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Reaction of Heterozygous Swiss Mice to Implantation of C_3H Myeloma X5563.* (32319)

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Myeloma X5563, which originated spontaneously in the cecum of a C_3H/HE mouse, is readily transplanted to mice of this strain (1) with practically 100% takes and uniformly progressive growth. This report deals with the reaction of Swiss mice to allogeneic implantation of this tumor.

Materials. Tumor-bearing C_3H mice were kindly furnished by Dr. George D. Sorenson. C_3H mice were obtained from the Jackson Memorial Laboratories, and random-bred white Swiss mice from a local dealer. All mice were males, 6-7 weeks old when tumors were implanted. They were caged in groups of 5-7, and given Purina laboratory chow and water *ad lib.*

Methods. Implantation. Viable appearing tumor tissue was minced in phosphate buffered saline, and selected fragments with an estimated volume of about 0.1 ml were placed subcutaneously in the right mid-dorsal area, using a 10 gauge trocar. (Trypsin-dispersed cells gave less uniform results.) Fragments from a particular tumor were usually transferred to two genetically different groups of mice: for example, a C_3H tumor to 6 C_3H and 6 Swiss; a tumor from a Swiss mouse to 7 Swiss and 6 C_3H, etc., until significant data for each of the 4 permutations became available (Table I). For implantation, tumors with a volume of 1.5 to 2.0 ml were used; these tumors were excluded from the data since their ultimate fate was unknown.

Progressively enlarging tumors were left undisturbed until their hosts became moribund or died with large (8-15 g) tumors. When rejection occurred, mice were maintained for 20 weeks unless death occurred sooner.

Histology. Selected tumors in C_3H and Swiss mice, and the rejection sites in Swiss mice, were studied by routine formalin fixation, paraffin embedding, and staining by hematoxylin-eosin and by the Giemsa method.

Electrophoretic studies of sera obtained by snipping the tails were carried out in normal, tumor-bearing, and tumor-rejecting Swiss mice, using the cellulose acetate strip method, as well as a Canalco disc electrophoresis apparatus (Model 200 power plant).

Results. From Table I it is seen that implants from C_3H to Swiss and from Swiss to Swiss mice, resulted in 47% and 44% takes, respectively, in contrast to 99+% takes with syngeneic implantation. About 40% of the tumors developing in Swiss mice were rejected after reaching an estimated volume of 0.5 to 4.5 ml. When returned from Swiss to C_3H mice, progressive tumor growth was noted in 100% of 74 mice. The latent period of tumor development was prolonged in the Swiss mice.

Rejection was most often manifested by blackening and extrusion of dried necrotic tumor tissue (Fig. 1) followed by rapid repair by granulation. Less often (with deeper tumors) internal resolution occurred, with unbroken skin. Small secondary tumors sometimes appeared adjacent to or near the rejection sites, but these in turn were always rapidly rejected. Tumors in Swiss mice larger than about 4.5 ml were not rejected, but grew progressively until death occurred.

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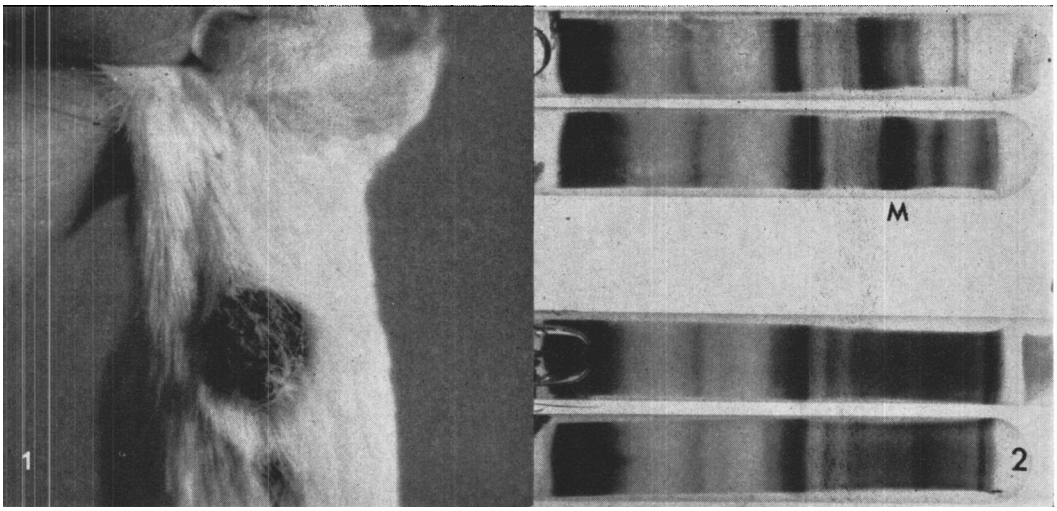

FIG. 1. A Swiss mouse starting to reject a blackened dry necrotic tumor. A smaller recurrent tumor below the main tumor is also being rejected. This mouse later developed runt disease and died.

FIG. 2. Pattern by disc electrophoresis of sera from a Swiss mouse carrying a large tumor (2 upper gel columns-M=myeloma globulin) and from a Swiss rejecter which later became fatally runted (2 lower columns).

TABLE I. Results of Syngeneic and Allogeneic Implantation of C_3H Myeloma X5563.

Donor	Recipient	Total mice	% with tumors*	Latent period	Rejection of tumors
C_3H	C_3H	104	99+	5-12 d.	0
C_3H	Swiss	98	47	8-22 d.	18
Swiss†	C_3H	74	100	6-12 d.	0
Swiss	Swiss†	58	44	8-16 d.	11

* Tumors 0.5 ml or larger.
† One to 5 transplant generations.

In one experiment, very large C_3H tumor implants (about 0.6 ml) gave takes in 8 out of 8 Swiss mice, without prolongation of the incubation period. (These mice are not included in Table I.)

End results in 29 Swiss rejecters (Table II). Though free from tumor, 25 of the 29 rejecting mice died 3-7 weeks later of "wasting syndrome," characterized by weight loss, ruffled fur, hunched posture, and marked atrophy of the spleen, thymus, and lymphoid tissue. The spleen was often represented by a thread-like structure 1.0-1.5 cm in length and less than 1 mm in greatest diameter. (Swiss mice in which tumors grew progressively until death did not show evidence of runting.) In 3 animals the spleens were 5-8 times normal size, and hyperplastic lymph nodes from 4-6 mm in diameter were present in axillary, inguinal and mesenteric regions.

TABLE II. End Results of 29 Swiss Rejectors.

Death in 2-8 wk	25
Survival in good health (20 wk)	1
Wasting syndrome (see text)	24
Amyloid in liver and kidneys	3
Atypical lymphoid hyperplasia	3*
Evidence of infection	0

* These mice did not show wasting, but were sacrificed for study.

Swiss mice not developing tumors. Most of the mice in this group were eliminated after 28-36 days. When one mouse was found to be runted on the 30th day, 26 apparent non-reactors were observed for longer periods, and in 6 of these a wasting illness, clinically and pathologically identical with that described in the rejecters, was observed. The runting was noticed on the 30th to 48th days, and death occurred 2-5 weeks thereafter.

Histology. Progressively growing tumors in C_3H and in Swiss mice were comparable in every way, being composed of neoplastic plasmacytes as described by others(1). Sites of tumor rejection showed necrotic material with "ghost" cells, surrounded and infiltrated by many lymphocytes, neutrophiles, mast cells and normal plasma cells. Two of the 25 mice with "wasting syndrome" showed extensive amyloid deposition in liver and kidneys, and one in the liver only. The atrophic spleens showed absence of follicles, and almost complete absence of lymphocytes from pulp. Thymic tissue, when identified, was largely replaced by adipose tissue.

The greatly enlarged lymph nodes and spleens found in 3 animals showed marked atypical hyperplasia, considered to be non-neoplastic. A leukemoid reaction was present in the liver of one of these 3, the infiltrating cells appearing to be large lymphocytes rather than plasmacytes. Microscopic evidence of bacterial infection was not seen in any of the mice studied.

Serum electrophoresis. Cellulose acetate patterns of sera from the Swiss rejecters showed, as compared with control mice, greatly elevated levels of globulins which migrated throughout the 7S gamma area. Characteristic results from disc electrophoresis are seen in Fig. 2. Heavy homogeneous myeloma globulin bands are noted in two separate runs from a tumor-bearing Swiss mouse. In the Swiss rejecter, this myeloma protein band had largely or entirely disappeared, and there was markedly increased deposition, both diffuse and zonal, in the 7S gamma area.

Discussion. The results suggest that the transplants growing in the Swiss mice are composed of C_3H myeloma cells, which are not genetically altered by residence in Swiss mice, since after 5 transplant generations there was no evidence of adaptation to this strain. Five passages in Swiss mice did not alter the syngeneic preference(2) manifested by the C_3H strain in which the tumor originated. The reduced incidence and frequent rejection of the tumor in Swiss mice is presumably an example of allogeneic inhibition(2) which apparently was overcome in one experiment by massive implantation.

The wasting syndrome, noted in 25 of the 29 Swiss rejecters was similar to that seen in graft-*versus*-host reactions, and this view was supported by histologic studies.

The gamma globulins, which were massively sedimented in association with tumor rejection, may represent antibody formation against the myeloma globulin or against some other component(s) of the tumor cells which are recognized as "foreign."

After completing these studies, our attention was called to a report by Frenkel *et al* (3) who found that 45.7% of rats neonatally conditioned with spleen homogenates from C_3H mice showed "runting syndrome" when implanted with neoplasm X5563, although the tumors were small and transient. Unconditioned rats showed neither tumors nor runting. Gel diffusion studies by these workers suggested that the globulins present in these "runts" originated largely from X5563 globulins.

Summary and conclusion. Random-bred Swiss mice reacting to implants of C_3H myeloma X5563 showed 46% takes, of which 40% were rejected after reaching a size of 0.5 to 4.5 ml, while 60% grew progressively. In C_3H mice, progressive growth of 99+% of the implants was noted and rejection was never seen. Adaptation apparently did not occur after 5 transplant generations in Swiss mice. When tumor tissue was returned from Swiss to C_3H mice, progressive growth always occurred. Most of the Swiss rejecters, though free from tumor, synthesized greatly elevated levels of 7S gamma globulins, and died in a few weeks from "wasting syndrome." Some mice in which palpable tumors did not develop also showed fatal runting. Three rejecters developed massive atypical lymphoid hyperplasia. It is suggested that these variable reactions may result, in part, from the heterozygous nature of the Swiss colony.

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Partial Isolation and Perfusion of Rat Submaxillary Gland.* (32320)

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Salivary gland separated from the systemic circulation and perfused with substituted fluid has provided a useful system for study of electrolyte secretion in dog(1) and cat(2-4), even though maintenance of the perfused gland in a normal functional state is frequently difficult to achieve. Perfusion of salivary gland in rat provides even greater technical difficulties. However, because of the potential usefulness of a method for separate perfusion of rat salivary gland, the possibilities of accomplishing this were investigated, using submaxillary gland. It was found that, although maintenance of functional and structural integrity in the completely isolated gland perfused with artificial serum was not readily feasible, the gland could be well maintained during partial isolation and artificial perfusion. The method involves the addition of an infusate of fortified Ringer's solution to the normally existing arterial supply to the submaxillary gland and deflection of the venous effluent from its normal return to the systemic system. Glands thus perfused were maintained for long periods in apparently normal structural and functional state.

Materials and methods. Adult male rats of the Long-Evans strain, 300-380 g in weight, were anesthetized by the intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The submaxillary gland was exposed through a midline neck incision and the external maxillary artery was located between the masseter and the anterior belly of the digastric muscles, distal to the origin of the glandular artery as schematically shown in Fig. 1. The vein emerging from the hilus of a gland was dissected free at the point where it unites with the anterior facial vein. The sublingual gland was eliminated from the preparation by ligation at its hilus. Just prior to cannulation of the artery, heparin (5 mg in 0.5 ml of isotonic NaCl) was administered intravenously through a cannula in the femoral vein. The external maxillary artery was then cannulated just distal to the origin of the glandular artery, using polyethylene tubing of 0.6 mm outside diameter (Clay-Adams PE 10). The glandular and femoral veins were cannulated with polyethylene tubing of 1.0 mm outside diameter. The infusate consisted of Krebs-Ringer bicarbonate solution containing 4% bovine albumin. A syringe-type infusion pump was used to deliver the infusate at a constant

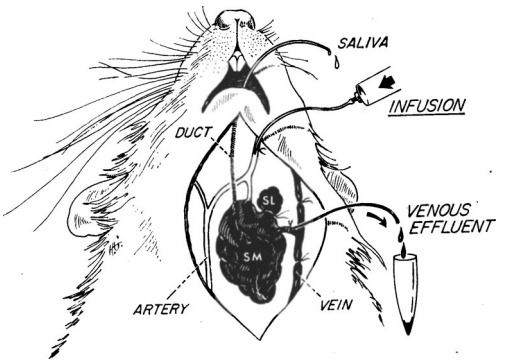

FIG. 1. Schematic representation of rat submaxillary gland (SM) prepared for infusion. Polyethylene tubing is inserted into the oral opening of the submaxillary duct for collection of saliva, into the external maxillary artery for infusion, and into the glandular vein for collection of venous effluent. The sublingual gland (SL) is ligated at the hilus.

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