malate	27	4

Results expressed as percentage incorporation of radioactivity excluding residual substrate.

The adult fluke utilized approximately twice as much of the labelled sugar and the radiocarbon was distributed among hexose phosphates, alanine and acids of the tricarboxylic acid cycle. In the immature fluke there was an increased incorporation of radioactivity in the hexose phosphate fraction suggesting that glycolysis was proceeding less efficiently than in the adult form. The decreased percentage incorporations of radiocarbon into the other fractions probably result from a decreased turnover of the phosphate fraction. The presence of carbon-14 in malate, fumarate and succinate indicates that the immature fluke resembles the adult form of the parasite in possessing enzyme systems capable of forming succinate from pyruvate via malate and fumarate[4].

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Maturation Rate of the Osteon of the Cat

SINCE the localization of tetracyclines in newly formed bone was first reported[1] these drugs have been used in investigations of bone activity[2,3] and bone growth[4]. In the course of an examination of bone activity in the mandible of the cat it was noticed that although successive injections of tetracycline were given at equal time-intervals successive labels were not evenly spaced. As the osteon formed, the rate of formation appeared to diminish. This 'maturation effect' has been noticed by other authors[5]. It was thought that some assessment of this rate of diminution might be interesting, particularly if this information could be used to shed light on factors influencing deposition of bone.

Two cats aged approximately 9 months and 2·5 years were used. The younger animal (*C*.4) received two doses of oxytetracycline (33 mg/kg) two weeks apart; the older animal (*C*.5) received three doses of oxytetracycline at four-weekly intervals. Both animals were killed 48 h after the final dose. The mandible and right femur of *C*.4, and the mandible, left humerus and right femur of *C*.5 were embedded in methyl methacrylate, sectioned at 30μ, and examined under ultra-violet light.

The osteon of the cat is approximately elliptical and most measurements were made on the short diameter where the labels tend to run parallel (some few measurements were made on the long diameter without apparently altering the results). The diameters of the external and internal labels were measured using an eyepiece micrometer, the measurements being taken from the centre of each label. In *C*.4 27 osteons in the femur and 74 in the mandible were measured, and in *C*.5 17 osteons in the humerus, 28 in the mandible and 29 in the femur were measured.

The radius of the internal label (r) was plotted against the radius of the external label (R) for each bone separately. For each bone a linear relationship obtained (Fig. 1). Statistical analysis has shown that in each case the results are consistent with the expression $r = kR$, and that the values of the constant for different bones from the

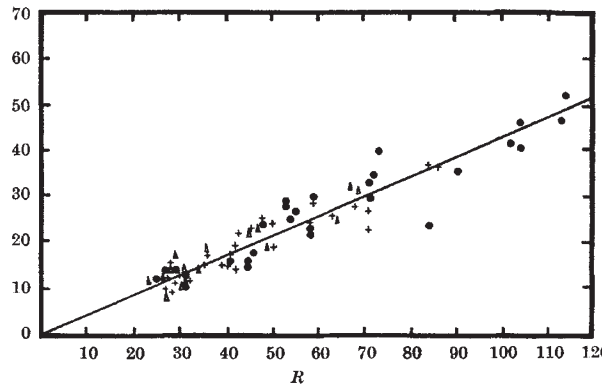

Fig. 1. Cat 5. ●, mandible; △, left humerus; +, femur