Irradiation Induced Neoplastic and Giant Cells in Earthworms

A tumor has been induced in earthworms (*Lumbricus terrestris* L.), that resembles the muscle tumor of vertebrates, termed myoblastoma[1]. These neoplastic cells may arise from degenerated adult striated muscle fibers. Granular cell myoblastomas contain acidophilic granules and have either rather large vesicular or small hyperchromatic nuclei.

These features can be demonstrated in earthworm myoblastomas. A few worms which had received 1000 R 23 days before sacrifice time developed masses with typical myoblastoma cells. The granular cells contained acidophilic material. Some of the cells had large vesicular nuclei and others possessed the small hyperchromatic nuclei. The striated muscle tissue had been lost. Sections through the seminal vesicles from a worm fixed 12 days after receiving 1000 R of X-irradiation showed myoblastoma cells proliferating around the calciferous gland and eroding the dorsal muscle layers to the exterior. The seminal vesicles lost their normal possession of a variety of forms of spermatogenic cells and instead were packed with a solid mass of cells resembling the myoblastoma cells, although the acidophilic elements were absent and only the hyperchromatic type nuclei were present. A worm that had received 400 R and killed 23 days after irradiation was found to have the coelum entirely filled with myoblastoma cells and much of the muscle tissue was missing. A worm that had received only 100 R and was sectioned 23 days afterwards showed myoblastoma cells, that had invaded the coelum and were seen in the lateral muscle tissues. Giant nuclei can also be found in the myoblastoma like masses in the altered areas of the seminal vesicles (Figure 1). Giant nuclei have been reported in the pharyngeal tumor of the earthworm by STOLK[2,3]. Giant nuclei were also described in 'normal' pharyngeal epithelium by HANCOCK[4]. Epithelial tumors in earthworms have been reported by GERSCH[5] which were induced by benzpyrene. HANCOCK[1] has also described several neoplasms in earthworms including an example of a myoblastoma.

Multinucleated giant cells have been seen in anaplastic sarcomas of rat muscle induced with nickel[6]. Experimental efforts with multinucleated giant cells were made by WAKAMATSU and SANKA[7], who reported on inducement of multiple nuclei in HeLa cells exposed to irradiation from Co^{60}. Furthermore, they showed that the greatest frequency of these multiple nucleated cells occurred with 2000 R 14 to 21 days post irradiation time. Many of these cells had 5 to 10 nuclei. To the writer's knowledge multinucleated giant cells in earthworms have not been previously reported. Multinucleated giant cells usually occurred in the coelum of earthworms after varying doses and postirradiation times. Earthworms that had received 1000 R and sacrificed 7 days later had cells with as many as 40 nuclei. Figures 2 and 3 are of earthworms that have received 600 R and were sectioned 12 days later. Figure 3 is an area in the muscle, which shows degeneration with hyalinization present. In the area immediately above,

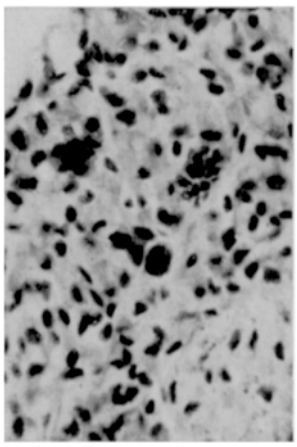

Fig. 1

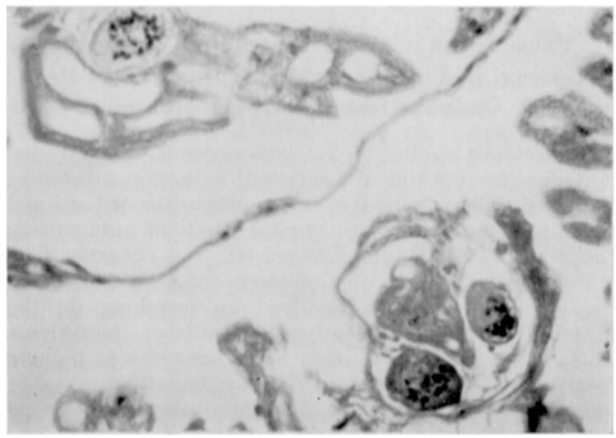

Fig. 2

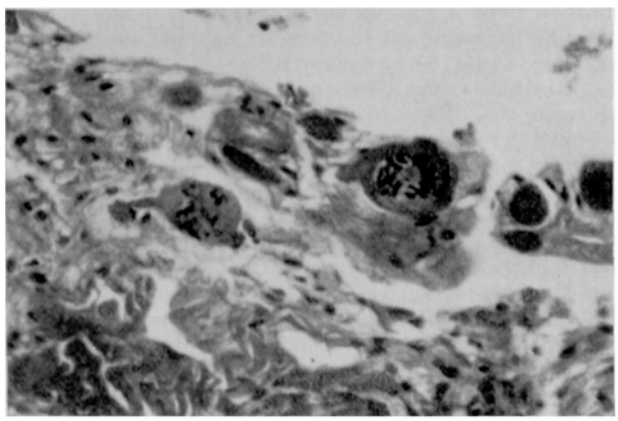

Fig. 3

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where no muscle fibers remain, the giant cells are imbedded in a region of fibroblasts. Such regions in the muscle tissue may be the site of origin for myoblastomas.

These responses in invertebrate tissues have particular significance, for they are reminiscent of changes seen in mammalian tissues and thereby support the concept that similar cell mechanisms occur in invertebrate pathology. The question, however, of whether invertebrate tumors are 'true' tumors remains an issue among cancer biologists.

Zusammenfassung. Verschiedenes, durch Röntgenbestrahlung hervorgerufenes Regenwurmneoplasma erweist sich den Vertebraten-Mioblastoma ähnlich. In solchen Tumoren werden zuweilen Riesenkerne gefunden. Bestrahlte Würmer weisen ebenfalls vielkernige Riesenzellen auf.

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Hulls Cove (Maine USA), July 9, 1964.

Presence of a Slow-Contraction Inducing Material in Fluid Collected from the Rat Paw Oedema Induced by Serotonin

In previous studies on rat paw oedema we succeeded in demonstrating that the different inducting substances appeared to determine the time course of the oedema and also its responsiveness to various kinds of antagonistic drugs[1–3]. These findings suggested the occurrence of manifold processes in this phenomenon. We decided to study the mechanisms involved by searching for the presence of biologically active material(s) in the oedema fluid of the swollen rat paw. Since serotonin as inducer resulted in a larger degree of swelling than did most other inducing materials, which facilitated the collection of sufficient amounts of oedema fluid, we decided to commence the investigation with this kind of oedema.

Male albino rats (100–110 g) from our own colony were given a subplantar injection of 2 μg serotonin creatinine sulphate dissolved in 0.9% saline in both hind paws. The injected volume was 0.1 ml in each paw and control animals received a corresponding volume of saline. 30 min after the injection, at the time of maximal swelling, the rats were sacrificed by decapitation; both hind paws were cut off distal to the tarso-crural articulation and pressed between the rollers of a small mangle, specially constructed for this purpose. This procedure enabled us to collect 0.2–0.5 ml of fluid from the two hind paws of rats that were given the subplantar injection of serotonin, whereas five or more of the rats which had received saline were needed to yield the same amount of fluid. The fluid was slightly opalescent and faintly pinkish. After filtration through cotton, a clear fluid was obtained. The fluid was collected and preserved in a test tube that was immersed in ice-cold water. Biological activity was tested: (a) On the isolated uterus of rats that were given 0.01 mg of estradiol benzoate one day before removing the organ. The temperature of the isolated organ bath (2.5 ml) was kept at 30°C and Jalon's solution was used as organ bath medium. Serotonin creatinine sulphate, acetylcholine chloride and synthetic bradykinin[4] were used as reference compounds, and LSD-25, atropine sulphate and phenylbutazone served as antagonists. In order to prevent tachyphylaxis when serotonin was used, the administration of this substance was alternated with addition of acetylcholine. (b) In other experiments the isolated guinea-pig ileum was used. This organ was suspended in Tyrode solution in an organ bath having a volume of 2.5 ml and kept at 37°C. Histamine dihydrochloride was used as reference substance and phenbenzamine (Antergan) as specific antagonist. The use of the isolated guinea-pig ileum was necessary in view of the non-responsiveness of the rat uterus to histamine. Irrespective of the use of either of the isolated organs, the lever was loaded with 0.5–0.85 g and the contractions were recorded on a smoked drum. The oedema fluid was tested as soon as possible after collection from the paws.

When the isolated organs were exposed to fluid collected from paws of rats receiving a subplantar injection of saline, no contractions were observed with doses up to 0.3 ml. The fluid originating from the swollen paws of rats treated by subplantar administration of serotonin, induced marked contractions of the isolated organs when added in a volume of 0.1 to 0.3 ml to the organ bath. In Figure 1 a record is presented demonstrating characteristic responses of the isolated rat uterus to various materials. In this particular experiment the kymograph was run at a higher speed than usual, in order to obtain more detached information on the qualitative character of the responses. Acetylcholine induced instantaneous contraction, whereas with serotonin a short lag period preceded the response. The lag period was even more pronounced with bradykinin, furthermore with this substance the contraction occurred at a slower rate than with either acetylcholine or serotonine. When the uterus was challenged with the oedema fluid, the lag period and speed of

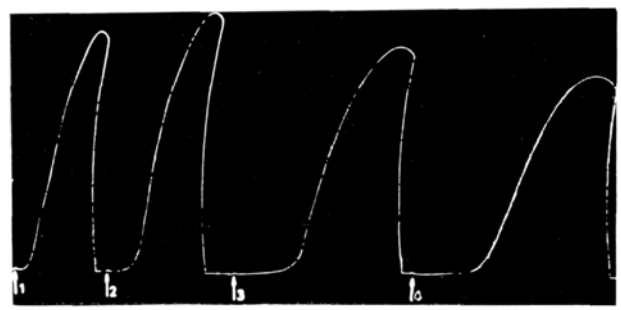

Fig. 1. Effect of various materials on the isolated uterus of the oestrus rat. 1 – Acetylcholine 0.75 μg. 2 – Serotonine 0.15 μg. 3 – Bradykinin (synthetic) 0.02 μg. 4 – Oedema fluid 0.1 ml.

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[3] I. L. BONTA and C. J. DE VOS, Acta endocr., in press (1964).
[4] Synthetic bradykinin was obtained through the courtesy of Prof. A. CERLETTI, Sandoz AG, Basel.