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# Mesenchyme-mediated Effect of Testosterone on Embryonic Mammary Epithelium[1]

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## Abstract

**The embryonic mammary rudiment of the mouse responds to testosterone with the formation of a condensation of mesenchymal cells around the gland bud and subsequent necrosis of the gland epithelium. Experimental combinations of epithelium and mesenchyme of this rudiment, by taking advantage of the androgen-insensitive mutant (*Tfm*, "testicular feminization"), have shown that the hormone acts directly on the mesenchyme, its effect on the epithelium being indirect and mediated by the surrounding mesenchyme. Only the mesenchymal cells at the epithelial surface are capable of initiating this reaction, and there is no oriented migration of specialized mesenchymal cells to the gland bud in response to the hormone. The mesenchyme forms this condensation only around mammary epithelium; in experimental association the epithelia of the embryonic lung, pancreas, or salivary gland remain unaffected. The experiments suggest further that association of mammary epithelium with "mammary" mesenchyme must exist for some time before hormone action to allow a response.**

**The finding that the destructive action of testosterone on mammary epithelium is mediated by the surrounding mesenchyme and requires organ-specific tissue interaction is discussed in regard to its possible significance for an androgen therapy of metastatic breast cancer (*i.e.*, mammary epithelium in nonmammary mesenchyme).**

## Introduction

The presence of receptors for androgenic hormones has been demonstrated in samples of human breast cancer (4, 14, 17) and in mammary carcinoma cell lines of human (MCF-7; Refs. 6 and 13) and mouse (Shionogi 115; Refs. 2 and 23) origin. Responsiveness to androgens was found in some cases of human breast cancer (1), in human (13) and mouse (2, 23) cell lines, as well as in dimethylbenzanthracene-induced mammary carcinomas of the rat (19, 24). Whereas the response of the rat tissue involved regression of the tumor, the mouse and human cell lines were found to be stimulated by and even dependent on androgens for their growth and maintenance in culture.

Since androgenic hormones have been used with some success in the treatment of human breast cancer (1), an understanding of the mechanism of action of androgens on mammary tissue would appear to be highly relevant for a possible endocrine therapy of breast cancer.

Extensive information is available on intracellular events that take place after steroid application. The action of the hormone is mediated by specific cytoplasmic receptors and involves the translocation of the hormone:receptor complex into the nucleus with subsequent modification of transcriptional activity (7). In our laboratory we are concerned with the intercellular processes that occur within the mammary gland in response to androgenic hormones. The model system is the embryonic rudiment of the gland in the mouse, which has long been known to develop differently in female and male fetuses (22, 25). Experiments *in vivo* have implied that fetal testicular hormones are the causative agents for this sexual dimorphism (20, 21), and we have shown in organ cultures of mammary rudiments that the gland responds directly to androgenic hormones with high specificity (9, 10).

The androgen-induced processes in the mammary gland take place on Day 14 of gestation, *i.e.*, at a time when the rudiment consists only of a small epithelial bud attached to the epidermis by a somewhat thinner "stalk." The first sign of the reaction is the appearance of a conspicuous condensation of mesenchymal cells around the epithelial bud. As this condensation progresses the stalk of the epithelial rudiment becomes stretched and finally ruptures, separating the mammary epithelium from the epidermis. This detached piece of gland epithelium, as well as the portion of the stalk connected to the epidermis, then undergoes massive necrosis (22). A more detailed description of the cellular events in response to androgenic hormones will be given elsewhere.[3,4]

From histological observations it is obvious that both tissues, gland epithelium and surrounding mesenchyme, are involved in the androgen-induced reaction (Fig. 1). However, the fact that testosterone was shown to act directly on the rudiment (in culture) does not necessarily imply that it also acts directly on both of its tissues. The gland could only have one target tissue for the hormone, which then influences the other component through processes referred to as "tissue interaction." The availability of an androgen-insensitive mutant of the mouse [*Tfm* for "testicular feminization" (16)] makes it possible to identify the tissue responding to the hormone.

## Materials and Methods

Normal mouse embryos were hybrids of BALB/c × C3Hf matings. The propagation of the X-chromosome carrying

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the *Tfm* mutation in a separate mouse colony (derived from 3 pairs donated by Mary F. Lyon) has been described (12). Only $X^{Tfm}/Y$ hemizygous embryos are androgen-insensitive, and these were distinguished from normal $X^+/Y$ males by testing 3 mammary rudiments of each embryo for androgen sensitivity. $X^{Tfm}/X^+$ heterozygous female embryos (derived from the same carrier females as $X^{Tfm}/Y$ embryos) could be distinguished from $X^+/X^+$ littermates by the incomplete response of their mammary glands to testosterone.

Dating of embryos and the organ culture of mammary rudiments has previously been described in detail (8), as well as the androgen response of intact glands *in vitro*. For the recombination technique see Ref. 12. Clean separation of epithelium from mesenchyme was carried out after 30 min incubation in a solution of 2.25% trypsin (1:250) plus 0.75% pancreatin (N. F., both Difco Laboratories, Detroit, Mich.). Mammary epithelia with few adhering mesenchymal cells were obtained by careful peeling of the skin of 12- or 13-day embryos (without previous enzymatic treatment). Mesenchyme-free epithelia of 11-day pancreas, 11-day lung, and 13-day submandibular salivary gland were obtained after a 5- to 10-min incubation in the same trypsin: pancreatin solution (5).

The androgen response of mammary rudiments as well as of experimental tissue combinations was observed in living explants and further documented in histological sections after glutaraldehyde fixation and embedding in Epon.

## Results

**Identification of the Target Tissue for Testosterone.** Explanted mammary rudiments (epithelium with surrounding mesenchyme) of normal mouse embryos respond to 1 nM testosterone in the medium (9). Histologically, this response *in vitro* is identical to the appearance of affected mammary rudiments in male fetuses. In contrast, glands of androgen-insensitive ($X^{Tfm}/Y$) embryos remain entirely unaffected even with 10 μM testosterone.

Experimental tissue combinations consisted of either (*a*) androgen-insensitive ($X^{Tfm}/Y$) gland epithelium and wild-type (BALB/c × C3H) mesenchyme or (*b*) wild-type epithelium and androgen-insensitive mesenchyme. Since $X^{Tfm}/Y$ embryos could not be distinguished from their $X^+/Y$ littermates at the stage of tissue separation and combination, we also prepared combinations of (*c*) $X^+/Y$ epithelium with BALB × C3H (wild-type) mesenchyme and (*d*) wild-type epithelium with $X^+/Y$ mesenchyme.

After 3 days in culture with medium containing 0.1 μM testosterone, we recovered 49 glands of the combination type *a*, 33 of which showed a typical androgen response despite the fact that the gland epithelium was from an androgen-insensitive embryo. In contrast, under the same conditions all 49 glands of the combination type *b*, containing androgen-insensitive mesenchyme, remained unaffected by the hormone. Both types of the combination involving tissues of $X^+/Y$ embryos yielded a reasonable percentage of responding glands, thus ruling out strain differences independent of the *Tfm* locus as the reason for the failure of combination *b* (Table 1).

This result shows that the response of an experimental tissue combination requires androgen-sensitive mesenchyme and that the genotype of the epithelium is irrelevant. We assume also that, in the intact gland, testosterone acts only on the glandular mesenchyme, which then, in a yet unknown fashion, causes epithelial necrosis (12).

Table 1

*The androgen response of 4 types of epitheliomesenchymal combinations*

The $X^{Tfm}/Y$ tissues are androgen-insensitive. All other tissues [$X^+/Y$ or wild type – (wt)] are normal with regard to the *Tfm* locus. Only combination type *b*, containing androgen-insensitive mesenchyme, did not yield a single responsive gland.

| Combination type | Androgen response observed |
|---|---|
| *a*. $\frac{X^{Tfm}/Y\text{-epithelium}}{\text{wt-mesenchyme}}$ | 33/49[a] |
| *b*. $\frac{\text{wt-epithelium}}{X^{Tfm}/Y\text{-mesenchyme}}$ | 0/49 |
| *c*. $\frac{X^+/Y\text{-epithelium}}{\text{wt-mesenchyme}}$ | 13/21 |
| *d*. $\frac{\text{wt-epithelium}}{X^+/Y\text{-mesenchyme}}$ | 26/30 |

[a] Number of respondings glands/total number of successful combinations.

**Origin of the Mesenchymal Cells Involved in the Androgen Response.** Virtually none of the mesenchymal cells exhibiting the condensation characteristic of the early stages in the androgen response showed thymidine incorporation in radioautographs. The mesenchymal condensation therefore arises by accumulation of cells rather than by localized proliferation. We wondered whether this accumulation was a strictly local phenomenon or whether some type of specialized mesenchymal cell would migrate from more distant sites to the gland bud in response to testosterone.

To test the latter possibility, we combined androgen-sensitive (wild type) glands containing only a thin coat (2 to 3 cell layers) of equally androgen-sensitive mesenchymal cells with a large mass of androgen-insensitive (*Tfm*) mesenchyme. All 62 of these combinations responded normally to testosterone, indicating that the mesenchymal cells required to initiate the reaction had already been at the epithelial surface at the time of combination (12- to 13-day stage). On the other hand, an equally thin coat of androgen-insensitive mesenchymal cells around the gland epithelium (of the same developmental stage) prevented the androgen reaction in all 82 glands associated with a large mass of normal, androgen-sensitive mesenchyme. This result also tends to exclude oriented migration of distant mesenchymal cells to the mammary rudiment as the source of the mesenchymal condensation. It appears that the mesenchymal reaction can only be initiated at the epitheliomesenchymal interface.

From the previous experiments it was not possible to deduce whether the entire mesenchymal condensation formed in the first type of combination was composed of androgen-sensitive cells or whether only a thin layer of such mesenchyme at the epithelial surface is required to initiate the reaction, which then spreads into more distant mesenchyme, irrespective of its capacity to respond to the hormone. A close look at the response of explanted glands taken from $X^{Tfm}/X^+$ heterozygous female embryos provided information pertinent to this problem.

Due to the inactivation of 1 X-chromosome at an early embryonic stage, each somatic cell of a female mammal contains only 1 functional X-chromosome (15). Inactivation occurs at random; but, after this occurs, all descendants of a cell have the same active X-chromosome. An X-chromosome-heterozygous female therefore is a mosaic composed of cell clones with different phenotypic properties specified by genes located on the X-chromosome. Consequently, an $X^{Tfm}/X^{+}$ animal consists of cones fully responsive to testosterone and of clones entirely unresponsive to the same hormone (3, 18). In the histological sections of $X^{Tfm}/X^{+}$ glands, we found heterogeneity within the mesenchyme after testosterone application: clusters of condensed cells next to apparently unaffected, loose mesenchyme (Fig. 2). We assume that the cell condensations, which are always in contact with mammary epithelium, represent clones of mesenchymal cells expressing the $X^{+}$-chromosome, whereas unaffected cells would be of *Tfm* phenotype. The presence of such unaffected mesenchymal cells at the epithelial periphery and next to condensing clones argues against the assumption that the testosterone reaction, once initiated, spreads within the mesenchyme independently of the hormone.

**"Recognition" of Mammary Epithelium.** In male fetuses the androgen-induced mesenchymal condensations form only around the mammary buds, and only mammary epithelium becomes necrotic. The adjacent epidermis with the first hair rudiments remains apparently unaffected.

We tested the capability of 12-day mammary (dermal?) mesenchyme to discriminate between mammary and various other types of epithelium by combining it with mesenchyme-free epithelia of the 11-day pancreas and lung and of the 13-day salivary gland. When testosterone was applied to these cultures, no mesenchymal reaction was observed to occur around these heterogenous epithelia. In a second series of experiments, 1 to 5 pieces of 12-day mammary epithelium were placed next to such a heterogenous epithelium on top of mammary mesenchyme. Frequently, the 2 different epithelia fused *in vitro*, but they remained distinguishable in histological sections due to different staining properties. After the addition of testosterone, a mesenchymal reaction (condensation) was found to occur exclusively around mammary epithelium (Fig. 3). No such reaction was seen around the other epithelium (pancreas, lung, or salivary). In the fused epithelia of dual origin, the mesenchymal cells condensed only around the mammary portion.

**Responsive Phases.** We know from earlier experiments that the mammary gland is responsive to testosterone only during a relatively short period of its development, extending from late Day 13 to very early Day 15 of gestation (11). In view of our finding that testosterone acts on the mesenchyme, which then affects the epithelium, this transient responsiveness of the entire rudiment need not necessarily reflect an equally short phase of hormone responsiveness of the mesenchyme. It is also conceivable that hormone-activated mesenchyme "attacks" mammary epithelium only in a certain developmental stage.

We had assumed that experimental combinations of epithelium and mesenchyme derived from embryos differing in their developmental stage ("heterochronic" combinations) would allow us to distinguish between these alternatives. Rather surprisingly, 14-day mesenchyme (*i.e.*, of the responsive stage) did not exhibit an androgen-induced reaction around mammary epithelium of any stage, including 14-day epithelium. Conversely, 14-day mammary epithelium did not elicit a testosterone response in mesenchyme of any stage between 11 and 15 days. Such a response was only seen when neither epithelium nor mesenchyme was older than 12 days at the time of experimental association. Even in "homochronic" combinations, 12-day epithelium associated with 12-day mesenchyme gave a response, whereas combinations of 13- and 14-day tissues yielded mostly negative results.

Heterochronic combinations of 11-day epithelium with 12-day mesenchyme responded to testosterone after 2 days *in vitro*, whereas the reciprocal association of 12-day epithelium with 11-day mesenchyme did so only after 3 days in culture. It appears therefore that the developmental stage of the mesenchyme is decisive for the earliest possible response in such heterochronous combinations.

## Discussion

The recombination experiments with trypsin-isolated, mesenchyme-free epithelium and mesenchyme of normal embryos and of androgen-insensitive embryos suggest that testosterone acts directly on the mesenchyme. Although an additional direct action on the epithelium cannot strictly be excluded, this would seem unlikely in view of the typical androgen response observed in combinations involving androgen-insensitive epithelium. Necrosis of the mammary epithelium as seen in 14-day male fetuses therefore does not represent hormone-induced cell death, but rather it represents hormone-induced cell "killing."

Although we are still ignorant of the mechanism by which testosterone-stimulated mesenchyme causes necrosis of the epithelium, our experiments have provided some information on the initiation of this hormone-induced tissue interaction. From the experiments in which a thin layer of normal mesenchymal cells was left around the mammary bud, which was then combined with androgen-insensitive mesenchyme, it appears that all mesenchymal cells required for the testosterone response are already at the epithelial surface, as early as in 12-day glands. Thus, the hormone does not cause a specialized type of mesenchymal cell to move to the mammary bud, a possibility that is also ruled out by the observed mesenchymal heterogeneity around $X^{Tfm}/X^{+}$ heterozygous glands. If a specialized type of mesenchymal cell migrated to the mammary bud in response to testosterone, these glands would be surrounded only by androgen-sensitive clones. The absence of a testosterone response in combinations in which a thin layer of androgen-insensitive mesenchymal cells separated the epithelium from a large mass of normal mesenchyme also reinforces this conclusion. The result of this experiment also indicates that the mesenchymal reaction can only be initiated at the epitheliomesenchymal interface and suggests that the mesenchymal cells starting the reaction require 2 "signals," testosterone and close vicinity to (or contact with?) mammary epithelium. As we have shown, the mesenchyme is able to distinguish between mammary

epithelium and at least 4 other types of epithelium: epidermis, salivary gland, lung, and pancreas.

We only very rarely observed an androgen response when recombination of mammary epithelium with underlying mesenchyme was done on Days 13 or 14 of embryonic development (*i.e.*, just before or during the responsive phase, respectively). The same experiment done on Day 12 consistently gave positive results. In such recombinations, it is technically impossible to place the mammary epithelia exactly on those few mesenchymal cells that had previously (*i.e.*, before tissue separation) been in contact with a gland bud. In these experiments a new association of mammary epithelium with mesechyme of the mammary region is created. Conceivably, either this association has to persist for at least 48 hr before hormone exposure, or the recognition of mammary epithelium by dermal mesenchyme occurs before Day 13. In any event the result suggests that the mesenchymal cells starting the androgen reaction need to be in close contact with mammary epithelium some time before they become responsive to the hormone.

Our current and rather limited understanding of the androgen response of the embryonic mammary rudiment of the mouse is that this process involves multiple and reciprocal epitheliomesenchymal tissue interaction. First, the epithelial bud exerts some influence on adjacent mesenchymal cells, which seems to be a prerequisite for their (later) response to testosterone. Once exposed to the hormone, these mesenchymal cells condense around the epithelial bud and, through a yet unknown mechanism, cause the eventual destruction of mammary epithelium.

Whether these findings have any relevance for the observed androgen response of mammary carcinomas is at present unclear. The presence of androgen receptors in epithelial cell lines (2, 6, 13) certainly points to the epithelium as the target tissue for the hormone in these cases. Our experiments, however, have shown not only that testosterone can act on the mesenchyme of the gland, but also that its effect on the epithelium is also mediated by this mesenchyme. Obviously, any kind of a comparable androgen-induced tissue interaction in the adult gland or in mammary carcinomas would be of fundamental importance for an endocrine treatment of metastatic breast cancer. Just as in our combination experiments, a distant metastasis of a mammary carcinoma represents a secondary, nonphysiological association of mammary epithelium with nonmammary mesenchyme. Such a situation would undoubtedly alter the nature of the epitheliomesenchymal interaction and thereby affect the response to the hormone.

## References


1. AMA Committee on Research. Androgens and Estrogens in the Treatment of Disseminated Mammary Carcinoma. J. Am. Med. Assoc., *172:* 1271–1274, 1960.
2. Bruchovsky, N., and Rennie, P.S. Classification of Dependent and Autonomous Variants of Shionogi Mammary Carcinoma Based on Heterogenous Patterns of Androgen Binding. Cell, *13:* 273–280, 1978.
3. Drews, U., and Alonso-Lozano, V. X-Inactivation Pattern in the Epididymis of Sex-Reversed Mice Heterozygous for Testicular Feminization. J. Embryol. Exptl. Morphol., *32:* 217–225, 1974.
4. Engelsman, E., Korsten, C. B., Persign, J. P., and Clenton, F. J. Estrogens and Androgen Receptors in Human Breast Cancer. Brit. J. Cancer, *30:* 177, 1974.
5. Grobstein, C. Inductive Epithelio-mesenchymal Interaction in Cultured Organ Rudiments of the Mouse. Science, *118:* 52–55, 1953.
6. Horwitz, K. B., Costlow, M. E., and McGuire, W. L. MCF-7; A Human Breast Cancer Cell Line with Estrogen, Androgen, Progesterone, and Glucocorticoid Receptors. Steroids, *26:* 785–796, 1975.
7. Jensen, E. V., and DeSombre, E. R. Estrogens and Progestins. *In:* G. Litwack (ed.), Biochemical Actions of Hormones, Vol. 2, pp. 215–255. New York: Academic Press, Inc., 1972.
8. Kratochwil, K. Organ Specificity in Mesenchymal Induction Demonstrated in the Embryonic Development of the Mammary Gland of the Mouse. Develop. Biol., *20:* 46–71, 1969.
9. Kratochwil, K. *In Vitro* Analysis of the Hormonal Basis for the Sexual Dimorphism in the Embryonic Development of the Mouse Mammary Gland. J. Embryol. Exptl. Morphol., *25:* 141–153, 1971.
10. Kratochwil, K. Experimental Analysis of the Prenatal Development of the Mammary Gland. Mod. Probl. Pediat., *15:* 1–15, 1975.
11. Kratochwil, K. Development and Loss of Androgen Responsiveness in the Embryonic Rudiment of the Mouse Mammary Gland. Develop. Biol., *61:* 358–365, 1977.
12. Kratochwil, K., and Schwartz, P. Tissue Interaction in Androgen Response of Embryonic Mammary Rudiment of Mouse: Identification of Target Tissue for Testosterone. Proc. Natl. Acad. Sci. U. S., *73:* 4041–4044, 1976.
13. Lippman, M., Bolan, G., and Huff, K. The Effects of Androgens and Antiandrogens on Hormone-Responsive Human Breast Cancer in Long-Term Tissue Culture. Cancer Res., *36:* 4610–4618, 1976.
14. Lippman, M. E., and Huff, K. A Demonstration of Androgen and Estrogen Receptors in Human Breast Cancer Using a New Protamine Sulfate Assay. Cancer, *38:* 868–874, 1976.
15. Lyon, M. F. Gene Action in the X-chromosome of the Mouse (*Mus musculus* L.). Nature, *190:* 372–373, 1961.
16. Lyon, M. F., and Hawkes, S. G. X-linked Gene for Testicular Feminization in the Mouse. Nature, *227:* 1217–1219, 1970.
17. Maass, H., Trams, G., Nowakowski, H., and Stolzenbach, G. Steroid Hormone Receptors in Human Breast Cancer and the Clinical Significance. J. Steroid Biochem., *6:* 743–749, 1975.
18. Ohno, S., Geller, L. N., and Young Lai, E. V. Tfm Mutation and Masculinization *versus* Feminization of the Mouse Central Nervous System. Cell, *3:* 235–242, 1974.
19. Quadri, S. K., Kledzik, G. S., and Meites, J. Enhanced Regression of DMBA-induced Mammary Cancers in Rats by Combination of Ergocornine with Ovariectomy or High Doses of Estrogen. Cancer Res., *34:* 499–501, 1974.
20. Raynaud, A. Effet des Injections d'Hormones Sexuelles à la Souris Gravide sur le Développement des Ébauches de la Glande Mammaire des Embryons. I. Action des Substances Androgènes. Ann. Endocrinol. Paris, *8:* 248–253, 1947.
21. Raynaud, A. Foetal Development of the Mammary Gland and Hormonal Effects on Its Morphogenesis. *In:* I. R. Falconer, (ed.), Lactation. Proceedings, International Symposium, Nottingham, pp. 3–29. London: Butterworth & Co., 1971.
22. Raynaud, A., and Raynaud, J. Les Principales Étapes de la Séparation, d'avec l'Épiderme, des Ébauches Mammaires des Foetus Mâles de Souris; Recherches sur les Processus de la Rupture de la Tige du Bourgeon Mammaire. Compt. Rend. Soc. Biol. *147:* 1872–1876, 1953.
23. Smith, J. A., and King, R. J. Effects of Steroids on Growth of an Androgen-Dependent Mouse Mammary Carcinoma in Cell Culture. Exptl. Cell Res., *73:* 351–359, 1972.
24. Teller, M. N., Stock, C. C., Stohr, G., Merker, P.C., Kaufman, R. J., Escher, G. C., and Bowie, M. Biologic Characteristics and Chemotherapy of 7,12-Dimethylbenz(*a*)anthracene-induced Tumors in Rats. Cancer Res., *26:* 245–254, 1966.
25. Turner, C. W., and Gomez, E. T. The Normal Development of the Mammary Gland of the Male and Female Albino Mouse. I. Intrauterine. Bull. Missouri Agr. Exptl. Sta., *182:* 3–20, 1933.

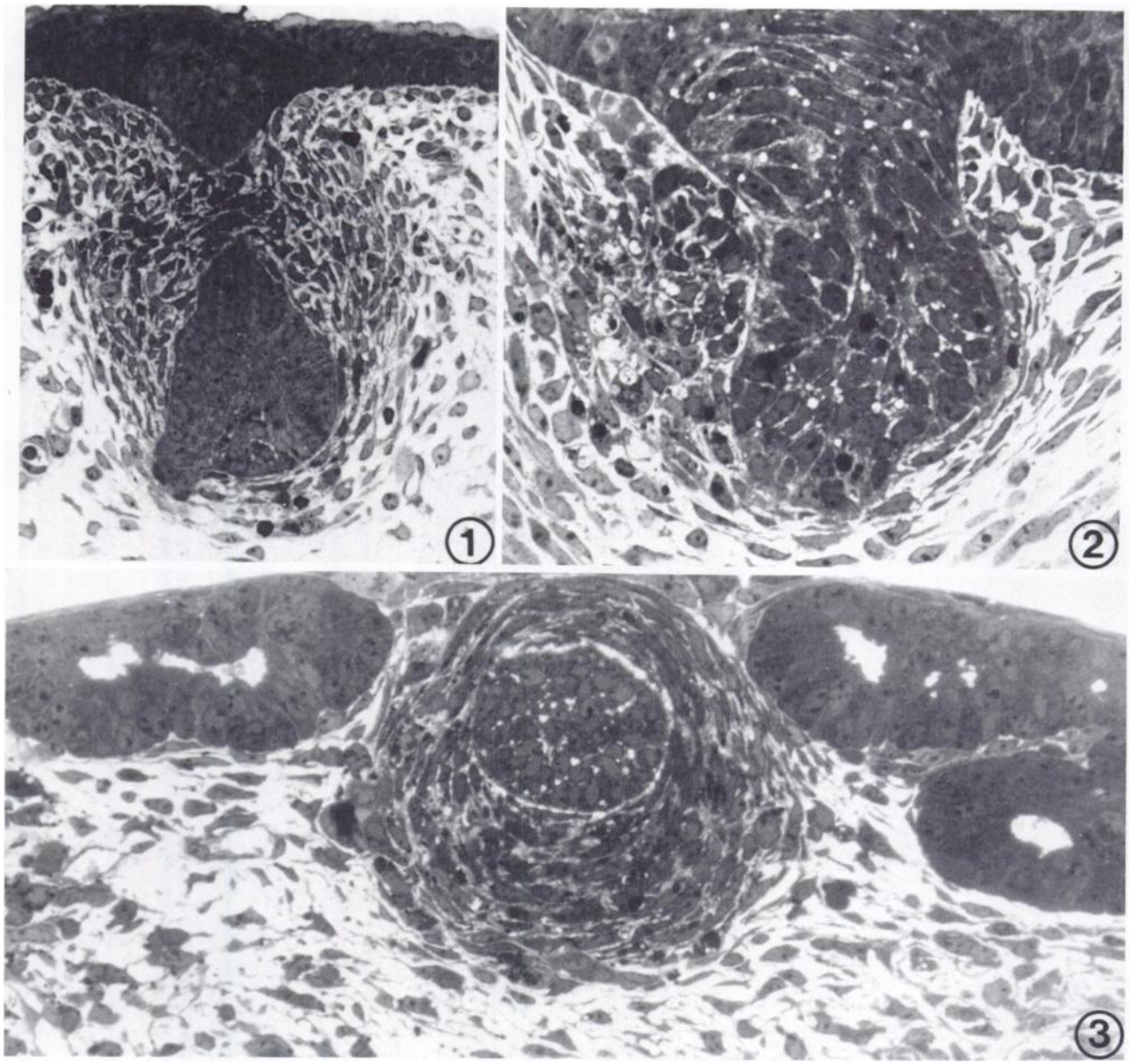


Fig. 1. Mammary gland rudiment of a 14-day male mouse embryo exhibiting the characteristic androgen response. Mesenchymal cells are seen to condense around the epithelial bud, which later separates from the epidermis and becomes necrotic. 1 $\mu$m Epon sections stained with toluidine blue. × 400.

Fig. 2. Mammary rudiment of an $X^{Tim}/X^{+}$ heterozygous female embryo exposed to testosterone for 35 hr in culture. A condensation of mesenchymal cells is seen only on the *left side* of the epithelium, whereas the remaining mesenchyme appears unaffected. It is assumed that the condensing cells represent an $X^{+}$ phenoclone (*i.e.*, being androgen-sensitive), whereas mesenchymal cells on the *right side* are androgen-insensitive due to expression of the $X^{Tim}$ chromosome. 1 $\mu$m Epon sections stained with toluidine blue. × 650.

Fig. 3. Mammary mesenchyme simultaneously combined with mammary epithelium (*center*) and with pancreas epithelium. After 30 hr of testosterone exposure, the characteristic mesenchymal condensation is formed exclusively around mammary epithelium. Pancreas epithelium, even in close vicinity to this condensation, does not provoke such a response. 1 $\mu$m Epon sections stained with toluidine blue. × 600.