

trophoresis, wound fluid had a higher per cent of albumin and lower alpha-2 and beta-1 globulin. An elevation of protein-bound hexose was found in beta and gamma globulin fractions at 3 to 4 weeks. Seromucoid was present at same level in wound fluid and serum. The lipid partition revealed especially low phosphatides but similar cholesterol:protein ratios. It appears that time-related changes occur in large molecular constituents of fluid found in wound area which may be significant in wound healing phenomenon.

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Alterations of Gamma Globulin with Plasma Cell Neoplasm in Mice.* (24938)

CHARLES D. COBAU[†] AND DONALD R. KORST (Introduced by John M. Weller)

*Dept. of Internal Medicine, University of Michigan Medical School and Radioisotope Service,
Vet. Admin. Hospital, Ann Arbor*

Potter, Fahey and Pilgram described a transmissible plasma cell neoplasm in C3H mice, which was distinguished by production of gamma globulin(1). Nathans, Fahey and Potter measured uptake of *l*-lysine-C¹⁴ by tumor and demonstrated that gamma globulin formed within tumor was released into general circulation(2). The following investigation was carried out with the intent of further characterizing alterations of plasma protein after partial ablation of tumor and during the

course of homologous transplantation of tumor.

Materials and methods. The plasma cell neoplasm, X5563, transfer generation 15, was obtained in its subcutaneous form in 2 living C3H mice.[‡] Continuous subcutaneous transmission for 5 generations has been carried out in C3H mice,[§] ranging from 6 weeks to 3 months in age at time of transplantation. Subcutaneous transplantation of minced tumor tissue was completed within 1 hour following removal from donor animal. Swiss mice have been used in homologous transplantation experiments. Blood was collected from tail vein in heparinized capillary tubes. Follow-

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[‡] From Nat. Cancer Inst., courtesy of Dr. M. Potter.

[§] Obtained from Jackson Memorial Lab., Bar Harbor, Me.

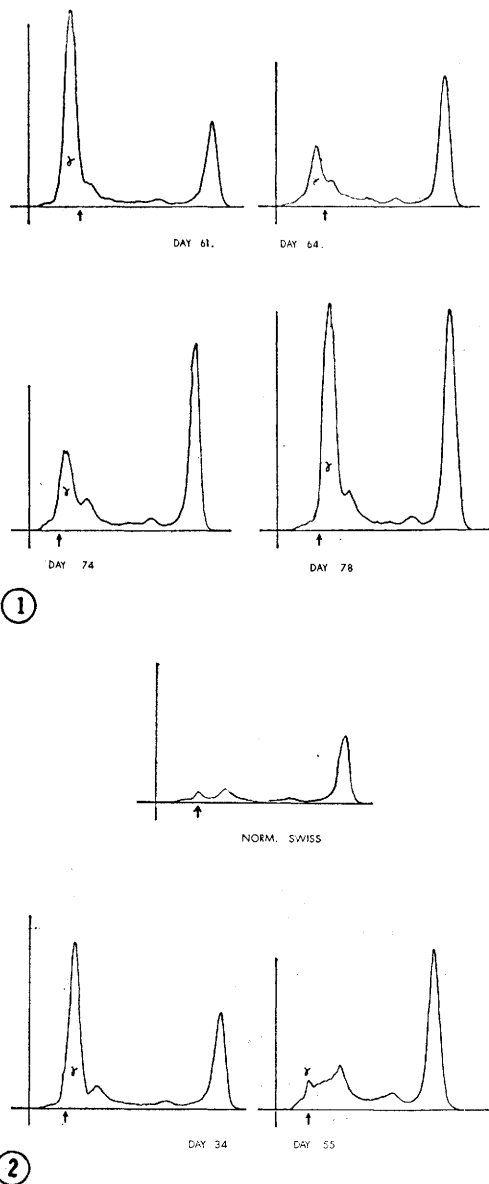


FIG. 1. 1.5 g tumor removed surgically 62 days after transplantation resulted in rapid fall of amount of gamma globulin within 48 hr. Twelve days after removal there was palpable recurrence of tumor and a return of abnormal plasma protein.

FIG. 2. Spontaneous regression of X5563 tumor in mouse given 600 r 2 days before transplantation. On 34th day following transplantation, there was a palpable flank tumor. Characteristic elevation of gamma globulin was present. Twenty-one days later (Day 55) there was a decrease in size of tumor and loss of high gamma globulin peak.

ing centrifugation, the tube was broken and plasma frozen until electrophoresis was carried out. Six lambda (0.006 ml) of plasma

was placed on standard Spinco Electrophoretic Apparatus in veronal buffer at pH 8.6. Paper strips were stained with bromphenol blue and graphed on Spinco Analytrol. Total body irradiation was administered from either 250 kv standard therapy unit or Cobalt-60 source of Phoenix Memorial Lab., Univ. of Michigan. Calculated dose was 300 or 600 r.

Results. X5563 is a poorly encapsulated neoplasm composed of relatively undifferentiated cells resembling plasma cells. It metastasizes to bone marrow and spleen. Survival longer than 3 months after subcutaneous transfer is unusual. In all animals in which tumor growth occurred, an abnormal gamma globulin peaking has been demonstrated in plasma by paper electrophoresis. This has been proportional in amount to tumor growth.

Following surgical removal of 0.4 to 1.6 g of subcutaneous tumor in 5 animals, a uniform fall in gamma globulin toward normal levels was noted. A representative experiment is shown in Fig. 1. We have effected no permanent cure by removal of subcutaneous tumor, however, recurrence was delayed in one instance 36 days during which time the electrophoretic pattern was essentially normal.

Ninety-six attempts over 5 generations have been made to transmit X5563 in C3H mice by the subcutaneous route. There were 66 (63%) successful transplantations. All successful transplants were confirmed by development of visible tumor and evidenced characteristic plasma electrophoretic changes. A subcutaneous nodule usually appears within one month of transplantation. The earliest electrophoretic abnormality occurred on 34th day following transplantation. Prior to this time, even in presence of small palpable tumor, no alterations in plasma proteins were seen. In 1 of 6 attempts it was possible to transmit this tumor by subcutaneous transplantation of splenic tissue from an animal sacrificed 74 days following subcutaneous transplant. One tenth ml blood from this and another similar animal injected intraperitoneally in each of 12 mice failed to yield successful transmission.

Homologous transplantation of X5563 tumor into Swiss mice by subcutaneous route

TABLE I. Summary of Attempted Homologous Transplantation of X5563 Tumor.

Group	No. of attempted transmissions	No. of deaths within 30 days	No. of successful transmissions
Non-irrad.	24	5	2
300 r	12	7	1
600 r	21	11	5
Total	57	23	8

has been attempted on 57 occasions. This is summarized in Table I. Total body irradiation was administered 48 hours prior to transplantation. Several animals dying within 30 days manifested hemorrhagic tendencies. Eight of 34 surviving animals developed palpable tumors and electrophoretic plasma changes typical of X5563. From 3 mice in which transplantation was successful, we were able to transmit X5563 to other C3H mice. Four other animals were followed with serial electrophoretic patterns, and in these spontaneous regression of tumor was observed. Fig. 2 illustrates electrophoretic changes occurring in these animals. One animal died of unknown cause.

Discussion. That removal of palpable subcutaneous tumor results in return of electrophoretic pattern to near normal is evidence that source of abnormal globulin is tumor. That this occurs with only partial removal indicates that there is a quantitative relationship between amount of tumor and degree of electrophoretic abnormality.

Observations regarding homologous trans-

plants and tumor regression illustrate genetics of transplantation as outlined by Snell(3). However, successful transplantation and subsequent regression of a tumor have been followed here for the first time without interval sacrifice of host. The number of homologous transplants has been inadequate to determine effect of irradiation, but based on other studies(4,5,6,7), enhanced success in homologous transplantation of X5563 should be expected with prior irradiation of host.

Summary. Experimental mouse plasma cell tumor, X5563, produces plasma gamma globulin alterations. Temporary disappearance of abnormal globulin occurs following partial removal of neoplasm. Homologous transplantation of tumor has been carried out. Because surviving tumor can be readily identified by changes in plasma proteins without animal sacrifice, it provides a new and useful tool for investigation of tumor immunity.

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Decrease of Enzymatic Synthesis of Hexosamine in Epiphyseal Plates of Aminoacetonitrile-Treated Rabbits.* (24939)

VITTORIO PEDRINI AND ANGIOLA PEDRINI-MILLE (Introduced by I. V. Ponseti)

Dept. of Orthopedic Surgery, College of Medicine, State University of Iowa, Iowa City

The most evident alterations of experimental lathyrism are in the epiphyseal plates of long bones that appear very widened and lack of cohesion and organization. The changes seem to be due to some defect in

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ground substance, but there is no agreement about the metabolic block produced by the lathrogenic agents. An alteration in mucopolysaccharides was suggested some years ago by Ponseti and Shepard(1), and more recently by Pyörälä *et al.*(2). Castellani *et al.*