ARE THE PROTEINS IN MALIGNANT CANCER CELLS OF DIVERSE ORIGIN SIMILAR OR DIFFERENT?

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The opinion has been frequently expressed that as cancers become increasingly malignant, they deviate more and more from those of the tissues of their origins and approach a common type, perhaps resembling embryonic tissues (Greenstein, 1947). From comparative studies of cancer cells of different etiology various generalizations have also been made. Thus all cancer cells resemble one another morphologically (Graham, 1972; Knox, 1967). They have similar (and relatively high) lactate production rates (Warburg, 1930); enzyme activities (Greenstein, 1947, 1956); free amino-acid accumulation patterns (Roberts and Tishkoff, 1949; Roberts and Frankel, 1949); and NMR relaxation times of their water protons (Damadian, 1971; Damadian, et al., 1973; Ling and Tucker, 1980). All of these generalizations about cancer cells agree with the statement of Greenstein made many years ago: "No matter how or from which tissue tumors arise, they more closely resemble each other chemically than they do normal tissues or than normal tissues resemble each other" (Greenstein, 1954, p. 589; see also 1956).

Another type of findings that have bearings on the subject of carcinogenesis came more recently from the demonstration of cancer genes, called oncogenes (Weinberg, 1981; Cooper, 1982, Goldfarb, et al., 1982; Barbacid (See Reddy, et al., 1982)). Stimulated by the studies of tumorigenic retroviruses (see Bishop, 1982, 1983), oncogenes were later detected in a varieity of human cancer cells and from cells chemically transformed in vitro. In those cases studied most extensively, the oncogenes were found to differ from their counterparts called proto-oncogenes, believed to be present in all normal cells, by only a single base (see Reddy, et al., 1982). How an oncogene and its protein product produce cancer is still not yet understood, although there are suggestions that they interfere with cellular differentiation (Beug, et al., 1982; Bishop, 1982) and that although the transformation of protooncogene to oncogene represents a single point mutation, this mutation may represent one of a series of essential steps in a multistage phenomenon comprising carcinogenesis (Weinstein, 1981; Moolgarkar and Knudsen, 1981; Farber and Cameron, 1980).

Each cell in a multicellular organism like the mammals, contains the same genome. Clearly the different tissue cells represent the product of different stable patterns of selective transcription and translation from the same pool of genes available to that multicellular organism. Yet before differentiation takes place, there is another stable pattern of transcription and translation specific to the ovum state of the organism. Greenstein's hypothesis in fact suggests a convergence backward toward this original pattern of transcription and translation. Since the end products of all transcription and translation are the cell proteins, it seemed to us that a more direct testing of the Greenstein hypothesis would be to analyze and compare the total (major) proteins of more or less pure cancer cells of widely diverse tissue origins. If an anticipated similarity can be demonstrated, then all the five types of similarities

based on the expression of one or a few of the proteins could be readily understood.

The first part of our task was to assemble a large variety of cancer cells. Departing from the practice of studying solid tumors which are complex organs unsuited for the present purpose, we chose only (pure) ascites cancer cells. In the end we succeeded in collecting a total of 15 types of well-established mouse cancer cells.

The primary technique used was one dimensional SDS polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedure of Laemmli (1970). Two dimensional gel electrophoresis was considered less suitable because the clarity and ready comprehensibility of the one dimensional data are more important at this stage of the game than the additional information that a two dimensional PAGE offers.

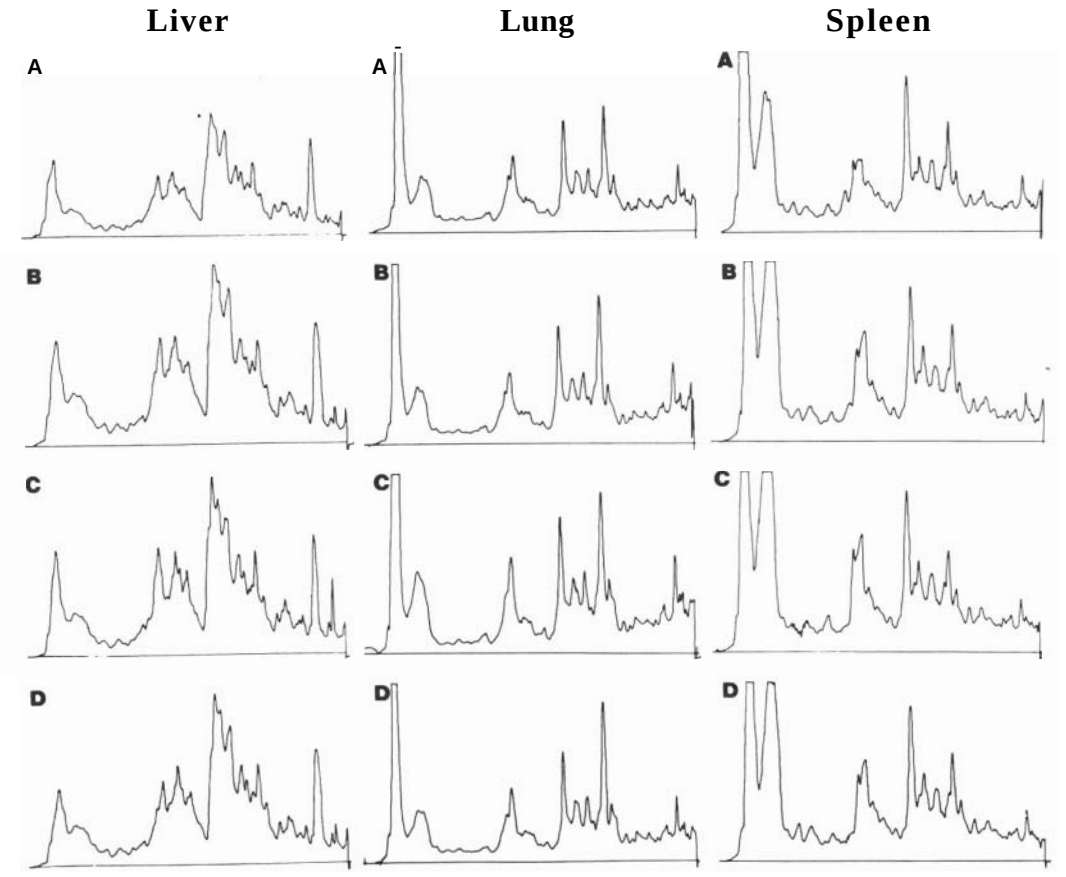


FIGURE I. ***Densitometer Tracings of SDS-PAGE Runs of Normal Liver, Lung and Spleen Tissues from Four Strains of Mice.*** 20-40 mg of normal tissues were frozen in liquid nitrogen and ground in cold stainless tube and grinder. 20 X tissue weight of 50% glycerin was added to the suspension homogenized. To 0.05 ml of the homogeneous suspension was added 0.2 ml of sample buffer containing 4.0 part 10% SDS, 2.5 part 0.5 M Tris-HCl at pH 6.8, 1.0 part 2 mercaptoethanol, 1.6 part 0.025% Bromphenol Blue, and 6.9 part water. The buffer-tissue mixture was heated in a boiling water bath for 2.5 min. 65 microliters of the cooled sample was placed in each well of the electrophoresis gel. It is absolutely important that for comparison, all samples and standards must be run on the same gel and their densitometer tracings taken at a single setting. The four strains of mice were A. Balb/c; B. $C_3D_2F_1/J$; C. C57BL/6J; D. DBA/2.

Figure 1 shows the densitometer tracing of SDS-PAGE of the proteins and polypeptides of three normal tissues (liver, lung, and spleen) from four different strains of mice (A: Balb/c; B: $C_3D_2F_1/J$; C: C57BL/6J; D: DBA/2). This figure provides an illustration of the variability among the protein composition of similar organs from the four different strains of host mice used to carry the cancer cells studied.

Figure 2 shows the densitometer tracing of the SDS acrylamide gel electrophoretic patterns of the proteins and polypeptides of the hearts and kidneys from representative animals belonging to each of the five vertebrate classes: fish (A); amphibian (B); reptile (C); bird (D); and mammal (E). In each case, the numbered stubs at the bottom of the tracing are molecular weight markers (see legend). Note that despite the wide evolutionary divergence of the different organ donors, a more or less consistent and distinctive pattern of protein and polypeptide contents can be detected for each organ.

As mentioned above, our main purpose was to compare the molecular weights and abundances of the major proteins in cancer cells from widely different origins. A technical difficulty lay in the minor (but nevertheless, bothersome) variations of densitometer tracings run on different gels of the same cell extract, despite all efforts taken to insure the highest possible uniformity. To make quantitative comparison more meaningful, some other steps had to be taken. Toward this, we finally chose to elect Ehrlich mammary adenocarcinoma cells as a standard and to routinely sandwich each run of a cancer cell extract between standard extracts of Ehrlich ascites cancer cells run on the same gel and to superpose the densitometer tracing of the Ehrlich ascites cell extract run on the neighboring slot on the densitometer tracing of each different cancer cell. Figure 3 shows the densitometer tracing of the extracts of Ehrlich ascites cancer cells. Of the 49 recognizable bands, we focused attention at present only on the 16 most prominent bands. Their molecular weights in kilodaltons are shown.

Figure 4 presents similar densitometer tracings of the SDS-PAGE of the proteins and polypeptides of the 15 "pure" mouse cancer cells, in the form of washed ascites cells from which blood cells had been carefully removed by prior treatment with saponin and washing. The 15 sets of tracings were from the following cancer cells: A, lymphocytic leukemia, L5178Y; B, pleomorphic leukocyte sarcoma (DMBA induced), T241; C, reticulum cell sarcoma, P4132; D, lymphoma, LSA; E, mammary adenocarcinoma, Klein TA3; F, Kreb's carcinoma of the inguinal region; G, lymphosarcoma, LSTRA; H, fibrosarcoma, Meth A; I, Hauschka; J, hepatoma, 134; K, myelogenous leukemia, P1081; L, pleomorphic leukocyte sarcoma; M, lymphoid leukemia, L1210; N, lymphocytic leukemia, P388; 0, mast cell leukemia, P815; P, mammary adenocarcinoma, Ehrlich. ***It is important to point out that all these cancers studied are all highly malignant and autonomous. That is, they are what Potter called "maximally deviated"*** with transplantation times of about one week (Potter, 1961).

As mentioned above, on each tracing in Figure 4 is superposed the tracing of an Ehrlich ascites tumor extract run next to the individual tracing on the same gel. When the peak is lower in the Ehrlich ascites tracing, the difference is marked in black; when the peak of the Ehrlich tracing is higher the difference is left blank.

The fact that the tracing of all 15 cancer cells of such widely different origin can all superpose on one standard Ehrlich adenocarcinoma tracing shows that in essence they are all mutually superposable. This means that the major proteins and polypeptides have similar molecular weights. Indeed, as shown in Table 1, the ratios of standard error

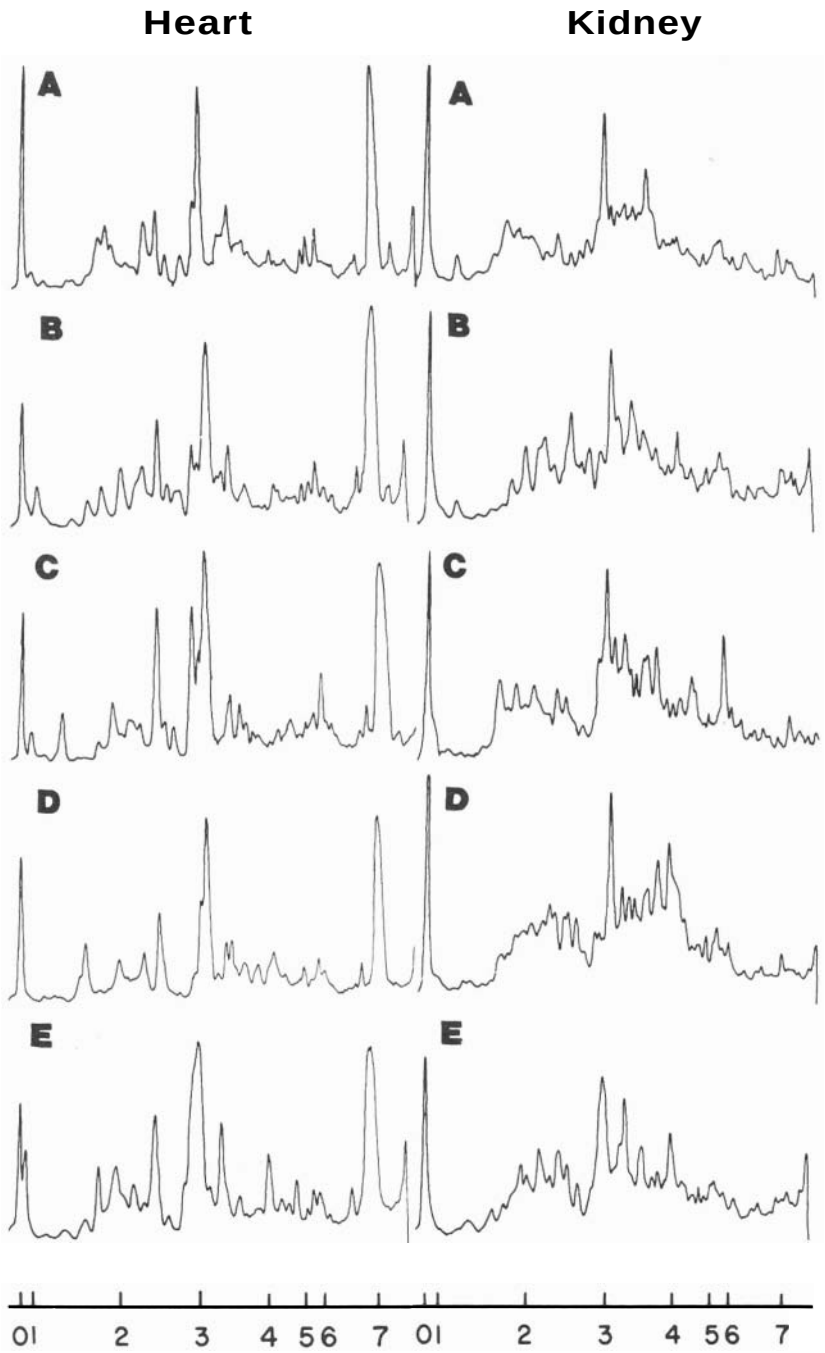


FIGURE 2. ***Densitometer Tracings of SDS-PAGE Runs of Normal Heart and Kidney Tissues from Representative Animals of Different Classes.*** A. Fish (gold fish, Carassius auratus); B. Amphibian (frog, Rana pipiens pipiens); C. Reptile (skink, Scincidae family); D. Bird (chicken, Gallus gallus); and E. Mammals (mouse, ICR). Methods same as described in Figure 1. Numbered stubs correspond to positions of standards from Sigma Chemical Co., St. Louis, MO: 0, lysozyme (MW 14,400 daltons); 1, soybean trypsin inhibitor, 21,500; 2, carbonic anhydrase, 31,000; 3, ovalbumin, 45,000; 4, bovine serum albumin, 66,200; 5, phosphorylase B, 92,500; 6, β-galactosidase, 116,250; and 7, myosin, 200,000.

over the means of the molecular weights of the 16 major peaks estimated is only 0.33% ± 0.042%.

In Figure 5A, the average abundance of each of the 16 major bands (as percentages of the total proteins) from the densitometer tracing of all 15 cancer cell types are arranged in order of decreasing abundance. Figure 5B is the average abundance of each of the bands of the Ehrlich ascites cancer cells in the 15 runs next to the 15 other cancer extracts. In both Figures 5A and B the heights of darkened area represent 2X standard error of the mean. Figures 5C to Q are abundance spectra of the different cancer cells. Although significant departures exist, by and large each spectrum resembles in general contour that of the average shown in Figure 5A.

The variations of the relative abundance of the 16 proteins or polypeptides is also given in Table 1. Here the (S.E./mean) ratios are 8.1% ± 0.79%, and thus much larger than those of the molecular weight distributions.

Figure 6 shows the relative abundance of different proteins and polypeptides with molecular weights equal to any one of those of the 16 major peaks of the cancer cells marked 18 to 199 kilodaltons present in each of the 17 types of normal mouse tissues: A, intestinal mucosa; B, gastric mucosa; C, ovary; D, thymus; E, brain; F, pancreas; G, liver; H, peritoneum; I, retina; J, lung; K,

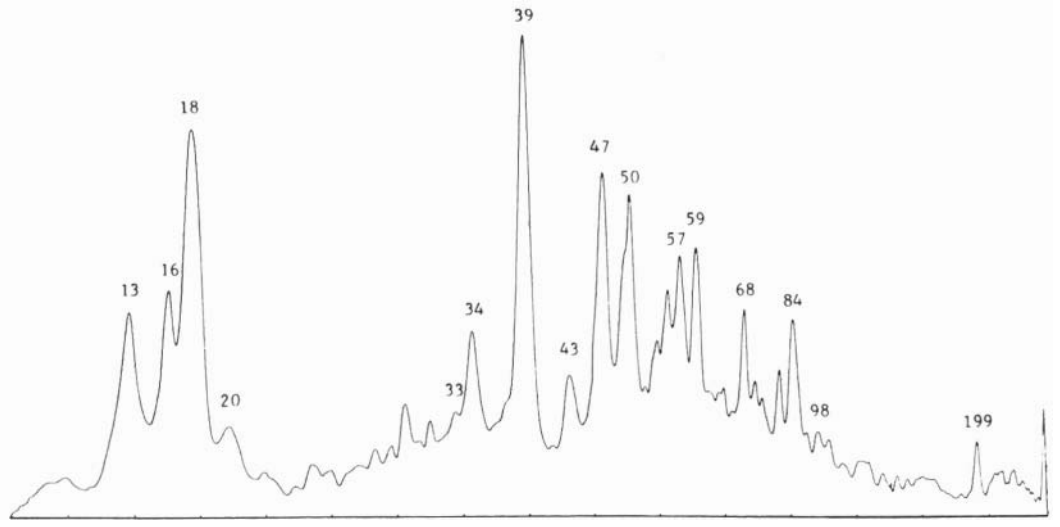


FIGURE 3. Densitometer tracing of SDS-PAGE separated protein bands of Ehrlich ascites cancer. Molecular weights (kilodaltons) of 16 easily recognizable protein band peaks are given as calculated relative to BioRad High and Low Molecular Weight Markers. (For details, see legend to Figure 2.)

	BioRad Molecular Weight Markers	
a	Lysozyme	14.4 kd
b	Soybean Trypsin Inhibitor	21.5
c	Carbonic Anhydrase	31.0
d	Ovalbumin	45 0
e	Bovine Serum Alumin	66.2
f	Phosphorylase B	92.5
g	β-Galactosidase	116.25
h	Myosin	200.0

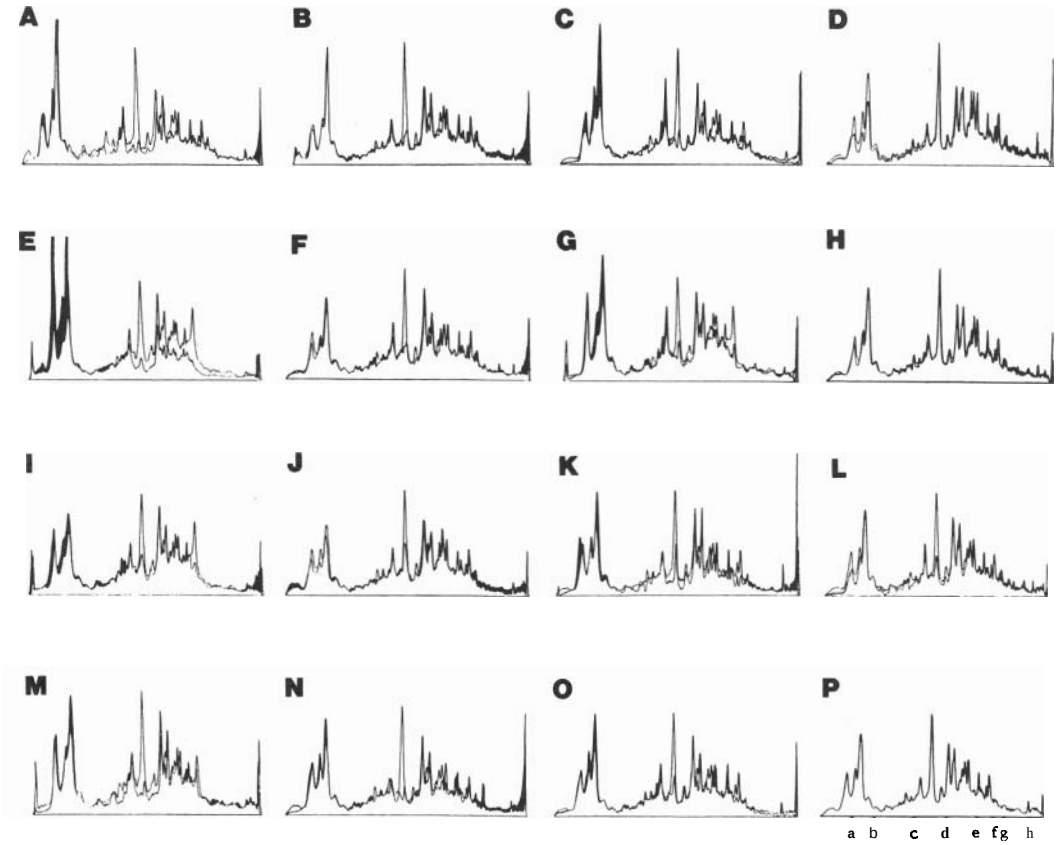


FIGURE 4. Densitometer tracings of the SDS-PAGE separated protein bands of 15 mouse ascites cancers. Tracings are superimposed upon tracings of Ehrlich ascites run adjacent to each cancer as an internal standard. Shaded areas represent the lesser abundance of Ehrlich proteins at those molecular weights, unshaded areas between the lines represent greater Ehrlich protein abundances. For molecular weights indicated by a-h see legend of Figure 3.

Graph	Cancer	Origin of Cancer
A	L5178Y	Lymphocytic Leukemia
B	T241	Plenomorphic Leukocyte Sarcoma (DMBA induced)
C	P4132	Reticulum Cell Sarcoma
D	LSA	Lymphoma
E	Klein TA3	Mammary Adenocarcinoma
F	Krebs	Carcinoma of the Inguinal Region
G	LSTRA	Lymphosarcoma
H	Meth A	Fibrosarcoma
I	Hauschka	(unknown)
J	Hepatoma 134	Hepatoma
K	P1081	Myelogenous Leukemia
L	Sarcoma 180	Pleomorphic Leukocyte Sarcoma
M	L1210	Lymphoid Leukemia
N	P388	Lymphocytic Leukemia
O	P815	Mast Cell Leukemia
P	Ehrlich	Mammary Adenocarcinoma

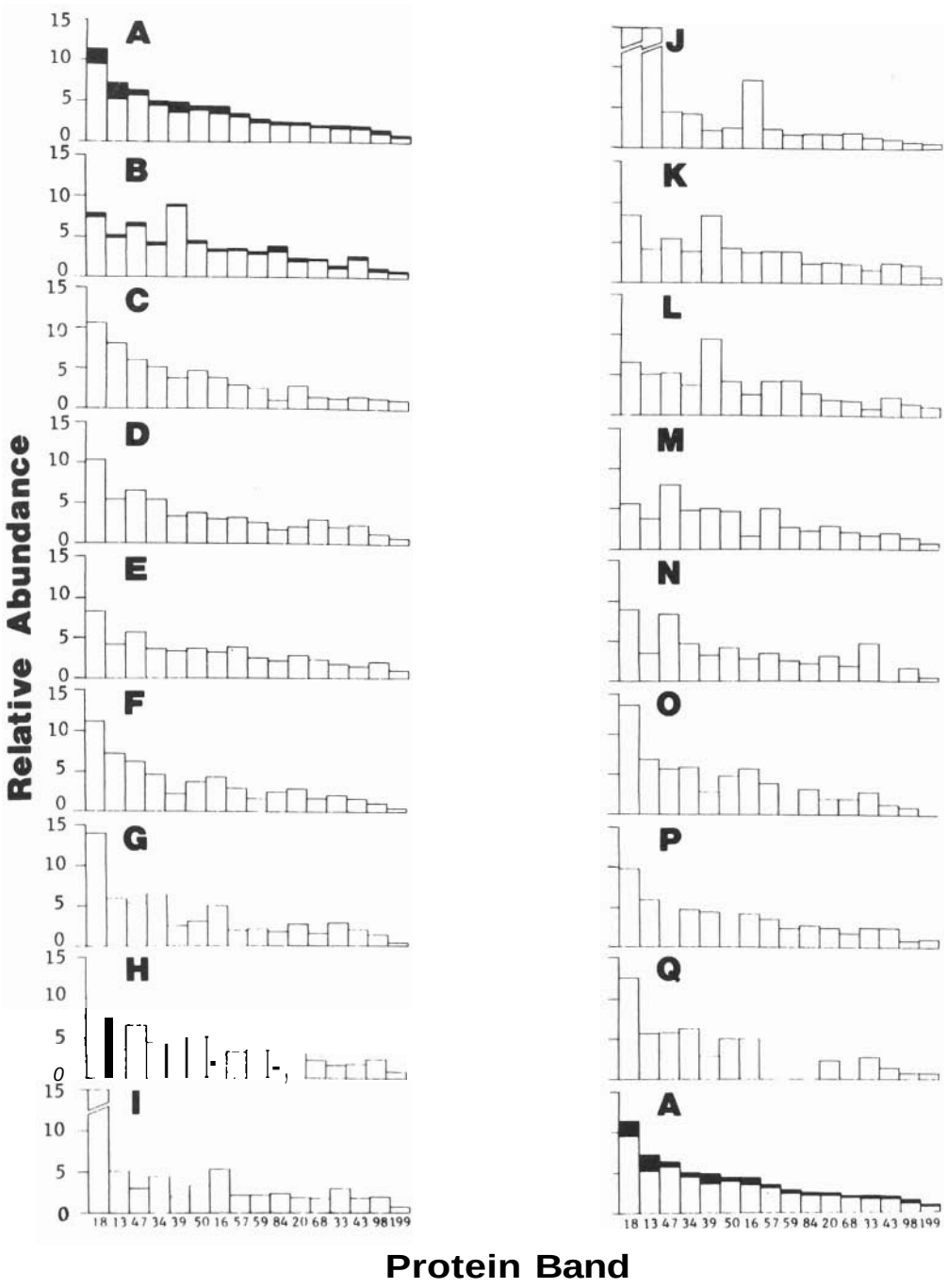
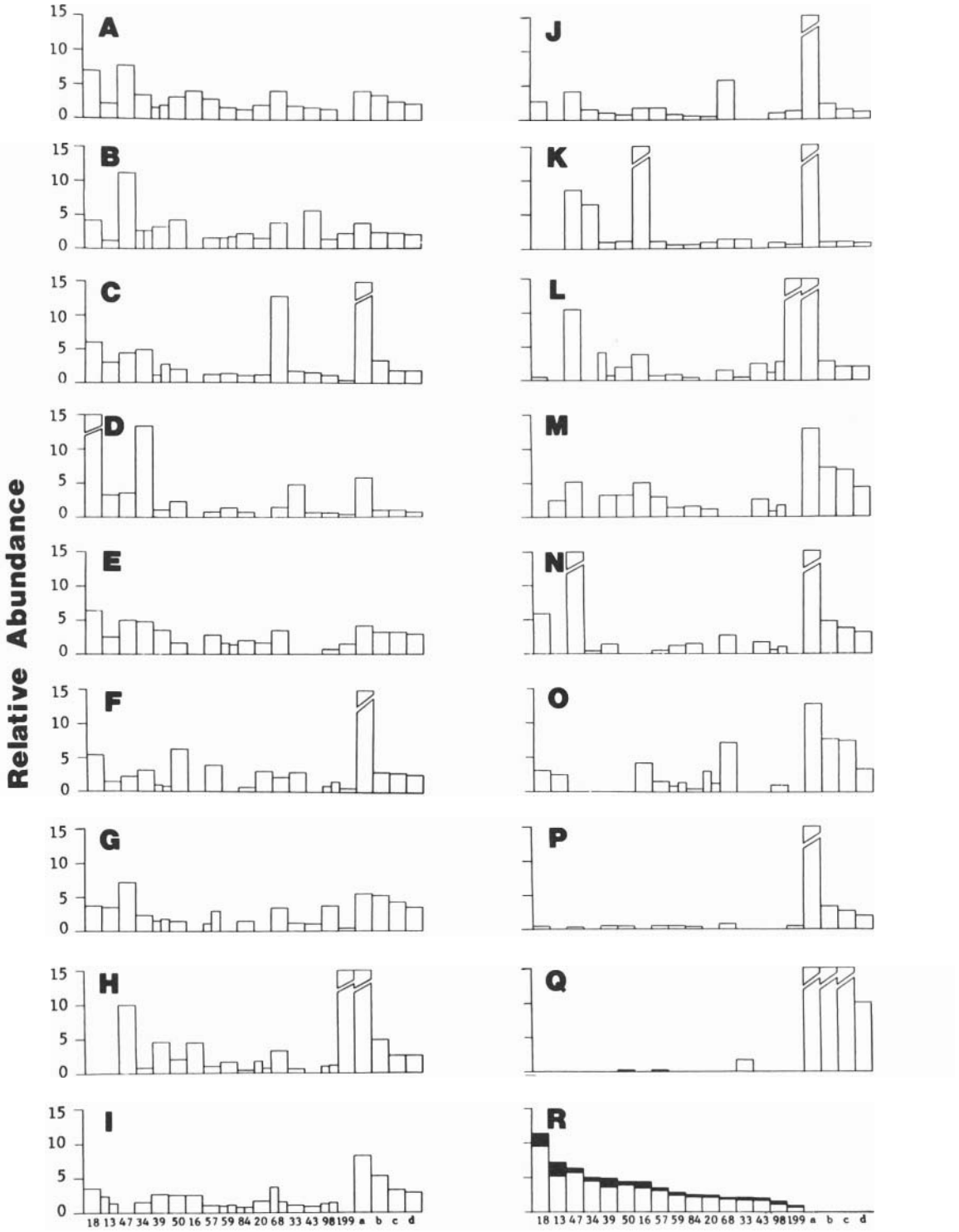


FIGURE 5. Bar graphs of the abundances of the SDS-PAGE separated protein bands of 15 mouse ascites cancers. Protein bands graphed had peaks with molecular weights inside two standard deviations of the mean values at the easily recognizable protein band peaks summarized in Table I, indicated in Figure 1, and shown here along the abscissa. Shaded blocks of Figures A and B indicate ± one standard error of the mean. Peaks with relative abundances greater than 15% are indicated by broken bar graphs and values are given below:

A. Summary of 16 mouse ascites cancers; *B.* Summary of Ehrlich cancer samples run adjacent to 15 other cancers; *C.* P1081; D. P815; *E.* T241; *F.* P388; G. P4132; *H.* Sarcoma 180; *I.* L5178Y (18 Kd = 16.6%); *J.* Klein TA3 (18 Kd = 16.2%, 13 Kd = 19.5%); K. Meth A; L. LSA; M. Hepatoma 134; N. Krebs; *O.* L1210; P. Hauschka; Q. LSTRA.


A
B
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L
M
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O
P
Q
R
15
10
5
0
Relative Abundance
18 13 47 34 39 50 16 57 59 84 20 68 33 43 98 199 a b c d
Protein Band

spleen; L, muscle; M, skin; N, heart; O, sciatic nerve; P, red blood cells; Q, lens.

In addition four other major peaks found in the normal tissues but not among those of the 16 peaks are listed under the heading a, b, c, d. the molecular weights of the extra proteins under a, b, c, or d for each tissue vary and are given in the legend of Figure 6. The spectra of abundance of each normal tissue is quite diverse and different from those of the cancer cells as shown in R which is a replica of spectrum A of Figure 5.

When viewed side by side with the convergence of both the molecular weights and abundance of the same tissues from animals of widely different genetic makeups the divergence of both the molecular weights and abundance of the 17 normal tissues offered sharp contrast to the data of Figure 4 which show even though 15 cancers came originally from different normal tissues, they have found high degree of similarity not with the normal tissues from which they developed but among other malignant cancers of totally different origins.

DISCUSSION

While recognizing the densitometer tracing is not always an accurate way to establish the identity of a specific protein or polypeptide and that each band may represent more than one protein or polypeptide, one can nevertheless state with assurance that the data presented here are in accord with the notion that all 15 mouse cancers of widely different etiology have essentially the same major proteins.

Two conclusions may thus be drawn tentatively from the present findings:

(1) when cancer cells have developed to their ultimate state of autonomy and malig-

FIGURE 6. Bar graphs of the abundances of the SDS-PAGE separated protein bands of 17 normal mouse tissues. Protein band peaks corresponding to easily recognizable cancer band peaks are graphed as in Figure 3 (when two corresponded, both were graphed in protein band slot). For each tissue, the four most abundant peaks not corresponding to highly visible cancer band peaks are also graphed and labeled a, b, c, and d along the abscissa. Molecular weights of these peaks are given below. (Due to rounding these molecular weights might appear to be the same as the summary values but not within two standard deviations*.) Peaks with relative abundances greater than 15% are indicated by broken bar graphs and values are given below:

	a	b	c	d
	Kilodalton			
A — Intestinal Mucosa	14 Kd	55 Kd	49 Kd	54 Kd
B — Gastric Mucosa	32	29	55	17
C — Ovaries	*18 (24.9%)	55	30	14
D — Thymus (18 Kd = 44.9%)	14	30	21	29
E — Brain	49	*16	32	>210
F — Pancreas	55 (18.9%)	46	49	32
G — Liver	54	12	166	48
H — Peritoneum (199 Kd = 22.9%)	45 (15.4%)	31	27	42
I — Retina	46	147	193	26
J — Lung	12 (58.7%)	55	32	30
K — Spleen (16 Kd = 29.6%)	12 (36.7%)	25	55	64
L — Muscle (199 Kd = 29.3)	45 (15.4%)	95	31	19
M — Skin	71	203	48	32
N — Heart (47 Kd = 15.7%)	12 (30.7%)	195	40	32
O — Sciatic nerve	185	31	46	38
P — Red blood cells	10 (77.2%)	<10	32	<10
Q — Lens	24 (35.2%)	30 (25.3%)	21 (15.2%)	26

TABLE I. Mean molecular weights (MW) and relative abundances (± standard errors) of the 16 easily recognizable protein band peaks (see Figure 1) from 16 different mouse ascites cancers. Abundances of each peak were relative to the total amount of protein band peaks (see Figure 1) from 16 different mouse ascites cancers. Abundances of each peak were relative to the total amount of protein in that cancer as calculated from integrated areas under densitometer tracings (see Figure 2).

Band	MW ± SE (kilodaltons)	Relative Abundance ± SE
13	13.09 ± .08	6.14 ± .95
16	15.90 ± .06	3.94 ± .45
18	17.56 ± .05	10.46 ± .83
20	19.77 ± .07	2.26 ± .16
33	33.18 ± .07	1.88 ± .17
34	34.46 ± .06	4.74 ± .22
39	39.04 ± .10	4.19 ± .62
43	43.06 ± .07	1.81 ± .12
47	46.79 ± .07	6.01 ± .35
50	50.44 ± .09	4.11 ± .19
57	57.04 ± .15	3.24 ± .21
59	59.32 ± .16	2.61 ± .19
68	68.30 ± .33	1.90 ± .10
84	83.69 ± .50	2.29 ± .15
98	97.50 ± .69	1.34 ± .12
199	199.06 ± .42	0.73 ± .06

nancy (i.e., "maximally deviated"), they become very similar;

(2) the carcinogensis process therefore involves a total or partial turning off of those regulatory and structural genes whose activities produce the spectra of proteins specific to each of the normal parent cell types and the turning on of a finite and common set of genes whose activities lead to production of all the proteins including those revealed by the 16 major bands.

The present findings confirm, extend, and round up the series of generalizations about the nature of cancer cells by Warburg, Greenstein, Roberts, Damadian, and others, if, as we here suggest that all maximally deviated cancer cells are themselves highly similar. Since all cells from the same living organs carry the same genome. that carcinogensis could lead eventually to a similar assembly of genes being activated and the suppression of all others is clearly feasible. The similarity of all cancers when "maximally deviated" (if future detailed study can confirm) clearly shows that the genes that determine the cancer cell proteins must be preexisting genes of the cell's normal genome and not new genes resulting from mutations. This view is, of course, in harmony with the repeated demonstration that cancer cells, when transplanted to the proper environments, can develop into normal healthy individuals (Braun, 1953; King and McKinnel, 1960; Mintz, 1978) facts which were in support of the maldifferentiation theory of carcinogenesis (Fischer and Wasels, 1927; Haldane, 1934; Needham, 1950; Pierce, 1967; Markert, 1968; Mintz, 1978).

On the other hand, paradoxically there is also compelling evidence showing that mutation plays a key step in carcinogenesis (Boveri, 1914; Lockhart-Mummery, 1934; Haddow, 1938; Burnet, 1957, 1974): First, almost all carcinogens are mutagens (McCann, et al., 1975); secondly, the activation of oncogenes represents a single point mutation. However, the paradox may be only apparent. Thus mutation may be an essential step leading to the eventual expression of the specific genes transcribed in all maximally deviated cancer cells.

That cancer may be related to the embryonic state of the living cell is a familiar conjecture. However, recent years have seen the actual demonstration of a number of proteins seen only in cancer cells and in embryonic tissues but not in adult tissues. Among these "carcinoembryonic" proteins are the a-fetoproteins (Abelev, 1968); carcinoembryonic antigen (Gold and Freedman, 1965); carcinofetal ferritin (Alpert, et al., 1973); etc. (for review, see Hirai, 1977). Thus it seems not entirely speculation that the maximally deviated cancer cells may in fact

represent the final stage in the reversion to the original embryo cells or even the original fertilized ovum. If this speculation has validity, then carcinogenesis and differentiation have the same destinies but are traversed in opposite directions.

In this light, the fact that protooncogenes are found in such a wide variety of eukaryotic living organs (Bishop, 1983) indicates that it had been carefuly conserved over vast genetic distances. If so, one would expect a key role of the protooncogene in the life history of eukaryotic organism. One would then like to ask the question, "Is it possible that the protooncogene in fact represents a master gene whose activity leads to the suppression of the transcription of the genes responsible for the unique set of embryo-cancer proteins and whose mutation releases this suppression?" (A brief abstract of this work was published earlier, Ling and Murphy, 1982).

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