steroids used may be in part indirectly mediated through the hypophysis and certainly would involve synergism with endogenous hypophyseal hormones(7). Inasmuch as nodules are generally identified for experimental studies in our laboratory by nature of their secretion of milk in response to a lactogenic hormone mixture, it is important to note that use of this response does not mean that we are selecting nodules with greater tumor-forming ability.

It would be incorrect to conclude that there is no relation between hormone sensitivity and neoplastic potential, but only that this particular criterion—the response of nodules to steroids in terms of milk secretion—indicates no particular relation, direct or inverse. It is possible that the degree of responsiveness of the nodules *in situ* to hormones and their tumor-forming ability are two independently variable characteristics; this would be in accord with studies on outgrowths from transplanted nodules, where there is no indication of a relation between degree of hormone sensitivity and frequency of tumor formation(8). Other properties of nodule outgrowths, including histologic architecture, secretory activity, growth rate, and chromosome number(5,9) also appear to be variables which may have no direct relation to neoplastic potential. Accordingly, the same may hold true for many properties of primary nodules *in situ*: morphology, mitotic index (Banerjee, unpubl.), dependence on insulin for glucose utilization (Moretti, unpubl.) and, as shown herein, degree of responsiveness to lactogenic steroids.

Summary. Transplantation of mammary hyperplastic alveolar nodules showing various degrees of milk secretion as a result of prior treatment with lactogenic steroids revealed no relationship between hormone sensitivity and tumor-forming ability.

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Methods for Reducing Duration of Parabiosis in Development of Tolerance to Skin Homografts in Mice. (28192)

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Parabiosis has been shown to induce tolerance of homologous skin grafts between inbred mice(1,2,3,4). This apparently results from a continuous transfer of all transplantation antigens, presumably in the form of intact cells(2,3,4,5,7,8). It has been shown that the parabiotic state must be preserved for a critical length of time to obtain tolerance of homologous tissue(7,8). This period appears to vary among strains and it is also dependent on the degree of histocompatability difference between the parabionts(7). If the

TABLE I. Effect of Talc Treatment and Skin Flap Parabiotic Techniques on Tolerance Production.

Combination	No. of pairs	Duration of parabiosis	Deaths during parabiosis	Deaths within 10 days from time of graft	Deaths after 10 days	No. of takes	No. of grafted long term survivors	%
		(days)	(pairs)					
Group I								
$C_3H:(AxC_3H)F_1$	18	8-10	2	4 C_3H	0	0	12	0
Group II								
$C_3H:(AxC_3H)F_1$	26	8-10	6	8 C_3H	1 C_3H (38 days)	3	12	25
Group III								
$C_3H:(AxC_3H)F_1$	10	8-10	1	2 C_3H	1 C_3H (20 days)	6	7	85.7

rate of transfer of transplantation antigens between parabionts could be increased it was hoped that exposure time could be reduced. Parabiosis between C_3H and (Ax C_3H)F_1 hybrid mice normally requires 20-30 days before tolerance becomes manifest(7).

In this study mechanical abrasion, talc powder and formation of large skin flaps were used to increase the vascular intercommunications between parabionts.

Methods. C_3H and (Ax C_3H)F_1 mice, 6-8 weeks old, weighing 18.0-24.0 g were put into parabiosis under intraperitoneal pentobarbital anesthesia by joining the peritoneum and the skin(2,3,4,5,6). Skin grafting was done immediately after termination of parabiotic union, the latter lasting 8-10 days.

The technique of skin grafting was standardized in all animals. A piece of full thickness skin measuring 1.5 cm × 2.0 cm was taken from the abdomen of the donor mice and transferred to a recipient area in the back of the C_3H host. The skin graft was secured by means of interrupted sutures of 5-0 silk after being turned 180° in relation to the host, so that the hair in the graft will grow in an opposite direction if the graft survives. This facilitates observation and determination of "take" or rejection. Each animal was placed in individual plastic cages throughout the observation period.

Three groups of mice were used. In the first or control group, 18 C_3H mice were joined by the above method to the same number of (Ax C_3H)F_1 mice and grafted with (Ax C_3H)F_1 skin upon termination of parabiont union. In the second group of animals consisting of 26 pairs, the same technique of union was used except that talc powder was sprinkled on the subcutaneous tissue and mechanical abrasion was employed. In the third group or "pre-treated" group of 10 pairs, talc powder was applied to the subcutaneous tissue through a lateral longitudinal incision 6-7 days prior to parabiosis. At time of parabiosis, in addition, skin flaps were created to increase the area of contact. Creation of skin flaps was accomplished by fashioning one skin flap from the dorsum of one animal and another flap from the ventral aspect of the other parabiont.

Results. Results are summarized in Table I. Of the 18 control pairs, 2 died during the period of parabiosis. Sixteen of these control animals were grafted and not one of the surviving 12 mice showed tolerance. In the second group, 26 pairs were joined initially and 6 pairs died during the period of parabiosis manifesting loss of weight and emaciation. Eight C_3H mice died within 10 days after grafting. Three of the remaining 12 C_3H grafted mice became tolerant (25%). One died 38 days after grafting with the graft showing no signs of rejection.

In the third group, one pair of animals died during the time of parabiosis and 2 C_3H mice died within 10 days after grafting. Six C_3H (85.7%) out of 7 surviving mice grafted were tolerant to (Ax C_3H)F_1 skin. One C_3H died of unknown cause 20 days after grafting with the homograft apparently viable. All of the tolerant mice have been observed for at least 3 months and there have been no indications of graft.

During this study, specifically at the time of separation and grafting, numerous dilated

and tortuous blood vessels were observed regularly in the subcutaneous layer of mice that belonged to Groups II and III in contrast to those in Group I.

Discussion. It has been repeatedly shown that acquired immunological tolerance of homologous skin grafts can be induced in adult mice of the parent strain by means of parabiosis with the F_1 hybrid(2,3,4,7). In previous studies, investigators have reported that the duration of parabiosis is very critical in the development of tolerance. It is obvious that sufficient time must be allowed for the exchange or transfer of antigens and development of immunologic tolerance(7). The antigen-dosage time relationship has been adequately shown to be critical in determining whether immunologic tolerance will be obtained or not(7).

This study suggests that if the antigen dosage or transfer of transplantation antigens between parabionts can be increased, the duration of parabiosis necessary for development of tolerance can be significantly reduced. This was accomplished by methods which promoted vascular communication between parabionts.

Summary. Using C_3H and (Ax C_3H)F_1 mice in parabiosis, this study was undertaken to find means of reducing the duration of parabiosis necessary for development of immunologic tolerance. Parabiosis in the usual way maintained for 8-10 days did not result in development of tolerance. When talc powder was sprinkled and mechanical abrasion employed, 25% of the grafted C_3H mice became tolerant to the hybrid tissue. If talc powder was applied 6-7 days prior to parabiosis and the area of contact between parabiosis increased by creation of skin flaps, 85.7% of grafted C_3H mice became tolerant to (Ax C_3H)F_1 skin.

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Effect of Parathyroid Hormone Administration on Bone Composition in Guinea Pigs.* (28193)

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In view of the role of collagen in nucleating mineral deposition(1), and the influence of polysaccharide on mineralization(1,2), the metabolism of bone matrix components apparently plays a fundamental role in normal and abnormal bone physiology. Several studies have shown an effect of parathyroid hormone on bone matrix metabolism which might be responsible for mineral mobilization. Johnston and Deiss reported a decrease in bone hexosamine concentration after parathyroid hormone administration to rats, suggesting a fall in matrix polysaccharide concentration(3). Hexosamine, however, is present in many proteins and polysaccharides, and its concentration is not a reliable measure of changes in the primary polysaccharide component of bone, chondroitin sulfate(4). Johnston, Miner, and Deiss(5) reported a suppression of hydroxyproline synthesis and stimulation of hexosamine synthesis by parathyroid hormone, support-

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