The Metabolism of Drugs by Hepatic Tumors*

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SUMMARY

Transformation from normal hepatic cell to tumor cell may be accompanied by a loss of ability to metabolize certain drugs by enzymes in the microsomal fraction. This change did not occur to any measurable extent during the "precancerous" stage; nor was it present in hepatic cells adjacent to the DAB-induced tumor. Only the neoplastic cell has lost this function—even severely damaged hepatic cells retained normal levels of metabolism of the drugs used in our experiments.

The loss of enzyme activity in DAB-induced tumors was probably caused by an actual deficit of enzyme protein and not by a cofactor deficiency or by the presence of inhibitors in such tumors.

Animals bearing hepatic tumors may be more "sensitive" than normal animals to some drugs. We have shown some tumor-bearing animals sleep longer after hexobarbital administration.

Possible therapeutic implications are mentioned.

This paper continues a study of the possible relation between the structure of the endoplasmic reticulum of liver and one of the functions of the microsomal fraction derived therefrom—the metabolism of drugs. Howatson and Ham (7), Porter and Bruni (19), and Novikoff (17), in studies with the electron microscope, have described deviations from normal in the endoplasmic reticulum of neoplastic hepatic tissue. The submicroscopic structure of tumor tissue may be similar to that of the embryo in some respects (7).

Conney et al. (3) have reported that certain hepatic tumors lack an enzyme system which demethylates 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB). This enzyme is localized in the microsomes of normal liver. Mascitelli-Coriandoli and Citterio (11, 12) described the decrease in activity of some microsomal reductases in N-

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flourenylacetamide-induced hepatic tumors as compared with normal tissue. In a recent paper, Neubert and Hoffmeister (16) compared the activities of certain microsomal drug-metabolizing systems in a transplanted hepatoma with those in normal liver.

We have previously shown that the newborn animal differs from the adult in its capacity to metabolize drugs (4). Others in our group have found differences in the metabolism of drugs by regenerating vs. normal liver tissue. Such results led us to study the metabolism of drugs in another type of rapidly growing hepatic tissue—the tumor.

MATERIALS AND METHODS

Source of tumors.—Hepatic neoplasms were induced by feeding male Sherman rats a Purina Laboratory Chow diet containing 0.06 per cent 4-dimethylaminoazobenzene (DAB). Tumors usually developed within 7 months in rats on this diet. Hepatic tumors were also induced in male Sprague-Dawley rats by feeding a Purina Laboratory Chow diet containing 0.04 per cent N-fluorenlylacetamide. In addition we studied two transplantable tumors, the Novikoff "hepatoma" and the Morris...
tumor (No. 5123). The Novikoff tumor was made available through the courtesy of Dr. Alan Sartorelli at the Samuel Roberts Noble Foundation in Ardmore, Oklahoma. This tumor was carried intramuscularly, intraperitoneally, or subcutaneously in male and female Holtzman rats. Dr. Harold Morris at the National Cancer Institute, Bethesda, Md., kindly supplied the transplantable hepatoma No. 5123 inoculated into Buffalo rats intraperitoneally and subcutaneously.

**Histology.**—Tissues were fixed in 10 per cent formaldehyde and stained with hematoxylin and eosin. Histological studies were carried out in the Pathology Department of the State University of Iowa. Several sections of each tumor or tissue used were examined.

**Time and area studies.**—Male Sherman rats, fed on the Purina Laboratory Chow containing 0.06 per cent DAB, were removed from this diet at various intervals to determine whether there was a change in the metabolism of drugs before a histologically identifiable tumor made its appearance. Studies on areas immediately adjacent to a tumor (as well as the tumor) were made with tumor-bearing rats which had been removed from the DAB-containing diet and fed only laboratory chow for at least 6 weeks prior to sacrifice.

**Preparation of tissue samples.**—Animals were killed by a blow on the head, and the livers (or tumors) were excised immediately. Care was taken to use non-necrotic tumor tissue in all assays. Any tumor with recognizable necrotic areas was discarded. Excision of tumors was made so as to avoid inclusion of any recognizable hepatic or muscle tissue surrounding the tumor. Samples were taken for histological examinations, and the remainder of the tissue was homogenized in 2 or 3 parts of cold isotonic KCl with a Potter homogenizer having a plastic pestle. The 9,000 × g supernatant fraction was prepared by spinning the homogenate in a high-speed angle centrifuge in the cold. Both homogenate and supernatant were used in assays of drug-metabolizing enzyme activity in vitro. Both fractions were studied in the event that the enzymes were distributed differently intracellularly in the tumor from the normal hepatic cell.

**Determination of enzyme activity.**—The metabolisms studied were side-chain oxidation of hexobarbital, hydroxylation of the aromatic ring of acetanilide, ring sulfur oxidation of chlorpromazine, O-dealkylation of codeine, N-dealkylation of aminopyrine, reduction of the aromatic nitro group of p-nitrobenzoic acid, and reductive cleavage of the azo linkage of neoprontosil. An end-product was measured to follow the metabolism of acetanilide, codeine, aminopyrine, p-nitrobenzoic acid, and neoprontosil, and disappearance of the substrate was measured to follow the metabolism of hexobarbital and chlorpromazine. Most methods used in these assays have been previously reported (13). The metabolisms of p-nitrobenzoic acid and neoprontosil were followed by methods used by Fouts et al. (5, 6). The cell fractions were incubated in a Dubnoff metabolic shaking incubator with oxygen or nitrogen as the gaseous phase. Oxidative reactions were incubated under oxygen for 2 hours, whereas reductive reactions were incubated for 1 hour under nitrogen. Cofactors added were triphosphopyridine nucleotide (TPN), glucose-6-phosphate, nicotinamide, and magnesium sulfate. Final concentrations of cofactors were the same as previously reported (13). The final volume of all incubation mixtures was 5.0 ml. Glucose-6-phosphate dehydrogenase activity was determined in both normal and neoplastic livers according to a modification of the method of Kornberg and Horecker (10) as described previously (13).

**In vivo studies.**—Some idea of the in vivo rate of the metabolism of drugs was obtained from sleeping times after hexobarbital administration. Sherman rats (normals and those with tumors induced by feeding DAB) were given injections intraperitoneally (I.P.) of 100 mg/kg of hexobarbital sodium. The time in minutes from the loss of the righting reflex until the regaining of this reflex was called the “sleeping time.” Statistical evaluations were made according to methods described by Snedecor (20).

**RESULTS**

**Histology**

Histological examination of the tumors obtained by feeding DAB revealed that they were hepatomas, cholangiomas, or hepatocholangiomas, whereas the tumors obtained by feeding N-2-fluorenylacetamide were primarily hepatomas.

Histological studies were made of all transplantable tumors (Novikoff and the tumor No. 5123). The findings were similar to those reported by others (15, 17). Such histological examination served additionally to assure us that samples used in enzyme assays were not contaminated with adjacent tissue (muscle or liver).

**Metabolism of Drugs by Various Tumors**

**Induced tumors.**—Table 1 shows that all types of neoplasm obtained from feeding DAB lacked the capacity to metabolize drugs in vitro. All oxidative pathways investigated were absent, whereas metabolisms by the two reductive pathways were considerably depressed.

**Novikoff transplantable “hepatoma.”**—The ca-
pacity to metabolize drugs in vitro was also absent in the Novikoff tumor (Table 2). All oxidative pathways studied were absent, one of the reductive pathways was diminished (azo cleavage), and the other reductive pathway was absent (aromatic nitro-group reduction). The loss of capacity to metabolize certain drugs occurred in this "hepatoma" regardless of whether it was carried intramuscularly, intraperitoneally, or subcutaneously.

Transplantable tumor No. 5123.—This transplantable "hepatoma," which was a slowly growing tumor as contrasted with the rapidly growing Novikoff "hepatoma," also lacked the oxidative pathways (metabolisms of hexobarbital, codeine, and aminopyrine), whereas the reductive pathway investigated (reduction of p-nitrobenzoic acid) was severely depressed (Table 2).

Enzyme activities depended on the diet fed. It should be noted that, in our experiments, rats were fed Laboratory Chow containing DAB. Miller et al. (14) administered 3'-Me-DAB in a "semipurified" diet. We feel the difference in effects of the azo dyes on the microsomal enzymes may be explained by the different diets in which these dyes were incorporated.

Metabolism of drugs by tissue adjacent to tumor.—The finding that "precancerous" liver metabolized drugs at a normal rate led us to study the enzyme activities in hepatic tissue surrounding the tumor. Table 4 reveals no significant difference between the metabolism of drugs by normal liver and by the area immediately adjacent to the hepatic neoplasm. Figures 2 and 3, respectively, picture a section of a tumor induced by DAB and the area

| TABLE 1 |
| METABOLISM OF DRUGS in Vitro BY NORMAL LIVER AND BY DAB-INDUCED HEPATIC TUMORS* |

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Normal supernatant</th>
<th>No. exps.</th>
<th>Tumor homogenate</th>
<th>No. exps.</th>
<th>Tumor supernatant</th>
<th>No. exps.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetanilide</td>
<td>1.1 ± 0.3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>1.5 ± 0.0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Codeine</td>
<td>1.7 ± 0.6</td>
<td>30</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Hexobarbital</td>
<td>5.5 ± 1.9</td>
<td>22</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Neoprontosil</td>
<td>6.3 ± 2.5</td>
<td>7</td>
<td>0.5 ± 0.2</td>
<td>5</td>
<td>0.4 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>p-Nitrobenzoic acid</td>
<td>1.5 ± 0.7</td>
<td>16</td>
<td>0.1 ± 0.1</td>
<td>5</td>
<td>0.1 ± 0.1</td>
<td>7</td>
</tr>
</tbody>
</table>

* Includes all types of tumors: hepatomas, cholangiomas, and hepatocholangiomas. Sherman rats.

Values in the table are averages ± standard deviations.

Relation between drug-metabolizing enzyme activity and tumor appearance.—The loss in the capacity of hepatic tumors to metabolize drugs resulted in a study to see whether this change was present only in the neoplastic state or if there was a gradual loss of this function as the normal tissue progressed to a tumor. Results presented in Table 3 indicated that no loss of drug-metabolizing enzyme activity occurred in the liver (even in "cirrhotic" livers) until the appearance of the neoplasm. Figure 1 shows a section from one of several "cirrhotic" livers which had normal levels of metabolism. Likewise, others have reported that esterase activity of rat liver appeared to remain at normal levels throughout the period of feeding the animals DAB, decreasing in value only when the tumor made its appearance (9).

However, Miller et al. (14) showed that 3'-Me-DAB fed to rats can cause a decrease in hepatic reductase and N-demethylase activities. These workers pointed out that the levels of these enzyme activities depended on the diet fed. It should be noted that, in our experiments, rats were fed Laboratory Chow containing DAB. Miller et al. (14) administered 3'-Me-DAB in a "semipurified" diet. We feel the difference in effects of the azo dyes on the microsomal enzymes may be explained by the different diets in which these dyes were incorporated.

| TABLE 2 |
| METABOLISM OF DRUGS in Vitro BY NOVIKOFF AND NO. 5123 TUMORS* |

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Normal supernatant†</th>
<th>Novikoff tumor homogenate‡</th>
<th>Novikoff tumor supernatant‡</th>
<th>No. 5123 tumor supernatant‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetanilide</td>
<td>1.4 ± 0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>1.1 ± 0.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Codeine</td>
<td>1.8 ± 0.2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hexobarbital</td>
<td>3.6 ± 0.4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neoprontosil</td>
<td>5.6 ± 0.8</td>
<td>0.9</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>p-Nitrobenzoic acid</td>
<td>1.5 ± 0.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aminopyrine</td>
<td>0.31 ± 0.03</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* These tumors were carried I.P.
† Average values of at least four determinations (duplicates in each determination) ± standard deviation.
‡ Values represent averages of two or more experiments (duplicates in each experiment).
surrounding it. This tumor lacked the capacity to metabolize codeine and hexobarbital, whereas the enzyme activity of the tissue surrounding the tumor was the same as normal liver.

In vivo study.—The metabolism of hexobarbital in vivo by normal rats and those with DAB-induced tumors was estimated from sleeping times. Data in Table 5 indicate that animals bearing such tumors slept longer after hexobarbital administration than did controls.

**TABLE 3**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Time (in months) fed diet containing 0.06 per cent DAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Codeine</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>Hexobarbital</td>
<td>4.7±1.3</td>
</tr>
<tr>
<td>p-Nitrobenzoic acid</td>
<td>1.1±0.5</td>
</tr>
</tbody>
</table>

* Values are from determinations on the supernatant fraction and are μmoles of substrate metabolized or product formed per gm. liver used.

**TABLE 4**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Metabolism in μmoles/gm liver used*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal supernatant</td>
</tr>
<tr>
<td>Codeine</td>
<td>1.7±0.5</td>
</tr>
<tr>
<td>Hexobarbital</td>
<td>4.7±1.3</td>
</tr>
<tr>
<td>p-Nitrobenzoic acid</td>
<td>1.2±0.3</td>
</tr>
</tbody>
</table>

* Values are averages ± standard deviations (duplicates in each determination). Sherman rats.

A "t" test showed no significant differences between metabolism by liver adjacent to tumor and normal liver (P > 0.4 for all pathways).

**POSSIBLE REASONS FOR LOSS OF METABOLISM OF DRUGS**

The apparent lack of enzyme activity in hepatic tumors might be caused by several factors. Among these are (a) a deficiency of cofactors (reduced TPN [TPNH] or glucose-6-phosphate), (b) the presence of inhibitors of the drug-metabolizing enzymes, or (c) an absence of enzyme protein.

The TPNH required for these metabolisms was provided by adding glucose-6-phosphate, TPN, magnesium sulfate, and nicotinamide in all determinations. In addition, we assayed endogenous glucose-6-phosphate dehydrogenase activity of normal and tumor tissue and found that this enzyme activity was always greater in the tumor tissue. Thus, as far as could be determined, the absence of drug-metabolizing enzyme activity in neoplasms was not related to a deficiency of the cofactor TPNH.

Inhibitors of the drug-metabolizing systems might include DAB or its metabolic products. Metabolites of DAB have been reported to inhibit a diphosphopyridine nucleotide-requiring system (8) and a rat liver transaminase (3). We studied the effects of DAB, N,N-dimethyl-p-phenylenediamine (DPD), and p-phenylenediamine (PD) on the metabolism of chlorpromazine, hexobarbital, and codeine by supernatant from normal liver. At 10^-4 M final concentration, DAB, DPD, and PD gave no inhibition of any pathway. At 10^-4 M, DPD inhibited the three pathways about 50-60 per cent, whereas PD gave approximately a 20-25 per cent inhibition. These concentrations of DAB, DPD, and PD should be far in excess of any level possible in livers or tumors from rats fed the diets used. It would seem unlikely that lack of enzyme activity could be caused by these compounds. Certainly the Novikoff tumor and the 5129 tumor would have no such azo dye or metabolites.

Thus, we are left with the possibility that the loss of enzyme activity is related to an actual deficiency of enzyme protein.

**DISCUSSION**

Our results show that: (a) certain kinds of neoplastic liver tissue lack the ability to metabolize
a variety of drugs—a function normally present in hepatic microsomes (1). This is true in several types of neoplasm—hepatoma, cholangioma, or hepatoccholangioma induced by feeding DAB, Novikoff transplantable tumor, or the hepatoma No. 5125. We have also studied the metabolism of hexobarbital, acetanilide, and aminopyrine in hepatic tumors induced by feeding N-fluorenylaceticamide. The results might be considered preliminary at this time. We have shown that the metabolism of hexobarbital was missing in three such tumors; also, the metabolism of aminopyrine was not present in one of these tumors. In another tumor the hydroxylation of acetanilide did not occur. (b) Precancerous liver from animals fed DAB retains normal activities of the enzymes studied even though such tissue may be rather severely damaged. (c) Areas immediately adjacent to a DAB-induced neoplasm have normal levels of the enzyme activities studied, even though such areas may not be normal histologically. (d) The lack of certain drug enzyme activities in the tumor is not caused by a lack of the co-factor TPNH, is unlikely to be caused by DAB or its metabolites in animals fed DAB, but may be related to an actual deficit in enzyme protein. (e) Animals bearing tumors induced by feeding DAB seem to be unable to metabolize hexobarbital as rapidly as do normal animals. This may be inferred from the fact that a given dose of hexobarbital results in tumors one might seek to prepare a carcinostatic compound which accumulates in the liver or can be limited to this site of action by various means, such as the liver metabolism of at least some drugs.

Certain therapeutic implications of our findings might be mentioned: (a) Antitumor drugs which are normally “detoxified” by the liver may be more active against the hepatic tumor (unable to “detoxify” the drug) than the normal hepatic cell. If means are available to direct the drug pre-

TABLE 5
EFFECTS OF HEXOBARBITAL ON NORMAL VS. TUMOR-BEARING RATS

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Tumor-bearing†</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLEEPING TIMES (MINUTES)</td>
<td>38 ± 4 (10)</td>
<td>61 ± 22 (6)</td>
</tr>
</tbody>
</table>

* Values in the table are averages ± standard deviations. Figures in parentheses are the numbers of animals in each group. Hexobarbital sodium, 100 mg/kg, was injected I.P. A "t" test showed a significant difference (at P = 0.05) between normal and tumor-bearing animals.† Tumor-bearing animals were killed after the experiment and their livers removed for histological examination of the various nodules present. Among this group of animals were all three types of tumor—hepatoma, cholangioma, and hepatoccholangioma. Tumors were excised carefully and weighed. Total grossly dissectible tumor varied from less than 0.5 gm. to 61 gm. The animal with the smallest total tumor mass slept 40 minutes; the animal with the largest mass slept 100 minutes. No animal shown to have a tumor slept less than 40 minutes.
REFERENCES


Fig. 1.—"Cirrhotic" liver with normal drug enzyme activity. Moderately severe hepatocellular damage and early cirrhosis may be seen. Hematoxylin-eosin (H. & E.), X150.

Fig. 2.—DAB-induced hepatoma next to area of Fig. 3. Hepatic-cell tumor, mitotic figures may be seen. H. & E., X150.

Fig. 3.—Area of liver adjacent to tumor of Fig. 2. This section shows diffuse, moderate hepatocellular damage. H. & E., X150.
was impossible to evaluate specific cytotoxic responses as described prior to drug addition or methods of culture response based on primary or metastatic lesion type in the group evaluated. There was no significant difference in sensitivity or resistance to any agent based on the type of tissue. Normal treated tissue cultures never displayed over-all sensitivity or resistance as a result of prior therapy. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard. The five agents under investigation produced objective cytological changes. All five agents caused mitotic inhibition and cytolysis. The antibiotic, actinomycin D, produced nuclear cleolar reduction. The antimetabolite, methotrexate, caused pyknosis to form narrow, elongated nuclei. The agents resembled changes seen after x-radiation—namely, aberrant chromosome structures and giant cells. The three alkylating agents, thioTEPA, actinomycin D, and chlorambucil; and the antimetabolites, methotrexate and phenylalanine mustard, produced direct objective cytological changes. The antimetabolites caused mitotic inhibition and cytolysis. The compounds in decreasing order of effectiveness were: thioTEPA, actinomycin D, chlorambucil, methotrexate, and phenylalanine mustard.

Three successive biopsies from a patient with lymphosarcoma indicated that there was no relation between migration and growth in tissue culture and response to drug. This conclusion was further apparent: tissues which survive therapy and apparently grow in vivo are able to respond like untreated tissues in vitro, in this study. An analysis of the experimental data failed to reveal over-all sensitivity or resistance to any test drug concentrations. These data may be useful in clinical procedures involving the use of agents administered at the tissue site as, for example, in perfusions. Studies based on a large group of malignant neoplasms indicated that there was no relation between exposure to actinomycin D, chlorambucil, and thioTEPA; apparent: the resistance of breast carcinomas to chlorambucil; the sensitivity of fibrosarcomas to direct exposure to chlorambucil; the sensitivity of lymphomas to thioTEPA; the resistance of lymphosarcomas to methotrexate; the resistance of lymphosarcomas to methotrexate; the resistance of all melanomas tested to methotrexate and phenylalanine mustard; the sensitivity of certain carcinoma types to thioTEPA and phenylalanine mustard; the resistance of all melanomas tested to methotrexate and phenylalanine mustard; the sensitivity of fibrosarcomas to direct exposure to chlorambucil; and the sensitivity of lymphomas to thioTEPA.

The relation of prior therapy, in vivo, to cellular migration and growth in tissue culture was investigated. Prior treatment included administration of carcinostatic agents to malignant, and benign neoplastic tissues tested.

DISCUSSION

Tumor types in general responded individually to the test agents, certain trends were evident. The relation of previous drug therapy, in vivo, to cellular migration and growth in tissue culture, prior to drug addition or methods of culture, was investigated. Prior treatment included administration of carcinostatic agents to malignant, and benign neoplastic tissues tested. To investigate the relation between the response of primary and metastatic tissues from the same patient, 154 specimens excised from patients treated with one or several of the drugs were compared with the responses of 311 specimens from patients with no history of prior therapy.

In vitro, there was no detectable correlation between the prior growth rate and response of individual specimens apparently were not related to each other. Prior treatment within 7 months prior to biopsy and/or radiation therapy, in vivo, within ~ months prior to biopsy and/or radiation therapy, was compared with the responses of 30 culture series of primary melanoma and carcinoma lesions were compared with the responses of 30 randomly selected culture series of metastatic melanoma lesions.
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