Effect of EDTA on Leakage of Proteins from Slices of Normal Rat Liver and DAB-induced Hepatoma*

H. Kalant, R. K. Murray, and Wilhelmina Mons

(Departments of Pharmacology and Biochemistry, University of Toronto, Toronto, Canada)

SUMMARY

The leakage of protein from slices of normal rat liver incubated in vitro was found to be significantly increased by the addition of 0.5 per cent ethylenediamine tetraacetate (EDTA) to the medium. The concentrated medium without EDTA revealed essentially the same electrophoretic pattern of soluble proteins as that demonstrable in whole liver, but the addition of EDTA to the medium specifically increased the leakage of an unidentified material, "basic protein 4," apparently derived from the microsomal fraction. Slices of p-dimethylaminoazobenzene-induced hepatoma showed much more variable leakage of protein, which was not consistently or significantly increased by EDTA. Almost all the soluble basic proteins, including "basic protein 4," were markedly diminished or absent from the hepatomas, and the presence of EDTA in the medium did not alter the electrophoretic pattern of the proteins which leaked. The tumors showed much less esterase activity than normal liver, and occasional differences in the electrophoretic pattern of esterases. The findings confirm the clear difference between the DAB-induced hepatoma cells and normal liver cells with respect to the complement of intracellular proteins and show a possible difference with respect to the influence of divalent cations on the permeability of the cell membranes.

It has long been known that calcium plays a role in the adhesion of normal tissue cells to one another. In contrast, Coman and his collaborators have shown in a variety of ways that certain malignant cells do not bind calcium as normal cells do and adhere to one another much less firmly (2–4). Tissue slices cut from normal rat livers perfused with Ringer solution containing EDTA or citrate (which presumably remove calcium) lose more of their intracellular potassium, ADP, and protein into the incubation medium than do slices from livers perfused with Ringer solution alone (11). These effects of perfusions with EDTA or citrate do not result from breakage of cell membranes (16) and have therefore been taken as evidence for a role of calcium in the maintenance of normal selective permeability. If calcium does not play a similar role in malignant cells, EDTA or citrate should not affect the permeability of malignant cells in the same manner as that of normal cells.

In previous work (11, 12) normal rat and mouse liver tissue showed alterations in the oxidation of succinate after perfusion of the livers with EDTA or citrate. These changes were interpreted as the result of altered cell permeability. Mouse ascites tumor cells treated with citrate did not show similar changes. In the present work a study was made of the effect of EDTA on the permeability of liver slices to protein. Slices of normal rat liver and of hepatomas induced by p-dimethylaminoazobenzene (DAB) were compared with respect to the amounts of proteins and esterases leaking into the incubation media in the presence and absence of EDTA. In addition, the electrophoretic patterns of proteins and esterases leaking into these media were compared with those of proteins and esterases in homogenates and fractions of normal rat liver and DAB-induced hepatomas. Esterases were studied primarily because they are soluble enzymes that may be readily detected by application of standard histochemical methods to starch gel electrophoretograms (9).

MATERIALS AND METHODS

Treatment of animals.—The rats employed in this study were of an original Wistar strain, bred in the laboratory. Normal rats were fed ad libitum on Purina chow and tap water. For the production of hepatomas rats were fed Diet No. 3 of Miller et al. (19), containing 12 per cent crude casein and 1 mg riboflavin/kg, to which was added...
or by successful transplantation in other rats. At the time of sacrifice the normal rats weighed 250-300 gm.; the normal liver no substrate was added to the medium. In each experiment, four to six samples of each tissue were incubated in KRP medium, and an equal number of samples of the same tissue were incubated in KRP medium containing 0.5 per cent EDTA. The pH of the medium was always adjusted to 7.4 before adding the tissue.

Concentration and measurement of proteins in medium.—After 90 minutes of incubation the tissue samples were removed, and the media from all the samples of the same tissue treated in the same manner were pooled. Each pooled sample was cleared of tissue particles by centrifugation at 600 X g for 10 minutes. The clear sample was transferred to a length of cellulose dialysis tubing of 1 cm. diameter, and the tubing was closed by means of a rubber-covered surgical forcep immediately above the level of the fluid, so that no increase in sample volume could occur during dialysis. The sample was then dialyzed overnight against 10 volumes of distilled water at 0-4°C. By this means, the salt concentration was reduced to a level which would not interfere with electrophoresis of the final concentrate but which was still sufficient to keep all the proteins in solution. The sample was transferred to a 25-ml. flask and lyophilized.

Each lyophilized sample was resuspended in 0.3 ml. of distilled water, and the protein concentration of the solution was measured by a modification1 of the biuret method of Gornall, Bardwill, and David (5). Depending upon the protein content indicated by the biuret method the remainder of each sample was diluted with sufficient distilled water to bring it to a final protein concentration of 3-5 per cent (w/v). Within a given experiment, the samples with and without EDTA were adjusted to the same protein concentration. Each solution was then used for starch gel electrophoresis.

Electrophoresis.—Vertical starch gel electrophoresis was performed according to the technic of Smithies (25). The buffer used was 0.021 M boric acid 30 per cent neutralized with sodium hydroxide (pH 8.2, μ = 0.006). Electrophoresis was carried out for a period of 5 hours at room temperature with a current of 20 milliamperes (8 volts/cm) passing through the gel. In every gel samples of normal rat serum were run as markers. After electrophoresis the gel was cut into two slices of equal thickness and routinely stained to locate proteins and esterases.

Protein and esterase stains: (a) Amido Black B was used to locate proteins. The relative amount of protein in each was estimated by visual examination of the intensity of staining.

1 The modified biuret reagent contains 1.2 per cent hydrated copper sulfate, 1.2 per cent Rochelle salt, and 3.0 per cent sodium hydroxide. After some weeks a precipitate may appear; this is easily removed by centrifugation or filtration with no change in color yield.
a) Lactic dehydrogenase was detected by the method of Lawrence et al. (15).

b) Acid phosphatase was detected by incubating the gel in a solution composed of 50 mg. sodium α-naphthyl phosphate, 20 mg. naphthanol diazo blue, and 100 ml. 0.2 M sodium acetate buffer, pH 5.0.

c) β-glucuronidase was detected by incubating the gel in a solution composed of 30 mg. 6-bromo-2-naphthyl-β-d-glucuronide, 20 mg. naphthanol diazo blue, and 100 ml. 0.2 M sodium phosphate buffer, pH 6.5.

The stained gels from the above three procedures were preserved in a methanol:water:glacial acetic acid (5:5:1 v/v) solution, and the mobility of the various isozymes was compared with the mobility of the liver soluble proteins detected by Amido Black B.

d) Benzidine reagent (25) was used in an attempt to locate cytochromes by their peroxidative activity.

e) Histones were extracted from whole rat liver by the procedure used by Neelin and Neelin (22) for whole calf thymus. Samples were analyzed by electrophoresis in 8 M urea-formate starch gels prepared according to the method of Smithies and Council (26). The buffer was 0.05 M formic acid 20 per cent neutralized with sodium hydroxide (pH 3.0, μ = 0.01). The pH of the urea-starch gel was 4.0. Samples of the soluble rat liver proteins leaking from slices were also run in these urea-formate gels for comparison. These gels were stained with Amido Black B and washed in 1 per cent acetic acid.

Chromatography of rat liver soluble proteins on DEAE-cellulose.—Preliminary steps were taken to purify the basic proteins which were demonstrated by starch gel electrophoresis at pH 8.2. Both Moore and Lee (21) and Whitcutt et al. (32) have described chromatographic methods for separating rat liver soluble proteins; we have followed exactly the method described by Moore and Lee. Approximately 30 per cent of a sample of the soluble protein of rat liver was found to pass through a DEAE-cellulose column equilibrated with 0.005 M tris-phosphate buffer at pH 8.0. This nonretained material was dialyzed overnight against distilled water and then lyophilized. The dried material was dissolved in water to a concentration of 5 per cent and examined by starch gel electrophoresis at pH 8.2 as described above.

Homogenization and fractionation of liver.—For certain of the experiments, samples of liver or tumor were homogenized in 0.25 M sucrose with a Potter-Elvehjem-type all-glass tissue grinder ("Duall" grinder, Kontes Glass Co.). Fractionation of the homogenate was carried out by the procedure described by Hogeboom (8). The fractions were dialyzed against 0.1 per cent sodium chloride solution at 4°C. The samples were then lyophilized, and an aliquot was weighed and its protein concentration determined with the biuret reagent. The remainder of the sample was then dissolved in distilled water to a concentration of 5 per cent and used for starch gel electrophoresis.

RESULTS

HISTOLOGY

The tumors produced had a variable histological appearance, most commonly adenocarcinomatous, but ranging from relatively anaplastic solid tumors to moderately organized structures resembling liver cell cords. This variability was found both between tumors and between areas of the same tumor. The tumors appeared clearly to be epithelial, but it was impossible to make a definitive distinction with respect to parenchymal versus biliary origin.

PROTEIN LEAKAGE INTO MEDIA

Comparative measurements of protein leakage into the media with and without EDTA were made in eleven experiments with normal liver slices and in seven experiments with slices of hepatoma (Table 1). Protein leakage from the normal liver slices was greater in the presence of EDTA in nine of the eleven experiments. The mean difference was highly significant as determined by the "t" test for pair comparisons (P < 0.01). In contrast,

<table>
<thead>
<tr>
<th>LEAKAGE INTO KREBS-RINGER PHOSPHATE*</th>
<th>LEAKAGE INTO KREBS-RINGER-PHOSPHATE +0.5% EDTA</th>
<th>(2) - (1)</th>
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<tbody>
<tr>
<td>Normal liver</td>
<td></td>
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<tr>
<td>Mean 18.8</td>
<td>23.6</td>
<td>4.7†</td>
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<tr>
<td>S.D. ±5.1</td>
<td>±5.3</td>
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DAB-induced hepatoma

| Mean 16.1                         | 17.3                                          | 0.6†    |
| S.D. ±6.6                         | ±7.7                                          |         |

* Results expressed as per cent of total protein in tissue sample which passed into the medium during 90-min. incubation. Each value shown is the mean value for at least four tissue samples per experiment.
† Mean difference between pairs is significant (P < 0.01) by "t" test for paired data.
‡ Mean difference between pairs is not significant (P > 0.80) by "t" test for paired data.
the protein loss from the tumor slices was smaller in the presence of EDTA in three of the seven experiments, and the mean value for all seven experiments showed no significant difference between the leakage in the presence and absence of EDTA (P > 0.80).

**Electrophoresis**

**Soluble proteins leaking from rat liver slices.**—The proteins leaking from liver slices incubated in KRP and KRP-EDTA for 90 minutes (eleven experiments) were examined by starch gel electrophoresis. Some sixteen soluble proteins were regularly detected by staining with Amido Black B (Fig. 1). Five of these were basic proteins—i.e., they migrated toward the cathode at pH 8.2. With longer periods of electrophoresis at least two other basic components could be resolved, but in the standard procedure the electrophoretic separation shown in Figure 1 was constant. The proteins migrating toward the anode at this pH will be referred to as acidic proteins. The proteins were not characterized individually—e.g., by the use of specific antisera or other methods. The electrophoretic resolution of normal rat serum is also shown in Figure 1. For purposes of nomenclature, the liver protein migrating with the mobility of rat serum albumin was assumed to be albumin. There were three components of rat liver which migrated toward the anode more rapidly than this protein; they are designated pre-albumins.

One consistent difference was observed between the electrophoretic patterns of media with and without EDTA in all experiments performed. The amount of basic protein 4 (the fourth from the origin) was consistently greater after incubation of liver slices in the medium with EDTA. No other consistent quantitative or qualitative electrophoretic difference between the two incubation media was observed. This difference is shown in Figs. 1 and 2.

**Soluble proteins of whole homogenate.**—Electrophoretic examination of homogenates of three normal livers revealed a pattern similar to that of the soluble proteins leaking from liver slices incubated in KRP-EDTA (Fig. 4, slot 1).

**Soluble proteins of subcellular fractions.**—To detect the site of origin of basic protein 4, whole liver was fractionated, and the resulting fractions were incubated in KRP-EDTA. The soluble proteins leaking out of the various fractions were examined by starch gel electrophoresis (Fig. 1). Relatively little protein from the nuclear and mitochondrial fractions was seen to migrate electrophoretically. Since all samples were adjusted to 5 per cent protein, this suggests that much of the protein in these fractions remained or precipitated at the origin of the gel. Both microsomal and supernatant fractions revealed a pattern of basic proteins comparable to that of whole liver homogenates. Basic protein 4 was particularly prominent in the microsomal fraction.

**Soluble proteins of tumor liver.**—

Soluble proteins leaking from tumor slices: Investigation of proteins leaking from slices of seven tumors incubated in KRP and KRP-EDTA revealed that the basic proteins were always diminished in amount and in some cases absent. A typical electrophoretogram from these experiments is shown in Figure 3. The acidic proteins showed no consistent change, although the relative amount of protein with the same migration as serum albumin was usually increased as compared with that of normal slices. There were no consistent differences between the electrophoretic patterns of proteins leaking from tumor slices incubated in KRP and from those in KRP-EDTA. Media in which tumor slices had been incubated contained little or no basic protein 4. No difference in the intensity of this band was found to result from incubation in KRP as compared with KRP-EDTA.

Soluble proteins of tumor homogenates: Electrophoretic examination of two tumor homogenates revealed that all the basic proteins were markedly diminished in concentration (Fig. 4, slot 6). All of them were affected, although zones 3 to 5 were particularly diminished and almost absent. The proteins migrating toward the anode were not altered, although the amount of protein with the mobility of serum albumin was relatively greater than in normal liver homogenates. Thus, the electrophoretic pattern of whole tumor homogenates was similar to that of the concentrated incubation medium of tumor slices incubated in KRP and KRP-EDTA. In Figure 4 is also shown the electrophoretic pattern of a homogenate from a macroscopically normal area of the same tumor liver. The faster migrating basic proteins are absent; the pattern of basic proteins appears intermediate between that of normal and tumor homogenates.

**Esterases.**—

Normal rat liver: At least eight esterases hydrolyzing β-naphthyl acetate were detected in rat liver and three in rat serum. All migrate toward the anode (Fig. 5). All the esterases detectable in whole rat liver homogenates could be detected in the concentrated medium after incubation of liver slices with KRP or KRP-EDTA. Subcellular fractionation revealed that the microsomal fraction had the greatest concentration of esterases. Nuclei and mitochondria were relatively poor in esterase content. The supernatant fraction contained at least six esterases. One esterase migrating faster toward the anode than the others was detectable only in the microsomal fraction. The esterase patterns of the concentrated media from slices incubated in KRP and KRP-EDTA contained features of both the microsomal and the supernatant fractions (Fig. 5).

Tumors: The pattern of esterases leaking from tumor slices incubated in KRP and KRP-EDTA was identical. In five of seven tumors there was a general diminution of all esterases, with no band being specifically affected. In two of seven tumors a different pattern of esterase was obtained (Fig. 6). Examination of the esterase pattern of two tumor homogenates revealed a marked diminution of esterase content.

**Other enzymes and proteins used in an attempt to identify basic protein 4.**—

Lactic dehydrogenase: Some four distinct lactic dehydrogenases were detected in normal rat liver homogenates. Three of them migrated toward the cathode (Chart 1). Lactic dehydrogenase was studied in one tumor homogenate; the band migrating fastest toward the catho-
ode was absent, and the amounts of the other lactic dehydrogenases were diminished. The migration of the lactic dehydrogenases did not correspond to that of basic protein 4.

Acid phosphatase: Acid phosphatase activity was detected in three zones migrating toward the anode (Chart 1); the background staining was relatively high, and the three zones tended to merge into one another. No acid phosphatase migrating to the cathode was detected.

β-glucuronidase: This enzyme was detected as two sharp zones migrating toward the anode (Chart 1); no basic β-glucuronidase was detected.

Benzidine stain: No immediate peroxidatic activity was detected on starch gels after electrophoretic resolution of the proteins of a homogenate of normal rat liver. Under the experimental conditions used, horse cytochrome c migrated toward the cathode and gave a rapid positive reaction.

Histones: The soluble proteins leaking from rat liver slices were compared with the components of a histone fraction from normal rat liver with urea-formate gels. Fifteen distinct bands were observed in urea gels of soluble proteins leaking from normal liver slices incubated in KRP and KRP-EDTA (Fig. 7). No proteins migrating toward the anode were observed (pH 4.0). The faster migrating bands were diminished or absent in the concentrated incubation medium of tumor slices (Fig. 7); these are presumably the basic components observed to be absent in ordinary starch gels.

The histone pattern of normal rat liver showed at least twelve components, almost all of which had mobilities different from those of soluble "leakage" proteins resolvable in urea-formate gels (Fig. 7). Little basic protein was resolved in the incubation medium of the nuclear fraction of a liver homogenate (Fig. 1); no soluble protein with the electrophoretic mobility of basic protein 4 was resolved from the nuclear fraction itself. Presumably histones would be expected to have appeared in this fraction in the greatest concentration, although at pH 8.2 histones would probably be aggregated in nonurea starch gels (22).

Thus, the above methods have failed to identify the possible nature of basic protein 4.

**Electrophoretic Analysis of Basic Proteins Isolated by Chromatography on DEAE-Cellulose**

The rat liver soluble proteins passing through a DEAE-cellulose column were examined by starch gel electrophoresis at pH 8.2. Examination of the stained gel (Fig. 8) revealed that only the basic proteins, migrating toward the cathode, were present. The acidic proteins had evidently been retained on the column. Thus, a clear separation of acidic from basic proteins can be achieved by the method of Moore and Lee (21).

**DISCUSSION**

When slices of rat liver are incubated in vitro, intracellular proteins leak into the medium not only from damaged cells at the surface but from intact cells within the slices (13). The rate of this leakage varies with the physiological state of the animal (13) and is increased by the use of an incubation medium devoid of calcium (13, 18) or by the addition of EDTA to the medium, as in the present work. This effect of EDTA presumably results from its chelation of calcium, since removal of magnesium was not found to affect the leakage of protein (13). It is not clear how the removal of calcium might affect protein leakage, but it evidently involves a general increase in membrane permeability to proteins, since all the soluble proteins and esterases demonstrable electrophoretically in whole liver homogenates were found to leak out in increased amount in the presence of EDTA in the present study.

In addition to this general effect on protein leakage, EDTA also produced a specific change in the electrophoretic pattern of proteins leaking from normal liver slices. This was an increase in the proportion of a fraction designated above as basic protein 4. Although this protein is not yet identified in terms of various known enzymatic activities or cellular components, it appears to be derived from either the soluble cytoplasm or the microsomal fraction, or both. There are several conceivable ways in which EDTA might produce the specific increase of this protein in the medium:

1. EDTA might inhibit a specific Ca++- or Mg++-dependent proteolysis;
2. EDTA might cause a greater increase in membrane permeability to this protein than to other intracellular proteins;
3. Basic protein 4 might be a constituent of the normal cell membrane, linked to Ca++ or some other divalent cation and detached by EDTA;
4. EDTA might hasten the solubilization of this protein from an aggregated form in which it is held intracellularly by a divalent cation.

Of these various possibilities, the last seems the most probable, because the removal of Mg++ has been shown to solubilize certain ribosomal proteins (7), and basic
of more immediate interest from tumor slices. However, in view of the marked histological variability of the tumors, the small number of samples, and the variation of results in individual experiments, no firm conclusion can be drawn. It would be desirable to repeat this work with a minimum-deviation tumor of more uniform character; such studies are now in progress with the Morris 5123 hepatoma.

It is hardly surprising that EDTA failed to increase the amount of basic protein 4 in the medium of the tumor slices, since the tumor homogenates contained very little or none of the basic proteins found in normal liver. Subcellular fractionation revealed the highest concentration of basic proteins in association with the microsomal fraction; this suggests a possible microsomal origin of these proteins, and the diminution of basic proteins in the DAB-induced hepatoma may correlate with the diminution of ribosomes in the 3'-methyl-DAB-induced tumor as observed by electron microscopy (24). Sorof and Cohen (27) and Sorof et al. (29) demonstrated by electrophoretic studies that primary liver tumors showed a marked reduction in a slow-moving “h” fraction, and components of this fraction have also been shown to bind certain azo dyes derivatives (28). The “h” fraction may be identical with the group of basic liver proteins resolved by starch gel electrophoresis in the present work. Whitcutt and Elson (31) have recently observed diminutions of basic proteins including aldolase, lactic dehydrogenase, and phosphoglucose isomerase in 4-dimethylamino-3'-methyl-azobenzene hepatomas. Moore and Lee (21) resolved the soluble proteins in a rat liver homogenate by chromatography on DEAE-cellulose and found nine different enzymatic activities (including the above) in the fraction which was not retained on the column at pH 8.0. Using their conditions we have found that this nonretained fraction corresponds to the bands migrating toward the cathode in starch gels at pH 8.2. This finding justifies the use of the term “basic proteins” to describe the latter bands, despite the possible effect of electroendosmosis.

Normal liver and DAB-induced hepatomas also differed with respect to their content of esterases. Liver was found to contain at least eight electrophoretically distinct proteins hydrolyzing β-naphthyl acetate, as reported previously by Paul and Fottrell (23). The microsomal fraction contained the greatest concentration of esterases, as found previously by Underhay et al. (30). However, this fraction would also contain lysosomes, which Burstone has shown to be rich in esterase activity (1). Five of the tumors examined in the present investigation showed a decrease in the amounts of esterases leaking into the incubation medium, whereas two other tumors showed an altered electrophoretic pattern of esterases. Electrophoretic examination of whole homogenates revealed that the amounts of esterases were much diminished. These findings are compatible with earlier reports of decreased esterase activity in hepatomas in mice (6) and in rats (10).

The present work corroborates the finding that normal rat liver and DAB-induced hepatomas differ with respect to the pattern of soluble proteins (27, 29, 31, 32) which they contain. It also shows a difference in the esterase patterns of liver and hepatomas and a possible difference with respect to the effect of EDTA on the permeability of their plasma membranes. It is by no means clear, however, whether these differences reflect changes inherent in the malignant state, or merely different cyto logical origins of the normal liver parenchymal cell and the DAB-induced hepatoma cell. The latter could possibly be derived from biliary epithelial rather than from hepatic parenchymal cells. It is hoped that further light on this problem will be provided by a similar study on “minimal-deviation” tumors.

REFERENCES


24. Porter, K. R., and Brun, C. An Electron Microscope Study

**Fig. 1.**—Starch gel electrophoretogram, 0.021 M borate, pH 8.2, stained with Amido Black.

Slots 1 and 3: Soluble proteins leaking from rat liver slice incubated in KRP-EDTA.

Slot 2: Soluble proteins leaking from rat liver slice incubated in KRP.

Slot 4: Rat liver, supernatant fraction of homogenate.

Slot 5: Rat liver microsomal fraction.

Slot 6: Rat liver mitochondrial fraction.

Slot 7: Rat liver nuclear fraction.

Slot 8: Rat serum.

The white dot indicates the position of rat serum albumin, OR, refers to the origin, and B.P. 4 to the position of basic protein 4.

All protein concentrations were adjusted to 5 gm. per cent except serum.

**Fig. 2.**—Starch gel electrophoretogram, 0.021 M borate, pH 8.2, stained with Amido Black. The basic proteins of rat liver slices leaking into KRP and KRP-EDTA are shown. The acidic proteins are not shown, as no significant difference between them was noted.

Slots 1, 3, and 5: Basic proteins leaking into KRP-EDTA, in 3 different experiments.

Slots 2, 4 and 6: Basic proteins leaking into KRP in three companion experiments.

Slot 7: Rat serum γ-globulin.

The total protein concentration of the liver samples was adjusted to 5 gm. per cent.

**Fig. 3.**—Starch gel electrophoretogram, 0.021 M borate, pH 8.2, stained with Amido Black. Electrophoretic comparison between soluble proteins leaking out of normal liver and hepatoma slices is shown.

Slot 1: Soluble proteins leaking from liver slice incubated in KRP.

Slot 2: Soluble proteins leaking from liver slice incubated in KRP-EDTA.

Slot 3: Soluble proteins leaking from tumor slice incubated in KRP-EDTA.

Slot 4: Soluble proteins leaking from rat liver slice incubated in KRP-EDTA.

Slot 5: Soluble proteins leaking from tumor slice incubated in KRP.

Slot 6: Soluble proteins leaking from tumor slice incubated in KRP-EDTA.

Slot 7: Rat serum. The white dot indicates the position of rat serum albumin.

**Fig. 4.**—Starch gel electrophoretogram, 0.021 M borate, pH 8.2, stained with Amido Black. Electrophoretic comparison between homogenate and supernatant fraction of normal and tumor livers is shown.

Slot 1: Homogenate of normal liver.

Slot 2: Supernatant fraction of homogenate of normal liver.

Slot 3: Supernatant fraction of macroscopically normal area of a tumor liver.

Slot 4: Homogenate of macroscopically normal area of a tumor liver.

Slot 5: Supernatant fraction of rat liver tumor.

Slot 6: Homogenate of rat liver tumor.

All protein concentrations were adjusted to 5 gm. per cent.

The white dot indicates the position to which rat serum albumin migrated.
Fig. 5.—Starch gel electrophoretogram, 0.021 M borate, pH 8.2, stained to detect esterases. This gel is the other half of the gel shown in Fig. 1.

Slots 1 and 3: Soluble esterases leaking from rat liver slices incubated in KRP-EDTA.
Slot 2: Soluble esterases leaking from rat liver slice incubated in KRP.
Slot 4: Rat liver, supernatant fraction of homogenate.
Slot 5: Rat liver microsomal fraction.
Slot 6: Rat liver mitochondrial fraction.
Slot 7: Rat liver nuclear fraction.
Slot 8: Rat serum.

All protein concentrations were adjusted to 5 gm. per cent except serum. The white dot indicates the position of rat serum albumin.

Fig. 6.—Starch gel electrophoretogram, 0.021 M borate, pH 8.2, stained to detect esterases.

Slot 1: Soluble esterases leaking from normal liver slice incubated in KRP.
Slot 2: Soluble esterases leaking from tumor slice incubated in KRP.
Slot 3: Soluble esterases leaking from tumor slice incubated in KRP-EDTA.

The white dot indicates the position to which rat serum albumin migrated.

Fig. 7.—Urea-formate gel, pH 4.0, of various preparations from normal rat livers and from hepatomas.

Slot 1: Soluble proteins of tumor slice leaking into KRP.
Slot 2: Soluble proteins of normal liver slice leaking into KRP.
Slot 3: Soluble proteins of normal liver slice leaking into KRP-EDTA.
Slot 4: Whole rat liver histone fraction.

The total protein concentration of the sample in Slot 1 was 3 per cent, and that of the samples in Slots 2 and 3 was 5 per cent. The protein concentration of the histone fraction was not measured.

Fig. 8.—Starch gel electrophoretogram, 0.021 M borate, pH 8.2, stained with Amido Black. The electrophoretic pattern of the basic proteins of rat liver supernatant fraction, purified by passage through a DEAE-cellulose column at pH 8.0 (for details see text) is shown.

Slot 1: Rat serum.
Slot 2: Supernatant fraction of homogenate of rat liver.
Slot 3: Proteins that passed through the DEAE-cellulose column at pH 8.0.

The total protein concentration of the samples in slots 2 and 3 was adjusted to 5 per cent.
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