Hepatic Tumors in Rats Following the Prolonged Ingestion of Milk and Egg Yolk*

DOROTHY NELSON, PAUL B. SZANTO, ROBERT WILLHEIM, AND A. C. IVY

(Department of Clinical Science, University of Illinois College of Medicine, Chicago, and from the Department of Pathology and the Helden Institute for Medical Research, Cook County Hospital, Chicago, Ill.)

While attempting to produce experimental dietary arteriosclerosis in rats, we observed neoplastic growths in animals that had been on the diet for more than a year and a half. Most of the tumors appeared to be hepatomas, and these are the subject of the present report. These lesions are of particular interest because of the striking difference between our animals, which were obese, and the malnourished individuals that characteristically develop tumors of the liver in the presence of a nutritional deficiency (3, 4).

PROCEDURE

Ten male and ten female albino rats were placed on a diet designed to produce arteriosclerosis. The diet consisted of 5 per cent powdered egg yolk (Armour's) in whole milk. The mixture was homogenized in a Waring Blender, but during the course of the day the yolk rose to the top of the crocks, so that the composition of the diet as ingested was more than 5 per cent egg yolk. Fresh diet was distributed morning and evening except on Sundays, when the 24-hour ration was given in the morning.

The animals were purposely chosen from different age groups. The males were 10-23 weeks old with an average age of 16 weeks. Their weights ranged from 169 to 340 gm., with an average of 251 gm. The females were 15-19 weeks of age, with an average of 17 weeks. The weight of the females was 170-197 gm., with an average of 180 gm. The animals were weighed once a month throughout the experiment. Wire-floored cages were used for the 1st year, at which time some of the animals developed sore feet, so they were all transferred to cages with soft wood shavings.

When animals appeared to be moribund they were sacrificed. Some died spontaneously, and all surviving animals were sacrificed at the end of a 2-year period. With the animals under light ether anesthesia, blood was drawn from the heart until death occurred. Material for histologic studies was fixed in buffered formalin (1:10).

The following chemical determinations were made on the blood serum: total cholesterol by the Chaney modification of the Sperry-Schoenheimer method, total serum lipid by a turbidimetric method of Huerga and Popper, phospholipid by the method of Youngburg and Youngburg, and nonprotein nitrogen (NPN) by a slight modification of the Folin-Wu technic, 10 per cent trichloroacetic acid being used for deproteinization. Total liver fat was determined by chloroform extraction of the dried tissue in the Soxhlet apparatus followed by a second extraction with petroleum ether.

RESULTS

Incidence of "hepatomas."—Tumors which appeared to be hepatomas developed in three out of four males which survived on the diet for over 1.5 years. Four other males which survived on the diet for more than 1 year but less than 1.5 years did not have hepatic tumors. The other two which survived less than 1 year did not have a tumor. Nine of the ten females survived on the diet for over a year, and six for more than a year and a half. Of these six, three developed hepatic tumors. That is, six of the ten animals surviving for over 1.5 years had hepatic tumors.

The average age of the tumor-bearing males was 111 weeks (range, 98-119); the average of the females was also 111 weeks (range, 102-119). Animals exceeding the age of 98 weeks without developing the hepatic tumors were one male, age 101 weeks, and four females having an average age of 116 weeks (range, 104-123). One of these females, age 116 weeks, had been removed from the regimen after 70 weeks so should not be considered in this group. Of the ten animals reaching an age of 98 weeks or more and ingesting the milk-egg

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1 I. C. Chaney, personal communication.

2 J. de la Huerga, and H. Popper, personal communication.
yolk diet for a minimum of 78 weeks, six developed hepatomas.

Because it has been so well established that rats on stock rations do not have atherosclerosis, no control animals receiving normal diets were specifically set up for this experiment. However, we had in our colony 25 rats of a comparable age (average, 114 weeks; range, 98–133), and none had tumors of the liver. In seventeen additional rats which lived an average of 115 weeks (range, 99–126) and received fairly normal diets plus 0.05 per cent thiouracil, there were no hepatic tumors. In 22 animals which lived an average of 117 weeks

**TABLE 1**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age (weeks) on diet</th>
<th>Body weights</th>
<th>Liver wt. (X100)</th>
<th>Final body weight</th>
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<td>112</td>
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* These animals had liver tumors.
† Milk-egg yolk diet removed after 70 weeks.

(range, 100–140) and had received 2 per cent cholesterol plus 0.05 per cent thiouracil in their diet, two developed hepatic tumors.

**General data on individual animals.**—Information on the ages, weights, liver weights, and tumor incidence of individual animals is presented in Table 1. Inspection of the table shows that there is a high correlation between the length of time that the animals ingested milk and egg yolk and the weight of their livers, and a fair correlation between liver weight and the presence or absence of hepatomas. The ratio of liver weight to final body weight shows a surprisingly poor correlation with the length of time the animals ingested the diet and the presence or absence of tumors. This is probably due to the abnormal obesity of most of the animals and to the severe weight loss suffered by some just prior to death. Disregarding the

in diameter replaced the liver parenchyma (Fig. 1). In one animal the tumor nodules were also observed on the surface of the stomach, pancreas, and urinary bladder. All the tumors were greyish white in color and friable in consistency. There was no evidence of cirrhosis.

The microscopic examination revealed marked fatty changes of the liver cells (Fig. 2) and focal necrosis. In some areas the hepatic parenchyma showed a moderate degree of bile duct proliferation in the portal fields. The proliferation of the cholangioles was frequently more pronounced around the tumors.

The tumor nodules, which were single or multiple, were usually circumscribed, but occasionally the transition from normal hepatic parenchyma to the tumor tissue was indistinct. In some areas the structure of the tumor nodule was similar to that of...
normal liver, but without the typical lobular pattern (Fig. 3). Degenerative changes, necrosis, and hemorrhage were present in the larger tumors.

The tumor cells showed varying degrees of differentiation. In well differentiated nodes the cells showed a tendency to be arranged in plates consisting of three or more rows. Other areas occupied by tumor tissue showed an anaplastic growth with cellular pleomorphism. In some nodes the cells were small with relatively large nuclei and very little cytoplasm (Fig. 4). In most of the tumor nodules, however, the cells were larger than the normal hepatic cells, and their nuclei were large, being either vesicular with large nucleoli or hyperchromatic (Fig. 5).

**DISCUSSION**

In a recent analysis of the etiology of cancer of the liver, Gillman et al. (4) stressed the importance of nutritional factors in clinical as well as experimental malignancy. They presented evidence of the high incidence of liver disease in people and animals suffering from malnutrition and pointed out that the forms of liver disease most frequently leading to cancer are those which result in bile duct proliferation. In the Witwatersrand laboratory extensive bile duct proliferation was observed in rats fed a diet high in carbohydrate, moderately high in protein, low in fat, and devoid of the fat-soluble vitamins.

This account of the usual nutritional background for the development of hepatic tumors caused surprise when hepatic tumors appeared in our overly nourished rats consuming a milk and egg yolk diet, high in fat and fat-soluble vitamins and low in carbohydrate.

Histologically, the tumors in our animals appear to be hepatomas, frequently of multicentric origin. We do not interpret the cytology in the tumors as representing regenerative hyperplasia, but as being more analogous to what Mulay and Firminger (5) call hepatoma of the liver-cell type. The cytology is otherwise not analogous to tumors induced by 4-dimethylaminoazobenzene, because the bile duct proliferation was only moderate, there was no cirrhosis, and there were only a few cysts. The degree of degenerative changes and necrosis was related to the size of the tumors and their de-differentiation. There was no evidence of cirrhosis, the tumors developing directly in fatty livers.

When tumors occur in the absence of a recognized carcinogen, the question of spontaneous origin always presents itself. In this experiment the tumors were observed in six of ten rats 98 weeks old or in more receiving a milk-egg yolk diet. Of 25 rats of comparable age on normal diets, none developed tumors. As computed by the \( \chi^2 \) technic (6), this difference is highly significant, the probability being less than 1 per cent that it could have occurred by chance. This indicates that the milk-egg yolk diet is related to the observed lesions and acts either as a carcinogen or to cause the tumor on a genetic or metabolic susceptibility.

A possible common factor involved in the genesis of the hepatic tumors in our obese rats and in the undernourished animals observed by others is an abnormality in bile metabolism. It seems more than likely that the high percentage of cholesterol in the milk-egg yolk diet might in time interfere with the normal metabolism of bile. This suggestion gains credence from the work of Fitzhugh and...
Nelson (1). They observed hepatomas in fourteen of 29 rats that were fed thiourea for 2 years. No animal dying in less than a year and a half had tumors of the liver. None of the eighteen control animals in their experiment had tumors, but in animals on other experiments approximately 1 per cent developed hepatic tumors after the age of 2 years. The relation of the thyroid hormone to cholesterol metabolism suggests a possible analogy between this investigation and ours. In this connection, it is of interest that tumors of the liver were found in two of 22 animals in our colony receiving in their diet 2 per cent cholesterol and 0.05 per cent thiouracil, whereas no tumors developed in seventeen animals which received only 0.05 per cent thiouracil.

SUMMARY

Tumors of the liver which resemble hepatomas of the liver-cell type were observed in six of ten obese rats that had been maintained for over a year and a half on a diet solely of milk and egg yolk. In one case peritoneal implants were observed. No hepatic tumors occurred in a group of 25 animals of the same age receiving normal diets. The tumors developed in fatty but noncirrhotic livers. Bile duct proliferation was slight.

REFERENCES


Fig. 1.—a. A portion of the liver of rat No. 1 showing sectioned tumor (right). Tumor 4.4 X 3.7 X 2.8 cm. b. Part of the tumor of rat No. 6 showing peduncular attachment to liver (right). Tumor 5.1 X 4.0 X 3.1 cm. c. A portion of the liver of rat No. 10 showing sectioned tumor (left). Tumor 2.4 X 2.1 X 1.6 cm.

Fig. 2.—a. Fatty changes of the liver. b. Circumscribed tumor nodule. From Rat No. 1 (X 180).

Fig. 3.—Cords comprised of cells imitating superficially the normal architecture of the liver. From rat No. 1 (X 180).

Fig. 4.—This nodule consists of small tumor cells with relatively large nuclei and little cytoplasm. From rat No. 6 (X 180).

Fig. 5.—These tumor cells are larger than normal liver cells. The large nuclei contain one or more large nucleoli. Several mitotic figures are present. From rat No. 8 (X 400).
The purpose of this paper was to study the primary growth of leukemic cells in peritoneal exudate. It was found with the help of organ assay that the tendency of tumor cells to implant from peritoneal exudate into abdominal organs from peritoneal exudate is paralleled by a difference in survival (Table 1). Thus, primary localization of tissue growth either at the site of inoculation or from the opposite tendency to spread extensively into organs is an important factor in the amount of tumor growth and to be implanted into connective tissue either at the site of inoculation or from the opposite tendency to spread extensively into organs by blood route at the expense of the primary growth and--indirectly and directly--in the death of the host.

This factor is least significant for leukemias reported above and those on other tumors. However shorter survival of all leukemic strains the difference in the amount of tumor growth earlier and to a greater extent than for nonleukemic tumors, inversely to mortality assay of leukemic strains showed high tendency to early spread in the blood (AK4); C1498 (granulocytic leukemia) tumors. An inverse proportion was found for the tendency of tumor cells on the 6th day) was higher for leukemic cells in serous body fluids (Table 1). Thus, primary localization of tissue growth 'from benign and malignant intraperitoneal growth was higher for leukemic cells in the blood, and to infiltrate into organs was the essential lethal factor. For this strain the role of implantation of organ invasion from various sites (charts) was overshadowed by peritoneal exudate.

It appears from the results on transition between various strains of leukemias compared to those bearing scantier local growths and brief mention of nonleukemic tumors with regard to their two tendencies pt to spread extensively into organs is paralleled by their trend to grow as free cells in body fluids, to be separated from the tissue, and to infiltrate into organs.

It may be presumed that tissue growth 'from benign and malignant tumors (6) that both tendencies vary only quantitatively. The relatively slight difference between peritoneal exudate was overshadowed by peritoneal exudate. It appears from the results on transition between various strains of leukemias (7). The relatively slight difference between peritoneal exudate was overshadowed by peritoneal exudate. It appears from the results on transition between various strains of leukemias (7). The relatively slight difference between peritoneal exudate was overshadowed by peritoneal exudate.

Eventually being the direct lethal factor.
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