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Cancer Detection and Therapy. Affinity of Neoplastic, Embryonic, and Traumatized Tissues for Porphyrins and Metalloporphyrins.*

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In the course of studies on the cocarcinogenic action of porphyrins[1,2] many of the mice that were injected with methylcholanthrene and porphyrins developed tumors. When these mice died or were sacrificed, it was noted that the porphyrin which had been injected intraperitoneally had accumulated and concentrated in the subcutaneous sarcomas to such a degree that the tumor had become red fluorescent while other normal tissues had not. A search of the literature revealed that other investigators[3] had also observed the tendency for injected hematoporphyrin to accumulate in neoplastic tissues of rats. They had, however, only recorded this observation without further attempts to study or interpret this phenomenon. The affinity of porphyrins for neoplastic tissue was so striking that we have studied this in some detail. It was realized at the outset that if this affinity of neoplastic tissues for porphyrins extended also to metalloporphyrins and thus to radioactive metalloporphyrins, and proved to be generally true for all tumors, then this class of substances could be utilized to improve the existing methods of cancer detection and therapy.

The present report is based on observations made on 240 tumor bearing and 50 non-tumor bearing mice. The neoplasms studied included methylcholanthrene-induced tumors (spindle-cell fibrosarcomas and rhabdomyosarcomas), transplanted fibrosarcomas, spontaneous mammary carcinomas and transplanted mammary adenocarcinomas (variable type) that developed in this laboratory. The observations on the first 30 mice were non-systematic and extended over a period of about 2 years. In the first systematic experiment the affinity of all available types of mouse neoplasms was tested for their ability to concentrate hematoporphyrin. Eighty mice were used in this experiment. Forty of these bore tumors of one of the types mentioned above. Twenty tumor-bearing and 20 non-tumor bearing mice were injected intraperitoneally with 1 mg of hematoporphyrin. Eight of these hematoporphyrin injected mice and 8 non-injected controls were sacrificed at 24 hour intervals to determine the rate of development of maximum concentration of hematoporphyrin in the tumors. Most of the porphyrin injected migrated to the tumor within a period of 24-48 hours. Some of the porphyrin was eliminated through the liver and appeared in the red fluorescent feces. The greater omentum became red fluorescent and remained so for several days. Lymph nodes also became red fluorescent in all mice. The other normal tissues do not concentrate the hematoporphyrin. Hematoporphyrin-injected mice with carcinomas or sarcomas when sacrificed at the end of 24, 48 or 72 hours exhibited brilliant red fluorescent tumors in contrast to the tumors of the controls.

These observations were repeated several times and in subsequent experiments it was established that the porphyrin concentration as indicated by the red fluorescence of the tumor remains high for 10 to 14 days but gradually decreases. If the tumors contained necrotic centers the concentrations of porphyrin was greatest in and near the necrotic areas but was also concentrated throughout the tumor and near the periphery. The spontaneous and transplanted carcinomas and the induced or the transplanted

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1 Figge, Frank H. J., *A.A.A.S. Research Conference on Cancer*, Science Press, 1945, 117-128.

2 Figge, Frank H. J., *Ann. Int. Med.*, 1947, **27**, 143.

3 Auler, Hans, and Banzer, George, *Z. Krebsforsch.*, 1942, **53**, 65.

sarcomas—in other words all types of tumors tested—concentrated the hematoporphyrin. This indicated that the affinity of the tumors for porphyrin was not specific but perhaps general for all tumors.

Since regenerating and embryonic tissues are similar in some respects to neoplastic tissues, it was desirable to know whether these tissues would also concentrate porphyrins. The injection of hematoporphyrin into pregnant mice revealed that much of the porphyrin accumulated in the placentae and in the embryos. When hematoporphyrin was injected into mice which had been incised or otherwise traumatized, the porphyrin became concentrated at the site of injury and near the regenerating margins of incisions. These experiments indicated that growing tissues in general have an affinity for hematoporphyrin.

It is therefore probable that all neoplasms will have an affinity for porphyrins similar to that observed for this limited number of mouse tumors. It was known from the numerous observations in the experiments on cocarcinogenesis that neoplastic tissue had an affinity for both hemato and protoporphyrin but it was desirable to know whether this extended to other porphyrins. Mesoporphyrin and coproporphyrin were also tried and these substances also concentrated in neoplastic tissue. At the pH of the tissues the fluorescence intensity of protoporphyrin and coproporphyrin is not as great as hematoporphyrin and the concentrating effect does not seem as spectacular. A number of other fluorescent substances (fluorescein, rhodamine, dihydrocollidine and riboflavin) were also tested. None of these exhibited the same affinity for the tumors.

Perhaps the most important question was whether the neoplastic tissues would have the same affinity for metalloporphyrins. Heme (iron-protoporphyrin) was tested intensively but no conclusions could be drawn because the heme does not fluoresce and therefore could not be distinguished easily from hemoglobin and other substances present in tumor tissues. Zinc hematoporphyrin was therefore prepared by one of us (G.W.) for the express purpose of testing the affinity of neoplastic tissue for metalloporphyrin. Zinc hematoporphyrin in contrast to heme and many other metalloporphyrins fluoresces with a characteristic fluorescence spectrum and can easily be traced through the bodies of mice. A repetition of the experiment described above using 10 mice bearing transplanted methylcholanthrene-induced sarcomas demonstrated that the zinc hematoporphyrin also accumulated in tumor tissues. Even though the fluorescence intensity of this compound is not as great as hematoporphyrin at the pH of the tissues it was possible to visually observe the fluorescence spectrum of this compound in tumor tissues whereas this could not be seen in adjacent normal tissues.

Summary and Conclusions. These experiments show that all porphyrins tested accumulate in neoplastic (induced and transplanted sarcomas, spontaneous and transplanted mammary carcinomas), embryonic and regenerating tissues. Introduction of a metal (zinc) into the porphyrin molecule did not destroy the tendency of the porphyrin to concentrate in tumors. The tendency of injected porphyrins and metalloporphyrins to accumulate in lymph nodes may limit the therapeutic usefulness of these compounds in all neoplastic diseases except lymphatic leukemias. The possibility of using small doses of radioactive metalloporphyrins for detecting deep cancer is being investigated.