Tolerance in Pure Strain Newborn Mice to Tumor Homografts.* (22373)

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Owen(1) reported that fraternal twin cattle, having a common placental circulation, have as adults 2 blood cell types; their own and that of their twin. Since red blood cells have a limited life span he postulated transfer of red cell precursors between the fetuses, which cells failed to undergo homograft rejection and persisted to propagate red cells of their own immunologic type. Dunsford *et al.* (2) observed a similar phenomenon in humans. On the basis of these observations and certain theoretical considerations, Burnet and Fenner(3,4) predicted that animals exposed to antigens during fetal life would be forever incapable of a specific immunological response to these antigens. However, they were unable to confirm this prediction using the chick embryo as the test animal. Following the lead of Owen, Anderson *et al.*(5) and Billingham *et al.*(6) established that fraternal twin cattle would accept reciprocal skin homografts but would reject skin homografts from siblings, mother, or other twin cattle. Billingham *et al.*(7) produced a similar state in mice, which they defined as "acquired tolerance" to subsequent homotransplantation, by the injection of cells (spleen, kidney, testes) from one inbred strain of mice (prospective donors) into members of another inbred strain (prospective recipients) during fetal life. The latest time in embryonic life at which this state could be produced was a day or two before birth. Injection of cells at or shortly after birth did not regularly establish acquired tolerance and there appeared to be a "null period" at birth during which such foreign cells produced neither "acquired tolerance" nor immunity(8). Woodruff(9) studied this phenomenon in rats and ascertained that in these animals "acquired tolerance" could be produced in the neonatal period up to 2 weeks after parturition. He established that the capacity to develop "acquired tolerance" was not lost abruptly but gradually during the newborn period. Studies by Hanan and Oyama(10), Dixon and Maurer(11), Cinader and Dubert(12), and Smith and Bridges(13) suggest that a similar capacity to develop "acquired tolerance" to simple protein antigens also features the neonatal period in rats and rabbits. It has been established that the rejection of tumor homografts in inbred strains of mice is a function of the genetics of histocompatibility and appears to be identical in mechanism to rejection of normal tissue homografts(14,15). "Acquired tolerance" to lymphoma has been produced by the injection of tumor cells as blood and spleen cells during embryonic life in homologous mice.(20).

It is the purpose of this communication to present observations on the behavior of mammary cancer homografts in inbred strains of mice during the newborn period. We have found it possible to effect successful homotransplantation of strain-specific tumor between each of three highly inbred strains of mice if the tumor transplants are made during the first 3 hours of life.

Material and methods. Mice of the "A", "Z" (C3H) and "Ce" strains were used. Transplantable mammary adenocarcinomas which arose spontaneously in the "Z" (C3H) and "A" strains were selected. The "Z" (C3H) tumor had been retransplanted 55 times and the "A" tumor 41 times, in their respective lines. Each tumor grows only in its specific strain. Fresh tumor suspensions were prepared at 5.0% concentration in saline, using a Potter Elvejhem glass homogenizer. A subcutaneous injection of 0.05 cc was made under the nape of the neck in newborn mice (0-3 hours old) of an homologous strain. In the earlier experiments these sites

* Aided by grants from Minnesota Division of American Cancer Society, and the U.S.P.H.S.

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TABLE I. Growth of Tumor Homografts in Inbred Strains.

Tumor strain	Host strain	No. of mice treated with .05 ml 5% tumor suspension	Mice growing foreign tumor No.	%
Z (C3H)	Newborn A	17	9	53
Z (C3H)	Newborn Ce	15	12	80
A	Newborn Z (C3H)	8	8	100
Z (C3H)	Mature A	9	0	0

TABLE II. Histocompatibility Characteristics of "Z" Tumor Grown in "A" Mice.

Strain	No. mice treated with .25 ml 5% "Z" tumor growing in "A" mice	No. taking tumor
A	9	0
Z (C3H)	9	9

leaked. To prevent the possible loss of tumor tissue, subsequent injection sites were covered with collodion. All mice were weaned at one month of age.

Results. The results as tabulated in Table I reveal that the majority of newborn "A" mice accepted "Z" (C3H) tumor. These tumors grew progressively and resulted in death of the animals 1.5 to 2.0 months after injection. At that time lung metastases were invariably present.

Groups of "Z" (C3H) mice injected with "A" tumor and "Ce" mice injected with "Z" (C3H) tumor also accepted the tumor transplants in the neonatal period. On the other hand, attempts at homotransplantation of "Z" (C3H) tumor into mature "A" mice uniformly failed.

To determine whether these tumor homografts retained their histocompatibility characteristics following transplantation and growth in a foreign host, the following experiment was performed. A portion of a "Z" (C3H) tumor growing in a newborn "A" was excised, a 5% tumor suspension prepared and 0.25 cc injected into the right groin of each of 9 adult "A" strain and 9 adult "Z" (C3H) mice. The tumor took in all "Z" (C3H) mice but in none of the "A" mice. This observation demonstrates that the tumor remains a "Z" (C3H) tumor and retains its normal genetic and transplantation characteristics.

Discussion. These experiments show that mammary carcinoma, highly specific for a given strain of mice, may be successfully homotransplanted into an otherwise resistant strain if transplantation is carried out within the first few hours after birth. The mechanism permitting this successful tumor homotransplantation is not yet clear to us, in view of Billingham's failure to produce acquired tolerance to skin homografts in mice by injecting cells from the prospective donor in the newborn period.

It is possible that a degree of tolerance may be achieved by early transplantation, sufficient to permit the tumor to establish itself, pass through a "critical period" and then grow progressively. Woodruff(9) found that some rats accepting initial homotransplants of skin after prior treatment with homologous suspensions of spleen, failed to accept a second homotransplant. These data were interpreted as evidence for the existence of a "critical period"(16). However, the concept of the neonatal period as a "null period" of immunological unresponsiveness may be sufficient to explain our observations. During this "null period" one might anticipate that tumor homotransplants would take, establish themselves, and possess sufficient growth potential to keep a step ahead of the developing immunological forces. A period of immunological unresponsiveness is known to occur in many newborn animals as well as humans (17-19).

A third explanation is that both "acquired tolerance" and the relative immunological unresponsiveness of the newborn have operated to produce the reported results. One might even postulate that the former is a function of the latter.

Summary. Homotransplantation of "Z" (C3H) strain mammary adenocarcinoma to "A" and "Ce" strain mice and "A" strain tumor to "Z" (C3H) strain mice has been accomplished by injecting these tumors within a few hours after birth. After progressive growth in the "A" strain host the "Z" (C3H)

tumors retain their histocompatibility characteristics and can be transplanted into adult "Z" (C3H) strain mice but are rejected by adult mice of the "A" strain.

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Received April 3, 1956. P.S.E.B.M., 1956, v92.

Effect of Calcium Disodium Ethylenediamine Tetraacetate on Hypercholesterolemic Rabbits (22374)

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The possible mediation of atherosclerosis by the salts of ethylenediamine tetraacetic acid (EDTA) has been explored recently from the standpoint of its effect on cholesterol metabolism(1,2). Curran has demonstrated also that the incorporation of C-14-carboxyl labeled acetate into cholesterol is increased in surviving liver tissue by the addition of 0.0001 M concentration of both the sodium and calcium forms of EDTA(3). It has been reported by Uhl *et al.* that a high cholesterol diet containing calcium EDTA resulted in lower serum cholesterol levels in rabbits than when the compound was administered as the sodium salt subcutaneously(4). In a recent report a contrary view has been advanced(5). Dietary induced hypercholesterolemia in rats was augmented by the inclusion of EDTA compounds in the diet. Further evidence was presented which indicated that parenterally administered EDTA was without effect on endogenous or exogenous cholesterol metabolism in the rat. The present study was carried out to determine whether parenteral calcium disodium EDTA would influence the course of dietary hypercholesterolemia in rabbits.

Materials and methods. Eight rabbits approximately 6 months old and of both sexes of the New Zealand white albino strain were maintained on a high cholesterol diet and water *ad libitum.* The cholesterol diet was prepared by an adaptation of the method of Wang(6) by dissolving one gram of cholesterol (Fisher) in 5.0 ml of ethyl ether (reagent grade, C. P.) and mixing with 100 g of Gleco rabbit pellets. The animals were fed 100 g of diet per day, 6 days per week. After 11 weeks on the high cholesterol diet, the

* From thesis submitted in partial fulfillment of requirements for Master of Science degree, Department of Chemistry, Georgetown University, Washington, D.C.