Technical Papers

The Relationship Between *Trichobilharzia oregonensis* and *T. elvae*, Etiological Agents of Schistosome Dermatitis in the Pacific Northwest[1]

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Considerable confusion exists as to the status of various European and American species of schistosomes which cause dermatitis in man. Technical difficulties involved in experimental work, especially the recovery of intact adult worms, are largely responsible for this state of affairs. Recently we have been able to clarify the relationship between two species found in the Pacific Northwest.

In 1946, *Cercaria oregonensis* was described as a new species of schistosome found at Portland, Oregon, capable of producing dermatitis in man (*1*). Although this cercaria is similar to that of *Trichobilharzia elvae* Miller, there are certain minor differences in measurements and behavior, and the snail host is *Physa ampullaria* instead of *Lymnaea stagnalis* and *Stagnicola palustris elodes* which are hosts of the former species. If *T. elvae* is synonymous with *T. ocellata*, as believed by McMullen and Beaver (*2*), then this species is found in Europe, in addition to a wide North American geographical range.

Snails infected with *Trichobilharzia elvae* were transported more than 300 miles from Cascade Lake on Orcas Island, Puget Sound, Washington, where one of us (R. W. M.) has studied the biology of the species (*3*). A series of Pekin ducklings was infected with cercariae of *T. elvae* from *Lymnaea stagnalis* and a second group of these birds was given cercariae of *T. oregonensis* from naturally infected *Physa* snails collected at Portland, Oregon. Representative cercariae from each snail were measured in order to exclude the possibility of mixed infections, especially with *T. physellae* which sometimes occurs in *Physa* in the vicinity of Portland.

As indicated by the use of McMullen-Beaver flasks (*2*), about two weeks were required for the maturation of both species of worms. Eggs of *T. elvae* imbedded in the intestinal wall of the ducks showed the typical curved axis, producing a moon shape, whereas those of *T. oregonensis* were found to be more nearly straight and closely resembled those of *T. physellae*. However both the adults and cercariae are strikingly different from those of the latter species. The small cercaria of *T. physellae*, which we have studied here, has fewer flame cells than those of either *T. elvae* or *T. oregonensis* and closely fits the description given by Talbot (*4*) for *T. elvae*.

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Adults of *Trichobilharzia oregonensis*, some of them undamaged, were laboriously recovered in small numbers from the venules of the intestinal wall of the ducklings and were found to differ markedly from those of *T. elvae* as described by McMullen and Beaver. Although the average body length is only a little larger than that of *T. elvae*, the acetabulum of *T. oregonensis* averages 0.046 mm in diameter, approximately the same as the oral sucker, and is about three to four times the diameter of the acetabulum of the former species. Further, the seminal vesicle of the male is followed by a still longer, similar structure, bordered on the posterior end by a prostate mass, and probably therefore is a very elongate cirrus sac. These relationships were constant in six males examined.

Our work clearly demonstrates that at least three distinct species of *Trichobilharzia* occur in the Pacific Northwest. Further details will be published elsewhere.

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Estrogenic Activity in a Commercial Animal Ration

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In the course of some experimental work involving castrated mice during the past year, it became apparent that the animals were showing the presence of an appreciable amount of estrogen. Observations revealed that the vaginal smears of these mice contained many cornified cells. Examination of the uteri of representative mice taken from a group of 100 castrated animals revealed uterine weights of more than 40 mg. Since it had been previously demonstrated in our colony that the uteri of mice castrated for about 2 wk weighed approximately 12 mg, it was apparent that the animals were being exposed to an estrogen.

These observations led us to suspect the presence of an estrogen in the food and as a result the mice were placed on a new commercial ration (A). Following this change in diet the vaginal smear became negative and contained only leucocytes and an occasional epithelial cell. In order to prove the source of the estrogen, 1 kg of the suspected diet (B) was extracted with petroleum ether and taken up in 30 ml of oil. The material was then tested for estrogenic activity by the uterine weight method, using castrated mice.

TABLE 1

ESTROGENIC ACTIVITY IN AN EXTRACT OF A COMMERCIAL FOOD RATION (B) FOR LABORATORY ANIMALS

No. of mice	Av. body wt., g	Treatment	Mean uterine wt., mg
8	28.6	16 days castrated	11.2 ± 1.4
8	31.2	23 days castrated	13.3 ± 1.3
10	30.5	Estradiol, 0.002 μg/day	23.1 ± 2.7
10	29.2	Estradiol, 0.005 μg/day	24.2 ± 1.9
10	31.5	Estradiol, 0.01 μg/day	40.0 ± 1.4
10	29.2	Estradiol, 0.1 μg/day	97.3 ± 8.7
10	29.1	Food extract, 0.05 ml/day	24.6 ± 2.1
9	28.7	Food extract, 0.1 ml/day	53.6 ± 2.0

The animals were rested for 15 days postcastration and then injected daily for 7 days. The mice were killed 24 hr after the last injection, the uteri dissected out, split longitudinally to remove luminal water, and weighed immediately on a torsion balance.

It may be seen from the data in Table 1 that castration atrophy was complete by the 16th day. The mean uterine weight of mice castrated for 16 days was found to be 11.2 mg and that from mice castrated for 23 days was 13.3 mg. Since the body weights averaged approximately the same for each group of mice, it was not deemed necessary to convert the uterine weights to a per cent body weight figure. A standard dose-response curve was established with estradiol over a dosage range of 0.002 μg to 0.1 μg/day; these concentrations gave an adequate curve for assay purposes. The extract was injected at two dose levels, and in all instances a constant volume of 0.1 ml was injected. The equivalent of 0.05 ml of the extract increased the mean uterine weight to 24.6 mg, whereas 0.1 ml resulted in a mean uterine weight of 53.6 mg. Analysis of the data revealed that 1 kg of food contained an estrogenic potency equivalent to 3.75 μg of estradiol.

Although no direct evidence is available for the nature of the estrogen, it would not appear to be estradiol since the substance showed high oral activity. If one speculates on the source of the estrogen as due to implanted pellets used in the poultry industry (*1, 2*) and as having gotten into the food as waste scraps of meat, then one could conclude that the substance is a synthetic estrogen since these are the substances used by poultry breeders. It is of interest to note that several years ago the feeding of neck scraps from implanted birds caused sterility in mink (*3*).

There is also a possibility that the estrogen came from a plant source. Investigators have shown the presence of estrogenic activity in glycyrrhiza (*4*), willow (*5*), wheat germ oil (*6*), date palm tree (*7*), clover (*8, 9*), and alfalfa (*9*). Recently an isoflavone derivative has been isolated from soybean oil meal with estrogenic activity (*10*). Thus it is highly possible that the source of the hormone could have been from added plant material.

Regardless of the source of the estrogen, an appreciable amount of the hormone was found in a commercial food ration. The concentration was sufficiently high to prevent castration atrophy as noted by changes in the uterus and the vagina. This could easily invalidate many experiments and would definitely interfere with any assay for estrogen or with the Hooker-Forbes test for progesterone (*11, 12*). It would thus appear essential that proper controls be maintained at all times for possible food contamination with an estrogen.

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Comparative Studies in the Uptake of Phosphorus by Tissues under Different Doses of Injected Radioactive Phosphorus P^{32} [1]

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Radioactive phosphorus (P^{32}) is one of the few radioisotopes that have been used in therapy (*1, 2*). When a radioisotope in soluble form is injected or administered by mouth, it is deposited with greater or less selectivity in different organs. A knowledge of this differential uptake is essential for a correct estimate of the radiation dosage and for successful radioisotope therapy (*3*). The results of early investigations on the relative concentration of P^{32} in different organs of experimental animals and of the time variation of the concentration have been summarized by Hevesy (*4*) and Chaikoff and Zilversmit (*5*).

In this work we have attempted to find out how the relative concentration of phosphorus by tissues is affected by different doses of the injected radioisotope. This seems to be of fundamental importance, not only for the correct estimation of the therapeutic dose of radioactive material, but also for showing the disturbances in metabolism produced by different doses of beta irradiation from P^{32}.

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