Oxidation of Parenterally Administered C^{14}-labeled Tripalmitin Emulsions[1]

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The question whether long-chain fatty acids can be utilized when administered by routes other than the gastrointestinal tract has aroused considerable interest in recent years. Dunham and Brunschwig (*2*) failed to observe protein-sparing effects in 9 of 11 dogs when a highly emulsified fat was injected intravenously for periods as long as one month. The emulsions used, however, were quite toxic. McKibbin, *et al.* (*4*), on the other hand, have drawn the conclusion that intravenously administered emulsions of fat are utilized, for not only did they find weight improvement and nitrogen retention in 2 dogs, but, in addition, they were unable to account for a considerable portion of the infused fat by finding it stored in an unmodified form. Meng and Freeman (*5*) also noted a gain in body weight in dogs that received fat emulsions intravenously, but they point out that such results furnish no direct proof of fat utilization.

The use of radioactive carbon provides for the first time a direct method for determining whether an animal can convert parenterally administered fatty acids to CO_2. Palmitic acid containing C^{14} in the sixth carbon atom

$$C_{10}H_{21}C^{14}H_2CH_2CH_2CH_2CH_2COOH \qquad (I)$$

(I) was synthesized as described in an earlier communication (*1*) and then esterified with glycerol by a modification of the method of Feuge, *et al.* (*3*). An emulsion of the tripalmitin was prepared with glycerol monostearate as the stabilizer and the fat dispersed into particles of less than 2 μ by means of supersonic energy. One or

TABLE 1

RECOVERY OF C^{14} IN THE EXPIRED CO_2 OF A RAT INJECTED INTRAVENOUSLY WITH A FAT EMULSION CONTAINING TRIPALMITIN IN WHICH THE PALMITIC ACID WAS LABELED WITH C^{14}*

Interval (hrs)		0–2	2–4	4–6	6–19	19–24	Total
C^{14} in expired CO_2	Rat 1	7.2	10.2	8.6	20.6	4.3	50.9
	Rat 2	6.4	13.7	7.7	24.2	4.5	56.5

* The values recorded are percentages of the total injected radioactivity.

1.5 cc of this emulsion containing 25 mg of tripalmitin was then injected into the foot vein of fasted rats weighing 175 gm. The expired CO_2 was collected and its C^{14} determined. Typical results are shown in Table 1.

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In the first 2 hrs approximately 7% of the injected C^{14} was found in the expired CO_2, and at the end of 24 hrs about one-half of the radioactivity was exhaled. The maximum rate of $C^{14}O_2$ exhalation was observed between the 2nd and 4th hrs. These results indicate that about one-half of the administered palmitic acid had been metabolized in 24 hrs.

The data presented here justify the conclusion that emulsified fat introduced directly into the blood stream is available for caloric purposes. Further evidence that parenterally administered emulsified fat pursues a normal metabolic path was provided by the finding that about 50% of the injected C^{14}-labeled fatty acids recovered in the liver had been incorporated into phospholipids at the end of 24 hrs.

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Life Cycle of *Postharmostomum laruei* McIntosh 1934 (Trematoda: Brachylaemidae)

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The adult trematode, *Postharmostomum laruei* McIntosh 1934 (Brachylaemidae), has been experimentally developed in this laboratory in the deer mouse, *Peromyscus maniculatus* (various subspecies), as the final host.

McIntosh (*1*) described the adult, specimens of which he obtained from the cecum of the chipmunk, *Tamias striatus lysteri* (Richardson). The metacercaria and adult of this species were described by Miller (*2*) in an abstract of an unpublished doctoral dissertation, in which he named this species *Brachylaima (Postharmostomum) sexconvolutum.* An examination of Miller's thesis, however, shows conclusively that his material included at least two species. Apparently aware of the synonymy and without referring directly to his published abstract, Miller (*3*) reported on the growth rate of this parasite, now referring to it as *Postharmostomum laruei* McIntosh.

My interest in the completion of the life cycle of this species was aroused when the metacercarial stages of the parasite were encountered repeatedly during examination of land snails in the vicinity of Ann Arbor, Michigan. The metacercariae are found within the pericardial cavity of the following land snails: *Anguispira alternata, Polygyra thyroides, P. profunda, P. multilineata, P. fraudulenta, P. hirsuta, Gastrodonta ligera,* and *Zonitoides ar-*

[1] Contribution from the Department of Zoology, University of Michigan, under the direction of Dr. George R. LaRue.

boreus. In slugs, too, the metacercariae occur within the pericardial cavity. Only two species of land snails thus far examined (*A. alternata* and *P. fraudulenta*) harbor this species to the exclusion of all other brachylaemids. The number of metacercariae found within the pericardial cavity varies from one to many. Particularly in *A. alternata*, the number per snail is frequently above 50, and one snail contained 83 fully developed metacercariae. In general, metacerariae within the pericardial cavity may be divided into three recognizable age groups. Those fully developed are ovoidal, with well-established genital fundaments and exhibiting little or no movement. Very young metacercariae are characterized by the presence of a rudimentary tail, whereas intermediate forms are more elongate and tailless and show a considerably greater motility. All specimens differ from other members of the family found in snails in this area by the lack of ciliated excretory tubules. All three age groups may be present within the pericardial cavity of the same snail, indicating that immunity to the cercariae is not established following infection.

Observation showed that entrance of cercariae to the pericardial cavity occurs via the respiratory aperture, From this point, they are assumed to migrate through the excretory duct to the kidney and thence, by way of the renopericardial connection, to the heart chamber itself. Growth of metacercariae is extremely slow; feeding experiments in progress show that at 18 weeks the metacercariae have not yet reached full size.

When fully developed metacercariae were fed to laboratory-reared *Peromyscus*, adults of varying ages were secured from the cecum of the host. Attachment to the cecal walls and ingestion of blood commence within a very few hours after feeding. Adult flukes have been maintained in *Peromyscus* for as long as 100 days. Egg production commences on the 8th or 9th day, while fully embryonated eggs appear in the feces about the 20th day. All attempts at securing discharge of eggs from living worms have been unsuccessful.

Published data dealing with members of this family have shown a conspicuous lack of information concerning the miracidial stage, primarily because of difficulties encountered in freeing the miracidia from the egg shells. Miller (*2*) concluded that the miracidial stage is nonexistent for this form. Hatching of the miracidium of *P. laruei* has been observed by me, and work is now in progress in an attempt to determine its morphology. Movement of the miracidia within the eggs can be seen after the crushed adults have been placed in saline; but emergence was noted only after the adults had been kept under refrigeration several days in normal saline and then transferred to tap water, crushed, and the eggs removed.

Eggs containing fully developed miracidia were fed to laboratory-reared specimens of *A. alternata* and various species of *Polygyra*. Branching sporocysts containing immature cercariae were found only in *A. alternata* after several weeks, while mature cercariae emerged between 3 and 7 months after exposure, depending upon temperature conditions. It is noteworthy that, although snails infected with the metacercarial stage are abundant in nature, averaging from 85 to 100% infection in localities near Ann Arbor where this parasite is found, the number of snails harboring sporocysts and cercariae is exceedingly small, since only 15 individuals of more than 3,200 examined to date by techniques involving the crushing of the snail have shown these stages. Moreover, fully developed cercariae emerged from only 5 of these 15. During the examination of a great variety of terrestrial snail hosts, sporocysts of other species of Brachylaemidae have been found, but those of *P. laruei* were found only in *A. alternata*, indicating specificity of this species of snail to infestation with the sporocyst of *P. laruei*.

A detailed account of this life cycle with complete descriptions of all stages involved is in preparation and will appear elsewhere.

References

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Distribution of Free Amino Acids in Mouse Epidermis in Various Phases of Growth as Determined by Paper Partition Chromatography

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This is part of a series of experiments the purpose of which is to characterize the nitrogen metabolism of mouse epidermis in various phases of growth.

In the experiments to be reported a survey was made of the free amino acids found in alcoholic extracts of whole epidermis obtained from newborn mice (0–4 and 7 days of age), from normal adult mice, and from mice receiving 3, 6, or 12 applications of methylcholanthrene in benzene or 3 applications of benzene alone. A transplantable squamous cell carcinoma originally derived from a carcinoma produced on the skin of a mouse by the application of the carcinogen was also studied. The method of treatment of the animals and preparation of the tissues has been described (*6*). The tissues were dried *in vacuo* over P_2O_5, ground, and redried to constant weight. Samples (200 mg) of the tissues studied were stirred thoroughly with 1 ml of 76% alcohol at room temperature, and determinations by the two-dimensional chromatographic method (*1, 2, 4*) were made on 75 μl of the untreated extract and on similar aliquots after oxidation with H_2O_2 and after hydrolysis with HCl. The amino

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