Some Characteristics of Transplantable Rat Hepatoma No. 5123 Induced by Ingestion of N-(2-fluorenyl)phthalamic Acid

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SUMMARY

A transplantable liver carcinoma No. 5123 induced by ingestion of N-(2-fluorenyl)phthalamic acid (2-FPA) in an inbred Buffalo-strain rat is described. Liver tumors were found in rats fed 2-FPA for 9.9 months but not until 8.1 months following their removal from the diet. In the tumor described the cells had the appearance of hepatic parenchymal cells. The primary tumor metastasized to the lungs. After fourteen serial transfers the tumor retained many of the morphological characteristics found in the primary tumor. Some biochemical activities of this tumor have been found to resemble those present in normal liver and are unlike those in most transplanted liver tumors that have been examined (4). Preliminary studies with colorimetric methods after a single oral dose indicate that the urinary metabolites of 2-FPA resemble those of N-2-fluorenylacetamide (2-FAA) in chemical behavior and rate of excretion.

The relation of molecular structure to carcinogenic activity has engaged our attention for some time. Through a rather simple modification of the molecular structure (1) of N-2-fluorenylacetamide (2-FAA), N-(2-fluorenyl)phthalamic acid (2-FPA), was synthesized. The induction of a transplantable liver carcinoma having many interesting properties has resulted from the testing of the carcinogenic activity of this new compound. This report relates some of the important findings derived from the induction and preliminary studies of this tumor.

PROCEDURES AND RESULTS

2-FPA dissolved in warm corn oil was mixed in diet 222-A at a level of 1.27 mmoles/kg. Seventeen inbred Buffalo-strain female rats (3) were fed this diet mixture for 9.9 months. All animals in the group were then kept on the basal diet without added 2-FPA for an additional period of 8.1 months, and the twelve survivors were then sacrificed. All twelve had liver tumors, whereas no liver tumors were found in five animals autopsied prior to the termination of the experiment at 18 months (4).

The average daily intake of 2-FPA for the group was 4.0 mg., with an average total intake of 1.12 gm/rat. Animal No. 5123, the animal that had a liver tumor which was used for transplantation, had ingested a daily average of 3.7 mg. of 2-FPA and a total of 1.12 gm. All twelve of the rats sacrificed at the end of the experiment had multiple raised dark red nodules scattered throughout the liver. Because of the large size and deep red color, one large nodule of the left lobe of the liver in animal No. 5123 was selected for transplantation. Three rats were given inoculations by

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1 G. M. Tompkins, personal communication.
2 Chemical abstracts nomenclature has been used. N-(2-fluorenyl) phthalamic acid could also be designated as 2-benzoylaminothiophene-2'-carboxylic acid or 2-benzoylaminothiophene-2'-carboxylate. N-2-fluorenylacetamide is also known as 2-acetylaminothiophene.

Diet 222-A was composed of the following constituents by weight, in grams: commercial casein, 3.00;degrade skim-milk powder, 28.75; whole wheat meal (hard spring), 60.50; brewer's dehydrated debittered yeast, 1.00; whole-liver powder 1.00; sodium chloride, 1.40; ferric citrate, 0.13; cod-liver oil, 2.00; corn oil, 8.80.

trocar of small pieces of the primary neoplasm. All subsequent transfers were made in a similar fashion. After 4-6 months the tumor was again transplanted. The third transplantation was made after 4 months, and the tumor was transferred at 2-4-month intervals thereafter. It has now been carried from February 7, 1956, to February 9, 1960, by serial transfer into the seventeenth generation. It is a relatively slowly growing tumor, reaching a diameter of 1.5-2.0 cm. in approximately 2 months after intraperitoneal inoculation. The tumor grows in almost 100 per cent of animals whether inoculated intraperitoneally or intramuscularly. After sixteen generations the tumor has retained its dark red color. Tumor No. 5123 has as yet not been tested for capacity to grow in other strains of rats.

Pieces from the original tumor and all transplant generations have been fixed in Zenker-formol, embedded in paraffin, and sectioned. Hematoxylin-eosin stains were made routinely.

Microscopically, the primary tumor was a hepatocellular carcinoma. The tumor contained double cords of cells separated by prominent vascular spaces. Some areas had a glandular pattern with acinar-like formation (Fig. 1). Eosinophilic-staining cellular debris was found in the center. The tumor cells were polygonal, eosinophilic, and variable in size, and they resembled hepatic parenchymal cells. The vesicular nuclei showed light variations in size, and the majority had a prominent nucleolus. Mitotic division was rarely found. Multiple metastatic nodules were found within the lungs. These areas of metastatic involvement closely resembled the primary tumor (Fig. 2).

Examinations of sections of transplants from the first through the fourteenth generations revealed that the tumor retained most of its original morphologic characteristics (Fig. 3). The only significant change was the occurrence of more frequent mitotic figures in sections from tumors of the later generations (Fig. 4).

In the colorimetric R-salt test of Westfall (5), this compound was found to act quantitatively like 2-FAA, indicating that hydrolysis of the phthalic acid from the N-2-fluoreneamine (2-FA) part of the molecule was complete under the conditions of this procedure. A single dose of 2-FPA administered by stomach tube to the rat was eliminated in the urine and feces in the same relative amounts of diazoizable R-salt-reacting material (measured as 2-FAA) and of nitrite-reacting material (estimated as N-[7-hydroxy] 2-fluorenylacetamide) (9) as was 2-FAA under the same experimental conditions. Equally small amounts of free amine were found in the urine following 2-FPA and 2-FAA. All the detectable urinary metabolites were excreted within 48 hours after oral administration. In the first 24 hours a larger percentage of the total was excreted following 2-FPA administration than after a similar dose of 2-FAA. Feces collected 72 hours after administration of 2-FAA contained little, if any, detectable material.

**DISCUSSION**

Steroid reductases that are present in normal liver of rats have been found by Tompkins (1) in transplanted tumor No. 5123. The activity of these enzymes in this tumor was similar to that present in normal rat liver and in much higher concentration than that observed in several other transplantable rat hepatomas. Potter et al. (4) have found this tumor to have thymine-degrading enzyme systems comparable in activity to that in normal rat liver but different from that in many other rat hepatomas. They also found that this tumor lacks enzymes to convert deoxycoptidone monophosphate to deoxurydine monophosphate.

The similarity in excretion of diazoizable and nitrite-reacting metabolites by the rat following administration of 2-FPA and 2-FAA suggests that 2-FPA is hydrolyzed and then acetylated to 2-FAA in vivo. If, indeed, 2-FPA is converted to 2-FAA in the rat, it would appear that these steps in the metabolism of the compound may be involved in differences in its carcinogenic behavior from that of 2-FAA.

The findings indicate further that the carcinogen probably did not remain in the organism for more than a few days after the last amount was ingested. Therefore, the alteration in liver cells leading to liver tumors must have occurred 8 or more months prior to the gross or microscopic detection of the tumors.

The findings relating to certain enzyme activities (1) of tumor 5123, its long initial induction period, and its slow rate of growth after many generations are of much interest. These initial observations suggest that this tumor behaves differently from other chemically induced transplantable rat liver tumors. From these preliminary studies tumor 5123 appears to be more like normal rat liver tissue than do the tissues of other transplantable rat liver tumors studied to date. Additional investigations are now in progress to determine whether other liver tumors induced by 2-FPA in rats of the Buffalo and other strains will demonstrate characteristics similar to those found in tumor No. 5123.
REFERENCES


Fig. 1.—Primary liver tumor (Rat No. 5138). Hepatocellular carcinoma composed of cords of cells resembling hepatic parenchymal cells with acinar formation and sinusoid-like vascular channels. Hematoxylin and eosin. X100.

Fig. 2.—Lung (Rat No. 5138). Metastatic tumor from liver retains morphologic characteristics of primary tumor. Hematoxylin and eosin. X100.
FIG. 3.—Transplant generation 13. Transplant retains most of the morphologic characteristics of primary tumor. Hematoxylin and eosin. X100.

FIG. 4.—Transplant generation 13. This is a higher magnification of transplant seen in Figure 3. Frequent mitotic figures are present. Hematoxylin and eosin. X780.
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