Observations on Hepatic Cell Hyperplasia, Adenoma, and Hepatoma of Rainbow Trout (*Salmo gairdnerii*)

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SUMMARY

Morphologic and biochemical features of non-neoplastic livers were compared with those of spontaneous hepatoma (hepatic carcinoma) in rainbow trout. More florid degenerative changes and lipochrome deposits were encountered in non-neoplastic livers of hatchery-reared trout than in those of wild trout of identical age. Cirrhosis was encountered only rarely.

The majority of hepatomas were well differentiated. Metastases were encountered in only four of 28 tumor-bearing fish. Histochemical studies showed increased cytoplasmic RNA and decrease to absence of glycogen in tumor cells. In five hepatomas there was an extracellular accumulation of protein which contained arginine and tyrosine. Electron microscopy of the tumor cells revealed hypertrophy of granular endoplasmic reticulum and Golgi apparatus and the accumulation of granules and amorphous material in the cytoplasm. The plasma proteins of trout with hepatoma were increased two- to threefold. The serum electrophoresis patterns showed increase of four protein components normally present in plasma of teleosts and a fifth component with a greater mobility than serum albumin. A comparison of enzyme patterns of non-neoplastic livers with adenomas and hepatomas indicates that neoplastic liver cells not only possess all the enzymes present in normal liver but augmented activity of many of the enzymes. Decreased activity in tumor cells was obtained only for succinic dehydrogenase, acid phosphatase, and glucose-6-phosphatase. Morphologic and biochemical characteristics of hepatoma in rainbow trout suggest that they should be classified as minimal-deviation tumors. On the basis of enzyme patterns adenomas were indistinguishable from hepatomas.

Prior to 1955 only four hepatic neoplasms had been reported in fish, all of them in trout (924, 84, 47). In 1955 a high incidence of hepatic tumors was found in rainbow trout in Italian hatcheries (9). Interest in these neoplasms was renewed recently when hepatic tumors were discovered in large numbers of hatchery-reared trout in the United States (928, 35, 61, 64), Italy (30), and France (3, 30).

The main varieties of tumors that occur in man and other warm-blooded vertebrates have also been observed in fish but have been largely neglected by oncologists (31, 56). The apparent recent increase in the incidence of hepatic tumors in trout may be attributable to natural or artificial carcinogenic agents but may also reflect more intensive studies of the diseases of fish.

The present study deals with gross, histologic, histochemical, biochemical, and electron microscopic observations on normal and tumorous livers of hatchery-reared rainbow trout and livers of wild rainbow and brook trout.

MATERIALS AND METHODS

The present study included fifteen normal yearlings and 135 brood hatchery-reared rainbow trout, both males and females. Livers from fifteen wild rainbow and ten brook trout were also available for histologic study. Hatchery-reared fish were all initially fed beef liver. As they matured they were placed on a diet of beef liver and spleen and dry meals. They were then maintained on a commercial dry pellet diet. The fish were anesthe-
tized in a 1:5000 solution of tricaine methanesulfonate, and complete autopsies were performed. One-half- to 1-cu. mm. cubes of normal and neoplastic liver tissue were fixed for 1 hour at 4°C. in osmium tetroxide buffered to pH 7.8 and containing 0.13 M sucrose. The tissue was embedded in methacrylate and in Epon 812 (8). Thin sections were stained with uranyl acetate or lead hydroxide and examined in an RCA EMU-8F electron microscope. Sections from 0.5 to 1 μ in thickness were cut from the same blocks, mounted on slides, and used for cytological, histochemical, and tinctorial studies. Paraffin sections 5 μ thick of formalin-fixed tissues were employed for histologic study and to check the effect of osmium fixation and methacrylate embedding on the various staining and chemical reactions. Carnoy-fixed tissue was used for the histochemical demonstration of ribonucleic acid (RNA). The histochemical methods employed were the Millon and Sakaguchi reactions for tyrosine- and arginine-containing proteins, respectively, the fast green stain at pH 9 for basic proteins, the azure B stain for RNA with ribonuclease (RNase)-digested controls, and the periodic acid-Schiff (PAS) reaction for 1,92-glycol groups digestion. A few rainbow trout showed considerable deposits of melanin and ceroid. The remainder of fish were dissected freshly from non-neoplastic livers expressed as per cent of body weight of these fish was 92.3. On microscopically, the hepatic units were composed of plates or muralia two cells thick, radiating from a central vein (Fig. 1). The liver lumen, and mitoses were exceedingly rare. This is characteristic of the tubulosinusoidal liver of teleosts as described by Elias and Bengelsdorf (13). These cells contained little glycogen as established by scattered PAS-positive cytoplasmic granules which were removed by previous diastase digestion. A few rainbow trout showed considerable deposits of melanin and ceroid. The remainder of the wild trout livers were free of fatty metamorphosis, ceroid deposits, inflammation, and fibrosis.

The following dephosphorylating enzymes were assayed by the methods of Novikoff et al. (41) and Novikoff (36); adenosine triphosphatase (ATPase), incubation for 20 minutes at 37°C. in 0.2 M tris-maleate, pH 7.2, 0.5 × 10⁻² M ATP, with and without the addition of either 1 × 10⁻² M calcium or magnesium chloride; acid phosphatase, incubation for 30 minutes at 37°C. in 2.4 × 10⁻² M sodium barbital, pH 4.5, 2 × 10⁻² M sodium β-glycerophosphate; alkaline phosphatase, incubation for 30 minutes at 37°C. in 2.4 × 10⁻² M sodium barbital, pH 9.4, 2 × 10⁻² M β-glycerophosphate, 1 × 10⁻² M magnesium chloride; glucose-6-phosphatase (7), incubation for 30 minutes at room temperature in 0.2 M Tris-HCl buffer, pH 6.8, 0.5 × 10⁻² M glucose-6-phosphate. At the end of incubation 2.5 ml. of 10 per cent trichloroacetic acid was added to each 5.0 ml. of incubation mixture. After centrifugation the concentration of inorganic phosphorus was determined in the supernatant by the method of Fiske and Subbarow (17).

Other enzymes assayed included glutamic-pyruvic and glutamic-oxalacetic transaminases (GPT and GOT, respectively) (65); lactic, malic, and glutamic dehydrogenases (29); isocitric (43), glyoxylate-phosphate (21), and succinic (58) dehydrogenases, and leucyl aminopeptidase (29) and aldolase (1). The total protein concentration of the homogenates was determined by the biuret method (23). Paper electrophoresis (4) of trout sera was accomplished with a Beckman Spinco Model R system utilizing barbital buffer, pH 8.6 (ionic strength, 0.01). Separation was obtained in 6–8 hours at 20°–25°C., 2.5 ma., and 275 volts. Dried paper strips were scanned with a Beckman Spinco Analytrol densitometer-integrator.

RESULTS

NON-NEOPLASTIC LIVERS

Wild rainbow and brook trout.—The mean weight of non-neoplastic livers expressed as per cent of body weight of fish ranging from 3 to 5 years of age was 1.2. The livers were reddish-tan and smooth. Microscopically, the hepatic units were composed of plates or muralia two cells thick, radiating from a central vein (Fig. 1). The liver cell nuclei were not oriented toward the sinusoidal lumen, and mitoses were exceedingly rare. This is characteristic of the tubulosinusoidal liver of teleosts as described by Elias and Bengelsdorf (13). These cells contained little glycogen as established by scattered PAS-positive cytoplasmic granules which were removed by previous diastase digestion. A few rainbow trout showed considerable deposits of melanin and ceroid. The remainder of the wild trout livers were free of fatty metamorphosis, ceroid deposits, inflammation, and fibrosis.

Hatchery-reared trout.—The majority of the livers from non-neoplastic hatchery-reared rainbow and brook trout ranging from 1 to 5 years of age were enlarged, smooth, yellowish-tan, and firm. The mean liver weight expressed as per cent of body weight of these fish was 2.8. On microscop-
ic examination the liver cells appeared uniformly vacuolated, hydropic (Fig. 2), and were so filled with glycogen that the nuclei were eccentrically placed (Fig. 3). Occasional liver cells contained large cytoplasmic vacuoles filled with neutral fat as indicated by oil red O staining of frozen sections. Except for the usual peribiliary fibrous connective tissue there was no evidence of cirrhosis. Considerable melanin and a granular golden-yellow pigment which was sudanophilic, PAS-positive, and acid-fast in paraffin sections had accumulated in the peribiliary and perivascular fibrous connective tissue. Occasional liver cells contained similar pigment. These histochemical results indicate that the yellow pigment was ceroid. These deposits of ceroid were often surrounded by an infiltrate of lymphocytic and monocytic cells. Hepatic deposits of melanin and ceroid first became apparent in 18-month-old fish, were present in larger amounts in older fish, and were absent in the livers of yearling fish. Ceroid deposits were more abundant in rainbow than in brook trout of comparable ages. Under the electron microscope ceroid pigment appeared as densely osmiophilic bodies.

The number and type of the hepatic lesions encountered, the age of the fish, and the mean average liver weight expressed as per cent of body weight are listed in Table 1. The livers showing cirrhosis were only slightly enlarged, and one was reduced in size. Their surfaces were studded with nodules of varying size separated by fine bands of fibrous tissue. Fibrosis was perimural, often compressing the muralia (Fig. 4), and was accompanied by an infiltrate of lymphocytes and monocytes. The amount of ceroid pigment did not appear to differ significantly from that observed in noncirrhotic livers. Glycogen was scant in these livers.

The abundant glycogen in the liver cells of 18-month-old trout studied by electron microscopy was localized in discrete deposits which were separate from other cytoplasmic components (Fig. 19). Occasionally mitochondria and lipide droplets were enmeshed in the deposits. In sections stained with lead hydroxide or uranium acetate the glycogen deposits consisted of discrete particles 40—60 μ in diameter. At the junction of the glycogen deposits and other cytoplasmic structures small tubules and vesicles of the agranular endoplasmic reticulum were interspersed among the granules (Fig. 20). Within the deposits glycogen particles appeared to be connected by thin membranes. The granular form of the endoplasmic reticulum occurred as numerous small arrays of parallel lamellae or as small vesicles (Figs. 20, 21); this difference is probably related to the plane of sectioning. Few free ribosomes were present. Mitochondria were numerous, often elongate with cristae generally parallel to the long axis of the organelle. Lysosomes were infrequently encountered.

The Golgi apparatus of these cells was composed of pairs of double membranes forming closed sacs and numerous vesicles of the same diameter as the cross-section of the lamellae (Fig. 21). The large vacuoles often described as a component of the Golgi apparatus of other cell types (10) were not present. Near the Golgi apparatus numerous osmiophilic bodies surrounded by a single membrane were present (Fig. 21). These often composed an assemblage of vesicles resembling Golgi vesicles.

### TABLE 1

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>No. fish</th>
<th>Age (years)</th>
<th>Liver wt/ body wt (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-neoplastic:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>4</td>
<td>3—5</td>
<td>2.3</td>
</tr>
<tr>
<td>Fatty nodules</td>
<td>4</td>
<td>3</td>
<td>2.3</td>
</tr>
<tr>
<td>Eosinophilic nodules</td>
<td>18</td>
<td>24—34</td>
<td>2.4</td>
</tr>
<tr>
<td>Eosinophilic-basophilic nodules</td>
<td>3</td>
<td>24—3</td>
<td>2.4</td>
</tr>
<tr>
<td>Neoplastic:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophilic nodules</td>
<td>4</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>Hyperplastic nodules</td>
<td>14</td>
<td>3—5</td>
<td>2.4</td>
</tr>
<tr>
<td>Adenomas</td>
<td>11</td>
<td>3—5</td>
<td>2.7</td>
</tr>
<tr>
<td>Hepatomas</td>
<td>28</td>
<td>3—5</td>
<td>3.5</td>
</tr>
<tr>
<td>Total:</td>
<td>81</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The granular form of the endoplasmic reticulum occurred as numerous small arrays of parallel lamellae or as small vesicles (Figs. 20, 21); this difference is probably related to the plane of sectioning. Few free ribosomes were present. Mitochondria were numerous, often elongate with cristae generally parallel to the long axis of the organelle. Lysosomes were infrequently encountered.

The Golgi apparatus of these cells was composed of pairs of double membranes forming closed sacs and numerous vesicles of the same diameter as the cross-section of the lamellae (Fig. 21). The large vacuoles often described as a component of the Golgi apparatus of other cell types (10) were not present. Near the Golgi apparatus numerous osmiophilic bodies surrounded by a single membrane were present (Fig. 21). These often composed an assemblage of vesicles resembling Golgi vesicles.

**Hepatic Nodules, Hyperplasia, Adenoma, and Hepatoma**

The glycogen content of the nodular, hyperplastic, adenomatous, and malignant hepatic lesions was scant to absent as contrasted to the glycogen-laden cells of surrounding liver.

**Fatty nodules.**—The livers were smooth, yellowish-tan, and mottled with numerous well-circumscribed, round, yellow to chalky-white areas 1—3 mm. in diameter. Microscopically, these areas consisted of markedly vacuolated cells whose cytoplasm was filled by oil red O positive, alcohol-labile droplets of neutral fat (Fig. 5). In two fish pure fatty nodules were present; in two others they were accompanied by basophilic nodules.

Figures 22 and 23 illustrate the cytologic appearance of these nodules. The intracellular vacuoles in Figure 22 represent areas from which most of the fat was removed during the preparatory procedures for electron microscopy, since similar

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areas in formalin-fixed frozen sections stained with oil red O. Otherwise the cytoplasmic components of these cells were qualitatively and quantitatively similar to those of the liver cells previously described.

In some areas of this tissue (see lower left corner of Figure 22 and Figure 23) degenerating liver cells contained large conglomerate deposits of osmiophilic vacuolated bodies resembling lysosomes. These deposits were PAS-positive and resistant to diastase digestion.

**Eosinophilic nodules.**—The livers were smooth, yellowish-tan, and mottled, with numerous well-circumscribed gray areas which in some regions became confluent; their consistency was identical to that of uninvolved liver. Microscopically, these nodules were composed of muralia still two cells thick and set apart from the surrounding liver cells by their intense eosinophilia. The nuclei of these cells were identical to those of uninvolved liver. Mitotic figures were absent. The cytoplasm was devoid of granules. The faint staining with azure B indicated scant RNA in these cells. The cytoplasm was also devoid of glycogen as demonstrated by the PAS reaction.

**Basophilic nodules.**—The livers were indistinguishable from those of the previous group. Microscopically, the nodules consisted of muralia of intensely basophilic cells two cells thick (Fig. 6). The nuclei and nucleoli were of normal size and appearance. Mitoses were absent. Because RNase digestion abolished cytoplasmic azure B basophilia, it is concluded that the basophilia was due to RNA.

**Mixed eosinophilic and basophilic nodules.**—The livers were grossly identical to those containing either the pure eosinophilic or the basophilic nodules. Microscopically, both types of nodules were morphologically and histochemically identical to those described in the pure lesions. In several instances eosinophilic and basophilic cells were present within the same nodule (Fig. 11). A single muralia contained both types of cells, and the transition from eosinophilia to basophilia was sharply demarcated.

**Basophilic hyperplastic nodules.**—Grossly, these livers were indistinguishable from those of the two preceding groups. Microscopically, the nodules consisted of thickened muralia three or more cells thick. The nuclei were larger, more vesicular, and possessed larger nucleoli than those of normal liver cells. Mitotic figures were rare. The cytoplasm was intensely basophilic following staining by azure B. The nodules were at random throughout the liver and apparently bore no consistent relation to the various components of the hepatic unit. The nodules often contained, or were closely associated with, foci of mononuclear cells resembling lymphocytes and monocytes, and accumulations of ceroid pigment. In some nodules proliferating capillaries and fibrous tissue appeared to originate from nearby bile ducts.

**Adenomas.**—The livers were studded with sharply circumscribed, tan-gray nodules which were larger than either the eosinophilic or basophilic nodules, the largest measuring 1.2 cm. in diameter, and were raised above the liver surface. In one instance the nodules were surrounded by fibrous tissue. The muralia were thickened, containing as many as ten rows of cells (Fig. 12). These cells were crowded, in disorderly arrangement, were intensely basophilic, and possessed hyperchromatic nuclei with prominent nucleoli. An occasional mitotic figure was evident. In one adenoma the tumor cells contained numerous cytoplasmic vesicles filled with an amorphous eosinophilic material which was also present in dilated sinusoids. This material was stained by the Millon and Sakaguchi reactions, and was intensely stained by fast green at pH 2.

Adenomatous nodules from the livers of two rainbow trout were studied by electron microscopy. Many cells contained numerous, round, dense bodies, some as large as 2.5 μ (Fig. 24), which were intensely osmiophilic, consisted of numerous whorled membranes resembling myelin, and were surrounded by a single membrane. Although Figure 27 suggests that the whorled membranes may be arising from the cytoplasm, their proximity to the Golgi apparatus makes this conclusion hazardous. Transitional stages between these bodies and mitochondria were not observed.

The endoplasmic reticulum of these cells was compact and extensive, and yet exhibited only a few areas of long double lamellae (Figs. 24–28). Mitochondria were numerous and occasionally contained a crystalline-like structure (Fig. 25). Occasional glycogen deposits and large lipid droplets were present. Lipid droplets were often surrounded by glycogen (Fig. 28).

The Golgi apparatus (Fig. 26) was prominent and was associated with many small osmiophilic vesicular bodies localized near the long, closely packed double membranes. These bodies were associated with the Golgi apparatus of the cells of all the trout livers examined. Occasionally material morphologically similar to these bodies was present between the layered Golgi membranes.

**Hepatomas.**—The tumors were grayish-tan, large, and multiple. The largest tumor nodule measured 5.7 cm. in diameter and consisted of numerous smaller nodules which had coalesced. The
tumors varied in consistency from sclerotic nodules to masses showing extensive central necrosis with hemorrhage (Figs. 7, 9). In seven fish the tumor nodules were cystic and were filled with a tan, gelatinous material resembling thyroid colloid. In one fish the liver contained a solitary 7-cm. cyst which almost completely replaced the liver (Fig. 8). The cyst wall consisted of a thin rim of neoplastic liver tissue. Metastases were found in four fish; two contained tumor nodules in the spleen, one in the kidney, and one in the gill (Fig. 10). Tumor dissemination by extension into the stomach and pylorocecum and hepatic portal veins (Fig. 13) was also observed.

The hepatomas were of three histologic types: (a) The trabecular type consisted of broad anastomosing trabeculae of neoplastic liver cells with moderately enlarged, hyperchromatic nuclei and nucleoli and basophilic cytoplasm (Fig. 14). Intertabecular spaces varied from thin slits to wide lacunae (Fig. 15). In five tumors the lacunae were filled with an eosinophilic proteinaceous material which was stained by the Millon and Sakaguchi reactions and by fast green at pH 2 (Fig. 16). In three of the five the basophilic cytoplasm of the liver cells was vacuolated (Fig. 17). In no instance was this material associated with degenerated tumor cells. In frozen sections the cytoplasmic vacuoles were stained by oil red O and Sudan black, indicating the presence of neutral fat (Fig. 17). The total serum proteins of these five fish were markedly elevated and will be more fully described subsequently. (b) A spindle-cell type consisted of elongate fusiform cells disposed in a whorled pattern sometimes forming primitive acinus-like structures. These cells resembled fibroblasts, so that to differentiate them from the bands of connective tissue was difficult. (c) A cholangiohepatoma consisted of both neoplastic liver and bile duct epithelial cells. The former cells formed trabeculae, the latter acini (Fig. 18). Bile secretion was not observed in these tumors.

Occasional mitoses were observed. Some of the tumors had a fibrous stroma, but in no instance was cirrhosis associated with them.

The intense basophilia of the tumor cells was associated with the extensive arrays of long cisternae of the granular endoplasmic reticulum (Figs. 29, 30). Such arrays resembled those of pancreatic acinar cells and other cell types synthesizing large amounts of protein. Distended sacs of this reticulum contained an amorphous material (Fig. 30). These cells also contained large lipide droplets (Figs. 29–31), some of which were associated with glycogen deposits. Scattered through the cytoplasm, and generally but not always between the interstices of the endoplasmic reticulum, were small osmiophilic droplets, most of which measured approximately 0.2 μ in diameter (Figs. 30, 31). These droplets were smaller than the dense bodies observed in the cells of fatty nodule and adenoma and were devoid of both vesicles and whorled membranes. Their small size precluded histochemical characterization. However, the matrix of the small droplets was identical in appearance and density to the large cytoplasmic droplets which have been shown histochemically to contain fat. In areas where the tumor cells lined a sinusoid containing fast-green-positive material (Fig. 30), dense deposits observed along the cell border and in the sinusoid suggested extrusion of the material from the cell into the sinusoid. Microvilli were numerous along the cell borders adjoining such lumen.

Figure 29 illustrates two other aspects of these tumor cells. For short distances along the adjacent cell membranes the cytoplasmic matrix was amorphous and free of endoplasmic reticulum and other cell components. This pattern was also observed along the cell membrane bordering a sinusoid. The mitochondria were numerous, were often much larger than those in normal liver, and occasionally exhibited cristae that were parallel to the long axis of the organelle.

The Golgi apparatus of the tumor cells (Figs. 29, 31) was associated not only with the multivesicular bodies but also with the small, dense droplets peculiar to these hepatoma cells. The tumor cells showed no alterations of fine structure indicative of degeneration or necrosis.

**Biochemical Studies**

**Plasma proteins.**——Four component bands were resolved by paper electrophoresis in sera of hatchery-reared rainbow trout free of neoplasm. Component 1 with the slowest mobility resembled γ-globulin; components 2 and 3, α and β-globulins; and components 4, albumin. Treatment of serum with saturated ammonium sulfate removed two components with electrophoretic mobilities comparable to those of α-globulins and albumin. The average total serum protein concentration of these fish was 3.6 gm/100 ml. In addition to the four major components a fifth component with a greater mobility than albumin was present in sera of trout with adenomas and hepatomas. The average total serum protein concentration of fish with adenomas was 6.1, and with hepatomas 9.8 gm/100 ml. The highest values were obtained in the sera of fish with protein-rich trabecular tumors. These results are summarized in Table 2 and Chart 1.

**Enzymes.**——Enzyme assays of homogenates of pooled nodules diagnosed as adenomas and three hepatomas revealed increased activities of GOT...
and GPT, lactic, malic, isocitric, and glucose-6-phosphate dehydrogenases, aldolase, leucyl aminopeptidase, and both magnesium- and calcium-activated adenosine triphosphatases. Decreased activities of succinic dehydrogenase, acid phosphatase and glucose-6-phosphatase were observed in all the hepatic tumors. These results are summarized in Table 3.

**DISCUSSION**

On the basis of morphological and histochemical characteristics, a spectrum of degenerative and proliferative hepatic lesions could be defined in hatchery-reared rainbow trout. The accumulations of ceroid pigment in the livers of hatchery-reared trout has been observed by other investigators (18, 48, 53, 54, 57). Although the exact etiology of excessive ceroid accumulation in trout livers is still obscure, evidence has been presented that the condition is a nutritional disease resulting from a diet rich in unsaturated fats (16, 19, 32). Ghittino (19) has reported that excessive ceroid accumulation in livers of trout in Italian hatcheries was often attended by cirrhosis. The infrequency with

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**TABLE 2**  
**COMPOSITION OF RAINBOW TROUT SERUM PROTEINS OF FISH WITH NON-NEOPLASTIC AND NEOPLASTIC LIVERS**

<table>
<thead>
<tr>
<th>Source of Serum</th>
<th>No. Serum Tested</th>
<th>Av. Total Serum Protein (mg. per cent)</th>
<th>Per cent Component in Serum</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Non-neoplastic</td>
<td>11</td>
<td>3.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Adenoma</td>
<td>4</td>
<td>6.1</td>
<td>6.7</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>9</td>
<td>9.8</td>
<td>4.8</td>
</tr>
</tbody>
</table>

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**TABLE 3**  
**ENZYME ASSAYS OF NON-NEOPLASTIC AND NEOPLASTIC LIVERS OF RAINBOW TROUT**

All values represent the average of three assays on a single specimen. All data without an asterisk are expressed as \( \mu \) moles of substrate utilized per gram protein per hour \( \times 100 \).

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Non-neoplastic</th>
<th>Adenomas</th>
<th>Trabecular hepatoma</th>
<th>Functional hepatoma</th>
</tr>
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<tbody>
<tr>
<td>Glutamic-oxaloacetic transaminase</td>
<td>2</td>
<td>5.8</td>
<td>32</td>
<td>44</td>
</tr>
<tr>
<td>Glutamic-pyruvic transaminase</td>
<td>9.7</td>
<td>11.5</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>8.5</td>
<td>10</td>
<td>3,470</td>
<td>3,980</td>
</tr>
<tr>
<td>Malic dehydrogenase</td>
<td>44</td>
<td>44</td>
<td>2,300</td>
<td>2,480</td>
</tr>
<tr>
<td>Isocitric dehydrogenase</td>
<td>1,700</td>
<td>1,700</td>
<td>1,775</td>
<td>1,285</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>40</td>
<td>40</td>
<td>194</td>
<td>165</td>
</tr>
<tr>
<td>Succinic dehydrogenase</td>
<td>117</td>
<td>39</td>
<td>2</td>
<td>1</td>
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<tr>
<td>Aldolase</td>
<td>17</td>
<td>19</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>Leucine aminopeptidase</td>
<td>10,000</td>
<td>4,050</td>
<td>58,000</td>
<td>58,000</td>
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<tr>
<td>Adenosine dehydrogenase</td>
<td>298</td>
<td>510</td>
<td>514</td>
<td>640</td>
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<tr>
<td>Adenosine triphosphatase (Mg**+)</td>
<td>370</td>
<td>606</td>
<td>560</td>
<td>780</td>
</tr>
<tr>
<td>Adenosine triphosphatase (Ca**+)</td>
<td>177</td>
<td>157</td>
<td>155</td>
<td>88</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>192</td>
<td>141</td>
<td>141</td>
<td>131</td>
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<tr>
<td>Acid phosphatase</td>
<td>780</td>
<td>921</td>
<td>308</td>
<td>394</td>
</tr>
</tbody>
</table>

* These data expressed as \( \mu \) moles of inorganic phosphate released per gram protein per hour.
which cirrhosis was encountered in the present study may be due to differences in dietary practices of hatcheries in the United States. According to Ghittino, sardine, anchovy, and mackerel fish meals constitute up to 50 per cent of the trout diet in Italy; in the United States dry pelleted meals and fresh meat diets are employed.

Halver suggests that the large aggregations of glycogen seen in the livers of hatchery-reared trout may result from the overfeeding employed to produce large fish quickly. The small deposits of hepatic glycogen which we encountered in wild trout support this view; in no instance were the accumulations of glycogen as large as those commonly found in hatchery-reared trout.

Although the spectrum of degenerative and proliferative hepatic lesions which has been observed in hatchery-reared rainbow trout may suggest various stages in the pathogenesis of hepatoma, such conclusion is at present pure conjecture, and confirmation must await further investigation. Nevertheless, certain striking similarities exist between the eosinophilic-basophilic nodules in livers of hatchery-reared trout and those described by Opie in dimethylaminoazobenzene-induced hepatoma in the rat. Opie concludes that the mobilization of cytoplasmic RNA or “chromatolysis” is due to progressive injury of liver cells by carcinogen and is followed by reappearance of cytoplasmic RNA, increased mitosis, and finally hepatoma. It is of interest that in the eosinophilic-basophilic hepatic nodules of rainbow trout mitoses were encountered only in the basophilic portions.

The large numbers of dense bodies morphologically similar to the residual bodies or lysosomes in cells with degenerative cytoplastic alterations in the fatty nodules and adenomas of trout liver are noteworthy. The precise role of dense bodies in cell damage is not clear, yet significant increases of these bodies in a variety of cells in diverse pathologic conditions have been reported. In the preneoplastic lesions of rainbow trout, groups of liver cells exhibiting increased cytoplasmic eosinophilia, hyaline bodies, and, at the electron microscope level, dense bodies, suggest that the hepatoma is preceded by focal hepatic degeneration.

Although a consideration of etiology is not the purpose of this report, the morphologic findings in livers of hatchery-reared trout suggest that they were damaged by a hepatotoxic substance or substances.

The true incidence of hepatoma in hatchery-reared rainbow trout is difficult to assess, since most of the reports represent random sampling of hatchery populations. However, it is well established that the livers of these fish are highly susceptible to neoplastic transformation. This is in contrast to the apparent resistance to hepatoma of brown and brook trout raised under identical conditions. Thus, hepatoma in rainbow trout may be regarded as a species-specific tumor as defined by Schlumberger. As he points out, species-specific tumors may have a genetic basis, yet the neoplastic transformation is often the result of interaction with environmental chemical, physical, or biological agents.

Our failure to find virus, virus-like particles, or inclusions in any of the trout hepatomas examined with the electron microscope is in harmony with the observations of other workers that hepatoma was not induced by injection of a cell-free extract into host fish. Recently, Halver and his associates have isolated by lipide extraction of a commercial trout ration a substance which they report is capable of inducing hepatoma in rainbow trout. Alterations of plasma proteins in rainbow trout with hepatomas in various stages of development were first appreciated by Snieszko, Miller, and Atherton. Our electrophoretic data indicate that the four components of plasma proteins in normal trout sera are increased in hepatoma-bearing trout. The appearance of a fifth component with a more rapid mobility than albumin in fish with hepatoma merits further investigation.

The demonstration of serum hyperproteinemia in hepatoma-bearing trout, of extracellular accumulations of protein, and of excessive fat storage in certain of the tumors may be a reflection of “function” in these neoplasms. The hypertrophied granular endoplasmic reticulum with the accumulation of material within it, the prominent Golgi apparatus, and the aggregates of secretory and fat droplets and their extrusion from the tumor cells tend to support this view. However, firmer proof that trout hepatic tumors are indeed functional must await biochemical demonstration of augmented synthesis. Similar hyperfunctional manifestations have also been observed in human hepatomas. These include hyperglobulinemias and albuminemias, excessive storage of glycogen, fat, and bile. The accumulation of fat in the liver should be interpreted with caution, since it is impossible to differentiate between mobilized depot fat and fat synthesized by the liver. Hypertrophy of the endoplasmic reticulum in most hepatomas is of interest, since

1 Personal communication.
overdevelopment of this organelle in tumor cells is the exception rather than the rule (54). Recently, Bruni (6) has reported that the endoplasmic reticulum of the slow-growing Morris hepatoma 5123 is more highly developed than the more rapidly growing Dunning hepatoma. These results suggest that the fine structural characteristics of tumor cells may be a reflection of their degree of differentiation and possibly of function.

The Golgi apparatus has been implicated in the cellular function of secretion (10). The morphology of this organelle in neoplastic cells is highly variable (42), and no correlative data concerning its morphology in functional neoplasms producing a secretory product are available.

The deletion theory of carcinogenesis first suggested by Potter (50) is based on the concept that cancer cells lack a certain enzyme or complement of enzymes that is present in their non-neoplastic counterparts. The validity of this theory has received support recently from the chemical induction of transplantable hepatomas displaying complete and partial deletion and, in a few instances, augmentation of enzyme activities. More significant still is the finding that the fewer and more attenuated the enzyme deletions the more closely the hepatomas resemble liver morphologically and the less rapid is their growth. From these points of view rainbow trout hepatomas may be classified as minimal-deviation tumors.

The gross and microscopic differentiation of regenerative, hyperplastic, and adenomatous nodules from hepatocarcinomas is often difficult (11, 26, 49), and the results of biochemical assays were as equivocal as the morphology. The small, gray hepatic nodules diagnosed as adenomas were indistinguishable enzymatically from unquestionable hepatomas with extrahepatic metastases. This again emphasizes the close relationship that exists between hyperplasia and neoplasia. Here the observations of Emmelot et al. (14) in rats fed dimethylaminoazobenzene are of interest. They suggested that the fine structural characteristics of tumor cells lack a certain enzyme or complement of enzymes that is present in their non-neoplastic counterparts. The validity of this theory has received support recently from the chemical induction of transplantable hepatomas displaying complete and partial deletion and, in a few instances, augmentation of enzyme activities. More significant still is the finding that the fewer and more attenuated the enzyme deletions the more closely the hepatomas resemble liver morphologically and the less rapid is their growth. From these points of view rainbow trout hepatomas may be classified as minimal-deviation tumors.

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In many respects the spontaneous hepatoma of the liver in rainbow trout resembles one another even in widely different animal classes, including poikilothersms. Future studies should concentrate on the transplantation and establishment of various trout hepatomas so that more detailed correlative studies on the growth rate, morphology, and enzyme patterns can be accomplished (31).

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REFERENCES

18. GASCIOVIT, O. Die verlustreichen Leberkrankungen der...


35. ———. Mobilization of Basophil Substance (Ribonucleic Acid) in the Cytoplasm of Liver Cells with the Production of Tumors by Butter Yellow. Ibid., 84:91-106, 1946.


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**Fig. 1.**—Liver of a wild rainbow trout showing the tubulo-sinusoidal structure characteristic of teleosts. Note that the muralia are two cells thick and radiate from the central vein. Hematoxylin & eosin (H. & E.), X150.

**Fig. 2.**—Liver of a non-neoplastic hatchery-reared rainbow trout. The cells are markedly vacuolated and appear swollen. H. & E. X150.

**Fig. 3.**—Liver of fish shown in Figure 2. The purplish PAS-positive cytoplasmic material is glycogen and appears black in the photograph. The nuclei are unstained, PAS, X1040.

**Fig. 4.**—Liver of a non-neoplastic hatchery-reared rainbow trout with cirrhosis. Note that the muralia though distorted are still two cells thick. Masson trichrome. X170.

**Fig. 5.**—Liver of a non-neoplastic hatchery-reared rainbow trout containing numerous small yellow and chalky-white nodules. The fatty nodules consist of lipid-laden cells. Note the oil red O positive perivascular accumulations of ceroid pigment. Oil red O, X45.

**Fig. 6.**—Liver of a hatchery-reared rainbow trout containing a basophilic nodule. The muralia are two cells thick. The basophilia was RNase-labile. Azure B, X45.
Figs. 7–10 are of hatchery-reared rainbow trout $\frac{3}{4}$–5 years of age.

**Fig. 7.**—Liver studded with gray nodules.

**Fig. 8.**—Liver showing a large solitary cyst filled with gelatinous material. Note the small gray nodules in residual tissue in the upper right portion of the cyst wall.

**Fig. 9.**—Representative tumorous livers encountered showing moderate enlargement with numerous small nodules at the upper left to massive hepatomegaly with multiple large nodules and hemorrhagic necrosis at the lower right.

**Fig. 10.**—Metastatic nodules of hepatoma in the gill involving a primary filament.
FIGS. 11–16 are of tumorous hatchery-reared rainbow trout.

Fig. 11.—Eosinophilic-basophilic hepatic nodule. Note the sharp line of demarcation between the basophilic portion at the lower left and the eosinophilic portion in the remainder of the nodule. Azure B, X30.

Fig. 12.—Adenoma. The muralia are widened and contain as many as ten cells. Azure B, X70.

Fig. 13.—Intraportal metastasis in a tumorous liver. Groups of dark-stained nucleated red cells are enmeshed in the tumor embolus. H. & E. X240.

Fig. 14.—Trabecular hepatoma. Mitoses are not present. H. & E., X240.

Fig. 15.—Trabecular hepatoma showing widened intercellular spaces filled with amorphous material. The cytoplasm of the tumor cells is vacuolated. H. & E., X240.

Fig. 16.—Trabecular hepatoma. The intercellular accumulations of amorphous material stain strongly for arginine. Sakaguchi reaction, X30.
Fig. 30.—An area of hepatoma cells adjoining a sinusoid that contains a protein deposit. The cell membranes abutting the sinusoid have many microvilli. Note the enlarged sacs of the endoplasmic reticulum in the cell at the left. The deposits at the arrow may be material being released from the cells X6,500.

Fig. 31.—A profile of the Golgi apparatus of a hepatoma cell. Parts of two large lipide droplets (l) are along the upper edge and a smaller one in the lower right of the micrograph. The dense material enclosed within the membranes differs from the vesicular bodies associated with the Golgi apparatus of Figures 21 and 26. These structures are present in various areas of the cytoplasm. X38,000.
Observations on Hepatic Cell Hyperplasia, Adenoma, and Hepatoma of Rainbow Trout (Salmo gairdnerii)

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