anaerobic glycolysis and could represent either a blockade or other loss of insulin action or result from a decrease in the glucose load. Either or both of these alternatives is compatible with decreased gluconeogenesis, enhanced glycogenation or decreased glycogenolysis as mechanisms whereby BZ-55 may lessen the insulin requirement in some diabetics.

Summary. Insulin tolerance tests in juvenile diabetics following administration of N-sulfanilyl-N′-butylurea (BZ-55 or Carbutamid) failed to show potentiation or prolongation of the usual insulin hypoglycemia and hypophosphatemia. Actually, the decrease in serum inorganic phosphorus which followed administration of insulin to juvenile diabetics was less pronounced, suggesting a diminution in anaerobic glycolysis, either as a result of interference with the usual action of insulin or as a consequence of a decreased glucose load. Both of these possibilities are compatible with decreased gluconeogenesis, increased glycogenation, or diminished glycogenolysis as factors in the decreased insulin requirement which follows administration of this agent to certain patients with diabetes mellitus.

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Abnormal Serum Protein and Bone Destruction in Transmissible Mouse Plasma Cell Neoplasm (Multiple Myeloma). (22935)

MICHAEL POTTER,* JOHN L. FAHEY,* AND H. IRA PILGRIM†
(Introduced by L. W. Law)
National Cancer Institute, Bethesda, Md.

Plasma cell neoplasms of the C3H/HE strain mouse are rare and most often originate in an ulcerated lesion in the ileocecum. Neoplasms of this type have previously been described pathologically and one has been transplanted in this laboratory successfully through 50 transfer generations(1,2,3,4). Plasma cell neoplasms of other mouse strains have been described(5,6,7). The resemblance of these neoplasms to multiple myeloma of man has been made on the basis of microscopic pathology only. The subject of this report is another successfully transplanted C3H strain plasma cell neoplasm of ileocecal origin (X5563), which resembles multiple myeloma of man, not only microscopically, but also by producing an abnormal serum electrophoretic pattern and multiple osteolytic lesions.

Materials and methods. 1. *Origin of tumor, serum sources and methods of transplantation.* The plasma cell neoplasm X5563 originated in a 22½-month-old female of C3H/HE/CRGL strain which had been gonadectomized at the age of 2 months. This animal developed an abdominal mass, and at autopsy there was found a large neoplasm in the wall of the ileocecum, and an enlarged

* National Institutes of Health, P.H.S., U. S. Dept. of Health, Education and Welfare.

† Department of Zoology, University of California, Berkeley.

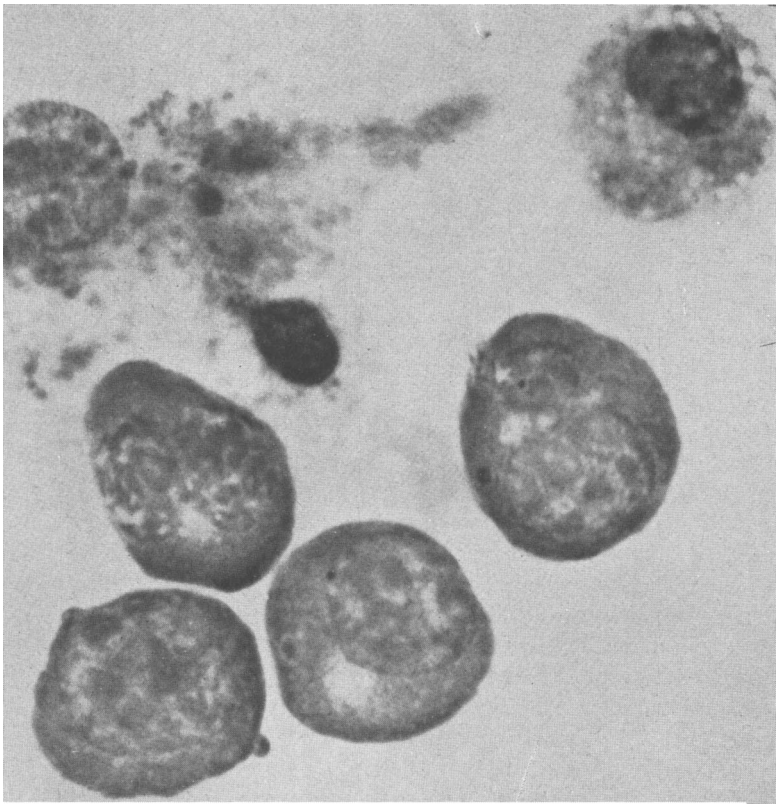

FIG. 1. Free neoplastic cells of neoplasm X5563, extruded into serous ascitic fluid which collects during intraperitoneal growth. Cytoplasm stained intensely blue. Cell in upper right part of the field is an histiocyte. Wrights' stained smear.

mesenteric node. Fragments of the ileocecal neoplasm were transplanted into subcutaneous connective tissues. Continuous subcutaneous transfer for 5 transfer generations has subsequently been maintained. Two methods of transfer have been employed; subcutaneous transfer employing 12 and 13 gauge trochars, and by intraperitoneal inoculation of homogenates prepared in Potter-Elvehjem tissue homogenizers. The subcutaneous trochar technic has proved the more desirable. Routine transfer can be carried out every 30 days. Serum for use in electrophoretic studies was obtained for the most part by cardiac puncture. Mice bearing neoplasm X5563 develop a moderate hypervolemia, and 2 to 3 ml of whole blood can be obtained by this method. 2. *Other serum sources.* Normal serum was obtained for controls from C3H/DE, C3Hf/Lw mice both derivatives of the C3H/HE strain(8). Serum was obtained from C3H mice bearing spontaneous and transplanted neoplasms as follows: 1) 2 spontaneous mammary tumors designated MT-1, MT-2 (original tumor bearing mice) arising in C3H/HE females, 2) an undifferentiated lymphocytic neoplasm designated L5649 (transfer generation 16) of C3Hf/Lw origin, 3) a spontaneous thymic lymphocytic neoplasm designated L7688 (transfer generation 6) of C3H/HE origin, 4) an unusual lymphoma designated L9380 (original tumor bearing animal) of C3Hf/Lw origin, and 5) serum from various animals bearing the ascitic plasma cell neoplasm 70429 (transfer generations 30 to 55) transplanted to C3Hf/HE, C3Hf/Lw and C3H/DE mice. Serum was obtained from mice bearing various spontaneous and methylcholanthrene induced transplantable DBA/2 lymphomata: 1) P329 (transfer generation 2) P632 (transfer generation 1) both type A reticulum cell sar-

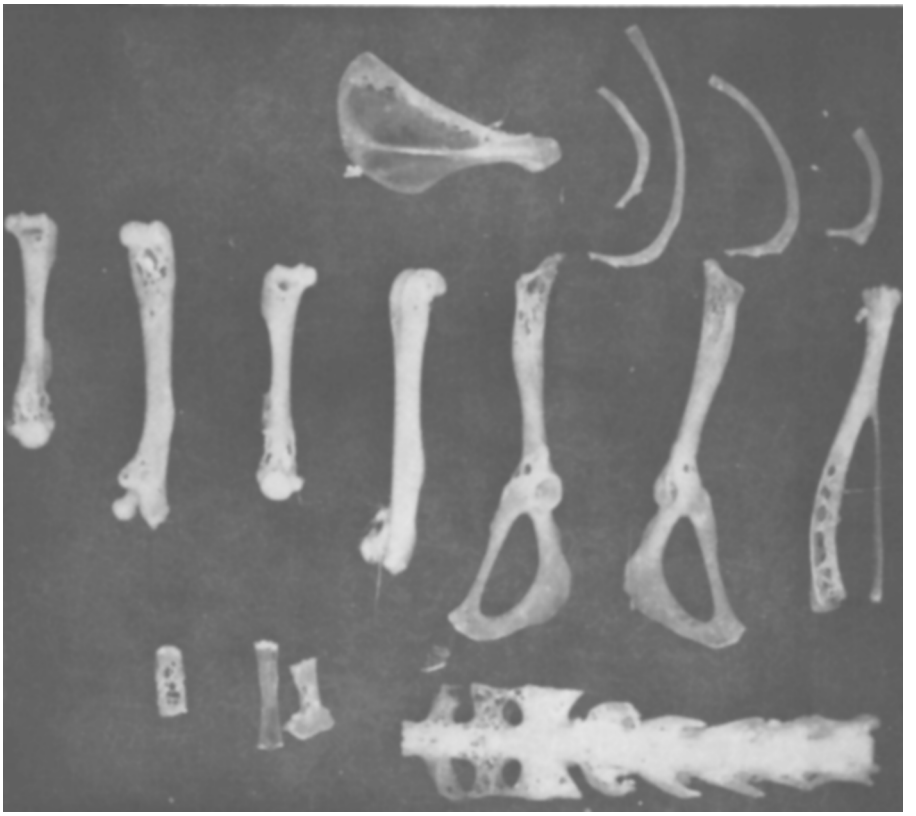

FIG. 2. Photogram of bones from a mouse bearing neoplasm X5563 for about 3½ months. (Papain digested carcass, magnification 2.85X.)

comata(3), 2) P195 (transfer generation 7) a type B reticulum cell sarcoma(3), and 3) P353 (transfer generation 6) an undifferentiated lymphocytic neoplasm(3). 3. Mice used for maintenance of the plasma cell tumors were C3Hf/HE mice or direct derivatives of this colony C3Hf/Lw or C3H/DE. These mice were fed Derwood chow and water *ad libitum*(9). 4. Preparations of skeletons were made by papain‡ digestion; by the method described by Searle(10). 5. Photograms§ of skeletons were made by passing the light of photographic enlarging apparatus through the bones with exposure of negative film. 6. Pathologic sections were fixed in Tellyesniczky's fluid (70% ethyl alcohol 20 parts, formalin 2 parts, glacial acetic acid 1 part) or Zenkers' solution and stained with hematoxylin and eosin. Smears were stained with Wrights' stain. 7. Paper electrophoretic determinations were made on 0.008 to 0.010 ml of serum in hanging strip cell(11) in 0.075 μ veronal buffer at pH 8.6. Eight Whatman 3 mm strips were run simultaneously in one cell for 18 hours at room temperature under constant current conditions of 6 m. amps per cell and under potential of 68 to 72 volts. The strips were stained with bromphenol blue under conditions recommended by Jencks *et al.*(12). The paper strip patterns were evaluated by transmission densitometry with the Analytrol‖ using a cam which provided linear response for albumin dye binding. Each electrophoretic run in a given cell included one strip which contained 0.008 ml from pooled

‡ Commercially obtained from Nutritional Biochemical Laboratories.

§ This method was devised by Mr. Vernon E. Taylor of the N.I.H., Scientific Reports Branch.

‖ Spinco Division, Beckman Instruments Inc., Belmont, Calif.

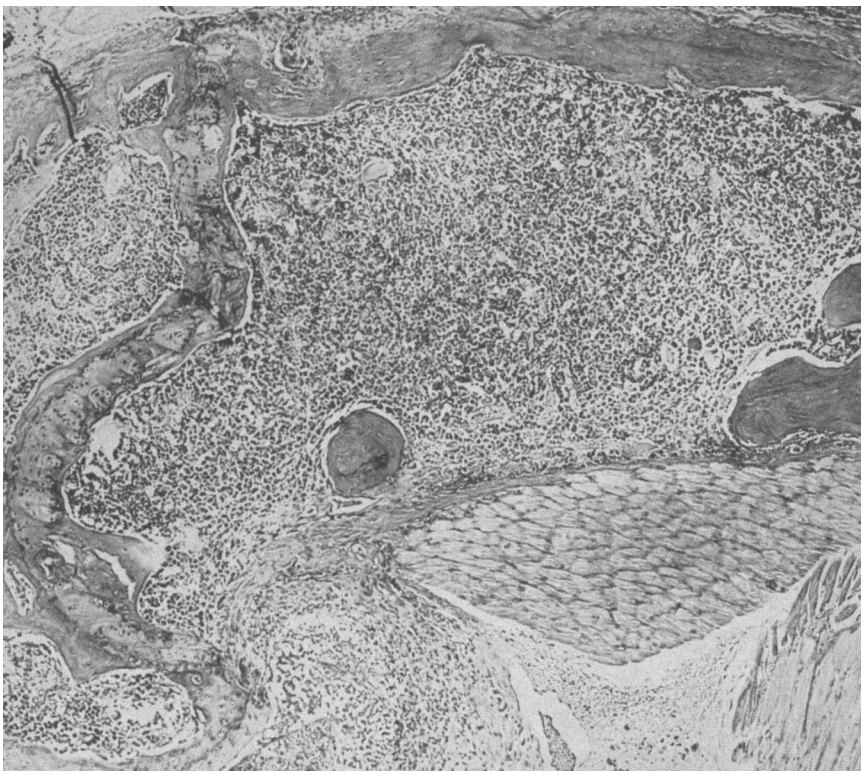

FIG. 3. Section through proximal end of humerus of mouse with multiple osteolytic lesions. Interruption of osseous tissue may be seen in lower portion of the field. Distal margin is bounded by rounded bone, proximal by an absence of bone at epiphyseal plate. H. and E. Stain magnification 70X.

human serum of known composition used as a control for all electrophoretic determinations. Migration distance (or "Mobility") of the abnormal protein was calculated relative to the movement of the albumin in each serum $\left(R_A = \frac{\text{mm myeloma protein moved}}{\text{mm albumin moved}} \right)$. Total serum protein determinations were made by the biuret method according to Gornall(13).

Results. 1. *Pathology.* Neoplasm X5563 grows locally in subcutaneous tissues and from these tissues disseminates very late to marrow cavities. Local growth is very firm, and has a fibrous character when cut; it is reddish in color, and is moderately vascular. Liver and kidneys are not involved with infiltrations of cells as they are in lymphocytic neoplasms. Rarely, rounded masses are seen in the spleen. Regional lymph nodes in the deep peritoneum are infiltrated. Intraperitoneal infiltrations progress from local sites, and usually accumulate in the mesentery and in the retroperitoneal regions. Intraperitoneal passage has not produced an ascitic tumor in the first two intraperitoneal transfers, though 2 to 3 ml of the slightly cloudy serous fluid does collect. This fluid contains a few cells, a normal flora of lymphocytes, mast cells and histiocytes; and tumor cells which are hyperchromatic cells of an immature type resembling plasma cells. Many of these are multilobulated and some of the smaller cells possess a spoke wheel arrangement of the chromatin.

An illustration of characteristic tumor cells found in the peritoneal fluid may be seen in Fig. 1. Such cells are unique with this neoplasm, similar cells have never been seen in other ascites lymphomata of the mouse including lymphocytic neoplasms, type A reticulum

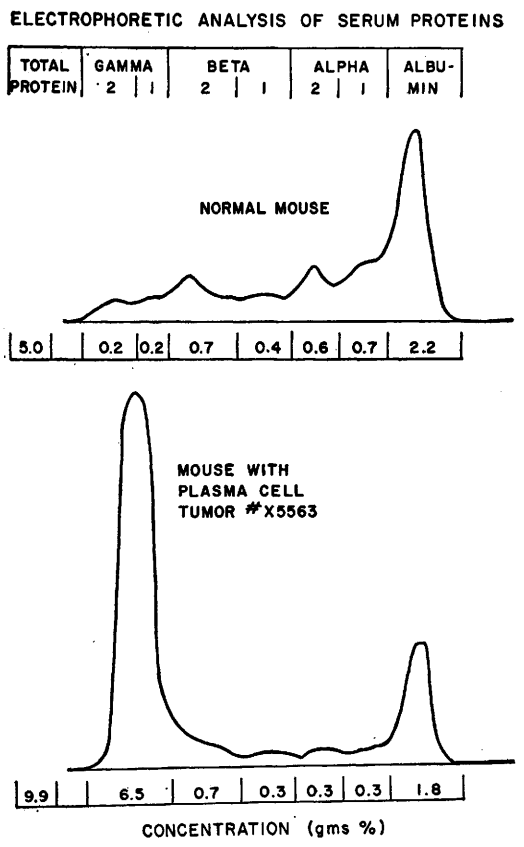

FIG. 4.

cell sarcomata, a mast cell neoplasm, or ascitic extrusions of undifferentiated lymphocytic neoplasms. Detailed tissue sections of this neoplasm will be presented by Dr. Thelma Dunn, National Cancer Institute, in a forthcoming publication.

By use of sections, and papain digested skeletons, it has been possible to study infiltration of bone marrow and subsequent bone destruction in mice bearing neoplasm X5563. In certain animals, bearing neoplasm X5563 for periods of 3 months and longer, invasion of the marrow cavity did occur, with subsequent eruption of some lesions into surrounding soft tissues. Three such sites have been observed. In 2 cases lesions were seen in the spine where paraplegia was the outcome, in one case in the sternum, and in the fourth case in the distal femur and proximal tibia where a large tumor mass was seen.

A more comprehensive view of the skeletal changes is observed in papain digested skeletons where multiple osteolytic lesions are seen. These are most marked in the distal femora, proximal tibiae, sacrum and lower thoracic spine, the sternum, scapula and ribs, (Fig. 2). A total of 26 skeletons of various animals bearing different age transplants were studied. Multiple osteolytic lesions similar to those shown in Fig. 2 were found in 8 of 11 mice which carried the tumor for a period of 90 days or longer. Most of these were found in animals bearing subcutaneous transplants.

Histologic section of the advanced bone lesions reveals infiltration by the neoplastic plasma cells. More precisely in areas where bone destruction is observed in tissue sections, and as can be seen from Fig. 3, there is a sudden interruption of the osseous tissue along the shaft of the bone. No osteoclastic activity is seen. The osseous edges of the lesion are rounded. These areas probably are the same as those rounded punched out areas seen in the papain digested skeletons. The marrow cavity under these lesions is filled with invading neoplastic plasma cells.

2. *Serum changes.* Serum protein analysis by zone electrophoresis revealed the presence of an abnormal protein with the mobility of a gamma globulin (Fig. 4). The abnormal serum protein was present in all animals with palpable tumors. It was not evident on examination of 28 sera from normal mice or mice bearing any of the 9 other tumors tested (Table I). The mobility of the abnormal

TABLE I. Electrophoretic Examination of Mouse Serum.

Animal and tumor type	No. tested	Abnormal protein present
C_3H mice		
Plasma cell tumor—X 5563	19	100%
Normal	4	0
Mammary carcinoma	2	0
Plasma cell tumor—70429	12	0
Lymphatic leukemia—67688	3	0
Lymphoma—L 5649	1	0
" L 9481	1	0
DBA f/2		
Lymphoma—P 195	2	0
" P 329	1	0
" P 353	1	0
" P 632	1	0

TABLE II. Serum Protein Changes Produced by Growth of Tumor X 5563.

Days after transplant	Tumor wt, mg	Serum proteins (g %)								
		Total	Normal component total	Individual components						
				A	α-1	α-2	β-1	β-2	M	γ-2
19	310	5.7	4.4	2.9	.3	.3	.2	.6	1.3	.1
33	830	7.1	4.1	2.2	.3	.4	.3	.7	3.0	.2
33	2590	9.0	3.6	2.0	.3	.3	.3	.6	5.4	.1
68	8185	11.6	3.4	1.6	.4	.4	.3	.6	8.2	.1

protein was uniform in the serum from all of the animals tested. The mobility relative to albumin (R_A) in consecutive serum samples from 13 individual tumor-bearing mice was .10 ± .01. The quantity of abnormal protein in the serum varied with the amount of tumor present. Table II illustrates the quantitative relationship of tumor to myeloma protein. The larger the tumor the greater the amount of the abnormal serum protein component. The other globulin components did not decrease, although albumin tended to fall with progression of tumor growth. These changes in the normal serum protein components are in general agreement with those previously described in tumor bearing mice(14).

Urine specimens from 4 animals with palpable tumors and the abnormal serum protein were concentrated by ultrafiltration and examined by electrophoresis. Two had no detectable protein, and the other 2 each had a single protein component with the mobility of albumin. No protein was present with a mobility the same or slightly faster than the abnormal serum protein. There was no evidence of a Bence-Jones protein by the usual heat precipitation-solubility test.

Because of the possibility that the abnormal serum proteins may be formed in the plasma cells of the tumor, a saline extract of the tumor was examined by electrophoresis. For comparison, electrophoretic analysis was also performed on serum from the same animal as well as a saline extract from a plasma cell neoplasm 70429 that is not associated with an abnormal serum protein. The results, illustrated in Fig. 5, clearly demonstrate the presence of a component in the saline extract of neoplasm X5563 that is not obtained from neoplasm 70429. The mobilities of this cellular protein and of the abnormal serum protein are the same. The demonstration of an abnormal protein in the tumor cells with electrical properties similar to the abnormal serum protein suggest that the abnormal protein of the serum may originate in the tumor cell.

Discussion. These findings reveal a mouse neoplasm (X5563) bearing a similar histologic picture to human plasma cell neoplasms that is also similar in abnormal globulin production and the pathologic predilection for

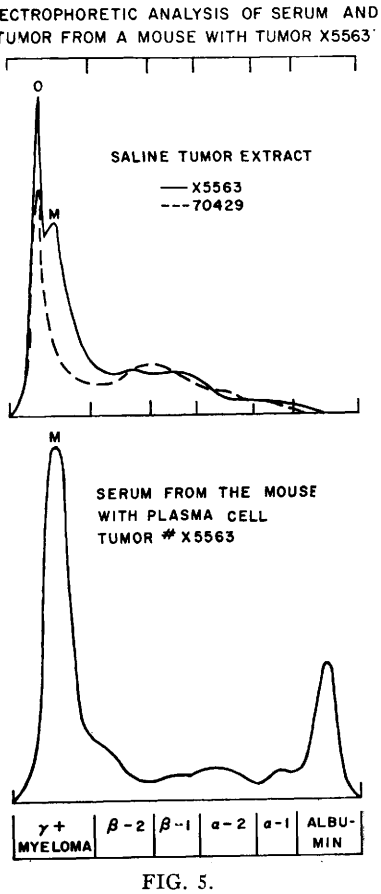

FIG. 5.

invasion of bone marrow with subsequent osteolysis and tumor formation. This triad of findings was found to be associated only with this relatively differentiated plasma cell neoplasm, and not with other lymphomata of the C3H and DBA/2 mouse, including the plasma cell neoplasm 70429 which had been transplanted over 50 transfer generations.

The concept that the plasma cells are the source of the abnormal serum protein is not new. The demonstration of a protein in the tumor cells with properties similar to the abnormal serum protein is suggestive of such a relationship. The transfer of cells to normal hosts, which later develop the abnormal serum protein, as the neoplasm grows, is again evidence to relate intimately the neoplastic plasma cell itself with abnormal serum protein production.

It is not possible at the present time to say whether the abnormal electrophoretic peak represents an abnormal amount of a normal serum component, or a protein not normally present in serum. Work is in progress to evaluate this point.

The striking uniformity of the abnormal serum protein found in the various animals bearing neoplasm X5563 would appear to differ sharply from the variety of abnormal proteins present among patients with multiple myeloma(15). However, this difference is not surprising when it is remembered that the transplants of neoplasm X5563 are all from one source and are introduced into animals of the same genetic background. In any individual human patient with multiple myeloma, the properties of the associated abnormal serum protein remain quite constant.¶ Thus in this property, plasma neoplasm X5563 resembles an individual human myeloma. The variety among plasma cell tumors in humans is paralleled by the difference between the two plasma cell tumors currently available in this laboratory, X5563 being associated with an abnormal serum protein and 70429 without such a protein. It is also possible that plasma cell neoplasm 70429 has lost the ability to form an abnormal serum protein in the process of laboratory transfers.

¶ Unpublished observations.

Summary. A plasma cell neoplasm X5563 of the C3H mouse has been described, which resembles human multiple myeloma, by similar histologic appearance of the neoplastic plasma cell; by similar development of osteolytic bone lesions after considerable residence in the host mouse, and by similar production of an abnormal serum globulin.

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