PLASMA CELL NEOPLASIA IN A SINGLE HOST: A MOSAIC OF DIFFERENT PROTEIN-PRODUCING CELL TYPES

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PLATES 35 AND 36

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In 1959 Merwin and Algire reported that strain BALB/c mice developed plasma cell neoplasms after the intraperitoneal implanation of Millipore diffusion chambers (1). This observation was of great importance for it focused attention on an inbred strain of mouse which before that time had not been associated with plasma-cell neoplasms and further indicated a unique and complex means for inducing this rare type of new growth. Since the initial findings of Merwin and Algire were published, other methods have been found to induce plasma cell neoplasms in strain BALB/c mice (2, 3). Specifically, plasma cell neoplasms have been induced after single intraperitoneal injections (0.5 ml per injection) of mineral oil (Bayol F) or mineral oil adjuvants. Two or three injections, spaced weeks or months apart, increased the yield of tumors and in some groups between 40 and 50 per cent of mice developed plasma cell neoplasms (3). Mice which have been injected intraperitoneally with mineral oil, or mineral oil adjuvants develop a lipogranulomatous reactive tissue on the peritoneal surfaces and it is in this pathologic tissue that the plasma cell neoplasms appear to arise (2). Two striking common properties between the Millipore diffusion chamber and the mineral oil techniques are the occurrence in the specific inbred strain and the intraperitoneal introduction of a non-removable, non-digestible, and only mildly irritating substance.

The induction of plasma cell neoplasms is of particular experimental and theoretical interest because this neoplastic cell is a functional cell type which secretes protein (4, 5). A matter of great practical value is the transplantability of the plasma cell neoplasm for the transplant continues to secrete protein in the new normal host. While it has been found that there is marked variation among different plasma cell neoplasms arising in different hosts, or even in the same host as will be shown here, the individuality of a given neoplasm (as judged by the protein secreted) does not change during transplantation (5, 6). The various characteristics of the neoplastic plasma cell; the stable heritable protein secretion, the variation among different neoplasms, the origin in an inbred strain which permits transplantation comprises a special genetic system, in which heritable characters that develop in a common somatic cell type, can be propagated (by transplantation) and characterized. This experimental system is a means for studying how genes function in somatic cells.

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In a study of the plasma cell neoplasias of mice that develop after intraperitoneal injection of a mixture of incomplete Freund's adjuvants and heat-killed staphylococci, we reported a case in which 2 different protein secreting transplant lines were established from a single primary host (7). Usually the primary plasma cell neoplasias developing after adjuvant staphylococcus mixtures consist of multiple nodules on the peritoneal surfaces. We have been unable with histologic sections to conclusively determine if the nodules represent separate primary neoplasms or metastases or both. This question can be resolved for some early cases by establishing parallel lines in transplant from separate nodules and then characterizing the abnormalities of serum and urinary proteins associated with each transplant line. Success in isolating individual lines depends upon 2 factors: Transplantation of nodules before overgrowth by another rapidly growing cell population masks the fine detail; and a sensitive transplantation technique. The availability of many primary plasma cell neoplasias has given us the opportunity to select cases for special transplantation studies.

In previous work we have been impressed by the failure to establish in transplant certain extensive primary plasma cell neoplasms and further by the fact that some transplantable neoplasms failed to grow in all recipients (2, 6). These experiences have led us to use as much neoplastic tissue as could be loaded in a 2½ inch long 13 gauge trochar in starting new transplant lines and maintaining existing ones. In the present experiments it was necessary to use small amounts of tissue. Tiny nodules were placed subcutaneously by trochars or inserted under the kidney capsule by an operative procedure.

Materials and Methods

Mice.—All mice used in these experiments were strain BALB/c An, and were obtained from the Animal Breeding Section of the Laboratory Aids Division, National Institutes of Health. Conditions of maintenance have been previously described (2).

Transplantation Techniques.—Two techniques were used for transplantation: (*a*) Trochar technique. The amount of tissue selected for transplantation was usually the smallest discrete nodule that could be found. On occasion pieces from larger masses were transplanted.

(*b*) Subcapsular implantation in the kidney (8). This technique is very suitable for nodules of 1 mm in diameter or less, and is particularly advantageous when tiny amounts of tissue are to be transplanted. The technique involved: the retroperitoneal exposure of the kidney (nembutal anesthesia 80 mg/kg B.W. was used): a small incision in the renal capsule; and insertion of the tiny piece of tissue with the aid of a dissection microscope. No suturing of the kidney capsule was necessary.

Electrophoresis.—Electrophoresis of undiluted serum and urine was done in agar gels on 3¼ x 4 inch glass plates. The agar gel plates were made by pouring 18 to 20 ml of a 1 per cent solution of molten agar suspended on 0.05 M veronal buffer (of pH 8.2 $\Gamma/2 = 0.037$) on to glass plates that fitted snugly in the bottom of a plastic tray device (7). Each plate had 13 wells (3/32 inch diameter) which were spaced 1 1/64 inch apart (intercircumferential distance). Each run was of 2 hours' duration. A potential of 35 to 40 volts was maintained between the 2 wicks (attached to agar block) by adjusting a regulated power supply (constant voltage) during the runs. Usually no adjustment was required after an equilibration period of

10 to 15 minutes. On occasion when wick to wick voltage drops occurred, an adjustment was necessary.

After electrophoresis the plates were immersed in 2 per cent acetic acid for 2 to 16 hours, washed in distilled water (15 to 60 minutes) overlayed with filter paper and dried in an incubator (37°–48°C) overnight. The dried plates were stained with amido-Schwartz by the method of Courcon and Uriel (9). Photographs were made from stained plates.

Immunoelectrophoresis.—The conditions of the electrophoresis were the same as just described. A modified template was used which cut maximally 6 wells ($\frac{3}{32}$ inch diameter) and 7 trenches ($2\frac{5}{16}$ x $\frac{1}{16}$ inches) with an intertrench distance of $\frac{9}{16}$ inch. Photographs were made from unstained plates using 2 substage refracted light sources.

Antisera.—Three antisera were used in the present studies. Two were obtained from rabbits immunized with washed microsomes extracted from the plasma cell neoplasms MPC 2 and ADJ PC 5. These antisera were designated MPC 2 Pw, and ADJ PC 5 Pw respectively. The ADJ PC 5 Pw antiserum was the same as described previously (7). The MPC 2 Pw antiserum was prepared by the method previously described with washed microsomes, extracted from cells of a later transfer generation. This MPC 2 Pw antiserum was slightly different from previously described MPC 2 Pw antiserum in that the previous antiserum formed only a single SI line instead of the double SI, and also precipitated the protein in the anodal end of the plate more clearly (7).

Representative sera from all the transplant lines were subjected to electrophoresis and confronted with ADJ PC 5 Pw and MPC 2 Pw antisera.

A third antiserum was used for only the MO PC 4 studies. This antiserum R6 euglobulin was obtained from a rabbit immunized with the euglobulin of the plasma cell neoplasm ADJ PC 6*A*. As described previously the myeloma globulin of this plasma cell neoplasm precipitates in low ionic strength media (7) and resolubilizes when the ionic strength is brought to 0.15 *u*. Using alternate precipitations and washings a relatively pure precipitate was obtained from whole ADJ PC 6A serum. A rabbit was immunized with 8.9 mg. of the euglobulin emulsified in complete Freund's adjuvants (7). Two weeks after the first injection a similar amount was given again and finally, 5 weeks after the initial injection a subcutaneous booster of 0.89 mg. of euglobulin fraction was given. This antiserum precipitated the SI, SII proteins in normal serum and also a third arc. Details concerning this serum are to be published separately. The ADJ PC 6*A* euglobulin was prepared by Dr. E. L. Kuff of the National Cancer Institute.

RESULTS

Description of Cases and Sources of Neoplastic Tissue.—The mice with primary plasma cell neoplasms characteristically developed hemorrhagic ascites which contained neoplastic plasma cells (Table I). The primary cases were detected by the presence of characteristic neoplastic plasma cells in Wright's stained smears of the ascites. One mouse (ADJ PC 34) developed very little ascites but had abdominal masses (Table I). Smears from this mouse contained a few neoplastic plasma cells. The presence of neoplastic cells in ascites indicates that one nodule could liberate cells which then seed on other peritoneal surfaces. In the past we have found the ascites cells to be an unreliable and poor means for establishing a neoplasm in transplant (though it can be used to maintain certain transplant lines). The presence of neoplastic plasma cells in ascites did not obviate demonstrating differences in individual fixed tissue nodules as will be seen below.

TABLE I

Origin of Primary Plasma Cell Neoplasias and Transplant Lines

Designation of primary	Age when plasma cell neoplasm appeared	Volume and material injected*	Nodules transplanted No. growing/ No. implanted	Site implanted‡	Appearance of primary plasma cell neoplasia	
					Ascites§ vol.	Character of plasma cell neoplasia
	mos.	*ml/material*				
ADJ PC 22	20	0.3/IF + S	4/5	s.c.	8–10 ml/h	Many small nodules, along mesentery, confluent in pancreatic region
ADJ PC 24	8	0.3/IF + S	4/5	s.k.c.	5 ml/h	Nodules and large and small masses in mesentery and pancreatic region. One large nodule on diaphram
ADJ PC 25	14	0.5/IF + S	3/4	s.c.	4 ml/h	Numerous small masses in mesentery confluent in pancreatic region
ADJ PC 30	9	0.3/IF + S	5/5	s.c.	2.5 ml/h	Numerous nodules in mesentery and on diaphram and peritoneal wall
ADJ PC 34	23	0.3/IF + S	3/3	s.c.	<0.5 ml/c	Many nodules in stomach region, mesentery, clear, large pedunculated uterine mass
MO PC 4	10	0.4/m.o./ S	6/12	s.k.c.‖	5 ml/h	Numerous, extremely small nodules in the mesentery

* All mice received single i.p. injection when 1 to 2 months of age. IF + S = mixture incomplete Freund's adjuvants 1 part + staphylococcus culture concentrate 1 part (2); m.o. = mineral oil (Bayol F).

‡ s.c., subcutaneous; s.k.c., subkidney capsule.

§ h, hemorrhagic. c, clear, neoplastic plasma cells were readily found in Wright's stained smears in all cases.

‖ Mice were implanted with nodules subcutaneously; none, however, grew.

The accumulations of neoplastic tissue varied greatly in size (Table I). In describing the material reported here I shall refer to a nodule as an amount of tissue that does not exceed 2 mm in diameter and larger accumulations as masses. The nodules selected for transplant were those which were the smallest discretely defined areas. These usually develop in the mesentery, on the diaphragm, or on the peritoneal surface of the abdominal wall. Another frequent

site is the pancreatic region. In this area the nodules often become confluent or appear as masses of tissue.

The most defined and yet the smallest nodulation is found in mice, that have been injected with mineral oil rather than adjuvant mixtures. Transplantation should be done as soon as the diagnosis is established. The appearance of a typical case is shown in Fig. 1. This figure may be contrasted with one shown in a previous publication (2) which is typical of the plasma cell neoplasm that appears after injection of the adjuvant staphylococcus mixture.

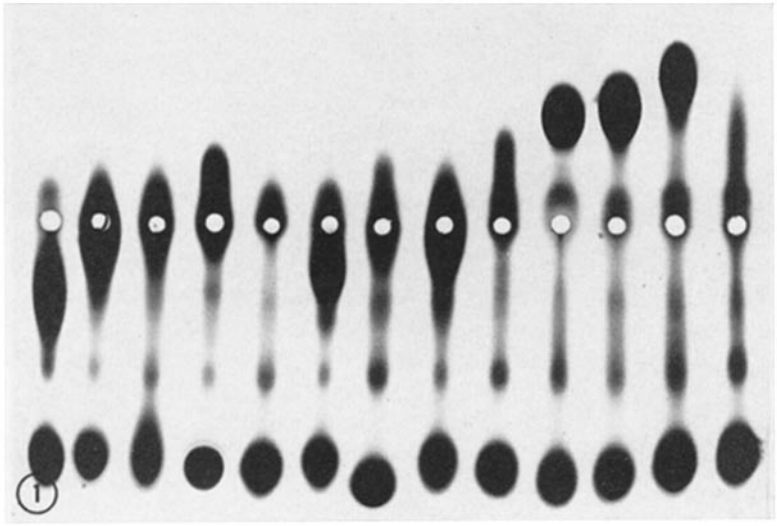
Histologic examination of the mesentery of the mouse with the primary plasma cell neoplasia MO PC 4 revealed accumulations of neoplastic plasma cells that were usually located near peritoneal surfaces (Figs. 2 to 4).

These accumulations may represent a primary plasma cell neoplasm or a seeding from another. Seeding may emanate from more than one primary source.

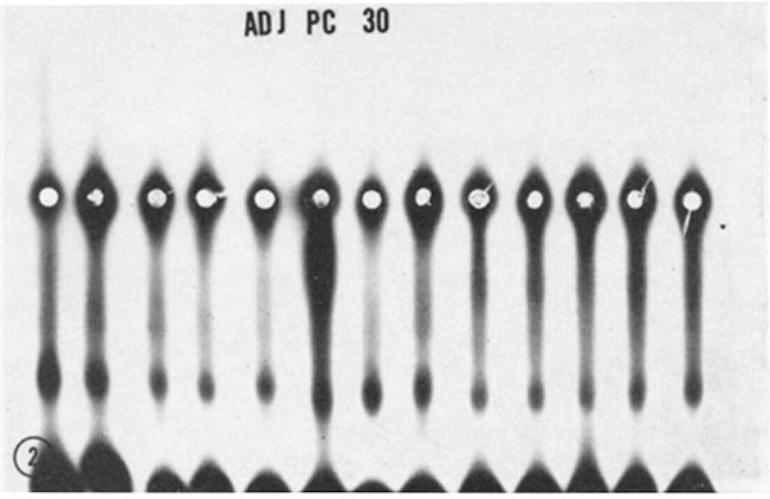
Transplantation of nodules under the kidney capsule resulted in growth in that region followed by metastases to the upper mesenteric lymph node. The mice then developed multiple peritoneal seedings with the formation of hemorrhagic ascites. An indication of the length of time for the various lines to produce large growths in the first transplantation generation is indicated in Table II where the time in days in the first transplant generation is given.

Characterization of Differences in Myeloma Proteins Associated with Transplant Lines.—The comparison of myeloma proteins associated with various transplant lines was made by agar gel electrophoresis and agar gel immunoelectrophoresis of whole serum. On a 3¼ x 4 inch plate overlayed with agar, 13 sera were subjected to electrophoresis when the plate was to be fixed and stained. On similar plates used for immunoelectrophoresis only 6 sera underwent electrophoresis. Uniformity and differences were established by many comparative runs. A summary of all the findings including a classification of the various lines is given in Table II. Thirty-one transplant lines from the 6 primary cases were examined. The transplant lines within the case were alike for ADJ PC 24 (5 lines) ADJ PC 25 (4 lines), and ADJ PC 30 (6 lines). Different myeloma protein types were found in each of the other 3 cases, 2 types among the 5 lines of ADJ PC 22, 2 types among the 4 lines of ADJ PC 34, and 5 types among the 7 lines of MO PC 4.

Electrophoretic Differences.—The myeloma proteins of mice have a wide range of electrophoretic mobilities in 0.05 M veronal buffer of pH 8.2. Examples of these differences are illustrated in Text-figure. 1. On this plate a hyperimmune serum was compared with 12 sera from mice bearing plasma cell neoplasms. Three cases (LPC-1, ADJ PC 5, and ADJ PC 23) not related to the present report were included for comparative purposes (Text-fig. 1). The masses of myeloma protein associated with these 3 illustrated examples were (*a*) very dense (*b*) round in shape like the albumin mass, (*c*) completely free



ADJ PC 30



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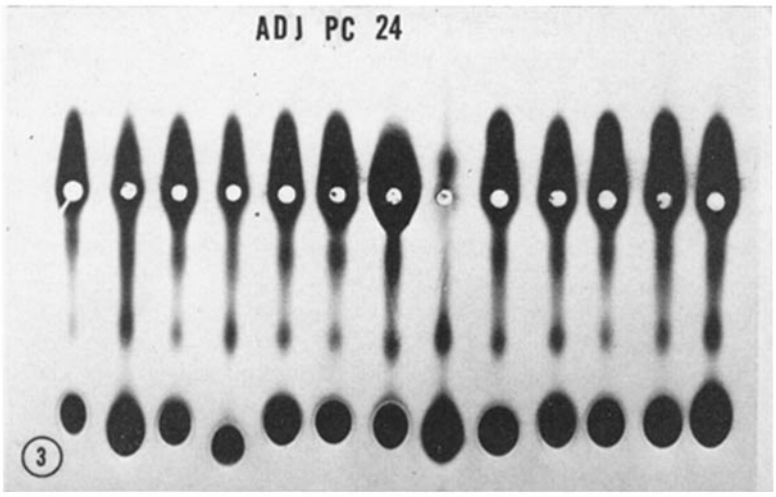


TABLE II

Characteristics of the Transplant Lines

Primary neoplasm	Designation of lines characterized from		No. protein types	Lines of similar type	Electrophoretic distribution	No. serum samples subjected to electrophoresis	No. transfer generations	Pathological urinary protein	Reaction with MPC 2 Pw
	Nodules*	Masses*							
ADJ PC 22	*A*(102) *C*(146) *D*(153) *E*(161)	*B*(130)	2	*A, C* *B, D, E*	*aoc* *al*	15 11	3–4 2–3	– –	++ ++
ADJ PC 24	*A*(63) *B*(98) *C*(113) *D*(114)	Pan(122)	1	—	*ocl*	13	2–3	–	++
ADJ PC 25	*A*(69) *B*(96) *C*(96) *D*(96)		1	—	*aoc*	23	2–3	–	++
ADJ PC 30	*N1*(83) *N2*(85) *N3*(85) *N4*(108)	*M1*(61) Pan(83)	1	—	*o*	17	2–3	–	++
ADJ PC 34	*N1*(36) *N2*(36) *N3*(36)	*UT*(36)	2	*N1, N2 UT* *N3*	*oal* *aoc*	17 6	3–6 4	– –	++ ++
MO PC 4	*A*(87) *C*(87) *D*(105) *B*(87) *F*(120) *G*(128)	*E*(119)	5	*A, E* *C, D* *B* *F* *G*	*ao* *aoc* *ocl* *ocl* *aoc, ao*	11 12 10 8 13	4–5 3–4 5 4 5	+ – – + +	+ ++ ++ + +

* Number in parentheses indicates number days duration in the first transplantation generation.

cathodally from the origin. (Immunochemically these 3 proteins have been shown to be SI myeloma proteins, 7).

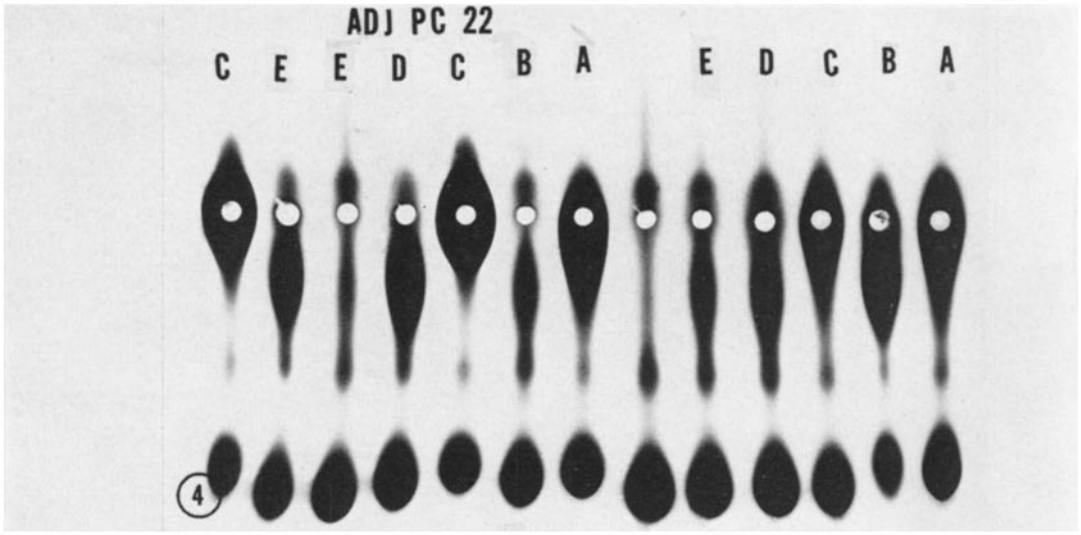
The other 9 examples shown in Text-fig. 1 were selected from the present series of cases. By comparison the myeloma protein masses were (*a*) less dense,

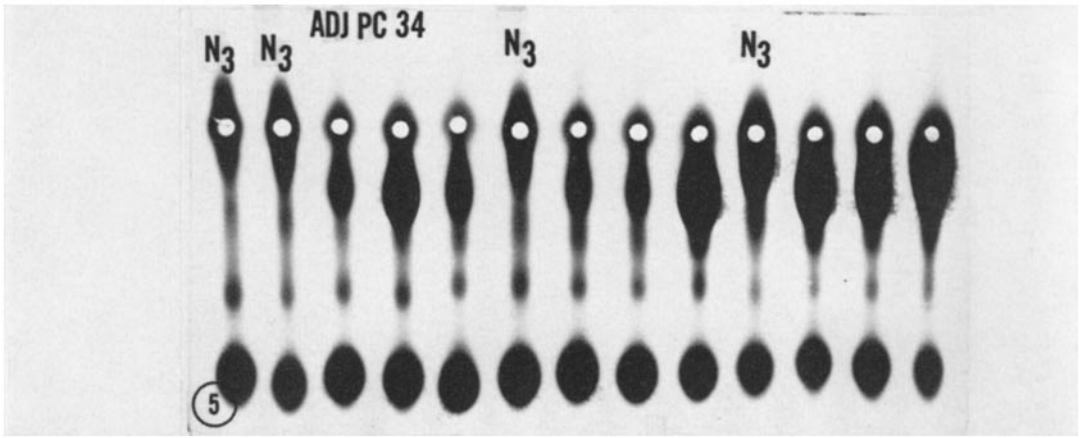
TEXT-FIGS. 1 to 11. Photographs of agar gel electrophoretic plates. In Text-figs. 1 to 7 the fixed, amido-Schwartz stained plate was photographed; only electrophoresis was done. Text-figs. 8 to 11, immunoelectrophoretic plates, the unfixed, unstained plates were photographed 18 to 24 hours after antiserum was placed in trenches. The round center wells are numbered from left to right. The cathodal end of the plate faces the top of the page. Numbers in parenthesis after serum samples indicate accession number of the sample. This is done only for ADJ PC 34 and MO PC 4 lines where occasionally the same sample may be observed on different plates.

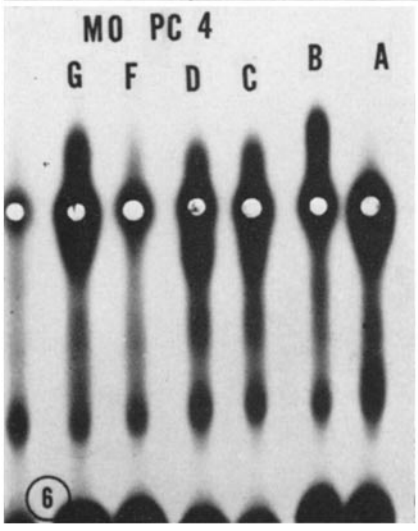
TEXT-FIG. 1. Electrophoresis: from left to right: ADJ PC 22*D*, 22*C*, 25, 24, 30, 34*N2* (1258), MO PC 4*C*(1149), ADJ PC 34*N3*(1157), MO PC 4*B*(1185), ADJ PC23, 5, LPC 1, and hyperimmune serum.

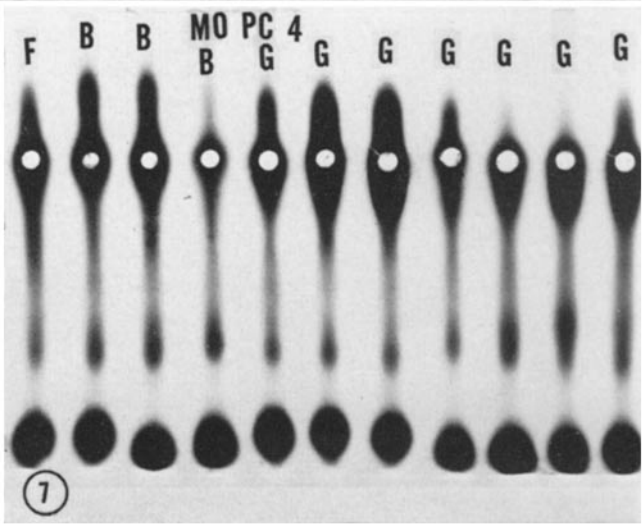
TEXT-FIG. 2. 12 sera from mice bearing the various ADJ PC 30 lines. From left to right: Normal serum, ADJ PC 30*N1*, 30*N2*, 30*N3*, 30*N4*, 30*M1*, 30Pan, 30Pan, 30*N1*, 30*N2*, 30*N4*, 30*M1*, 30Pan. The *M1* sample on the left was hemolyzed.

TEXT-FIG. 3. Electrophoretic plate: 12 different sera from mice bearing the various ADJ PC 24 lines. From left to right: ADJ PC 24*C* ascites serum, 24*A*, 24Pan, 24*B*, 24*B*, 24*B*, 24*D*, Normal, ADJ PC 24*C*, 24*B*, 24*D*, 24*C*, 24*B*.


ADJ PC 22
C E E D C B A E D C B A
4


ADJ PC 34
N3 N3 N3 N3
5


MO PC 4
G F D C B A
6


MO PC 4
F B B B G G G G G G G
7

(*b*) usually ellipsoidal or asymmetrical in shape along axis of migration, and (*c*) never free cathodally from the origin, though one was anodally free, the others migrated in part anodally and, or, cathodally with a major portion of the mass remaining at the origin (immunochemically these proteins were not SI (7) myeloma globulins, see below).

Most of the myeloma proteins of this later group were electrophoretically heterogeneous and some electrophoretically overlapped each other.

An electrophoretic mobility cannot be used to characterize a heterogenous mass of protein in a zone type electrophoresis such as was used here. In this report (where the major concern is comparative) three letters *a* (anodal), and *o* (origin), and *c* (cathodal) have been used to characterize the electrophoretic distribution of the protein masses. Subscripts under letters indicate the distribution of the protein mass migrated comparatively further; *i.e.*, c_1 consistently appeared more cathodal that *c*.

The various letterings assigned to myeloma proteins based on many comparative runs are tabulated in Table II.

By using electrophoretic criteria alone the following results were obtained. In three cases, (ADJ PC 24, 25, and 30) the myeloma proteins of the various transplant lines were the same within the case (Text-figs. 2 and 3). Electrophoresis alone established similarities of the lines, and no further differences were found in the immunoelectrophoretic study.

In the other 3 cases ADJ PC 22, 34, and MO PC 4 different electrophoretic types were found among the transplant lines (Text-figs. 4 to 7). For ADJ PC 22, two types were found, the *A* and *C* lines resembled each other and had an *aoc* electrophoretic distribution (Text-fig. 4). While the *B*, *D*, and *E* lines had an a_1o electrophoretic distribution. For ADJ PC 34, 3 lines had an *ao* distribution and 1 line, N_3, had an *aoc* distribution (Text-fig. 5).

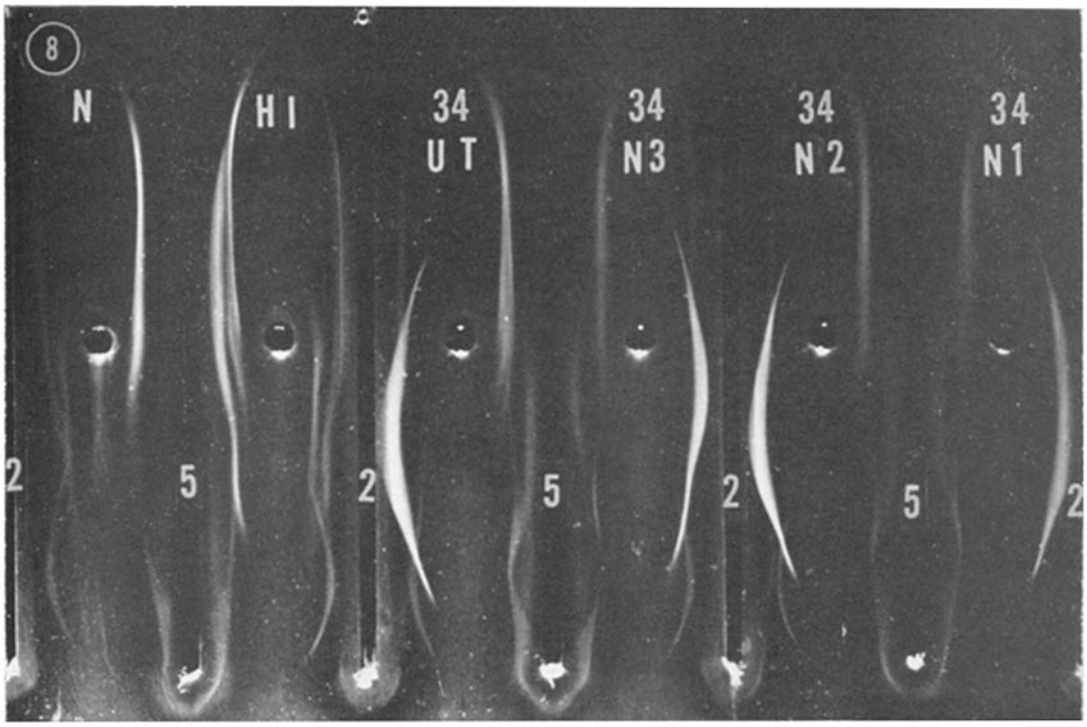
There were 3 electrophoretic distribution types among the 7 MO PC 4 lines (Table II) (Text-figs. 6 and 7). The MO PC 4 *G* line was exceptional. Two electrophoretic distributions were associated with this line, an *ao* distribution and an *aoc* (Text-fig. 7). In the first transfer generation serum sample the

TEXT-FIG. 4. Electrophoretic plate. 12 different sera from mice bearing the various ADJ PC 22 lines. From left to right: ADJ PC 22*C*, 22*E*, 22*E*, 22*D*, 22*C*, 22*B*, 22*A*, Normal, 22*E*, 22*D*, 22*C*, 22*B*, 22*A*.

TEXT-FIG. 5. Electrophoretic plate. 12 different sera from mice bearing the various ADJ PC 34 lines. From left to right: ADJ PC 34*N3*(1381), 34*N3*(1321), 34*N2*(1428), 34*N1*(1413), 34*UT*(1260), 34*N3*(1259), 34*N2*(1262), 34*N1*(1261), 34*UT*(1232), 34*N3*(1158), 34*N2*(1243), 34*N1*(1242), serum from mouse with the primary ADJ PC 34.

TEXT-FIG. 6. Electrophoretic plate. 6 sera from mice bearing various MO PC 4 lines. From left to right: Normal, MO PC 4*G*(1278), 4*F*(1108), 4*D*(1097), 4*C*(1149), 4*B*(1185), 4*A*(1101). MO PC 4*F*(1108) sample had little or no myeloma protein.

TEXT-FIG. 7. Electrophoretic plate. 11 sera from mice bearing various MO PC 4 lines. From left to right: MO PC 4*F*(1109), 4*B*(1375), 4*B*(1374), 4*B*(1372), 4*G*(1391), 4*G*(1390), 4*G*(1389), 4*G*(1280), 4*G*(1161), 4*G*(1279), 4*G*(1278). The MO PC 4*B*(1372) had little or no myeloma protein.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N
HI
34
UT
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N3
34
N2
34
N1
2
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2
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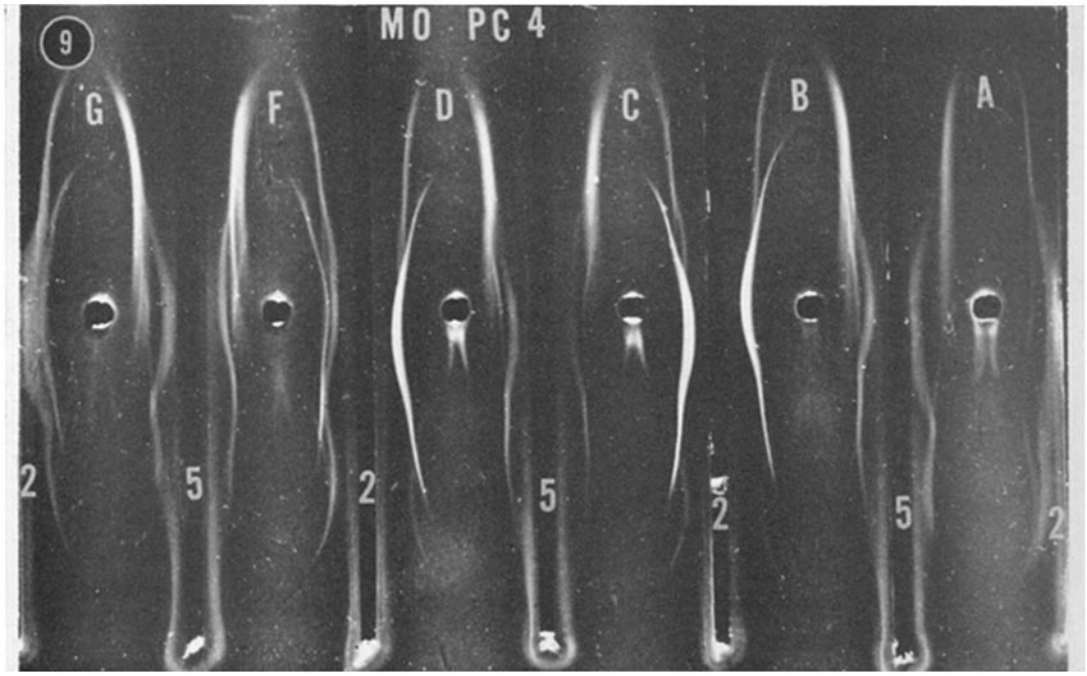
5
2


9
MO PC 4
G
F
D
C
B
A
2
5
2
5
2
5
2

myeloma protein was of *ao* electrophoretic distribution, thereafter, in the 2nd to 5th transfer generation samples, the myeloma protein was either *ao* or *aoc*. No correlation was established between pattern and the time (after transplantation) when the serum sample was harvested. Routine transfer of the new transfer generation was begun from a single donor indicating the MO PC 4 *G* line was continuously changing.

Another variation within the transplant history was the occasional failure of the myeloma protein to appear in sufficient concentration to be recognized in the stained plate. In the MO PC 4 collection consisting of 54 samples, this happened 7 times and occurred only for lines *B*, *E*, and *F* (Text-figs. 6 and 7). Failure to detect the myeloma globulin in serum was not the result of premature sampling of the serum for the time in transplant varied from 32 to 119 days and all the mice had large growths.

Immunoelectrophoresis

ADJ PC 22–24, 25, 30 and 34.—No further differences could be established between the similar and different lines of the five neoplasms using MPC 2 Pw and ADJ PC 5 Pw antisera in electrophoretic plates. Each of the myeloma proteins in these lines was precipitated sharply by MPC 2 Pw antiserum. The ADJ PC 5 Pw antiserum did not precipitate the myeloma proteins of the ADJ PC 22 ADJ PC 30 lines, however, this antiserum did form ill-defined "hazy" precipitin arcs with the myeloma proteins from the various lines of ADJ PC 24–25 and 34. A single example is shown (Text-fig. 8). The R6 euglobulin antiserum was not used in the study of these transplant lines.

MO PC 4.—An immunochemical difference was found to exist among the various MO PC 4 lines. Lines *A*, *E*, *F*, and *G* fell into one group and lines *B*, *C*, and *D* into another. Lines *B*, *C*, and *D* resembled immunoelectrophoretically the myeloma proteins of the other five cases. In this series in that they were sharply precipitated by MPC 2 Pw antiserum and very poorly if at all by the ADJ PC 5 Pw antiserum (Text-fig. 9).

The antiserum ADJ PC 5 Pw did not form sharply defined dense precipitin arcs with the myeloma globulins of any of the MO PC 4 lines. MPC 2 Pw antiserum, formed sharply defined dense precipitin arcs with the myeloma

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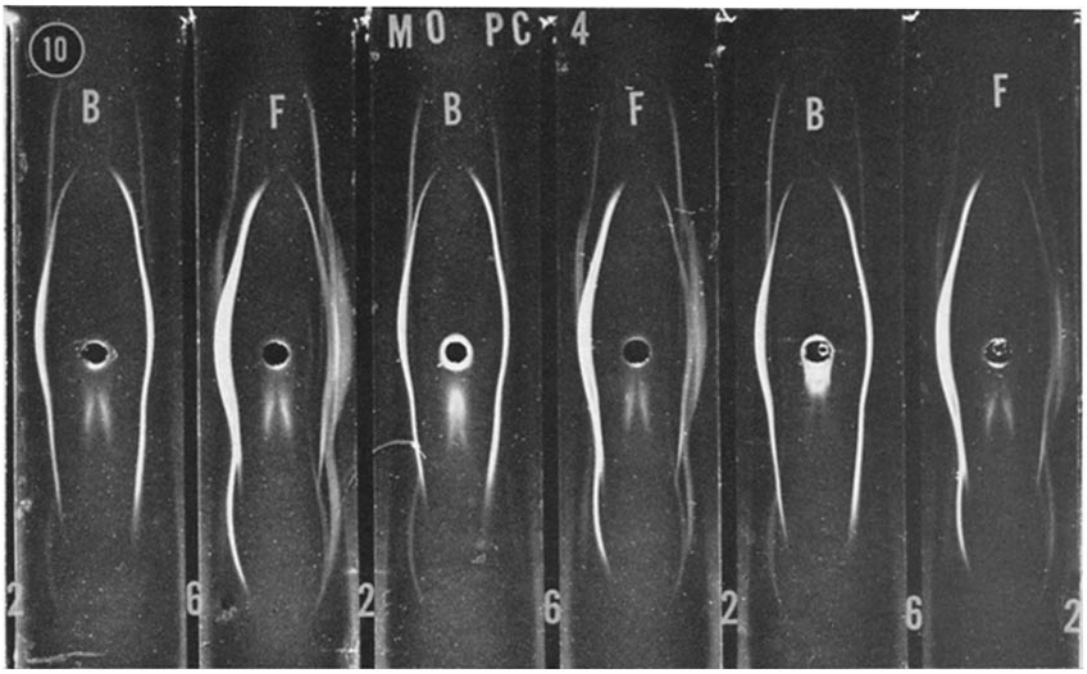
TEXT-FIG. 8. Immunoelectrophoretic plate. In the wells(w) from left to right: w1 normal serum (*N*), w2 hyperimmune serum (*HI*), w3 ADJ PC 34*UT*(1232), w4 ADJ PC 34*N3*(1263), w5 ADJ PC 34*N2*(1258), w6 ADJ PC 4*N1*(1242). In trenches (t), t1, t3, t5, and t7 MPC 2Pw antiserum (labeled *2* on photographs); in t2, t4, and t6 ADJ PC 5Pw antiserum (labeled *5* on photographs).

TEXT-FIG. 9. Immunoelectrophoretic plate. In wells from left to right: w1 MO PC 4*G* (1278), w2 MO PC 4*F*(1108), w3 MO PC 4*D*(1097), w4 MO PC 4*C*(1149), w5 MO PC 4*B* (1185), w6 MO PC 4*A*(1101). In t1, t3, t5, and t7 MPC 2Pw antiserum; in t2, t4, and t6 ADJ PC 5Pw antiserum. Note clarity and sharpness of precipitate formed with MPC 2Pw antiserum and the *B*, *C*, and *D* lines. Note hazy precipitin arc which the MPC *2* antiserum forms with lines *A*, *F*, and *G*.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MO PC 4
B
F
B
F
B
F
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6
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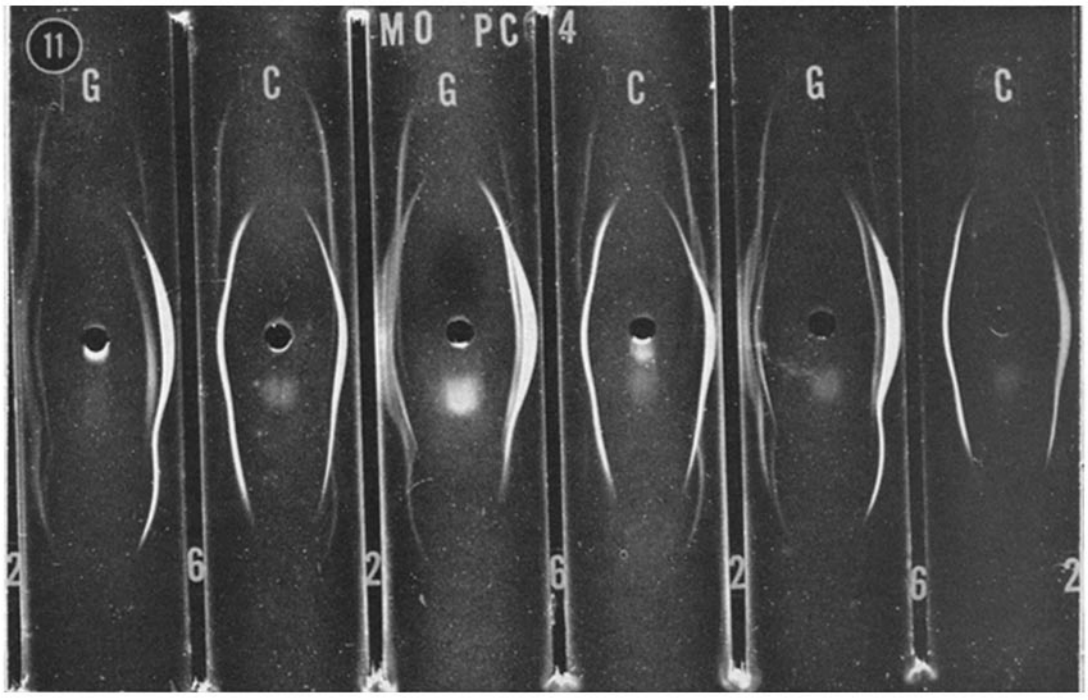
6
2
6
2


11
MO PC 4
G
C
G
C
G
C
2
6
2
6
2
6
2

proteins of lines *B*, *C*, and *D* lines of MO PC 4 (Text-figs. 9 to 11). The R6 euglobulin antiserum, on the other hand, formed sharply defined dense precipitin arcs with the myeloma proteins of lines *A*, *E*, *F*, and *G*. Further, the R6 euglobulin antiserum formed precipitin arcs with lines *F* (Text-fig. 10) and *G* (Text-fig. 11) that differed in contour from the precipitin arcs formed with lines *B* and *C*, respectively. With lines *F* and *G* the precipitin arcs consisted of two bows, a large bow in the origin and near-cathodal region and a smaller bow in the anodal region. The R6 euglobulin antiserum, developed a second arc with *F* and *G* that appeared closer (to the antigen source) than the larger double-waved arc. This 2nd arc was very dense in the line *F* samples but less dense in the line *G* samples. On the other hand R6 euglobulin formed only a single arc with lines *B* and *C*. The 2 electrophoretic types of myeloma protein of MO PC 4 *G* had a similar specificity for MPC 2 Pw, and R6 euglobulin antisera (Text-fig. 11).

The R6 euglobulin antiserum had different specificities from the MPC 2 Pw antiserum. Individual myeloma proteins which varied in antigenic constitution, reacted differently with these 2 antisera. Thus two pairs of myeloma proteins each with similar electrophoretic distributions (Text-figs. 5 and 6, 10 and 11) were separated into unlike types. This division created a total of 5 types of myeloma proteins among the MO PC 4 transplant lines (Table II).

DISCUSSION

The data presented here extend previous findings on plasma cell neoplasms in mice (2, 6, 7). The characteristic protein production of an individual neoplasm remains qualitatively fixed (heritable) and the proteins made by these cells do not change during the transplant history (with the one exception, the MO PC 4 *G* line described here). All plasma-cell neoplasms, however, are not alike in regard to protein secretion. There are many types of proteins produced by these neoplasms as a group. The cause of the variation in proteins is not related to host genetic factors but is a property of the cell itself, for cells of plasma cell neoplasms arising in the same inbred strain of mouse (2) or in a single host (7) may produce different types of proteins.

The characteristic protein associated with a plasma cell neoplasm, whether it is a serum (myeloma) or urinary (Bence-Jones) protein is the phenotypic

TEXT-FIG. 10. Immunoelectrophoretic plate. Comparison of the MO PC 4*B* and *F* lines. MO PC 4*B* samples in w1(1185), w3(1324) and w5(946). MO PC 4*F* samples in w2(1096), w4(1111), and w6(1276). MPC 2Pw antiserum in t1, t3, t5, and t7; R6 euglobulin antiserum in t2, t4, and t6 (labelled 6 on photographs). Note that the precipitin arcs formed by both antisera with the MOPC-4*B* lines are similar. Note difference in pattern the 2 antisera form with the line *F* samples.

TEXT-FIG. 11. Immunoelectrophoretic plate. Comparison of MO PC 4*G*, and MO PC 4*C*. MO PC 4*G* samples in w1(1161ao), w3(1390aoc), and w5(1278aoc). The MO PC 4*C* samples in w2(1006), w4(1149), and w6(1327). MPC 2Pw antiserum in t1, t3, t5, and t7; R6 euglobulin antiserum in t2, t4, and t6.

expression of a *protein-secreting apparatus*. It is of particular importance to define the structural components of the protein-secreting apparatus and determine whether the apparatus, as a whole, or in part, has been altered by the neoplastic transformation. Currently, details on the structure and physiology of the complex protein-synthesizing apparatus in neoplastic plasma cells are lacking. Some information has come from studies on the ultrastructure (10–12) and the microsomes (5, 7) of these cells. It is known that most of the cytoplasm of the neoplastic plasma cells consists of a system of membranes, sacs, and ribosomes (free and attached); *i.e.*, an ergastoplasm. This complex structure is associated with protein synthesis in normal plasma cells (13, 14) and other active, protein-forming (15) cell types. One of the important questions regarding protein formation in neoplastic plasma cells, is what role the nucleus (DNA) plays.

The nucleus by containing the chromosomes and genes is the ultimate source of information for protein synthesis in the cell. The ergastoplasm is intermediate between gene and protein, and serves 2 important functions, it carries out specifically the instructions of the nucleus, while greatly amplifying the capabilities for quantitative synthesis. The dividing neoplastic plasma cell with its heritable, fixed pattern of myeloma protein synthesis regenerates enormous quantities of specific new ergastoplasm during growth. It is not known how specific ergastoplasm replicates. It may replicate by a purely cytoplasmic process without nuclear control, or by a process mediated from the nucleus. If specific ergastoplasm can replicate without nuclear control it is clear that in some somatic cells, nuclear instruction is carried out continuously and independently in cytoplasm. The ability of the nucleus to change specific ergastoplasm is certainly curtailed in the transplanted plasma cell neoplasm.

The evolution of a specific ergastoplasm in a neoplastic plasma cell appears to be a separate process from the replication of specific ergastoplasm during growth of the transplantable tumor, and further appears to be a process which resembles a differentiation. A discussion of the differentiation of protein formation in neoplastic plasma cells is always complicated by the possible explanation that the differentiation itself is pathologic rather than physiologic. However, the present data bring up the question about what relationship neoplastic transformation (carcinogenesis) has to the differentiation of a specific pattern of protein synthesis in neoplastic plasma cells. One purpose for studying carcinogenesis in highly functional somatic cells is to advantageously use the performance of the function, as an indicator of the condition of intracellular organization and physiology in the hope that this may shed some light on the basic intracellular alterations of neoplasia.

One aspect of the relationship between carcinogenesis and differentiation of protein synthesis, particularly relevant to the present data, is the temporal relationship of these two events.

Does the neoplastic change precede, follow or occur simultaneously with the

differentiation of specific protein formation? It is considered profitable to comment on these 3 relationships even though it cannot be resolved at this time, which one is correct.

The first relationship considers that differentiation of the protein-forming apparatus precedes neoplastic transformation. Implicit in this explanation is that, normally there is a division of labor in protein formation. The chief complication to accepting this relationship is the fact that the proteins made by some plasma cell neoplasms produce pathologic effects, notably the Bence Jones proteins that cause renal tubular obstruction. Another reason for questioning the validity of this temporal relationship is the rarity of System I myeloma globulins. The 2 principal immunoelectrophoretic systems of globulins in mice elaborated by plasma cells are: System I globulins (SI, corresponds to gamma 2 globulin in man) and System II globulin (SII, corresponds to B-2A globulins in man) (7). SI globulins are found in greatest concentration in serum normally, and appear to be the types produced in the hyperimmune state. (Text-fig. 8). The fact that most plasma cell neoplasms in mice are not producing SI myeloma globulins, suggests those myeloma globulins may not be physiologic; *i.e.*, participate in antigen-antibody reactions.

Second, if the differentiation of protein secretion followed neoplastic transformation it would be expected that the line of cells undergoing neoplastic transformation would have multipotential protein-secreting capabilities. To explain the present findings, then, when the neoplasms were spatially separated, it would be necessary to postulate that the neoplastic transformation occurred in a mobile cell which seeded daughter cells on the peritoneum. These seeded cells gave rise to colonies of cells, futher progression of neoplasia, and the development of a specific pattern of protein secretion occurred, randomly in the small populations in the general manner, described by Foulds for tumor progression (16). Clones of cells which had one or another type of protein-producing pattern, progressed to a state where they could be transplanted and were selected by chance. The 2 protein types found in association with MO PC 4 *G* in the early transfers suggest (*a*) this line was composed of an unbalanced mosaic of cells, which had not yet adjusted during transplantation, or (*b*) this more transplantable cell type with a different protein-secreting mechanism was continually differentiating during transplantation. The MO PC 4 *G* line has been the only example of a change in protein-producing behavior observed during transplantation, thus far observed.

The third relationship considers the simultaneous occurrence of differentiation of protein synthesis and of the transformation to neoplasia. Both processes occur side by side in the same cell and attain later their respective phenotypic expressions in the progeny of that cell. Several alternative ways of simultaneous occurrence of both processes may be postulated (*a*) occurrence of both by chance in the same cell; (*b*) the vulnerable cell for neoplastic transformation is a multipotential protein-secreting cell, that was in the process differentiating

when neoplastic transformation began; (*c*) a single pathological event, caused both neoplastic transformation, and protein differentiation.

In the last postulate, the multipotential protein-forming cell, in order to give rise to 2 unequal cell types during mitosis, (one remaining multipotential, the other now acquiring a specialized protein-synthesizing apparatus) must undergo some intracellular changes or adjustments in its program. This particular postulate is a speculation, that neoplasia may be an error in programming or differentiating; coincidentally, the error in the case of the neoplastic plasma cell also results in pathologic alterations of protein secretion. Differentiation would be defined in this particular discussion as a process in which a particular, stable, heritable somatotype evolves from a precursor cell. This evolution probably would require the activation, of a non-pleiotropic gene or genes, to direct the cytoplasm into a particular type of activity.

The common plasma cell neoplasia in man, multiple myeloma, usually appears clinically as numerous lesions in the bone marrow cavities. The term "multiple" may be interpreted to mean multiple autochthonous plasmocytomas or multiple intramedullary metastases. Usually the serum of individuals with this disease contains only a single type of myeloma globulin which remains the same throughout the course of the disease (17). This fact suggests that most of the neoplastic tissue is of a common protein-producing type and favors the view that most of the intramedullary lesions are not autochthonous, but metastases. For if the lesions were different, it would be expected, that, a very heterogeneous collection of myeloma proteins would be found in the serum of each case. The findings of this study clearly indicate that in early experimental cases multiple protein-producing cell types are found. The important pathological question concerning multiple myeloma in man, is where is the site of origin of this neoplasia.

SUMMARY

The peritoneal plasma cell neoplasias that develop in strain BALB/c mice after the injection of adjuvant-staphylococcus mixtures or mineral oil alone appear in the form of multiple nodules in the mesentery and on peritoneal surfaces. Experiments were done to determine if these nodules were metastases or multiple primary neoplasms. Nodules or pieces of masses were transplanted subcutaneously by the trochar method or by insertion of tissue under the kidney capsule from 6 primary cases and parallel transplant lines were established. The serum and urinary protein abnormality (a stable heritable characteristic) of each of the various transplant lines was characterized by agar gel electrophoresis and immunoelectrophoresis. Different protein-producing lines were found in 3 cases; in one case 5 different protein-producing lines were isolated. Two different lines were found for each of the other 2 cases. When transplantation studies were begun early, it was demonstrated that the nodules were

multiple primary plasma cell neoplasms; when delayed, only one protein-producing plasma cell neoplasm was found.

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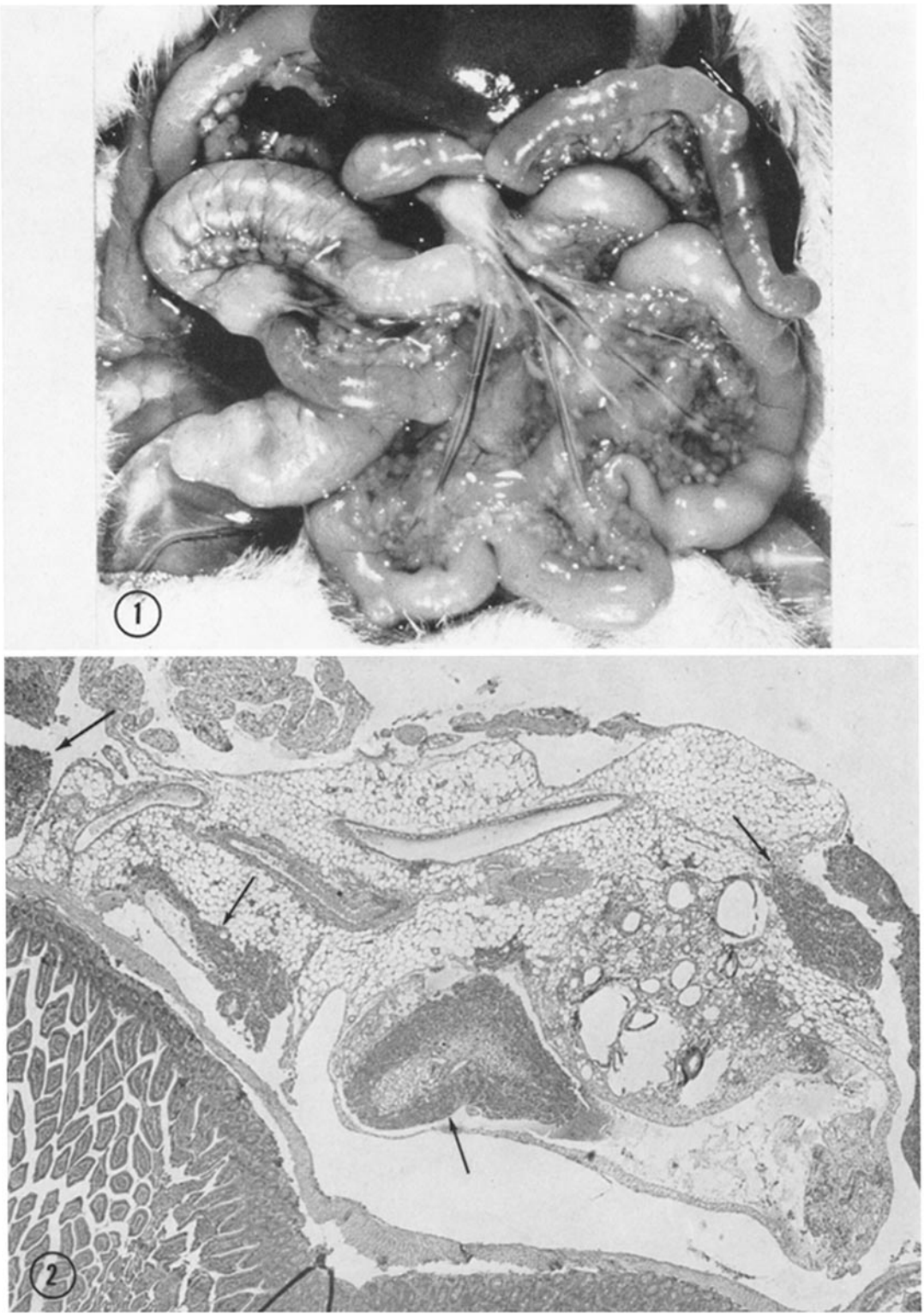
EXPLANATION OF PLATES

PLATE 35

FIG. 1. BALB/c mouse with primary plasma cell neoplasia MO PC 8 which had developed during 14½ months after intraperitoneal injection of 0.4 ml of mineral oil. Note the many small nodules of tumor tissue in the mesentery. × 4.

FIG. 2. Photomicrograph of a section of mesentery from the mouse with the primary plasma cell neoplasia MO PC 4. Arrows indicate regions of plasma cell neoplasm. Bouin's fixative, hematoxylin and eosin stain, × 45.

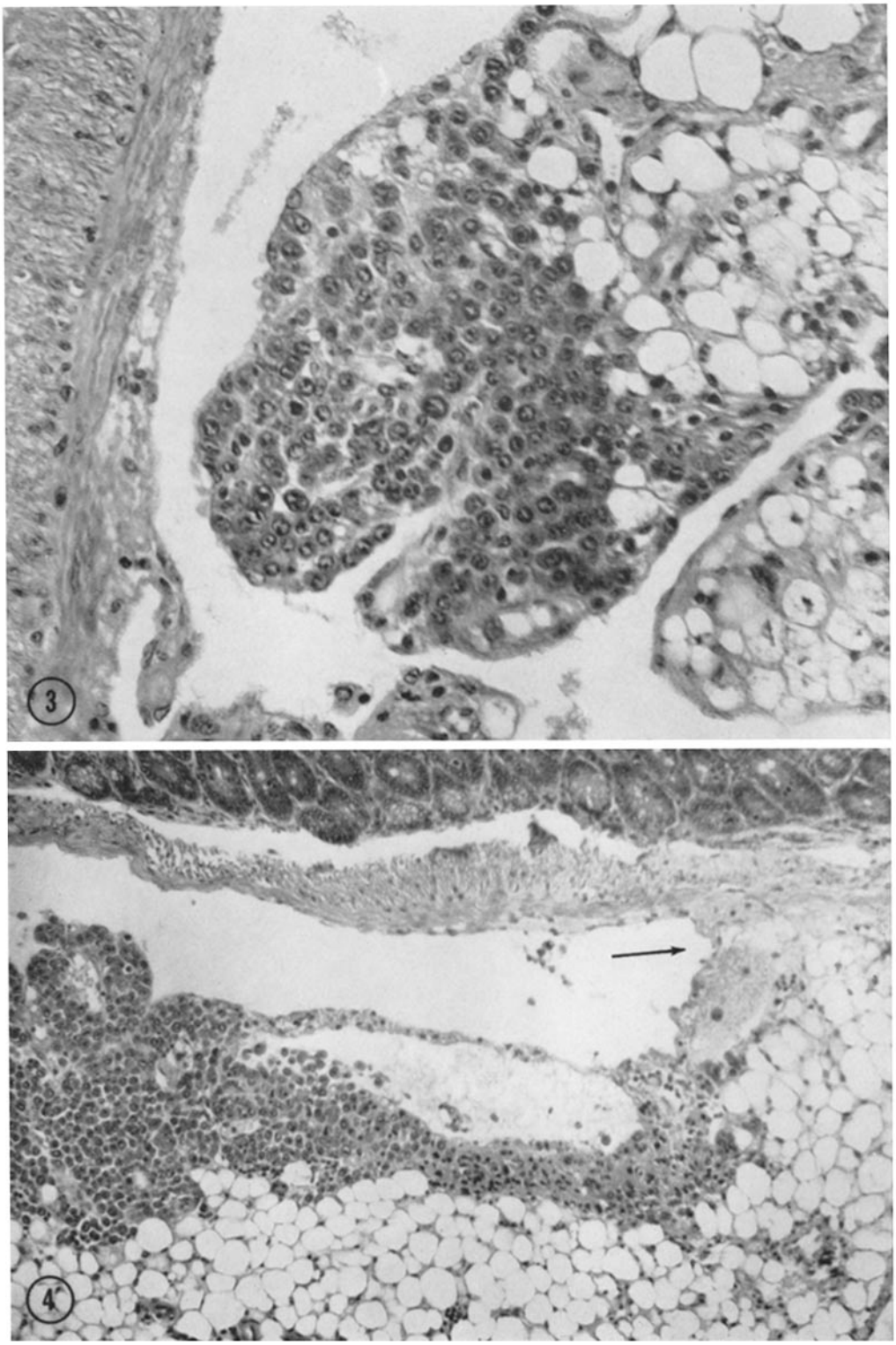


(Potter: Plasma cell neoplasia in single host)

PLATE 36

FIG. 3. Same as Fig. 2 showing infiltrate of neoplastic plasma cells near serosal surface of mesentery. × 400.

FIG. 4. Same as Fig. 2. Neoplastic plasma cells infiltrating surface of the mesentery. Note attachment to the serosal surface of the small intestine. × 163.



(Potter: Plasma cell neoplasia in single host)