Studies on Composition and Leakage of Proteins and Esterases of Normal Rat Liver and Morris Hepatoma 5123 t.c.¹

R. K. MURRAY,² H. KALANT, M. GUTTMAN, and H. P. MORRIS

Departments of Biochemistry and Pharmacology, University of Toronto, Toronto, Canada, and the Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, Maryland

Summary

The addition of 0.5% ethylenediaminetetraacetate (EDTA) to the incubation medium caused a significantly greater increase in protein leakage from normal Buffalo-strain rat liver slices (55%) than from slices of Morris 5123 t.c. hepatoma (33%) or of liver of tumor-bearing animals (25%). Electrophoresis in urea and non-urea starch gels revealed that the pattern of soluble proteins leaking from slices of the 5123 t.c. hepatoma was very similar to that of normal liver. The more basic soluble proteins, previously found to be markedly reduced in primary p-dimethylaminoazo-benzene-induced hepatoma, were only moderately reduced in homogenates of the Morris hepatoma. Addition of EDTA to the incubation medium increased the leakage of a fraction designated “basic protein 4” from normal liver slices, but this effect was not demonstrable with the tumor slices.

The leakage of esterases into the incubation medium was also studied. All of the esterases present in homogenates of normal liver and the hepatoma were found to leak into the incubation medium. However, the tumor homogenates showed a marked reduction in a fast-migrating esterase (Zone 1). Electrophoresis of plasma proteins and esterases revealed frequently-occurring changes in samples from tumor-bearing animals, including an extension of the albumin front, a decrease in the fast α-globulin, a marked increase in albumin esterase activity and a decrease in cholinesterase.

The findings demonstrate a possible difference with respect to the influence of divergent cations on the permeability of the cell membranes of normal liver, host liver, and this hepatoma, as well as differences between their soluble protein and esterase profiles.

Introduction

In a previous study (17) it was demonstrated that the leakage of protein from slices of normal rat liver incubated in vitro was significantly increased by the addition of 0.5% EDTA² to the medium. In contrast, slices of primary hepatoma induced by DAB showed much more variable leakage of protein, which was not consistently or significantly increased by EDTA. SGE of the soluble tissue proteins revealed in the DAB-induced hepatoma a marked decrease in the amounts of soluble basic proteins, which possibly corresponds to the decrease in “h” proteins described by Sorof et al. (31, 32, 34).

The primary DAB-induced hepatomas were quite varied in histologic appearance, and it was impossible to decide whether they were primarily of hepatocellular or other origin. For this reason, it could not be concluded whether the above-noted changes in protein leakage, EDTA effect, and pattern of soluble basic proteins reflected changes inherent in the malignant state or merely differences in cell population between normal liver and the DAB-induced hepatoma. Since 1960, hepatomas have become available which are both morphologically and biochemically much closer to normal parenchymal cells than previously available hepatomas (23, 24). For these reasons, they have been named minimal deviation hepatomas (27). It was felt that a study similar to that previously performed on the DAB-induced hepatoma, but using the relatively slow-growing and better-differentiated Morris hepatoma 5123 t.c., might indicate whether the altered permeability and profile of soluble proteins were true concomitants of malignancy of merely reflections or changes in cell population. The esterase profile of the 5123 t.c. hepatoma has also been studied as the previous investigation had indicated alterations of esterase pattern in the DAB-induced hepatoma. The electrophoretic patterns of the proteins and esterases of the plasma of rats carrying this tumor were also studied in an attempt to correlate them with the protein and esterase profiles of the hepatoma.

Materials and Methods

TREATMENT OF ANIMALS. The rats employed in this study were males and females of the highly-inbred Buffalo strain. Most had been bred and raised in the National Cancer Institute, Bethesda, Md., but some were purchased from Simonson Laboratories, Gilroy, California. At 8–10 weeks of age, the tumor-bearing rats were inoculated via trocar either i.p. or s.c. and i.m. with small pieces of hepatoma 5123 t.c. This tumor is a subline from 5123 C Generation 20, grown in tissue culture for 5 months at the McArdle Memorial Laboratory and then returned to the animal. The tumors used in these studies were transfer Generations 19–21 after return into rats. All rats were fed chow and tap water ad libitum. The tumor-bearing animals were sacrificed some 5–7 weeks after inoculation, together with their paired controls.

¹ Supported by grants from the Medical Research Council of Canada (R.K.M.) and the National Cancer Institute of Canada (H.K.). A preliminary report of this work was presented at the meeting of the Federation of American Societies of Experimental Biology, Chicago, Ill., in April 1964.
² Present address: McArdle Memorial Laboratory, Madison, Wisc.
³ Abbreviations: EDTA, ethylenediaminetetraacetate; DAB, p-dimethylaminoazo-benzene; SGE, starch gel electrophoresis; KRP, Krebs-Ringer-phosphate.

Received January 3, 1966; accepted September 28, 1966.

FEBRUARY 1967 403

Downloaded from cancerres.aacrjournals.org on May 28, 2017. © 1967 American Association for Cancer Research.
The animals were sacrificed by decapitation and decapitated. Samples of blood were collected in lighted heparinized centrifuge tubes. It is possible that heparin may have altered the mobility of some of the plasma proteins; however, samples from normal and tumor-bearing rats were treated in the same way. Plasma was collected after centrifugation and stored at -20°C for subsequent SGE. Immediately after the rat was decapitated, the liver was removed and chilled in ice-cold KRP medium (20) containing one-third the usual amount of Ca++. Slices approximately 0.3 mm thick were taken with the method of McIlwain (22) and washed in ice-cold KRP medium to remove any blood remaining in the liver. Tumor slices were obtained similarly. Portions of tumor and liver tissue were fixed in calcium-formol for histologic examination.

**INCUBATION AND PROTEIN MEASUREMENT.** Approximately 100-150 mg fresh weight of tissue and 2.0 ml of medium were used in each flask. In each experiment, 4 samples of tissue were incubated in KRP, and 4 samples in KRP + 0.5% EDTA disodium salt, all at pH 7.4, in a Dubnoff shaker bath at 35°C under pure oxygen, for 90 min. Following this, the slices were washed in fresh KRP to remove any adhering protein or EDTA. The samples were centrifuged at 35,000 × g for 60 min at 0°C in a Servalli RC-2 centrifuge. The supernatants were decanted, and 0.2- to 0.5-ml samples were taken for protein measurement by the biuret method (12), using the high-copper reagent described previously (17). With KRP samples, sufficient EDTA was added to the biuret reagent to give the same final concentration of EDTA as that obtained with the corresponding KRP-EDTA samples, and the same EDTA-biuret solution was used with the protein standards. The precipitated tissue particles were washed in fresh KRP, recentrifuged, and added to the corresponding tissue slices for micro-Kjeldahl digestion and total N determination. Final tissue protein content was taken as 6.25 × N content. Since all protein in the medium must have come originally from the tissue slices, leakage during incubation was expressed as (protein in medium)/(protein in medium + slices) × 100%.

**PREPARATION OF MEDIA FOR ELECTROPHORESIS.** Following the removal of portions for protein measurements, the remainder of the media from samples of the same tissue treated in the same manner were pooled and dialyzed against 8 volumes of distilled water at 0°-4°C, and then lyophilized. The dry samples were then made up in concentrated solution (approximately 5% unless otherwise stated) for electrophoresis, as described previously (17).

**HOMOGENIZATION OF LIVERS AND TUMOR SAMPLES.** Fresh chilled livers from normal rats, and tumors and livers from tumor-bearing rats, were homogenized in 9 volumes of ice-cold 0.9% sodium chloride. The samples were then centrifuged at 35,000 × g for 60 min at 0°C in a Servalli RC-2 centrifuge. The supernatants were collected, transferred to dialysis tubing, dialyzed for 24 hr against 8 volumes of distilled water at 0°-4°C, and then lyophilized. The protein content of the lyophilized samples was determined by the biuret method, and the samples were dissolved in distilled water sufficient to give a final protein concentration of 5%. Each solution was then used for SGE.

**STARCH GEL ELECTROPHORESIS.** Vertical SGE was performed according to Smithies (29) using Starch-Hydrolyzed (Connaught Laboratories, Toronto), 0.021 M sodium borate buffer (pH 8.4, μ = 0.006), and a current of 10 ma (4 volts/cm) for 16 hr at room temperature. Samples of normal rat plasma were usually run as markers. After electrophoresis, the gel was cut into 2 slices of equal thickness. One slice was stained with AmidoBlack B to locate proteins. The other was used to detect esterases by the method of Ecobichon and Kalow (11) using β-naphthyl acetate as substrate. Identification of the electrophoretic location of plasma cholinesterase was kindly performed by Dr. D. J. Ecobichon, using β-carbonaphthoxycyline iodide as the synthetic substrate (11). The system of nomenclature of the plasma proteins is based on that used by Beaton et al. (4).

**UREA-STARCH GEL ELECTROPHORESIS.** This was performed as described previously (17) using the method of Smithies and Connell (30).

**RESULTS**

**HISTOLOGY.** Hematoxylin and eosin sections of hepatoma 5123 t. c. showed a combination of blunt cell cords, 2-3 cells thick, and acinar structures also of 2-3 cells thickness, closely resembling the original 5123 hepatoma (23). Some of the larger pseudo-acinar structures contained pale-staining degenerated cells in the center. The typical tumor cells were fairly regular and normal in size, with few or no mitotic figures visible.

**PROTEIN LEAKAGE INTO MEDIA.** In Table 1 are shown the individual results for protein leakage from slices of 8 normal livers, 10 livers from rats with s.c. and i.m. implanted tumors ("host livers"), and 11 hepatomas, in media with and without added EDTA. Without EDTA, protein leakage from the tumor slices was greater than that from normal liver slices. In the presence of added EDTA, leakage from both increased, but the increase was proportionately greater in the case of the liver slices, so that the actual leakage values became virtually equal for liver and hepatoma. In contrast, leakage from slices of host liver was equal to that from normal liver, in KRP medium; but in KRP-EDTA the leakage from host liver was significantly less than that from normal liver and hepatoma, so that the percentage increase on addition of EDTA was the same as that found with tumor slices.

**Electrophoresis**

**SOLUBLE PROTEINS FROM NORMAL AND HOST LIVERS.** The soluble proteins present in the supernatant fraction of homogenates of 12 rat livers were analyzed by SGE in borate buffer. A typical pattern is shown in Fig. 1 (Slots 4 and 6). The resolution of zones migrating toward the anode was generally rather poor, only 3 distinct zones appearing. In some preparations, somewhat better resolution of the anodal zones was obtained (see Fig. 2, Slot 2). Five zones were regularly detected migrating toward the cathode as shown in Fig. 1, but occasionally a sharper resolution yielded 2 or 3 additional zones (see Fig. 2, Slot 2). These differences in electrophoretic resolution may be attributable to slight variations in the quality of the individual batches of starch used. The electrophoretic pattern of soluble proteins in the supernatant fractions of homogenates of 6 host livers did not differ from the pattern obtained with the livers of normal rats.
Proteins and Esterases of Normal Liver and Hepatomas

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Leakage in KRP*</th>
<th>Leakage in KRP-EDTA*</th>
<th>EDTA effect (% increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal livers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.1</td>
<td>17.8</td>
<td>47.6</td>
<td></td>
</tr>
<tr>
<td>10.4</td>
<td>18.0</td>
<td>72.1</td>
<td></td>
</tr>
<tr>
<td>13.7</td>
<td>21.4</td>
<td>56.8</td>
<td></td>
</tr>
<tr>
<td>13.4</td>
<td>20.0</td>
<td>49.4</td>
<td></td>
</tr>
<tr>
<td>10.9</td>
<td>15.0</td>
<td>37.6</td>
<td></td>
</tr>
<tr>
<td>10.5</td>
<td>15.5</td>
<td>47.6</td>
<td></td>
</tr>
<tr>
<td>11.1</td>
<td>18.7</td>
<td>68.5</td>
<td></td>
</tr>
<tr>
<td>10.6</td>
<td>18.9</td>
<td>63.5</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>11.6 ± 0.47</td>
<td>18.2 ± 0.76</td>
<td>55.4 ± 4.3*</td>
</tr>
<tr>
<td>Host livers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.8</td>
<td>16.1</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>9.3</td>
<td>14.2</td>
<td>51.6</td>
<td></td>
</tr>
<tr>
<td>10.1</td>
<td>11.0</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>9.9</td>
<td>13.2</td>
<td>32.6</td>
<td></td>
</tr>
<tr>
<td>10.2</td>
<td>15.3</td>
<td>49.1</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>18.5</td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>10.4</td>
<td>13.7</td>
<td>31.4</td>
<td></td>
</tr>
<tr>
<td>12.7</td>
<td>13.6</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>11.2</td>
<td>11.8</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>10.8</td>
<td>11.8</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>11.2 ± 0.53</td>
<td>13.9 ± 0.71</td>
<td>25.3 ± 6.2*</td>
</tr>
<tr>
<td>Morris 5123 t.c. hepatomata</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.6</td>
<td>16.1</td>
<td>67.9</td>
<td></td>
</tr>
<tr>
<td>14.6</td>
<td>20.0</td>
<td>37.0</td>
<td></td>
</tr>
<tr>
<td>16.2</td>
<td>18.0</td>
<td>11.3</td>
<td></td>
</tr>
<tr>
<td>14.8</td>
<td>19.8</td>
<td>33.9</td>
<td></td>
</tr>
<tr>
<td>13.3</td>
<td>19.4</td>
<td>45.4</td>
<td></td>
</tr>
<tr>
<td>17.4</td>
<td>24.3</td>
<td>39.7</td>
<td></td>
</tr>
<tr>
<td>15.8</td>
<td>17.6</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>10.6</td>
<td>16.3</td>
<td>53.4</td>
<td></td>
</tr>
<tr>
<td>11.6</td>
<td>15.7</td>
<td>35.6</td>
<td></td>
</tr>
<tr>
<td>13.0</td>
<td>15.4</td>
<td>18.4</td>
<td></td>
</tr>
<tr>
<td>12.9</td>
<td>13.4</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>13.6 ± 0.73</td>
<td>17.9 ± 0.86</td>
<td>32.5 ± 5.9*</td>
</tr>
</tbody>
</table>

* Values shown indicate % of initial protein of slices which leaked into medium during incubation. KRP, Krebs-Ringer phosphate solution; KRP-EDTA, KRP + 0.5% added EDTA; EDTA, ethylenediaminetetraacetate.

**Values shown indicate % of initial protein of slices which leaked into medium during incubation. KRP, Krebs-Ringer phosphate solution; KRP-EDTA, KRP + 0.5% added EDTA; EDTA, ethylenediaminetetraacetate.**

α P < 0.01 by "t" test for paired data.

β % increase with EDTA in slices of tumor and of host liver is significantly smaller than that with normal liver slices (P < 0.01 in both cases). Increase in host liver slices was not significantly different from that in tumor slices (P > 0.40).

Soluble Proteins of Hepatoma 5123 t.c. The electrophoretic pattern of the proteins in the supernatant fractions of homogenates of 3 of 6 individual hepatomas studied is shown in Fig. 1 (Slots 2, 3, 5, 7 and 8). In comparison with the pattern of soluble proteins from normal liver (Slot 4), a moderate reduction in the intensity of the zones migrating toward the cathode is seen. However, all of the cathodal zones are present. These are the zones which were markedly reduced or absent in the DAB-hepatoma (17).

Protein leaking from slices of normal liver and hepatoma. The pattern of proteins leaking into the incubation medium from slices of normal liver and hepatoma was investig-
abundance of Zone 1 in the hepatoma and the absence of Zones 2 and 3 in the host liver. The absence (or marked diminution) of Zone 1 is a constant feature of the hepatoma (15 individual tumor samples). The reduction in Zones 2 and 3 of the host liver was observed in the livers of 6 rats injected with tumor i.p. but not in the livers of 6 other rats injected with the tumor i.m. and s.c.

**PLASMA PROTEIN PATTERNS.** A typical electrophoretogram of plasma from normal and tumor-bearing rats is shown in Fig. 6. The most consistent abnormalities found in the plasma of tumor-bearing animals were an extension of the albumin front and a decrease of the intensity of the fast α-globulin zone. The 1st difference is seen in all 4 of the plasma samples from tumor-bearing rats shown in Fig. 6, and the 2nd difference is seen in 3 of them (Slots 1, 3, 5). Each of these abnormalities was found in 18 of 22 plasma samples from tumor-bearing animals examined, although the 2 abnormalities did not occur together in all cases. Both of these changes were found in male and female rats, and appeared to be independent of the site of inoculation of the tumor.

**PLASMA ESTERASE PATTERNS.** Typical electrophoretograms from normal and tumor-bearing rats are shown in Figs. 7 and 8. Fig. 7 represents a typical finding after approximately 10 min incubation in the esterase medium, and Fig. 8 shows the findings after approximately 1 hr. The most striking abnormalities found in the plasma of tumor-bearing animals were the marked increase in albumin esterase and the relative reduction of cholinesterase activity. The 1st change was observed in 18 of 23 samples examined, and the 2nd in 13 of 21 samples. No correlation with the site of implantation of the tumor was observed. As can be seen in Fig. 8, plasma samples from normal and tumor-bearing animals also differed with respect to the pattern of the esterase zones migrating between albumin and cholinesterase (intermediate zones). However, because these zones were considerably less intense than the albumin and cholinesterase zones and did not always stain satisfactorily in different electrophoretic runs, they were not studied systematically.

**Discussion**

It has long been known that divalent cations, especially Ca++, have an important influence in maintaining the adhesive and selective permeability of normal cell membranes (28). There are diverse hypotheses concerning the mechanism of this influence (1, 8, 35, 38), but most are agreed that Ca++ probably binds to anionic groups on the cell surface and diminishes intercellular repulsion, either by cross-bonding between cells ("calcium bridging") or by diminishing the electrostatic repulsion between anionic surface charges. The influence of Ca++ on membrane permeability is thought to be exerted, at least in part, through a reduction in the size of membrane "pores" through which hydrophilic substances can pass (7).

While much of the information on Ca++ has been obtained from studies with simple aquatic organisms and embryonic cells of higher metazoa, it has been shown repeatedly that EDTA can bring about separation of cells from the tissues of adult animals, and that it modifies their permeability at the same time (2, 16, 18). Studies with 4Ca and portal perfusion with EDTA solution have shown that these actions of EDTA are very probably due to removal of Ca++ (14).

Conan and his co-workers (6, 9) suggested that malignant cells differ from their normal counterparts with respect to the Ca++-binding groups on the cell surface. They believe that a decreased concentration of such groups on the cells of epidermoid carcinomas leads to a decrease in Ca++-binding, and therefore to decreased intercellular adhesiveness, resulting in a tendency to metastasize. If this is true of malignant cells generally, it should follow that EDTA would have less effect on them than on corresponding normal cells. Earlier studies (17) showed that EDTA, acting on tissue slices in vitro, did increase generally the leakage of protein from normal rat liver, and more specifically the leakage of a relatively basic protein ("basic protein 4") derived from the "microsomal" fraction, which has been shown to contain cell membrane fragments (13). The same concentration of EDTA did not significantly affect protein leakage from slices of DAB-induced hepatoma, but, as mentioned earlier, uncertainty concerning the cell population of these tumors made conclusions difficult.

Streffer and Williamson (36) have recently reported that EDTA failed to increase protein leakage from normal rat liver slices in vitro, even though removal of Ca++ from the medium did so. The reason for this discrepancy may be related to the fact that Streffer and Williamson used only 1 mM EDTA, while we have routinely employed approximately 13.5 mM.

The Morris 5123 hepatoma is so clearly of hepatic parenchymal origin (23) that it provides a much better material for comparison with normal liver. In the present study, EDTA again produced a highly significant increase in protein leakage from normal liver (Table 1). It also increased leakage from the 5123 t.c. hepatoma, but the increase was significantly smaller than with normal liver. While the leakage from the hepatoma was greater than that from normal liver in the control incubations, the addition of EDTA brought the leakage in both tissues to the same level. It seems reasonable to suggest that there is a limiting value for the permeability to protein in the complete absence of Ca++, and that the hepatoma has less membrane-bound Ca++ than normal and is therefore closer to this limiting value. It should be noted that the passage of intracellular proteins from tissue slices into the medium is not a matter of simple diffusion, but a complex process dependent upon the metabolic state of the animal and the tissue (19). In the present context, therefore, the terms "leakage" and "permeability" are used only to signify an outward movement of protein which is in some way dependent upon the functional state of the cell membrane. Atomic absorption spectrophotometry studies (R. A. Hickie and H. Kalant, in preparation) indicate that the Morris hepatoma 5123 t.c. actually has a higher total calcium concentration than normal liver. There is evidence to suggest that this is due to intracellular accumulation of Ca++ in the tumor, and does not invalidate the hypothesis that the cell membrane concentration of Ca++ is less than normal. A final conclusion, however, will require exact cytochemical localization studies, possibly by 4Ca radioautography with electron microscopy or by the microincineration technic.

The results with host liver slices were rather ambiguous, in that the protein leakage in KRP medium was the same as that of normal liver slices, while the EDTA effect was the same as that found with hepatoma slices. While host liver has also been found to contain more calcium than normal liver (R. A. Hickie and H. Kalant, in preparation), the difference is too small to account for the decreased EDTA effect on host liver on the basis of stoichiom-
Proteins and Esterases of Normal Liver and Hepatomas

The electrophoretic studies presented above reveal that the Morris hepatoma 5123 c.f. has a pattern of soluble proteins which is quite similar to that of normal liver. In particular, the basic proteins which were found to be markedly reduced in the DAB-induced hepatoma, were only moderately reduced in this hepatoma. It is of interest that Sorof [quoted by Pitot (26)] has previously observed that the “h” proteins, which probably correspond at least in part to the cathodally-migrating proteins of our study, are present in the Morris 5123 hepatoma. Recently, Sorof et al. (33) have shown that the h2 proteins of hepatoma 5123 C cannot serve in vivo as receptors for the binding of fluorenlyl carcinogen metabolites, in contrast to the corresponding class of proteins in normal liver. Further studies on the pattern of basic proteins of a variety of slow and fast-growing minimal deviation hepatomas should reveal whether there is any correlation between diminution of this fraction and the growth-rate of the tumors.

The electrophoretic results on the proteins leaking from slices of normal liver and hepatoma 5123 c.f. are similar to those obtained in our previous studies on slices of Wistar rat liver and of the DAB-induced hepatoma, except that the pattern of leakage from that hepatoma lacked all of the basic proteins. These results indicate that under our incubation conditions, the leakage of soluble proteins from either normal liver or the hepatoma is relatively unsselective and that EDTA influences mainly the quantitative rather than the qualitative pattern of protein leakage. The failure to detect increased leakage of “basic protein 4” from the hepatoma in the presence of EDTA may be due to its relatively low concentration in the tumor. Our previous study suggested that “basic protein 4” present in the medium was derived from the microsomal fraction, and an increase in its amount might represent increased solubilization of a ribosomal protein due to chelation of Mg++ by EDTA (15). However, EDTA produces a relatively small decrease in the Mg++ content of normal liver (14), and the Mg++ content of the hepatoma is greater than that of liver (R. A. Hickie and H. Kalant, in preparation). Moreover, Novikoff (25) has shown that hepatoma 5123 possesses a well-developed endoplasmic reticulum, with an almost normal complement of ribosomes, so that a general reduction in ribosomal protein can not explain the deficiency of “basic protein 4” in this tumor. The possibility remains that “basic protein 4” is a membrane constituent, reduced in amount in the hepatoma; however, there is no proof at present.

The reduction of esterase Zones 2 and 3 found in the host liver may be related to the nutritional status of these animals, as it was observed only in the livers of rats injected with tumor i.p. With extensive dissemination and growth of tumor nodules in the peritoneal cavity, these animals began to lose weight fairly rapidly. At autopsy, they showed small empty intestines and virtual absence of body fat. In contrast, rats with s.c. and i.m. tumors remained healthy and well-nourished. Other results (D. J. Ecobichon, R. K. Murray, and H. P. Morris, in preparation) from experiments designed to characterize the esterases of normal rat liver and of this hepatoma have shown that Zones 1, 2, and 3 are nonspecific ali-esterases. It will be of interest to observe whether Esterase 1 is also deficient in other minimal deviation hepatomas.

The most constant changes in the plasma protein pattern of rats carrying this hepatoma were a reduction in the fast α1-globulin zone and an extension of the albumin front. The significance of the first observation is not clear as the function of this protein has not been established. Beaton et al. (3) noted an increase of slow α2-globulin in rats carrying the Novikoff hepatoma and also in rats carrying a methylcholanthrene-induced hepatoma. This change was not noted in our present study. The extension of the albumin front may be due either to the presence of an abnormal species of albumin, or to an abnormal protein at the albumin front which is not resolved in the electrophoretic system used in the present study. The presence of an abnormal albumin in the plasma of tumor-bearing rats has been reported by Dufour et al. (10). It is of interest that another frequent change in the plasma of the tumor-bearing rats was also related to albumin, viz. an increased albumin esterase activity. This change was not due to an increase of total albumin in the plasma of the tumor-bearing animals as the albumin concentration was approximately normal (R. K. Murray, unpublished observations). The esterase activity associated with albumin is well-documented (5, 37), but no prior observation of increased albumin esterase in pathologic states has been reported. Reduction of serum cholinesterase has been demonstrated in a variety of pathologic conditions and is thus not a serum enzyme change specifically related to the tumor-bearing state (21).

References

6. Curran, P. F. The Biophysical Nature of Biological Mem-
R. K. Murray, H. Kalant, M. Guttman, and H. P. Morris


Fig. 1. Starch gel electrophoretogram, 0.021 M borate, pH 8.4, stained with Amidoblack. Comparison between proteins of supranatant fractions of homogenates of 2 normal livers and 3 hepatomas is shown. Slot 1, Normal rat plasma; Slots 2 and 3, duplicate samples of supranatant fraction of Hepatoma 1; Slot 4, supranatant fraction of Normal Liver 1; Slot 5, supranatant fraction of Hepatoma 2; Slot 6, supranatant fraction of Normal Liver 2; Slots 7 and 8, duplicate samples of supranatant fraction of Hepatoma 3. All protein concentrations were adjusted to 5% except plasma. OR indicates the origin and ALB the electrophoretic position of serum albumin.

Fig. 2. Starch gel electrophoretogram, 0.021 M borate, pH 8.4, stained with Amidoblack. Comparison between proteins of supranatant fractions of homogenates of normal liver and hepatoma, and proteins leaking from slices of normal liver and hepatoma 5123 t.c. Slot 1, Normal rat plasma; Slot 2, supranatant fraction of normal liver; Slot 3, proteins leaking from slices of normal liver into KRP-EDTA; Slot 4, proteins leaking from slices of normal liver into KRP; Slot 5, supranatant fraction of hepatoma; Slot 6, proteins leaking from slices of the hepatoma into KRP; Slot 7, proteins leaking from slices of the hepatoma into KRP-EDTA; Slot 8, normal rat plasma. * indicates the position of “basic protein 4.” All protein concentrations were adjusted to 5% except plasma. KRP, Krebs-Ringer-phosphate; EDTA, ethylenediaminetetraacetate; OR, origin; ALB, electrophoretic position of serum albumin.

Fig. 3. Urea-formate starch gel, pH 4.0, stained with Amidoblack. Comparison between proteins leaking from normal liver slices and hepatoma slices is shown. Slot 1, Normal rat plasma; Slot 2, proteins leaking from slice of Liver A incubated in KRP; Slot 3, proteins leaking from slice of Liver B incubated in KRP; Slot 4, proteins leaking from slice of Liver B incubated in KRP-EDTA; Slot 5, proteins leaking from slice of Liver C incubated in KRP; Slot 6, proteins leaking from slice of Liver C incubated in KRP-EDTA; Slot 7, proteins leaking from slice of Tumor A incubated in KRP; Slot 8, proteins leaking from slice of Tumor A incubated in KRP-EDTA; Slot 9, proteins leaking from slice of Liver D incubated in KRP; Slot 10, proteins leaking from slice of Liver D incubated in KRP-EDTA; Slot 11, proteins leaking from slice of Tumor B incubated in KRP; Slot 12, proteins leaking from slice of Tumor B incubated in KRP-EDTA. The protein concentrations of the samples applied to the gel varied from 2 to 4%, but for each pair of samples from the same liver or tumor the same concentration was employed. KRP, Krebs-Ringer-phosphate; EDTA, ethylenediaminetetraacetate; OR, origin.
FIG. 4. Starch gel electrophoretogram, 0.21 M borate, pH 8.4, stained for esterases. Slot 1, Esterases of supernatant fraction of host liver; Slot 2, esterases of supernatant fraction of normal liver; Slots 3-7, esterases of supernatant fractions of 5 hepatomas. All protein concentrations were adjusted to 5% before application of samples to the gel. OR, origin.

FIG. 5. Starch gel electrophoretogram, 0.21 M borate, pH 8.4, stained for esterases. Comparison between esterases of supernatant fractions of normal and host liver and of the hepatoma is shown. Slot 1, tumor plasma sample; Slot 2, esterases of hepatoma; Slot 3, esterases of Normal Liver 1; Slot 4, esterases of Host Liver 1; Slot 5, esterases of Normal Liver 2; Slot 6, esterases of Host Liver 2; Slot 7, esterases of Normal Liver 3. Protein concentrations of all samples except plasma were adjusted to 5% before application to the gel. Ch.E. indicates the electrophoretic position of cholinesterase; ALB, electrophoretic position of serum albumin; OR, origin.
FIG. 6. Starch gel electrophoretogram, 0.021 M borate, pH 8.4, stained with Amidoblack. Slots 1, 3, 5, 7, Plasma from 4 tumor-bearing rats; Slots 2, 4, 6, 8, plasma from 4 normal rats. F.G. indicates the electrophoretic position of fast α1-globulin; ALB, electrophoretic position of serum albumin; OR, origin.

FIG. 7. Starch gel electrophoretogram, 0.021 M borate, pH 8.4, stained for esterases. Time in the esterase incubation medium approximately 10 min. Slots 1, 3, 5, 7, Plasma from 4 tumor-bearing rats; Slots 2, 4, 6, 8, plasma from 4 normal rats; ALB, electrophoretic position of serum albumin; Ch.E., electrophoretic position of cholinesterase; OR, origin.

FIG. 8. Starch gel electrophoretogram, 0.021 M borate, pH 8.4, stained for esterases. This is the same gel shown in Fig. 7, but the time of incubation in the staining medium was increased to 1 hr to bring out the more slowly staining intermediate zones of esterase activity. Slots 1, 3, 5, 7, Plasma from 4 tumor-bearing rats; Slots 2, 4, 6, 8, plasma from 4 normal rats.
Studies on Composition and Leakage of Proteins and Esterases of Normal Rat Liver and Morris Hepatoma 5123 t.c.


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/27/2_Part_1/403