Notes on the Life History of a Brachylaemid Trematode

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All stages in the life cycle of a brachylaemid trematode have been observed in experimental infections. The cercariae are produced in branched sporocysts in the digestive gland of *Anguispira alternata*; they emerge from the snail and penetrate the same or another individual of *A. alternata* or *Polygyra thyroidus*, migrate to the pericardium, but do not encyst. Developmental and sexually mature stages have been recovered from *Peromyscus leucopus* and from the white mouse. Eggs are produced at about 13 days after ingestion of metacercariae by a white mouse and appear in the feces at about 21 days.

The adult measures 3.5×2.0 mm. The oral sucker is 500×580 μ, and the ventral sucker, 460×560 μ. The pharynx is muscular; the caeca loop back and forth, extend to the posterior end of the body, and have numerous evaginations on both lateral and medial sides. The gonads lie close together, forming a triangle in the most posterior portion of the worm, with one testis on the left side, one in the midline and posterior, and the ovary on the right. The testes are indented. In a mature worm from *P. leucopus* the left testis measures 420×460 μ; the posterior one, 460×540 μ. The ovary is smooth and measures 650×550 μ. The genital pore is median, at the level of the anterior testis. The vitellaria are lateral, follicular and sparse, and extend from the pharynx to the ovary. The uterus fills the body between the caeca from the oral sucker to the anterior testis. Both ascending and descending loops are much convoluted; the descending loop is conspicuous in the dorsal midline. The main collecting ducts of the excretory system are lateral to the caeca, and the bladder empties through a posterior pore.

The thin-shelled but somewhat yellowish egg measures 32×18 μ. The shell is thicker at the adopercular end than elsewhere. Miracidia are visible in the eggs when they are passed by the mice. The larvae do not move, but the adopercular half appears to be ciliated.

The sporocyst is much branched and dendritic and ramifies through the entire liver of the snail. The branching of a fragment is irregular, starting from a roughly defined main trunk. Each branch, but not the main trunk, is divided by constrictions, and may end in a filament with a terminal knob. All parts of the sporocyst except the constrictions are filled with cercariae in different stages of development. Some portions of the sporocyst contain but one cercaria between constrictions; others, from two to many. No birth pore has been observed.

Cercariae washed from the surface of infected snails are about 250 μ long when extended. The tail is a small protuberance in a posteroventral position; the caeca are broad and extend to the level of the center of the ventral sucker; and a prepharynx and short esophagus are visible in a fully extended cercaria. The cuticle bears a few widely-scattered, conspicuous projections, possibly sensory. Just posterior to the ventral sucker is a germinal mass. The flame cell pattern has not been entirely worked out, but there appear to be 5 flame cells in each anterior quadrant and 4 in each posterior one. The vesicular bladder empties through two ducts leading to pores on the tail.

The metacercariae are over 1 mm long at maximum size, broad, white and somewhat concave ventrally. The caeca extend to the posterior end and are looped back and forth. There is a thick cuticle which, in the living worm, is transversely folded and may present a spinous or scale-like appearance. Three distinct gonad rudiments are present. After three weeks in the second intermediate host the worms still have the cercarial tail, although they are larger and the caeca have increased in length.

This trematode possibly is identical with the metacercaria described by Leidy in 1847 as *Distoma helicis* (renamed *D. pericardium* by Creplin, 1849), and with *D. vagans* Leidy (1850). The morphological and taxonomic features will be presented in a later publication.

Vitamin B_{14} and Cell Proliferation

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A crystalline compound has been isolated from urine which had a very high activity on cell proliferation *in vitro* and on hemopoiesis *in vivo*.

From 100 liters of normal human urine 33 mg of a material was obtained as balls of small brown crystals which is designated here as vitamin B_{14}, although its origin and function is probably more that of a hormone than a vitamin. On analysis the material was found to contain 19.6% nitrogen and 4.0% phosphorus. Sulfur was not present. Cobalt was absent, or, if present, there was so small a fraction of a per cent that it could not be considered anything but an impurity.

Rickes, *et al.* (*4*), Shorb (*5*), and West (*7*) have reported a cobalt-containing compound (vitamin B_{12}) isolated from liver, which had a hematological activity in cases of addisonian pernicious anemia. Smith (*6*) described a pigment from liver as an antipernicious anemia factor which may be identical with vitamin B_{12}.

The activity of vitamin B_{14} was tested on bone marrow cultures by the technique previously described (*1*). The results are given in Table 1. The table shows that in bone marrow cultures vitamin B_{14} is at least 5,000,000 times as effective as xanthopterin, or 1×10^{-6} γ/ml of cell suspension gives an increase in the rate of cell proliferation similar to 5 γ/ml of xanthopterin. Vitamin B_{14} was not toxic in concentrations up to 100 γ/ml. Its effect in accelerating cell proliferation is counteracted by 2-amino-4-hydroxy-7-methyl pteridine (7MP), which also counteracts the effect of xanthopterin.

The effect of pterins on cell proliferation in a suspension of cells of neoplastic tissue is opposite to the effect on cells of bone marrow suspension or of normal tissue (*3*). Xanthopterin accelerates proliferation of bone marrow cells and inhibits neoplastic cells. 7MP inhibits bone marrow cell proliferation and accelerates proliferation of cells of neoplastic tissue. Table 2 shows the effect of vitamin B_{14} on a cell suspension of Brown Pearce rabbit tumor.

TABLE 1

EFFECT OF VITAMIN B_{14} ON CELL PROLIFERATION *in vitro* IN A BEEF BONE MARROW SUSPENSION*

Supplement/ml of suspension	Final concentration (RBC/mm³)	Increase in RBC (%)	Final concentration (NC/mm³)	Increase in NC (%)	Retic. /1,000 RBC
None	5,850	19	4,720	64	15
5γ xanthopterin	10,900	120	8,170	184	33
5γ 7MP	2,680	−46	1,680	−42	5
1×10^{-6}γ vit. B_{14}	12,800	161	6,850	138	39
1×10^{-5}γ vit. B_{14}	24,500	400	19,800	665	37
1×10^{-4}γ vit. B_{14}	39,000	695	33,900	1,080	50
1×10^{-2}γ vit. B_{14}	40,200	720	34,200	1,090	46
10γ vit. B_{14}	42,500	766	33,800	1,070	63
100γ vit. B_{14}	41,500	750	32,400	1,020	53
1×10^{-4}γ vit. B_{14} plus 5γ 7MP	28,100	472	23,800	760	40
1×10^{-4}γ vit. B_{14} plus 10γ 7MP	15,600	238	10,800	276	25

* Initial concentration of cells in the suspension: RBC, 4,910/mm³; nucleated cells (NC), 2,880/mm³; reticulocytes, 9/1,000 RBC. Time of incubation, 7.5 hrs at 37° C.

TABLE 2

EFFECT OF VITAMIN B_{14} ON CELL PROLIFERATION *in vitro* IN A SUSPENSION OF BROWN PEARCE TUMOR CELLS*

Supplement/ml of suspension	Final concentration (cells/mm³)	Increase in cells (%)
None	11,200	26
5γ 7MP	15,000	68
10γ 7MP	17,500	96
5γ xanthopterin	6,780	−24
10γ xanthopterin	4,600	−48
1×10^{-7}γ vit. B_{14}	7,960	−11
1×10^{-6}γ vit. B_{14}	4,850	−46
1×10^{-4}γ vit. B_{14}	3,120	−65
1×10^{-1}γ vit. B_{14}	1,320	−85
1×10^{-6}γ vit. B_{14} plus 5γ 7MP	9,100	2
1×10^{-6}γ vit. B_{14} plus 10γ 7MP	11,200	26

* Initial concentration of suspension: 8,920 cells/mm³. Time of incubation, 5 hrs at 37° C.

The technique used in culturing the tumor cells was the same as that previously described (*3*). Vitamin B_{14} is about 10,000,000 times as effective as xanthopterin in inhibiting cell proliferation *in vitro* in a suspension of tumor cells, or 1×10^{-6} γ/ml of vitamin B_{14} had about the same effect as 10 γ/ml of xanthopterin. The effect of 1×10^{-6} γ/ml of vitamin B_{14} was completely counteracted by 10 γ/ml of 7MP, or 1×10^{-6} γ/ml of vitamin B_{14} plus 10 γ/ml of 7MP gave the same rate of proliferation as with no supplement.

In rats made anemic with sulfathiazole by the procedure previously described (*2*), a single injection of 0.01 γ of vitamin B_{14} was as effective in alleviating the anemia and leukopenia as a single injection of 1 mg of xanthopterin.

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Action of Enzymes on Vitamin B_{14} and Pteridines

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The activity of xanthopterin and pteroyl derivatives in accelerating the rate of cell proliferation *in vitro* and in producing hemopoiesis in anemic rats has been found to be greatly increased by the action of certain enzymes.

Xanthine oxidase was prepared from milk and purified by repeated precipitation with ammonium sulfate. The enzyme preparation was very active when measured in a Thunberg tube with methylene blue, using either xanthine or xanthopterin as the substrate. Xanthopterin and folic acid (pteroylglutamic acid) were incubated with the enzyme, under toluene, at 37° C with a phosphate buffer of pH 7.4. Aliquots were removed at intervals for analysis. There was a very marked change in the absorption spectrum, which will be reported later. Kalckar, *et al.* (*1, 2*) have studied some phases of the action of the milk enzyme on xanthopterin.

The effect on cell proliferation of the products resulting from the enzyme action was measured by the technique previously described (*3*). Table 1 gives a summary of typical results obtained.

The activity of both xanthopterin and folic acid was increased by the action of the enzyme. The effect is most noticeable in the case of folic acid. At zero time the number of cells produced in the incubation of the bone marrow culture were the same with folic acid present as with no supplement. After the action of the enzyme the product formed from folic acid very greatly increased the rate of proliferation of both RBC and