Membrane conductance decreased during the late slow EPSP (Fig. 2A) and, as with MCh responses, the magnitude of the conductance decrease varied from cell to cell. The amplitude and duration of the fast EPSP increased during the late slow EPSP (Fig. 2B), and the increased amplitude fast EPSP's often initiated action potential generation (Fig. 2B). In the experiment shown in Fig. 2C, prior to eighth nerve stimulation, no fast EPSP's fired spikes, but after stimulation the fast EPSP's generated spikes for about 2.5 minutes. At the peak of the response, five out of six fast EPSP's fired action potentials, which is a clear demonstration of the facilitation of transmission in one synaptic pathway by activation of another.

In the preceding experiments, when the pharmacological or physiological stimuli produced large decreases in postsynaptic membrane conductance, they increased fast EPSP size. This suggests that the potentiation of synaptic transmission is caused by changes in postsynaptic membrane properties (*18*). Our findings indicate that different types of depolarizations have different consequences for neuronal interaction. That is, conductance increases (*19*) and electrical depolarization of the membrane (*11*) decrease synaptic potentials generated by a given excitatory input, whereas conductance decreases potentiate them. By augmenting, rather than diminishing, a synaptic input, a conductance decrease makes that input more effective in the generation of action potentials.

This control of the efficacy of synaptic transmission may be important in the function of neurons of the central nervous system. Central neurons receive an abundance of converging inputs, and several types of central neurons have conductance-decrease responses (*4, 5*). It is thus possible that increasing transmission in a synaptic pathway by a conductance-decrease response may have a function in information processing in the central nervous system. A synaptically evoked conductance decrease has been implicated in a behavioral response in the marine mollusc *Aplysia* (*8*). Further, lasting modification of responsiveness to a synaptic input might be a mechanism for storage of information at the cellular level. Long-term control of postsynaptic membrane properties might provide such a modification of responsiveness.

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13. Larger conductance decreases were measured in fall and spring than in winter.
14. Different cells produce depolarizations with different size resistance changes. When a large depolarization accompanies a small resistance change, the depressing effect of depolarization on fast EPSP size presumably overcomes the potentiating effect of the resistance increase, resulting in a net decrease of fast EPSP size. For fast EPSP's in the 2 to 4 mv range, increases of amplitude were observed for MCh-induced resistance increases (conductance decreases) of greater than 8 to 10 megohms and decreases in amplitude were observed for resistance increases of 4 megohms or less. With larger fast EPSP's (6 to 8 mv) potentiation could occasionally be observed even for small conductance decreases, perhaps because of activation of local responses. B. L. Ginsborg [*J. Pharmacol. Exp. Ther.* **150**, 217 (1965)] reported that MCh added to Ringers solution bathing the whole ganglion depressed synaptic transmission in these cells. Desensitization of receptors, depression of transmitter release, depolarization of the membrane, and small conductance changes are possible explanations for these findings.
15. Voltage-clamp analysis shows that decay of muscle end-plate potentials is determined by membrane time constant [A. Takeuchi and N. Takeuchi, *J. Neurophysiol.* **22**, 395 (1959)]. Fast EPSP's in ganglion cells of *Rana pipiens* are reported to decay slightly more slowly than electronic potentials generated by square current pulses (*11*).
16. The increased amplitude and duration of the fast EPSP may activate voltage- and time-dependent sodium conductances that may in turn contribute further to the increased duration of the EPSP. Activation of Na^+ conductances might also be triggered by a decrease in voltage-threshold caused by conductance decreases [see M. V. L. Bennett, B. Hille, S. Obara, *J. Neurophysiol.* **33**, 585 (1970)].
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18. Other possible mechanisms seem unlikely for several reasons. First, the potentiation of synaptic transmission cannot be mediated by interneurons because the test pathway is known to be monosynaptic. Second, changes of fast EPSP size depend on the magnitude of the slow conductance change. This argues strongly against the importance of presynaptic effects. In addition, there is no evidence of synaptic endings on synaptic terminals (H. Weitsen and F. Weight, *Brain Res.*, in press). Third, cooperativity in postsynaptic receptor activation seems unlikely, since the late slow EPSP is noncholinergic (*17*).
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Mesenchyme-Dependent Morphogenesis and Epithelium-Specific Cytodifferentiation in Mouse Mammary Gland

Abstract. *Isografts of heterotypic recombinants of embryonic mammary epithelium with salivary mesenchyme undergo development morphogenetically resembling that of salivary gland. However, cytodifferentiation of the epithelium is like that of mammary gland. In lactating hosts these isografts respond to endogenous hormonal stimulation and synthesize a milk protein, α-lactalbumin.*

Many studies have shown that interactions between epithelium and stroma are required for organogenesis and, ultimately, cytodifferentiation to proceed (*1–4*).

To clarify the respective roles of epithelium and stroma in normal morphogenesis and cytodifferentiation, investigations of heterotypic recombinations offer a useful approach. Much has already been done in this area (*2*). Previous studies have tended to support the general principle that mesenchyme largely controls development of form (morphogenesis), while epithelium is the determinant of the type of cytodifferentiation to be expressed. To exemplify, in an early study of feather regeneration Wang (*5*) found

Table 1. Development of 16-day mammary gland epithelia combined with mammary and salivary mesenchyme, 3 weeks after transplantation under the kidney capsule in 4-week-old syngeneic female mice.

Source of mesenchyme	No. of explants	No. of grafts recovered	Developmental response of mammary epithelium
Renal stroma	20	4 (20%)	Degeneration
Mammary (16-day)	18	11 (61%)	Mammary gland-like
Salivary (14-day)	24	18 (75%)	Salivary gland-like

that the dermal component of the feather follicle controlled symmetry and form of the developing feather, while, as might be expected, the follicle epithelium was responsible for feather keratin synthesis.

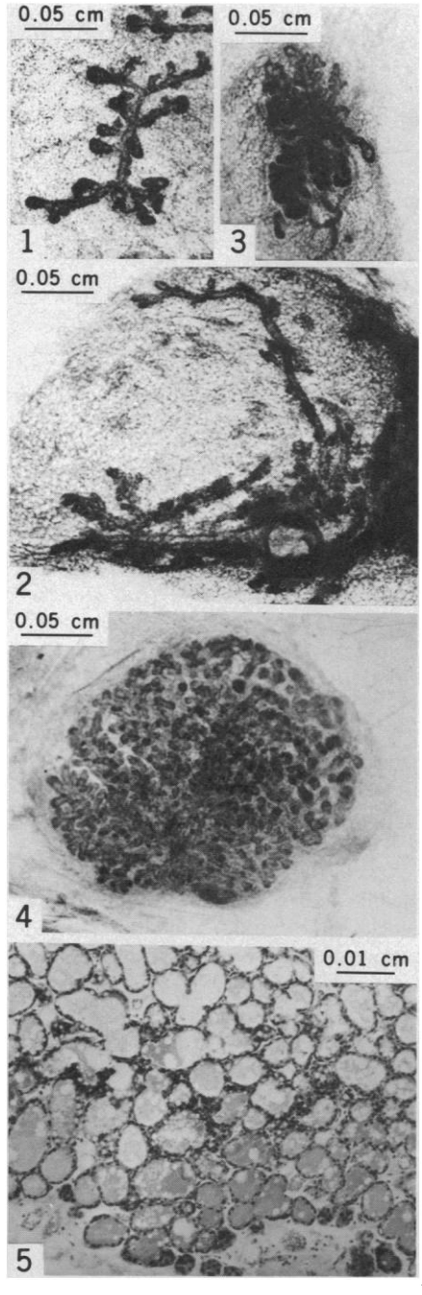
Specificities of various epithelia and mesenchyme vary widely. For example, until recently the evidence indicated that salivary epithelium could not be induced to undergo morphogenesis by any mesenchyme other than salivary (*2*). Cunha has shown, however, that mesenchyme of urogenital tract origin can support morphogenesis in salivary epithelium (*6*). Other epithelia, such as those of thymus, pancreas, and lung, appear to be less specific than salivary epithelium in their mesenchymal requirements (*2*).

There is little or no evidence that epithelium can be induced by heterotypic mesenchyme to synthesize in quantity any product "strange" to the organ from which that epithelial anlage was taken. For example, combinations of pancreatic epithelium with salivary mesenchyme secrete pancreatic enzymes, not salivary enzymes (*4*).

With respect to the mammary gland in particular, Kratochwil (*7*) has shown in vitro that combination of salivary mesenchyme with mammary epithelium results in morphogenetic patterns more closely resembling those of salivary gland than of mammary gland.

Our studies in vivo reinforce Kratochwil's observations regarding morphogenesis. In addition, we report here that at least one enzyme (B protein, or α-lactalbumin) normally present in milk is synthesized in heterotypic combinations of salivary mesenchyme with mammary epithelium subjected to the hormonal milieu of pregnancy and lactation.

Embryonic C3H/HeMs mice were aseptically removed from pregnant females and placed in 10 percent fetal calf serum (Difco) in Hanks solution. The rudiments of the submandibular salivary glands were dissected from 14-day embryos of both sexes (observation of vaginal plug = day 0), and the rudiments of the mammary glands were from 16-day female embryos. Sex of the embryos was determined by inspection of gonads. All rudiment excisions were performed in Grobstein dishes containing 10 percent fetal calf serum in Hanks balanced salt solution at room temperature. The dishes were kept in an atmosphere of 5 percent CO_2 in air. Tryptic separation and recombination of the isolated components were performed by a modification of the method of Grobstein (*1*). Separation of the epithelial and mesenchymal components was achieved under a dissecting microscope by gentle pipetting after incubation in Tyrode's solution containing 3 percent trypsin (Difco) for 10 minutes at room temperature. The isolated epithelial and mesenchymal components were then transferred to 50 percent fetal calf serum in Hanks solution for 2 hours at room temperature. After trypsin deactivation, isolated epithelium and isolated mesenchyme were placed together in a dish containing 50 percent fetal calf serum in Hanks solution and incubated overnight at 37° C in a humidified incubator (5 percent CO_2, 95 percent air), allowing recombination through direct contact.



Figs. 1 and 2. Sixteen-day mammary epithelium recombined with mammary mesenchyme, 7 days (Fig. 1) and 21 days (Fig. 2) after transplantation. Typical mammary morphogenesis with monopodial branching of ducts (×20). Figs. 3 and 4. Sixteen-day mammary epithelium recombined with 14-day salivary mesenchyme, 7 days (Fig. 3) and 14 days (Fig. 4) after transplantation. Extensive growth and compact, dichotomous branching of ducts. Adenomeres typical of salivary gland have developed (Fig. 4) (×20). Fig. 5. Lactation in 16-day mammary epithelium recombined with 14-day salivary mesenchyme, in a host 10 days postpartum (×80).

Syngeneic female mice of 4 weeks of age were used as recipients of recombined organ rudiments. Recombinants were transplanted under the kidney capsule with an orally controlled micropipette introduced through a dorsal incision exposing the kidney. All mice were anesthetized by Nembutal (Abbott). Transplants were allowed to grow for 1 to 3 weeks and were prepared for histological examination as whole mounts or in serial sections. Some mice were mated with males and allowed to become pregnant and to lactate. Transplants were recovered by carefully peeling off the capsules of kidneys under a dissecting microscope. During the lactating period (5 to 10 days postpartum) recombinants were examined for the presence of lactose synthetase. The levels of B proteins of lactose synthetase were measured in recombinants and in the indigenous mammary tissues by the method of Brew *et al.* (*8*) as modified by Vonderhaar *et al.* (*9*). All data are expressed as picomoles of lactose formed per milligram (wet weight) of tissue per 30 minutes.

Table 1 shows that the capsular and subcapsular renal mesenchyme did not support the development of mammary epithelium; no mammary duct system developed without mammary or salivary mesenchyme, and more than half of the epithelial rudiments showed abortive development or degeneration even when they were recovered. When 16-day mammary epithelium was recombined with mammary mesenchyme, the developmental response was morphogenetically typical of mammary gland, with a characteristic monopodial branching pattern (Fig. 1). End buds did not form, possibly because of insufficient hormonal influence in the young virgin host animals. Outgrowths of recombinations of mammary epithelium and mammary mesenchyme 3 weeks after transplantation were limited to sparsely branched monopodial duct systems (Fig. 2). On the oth-

er hand, when 16-day mammary epithelium was associated with salivary mesenchyme, salivary glandlike development was observed. The branching pattern was dichotomous rather than monopodial, and closely grouped complexes of adenomeres resembling salivary gland structure were found (Figs. 3 and 4). These observations are consistent with the finding of Kratochwil (*7*) that the morphogenetic pattern of mammary gland epithelium in vitro is controlled by the type of mesenchymal tissue with which it interacts. During pregnancy and lactation, remarkable lobuloalveolar development occurred in these grafts. Many new end buds and alveoli appeared along elongated ducts. Proliferation and enlargement of alveoli with increase in lumen sizes occurred during lactation, and dilated alveoli filled with milk were observed (Fig. 5).

Biochemical analysis of recombinants of mammary epithelium with salivary mesenchyme revealed significant quantities of the B protein of lactose synthetase in the grafts, relative to quantities found in lactating mammary glands in situ (Table 2). While values for two of the four recombinant samples were only about one-tenth those for normal lactating gland, the level in one recombinant was more than 60 percent of that in the normal lactating gland, and in another it was well above that in the normal lactating gland. Turkington *et al.* (*10*) have used the B protein of lactose synthetase as an indicator of lactational function, and by this criterion recombinants of the salivary mesenchyme with mammary epithelium clearly functioned as mammary glands, given the proper hormonal stimuli. It is probable that some of the variability among the recombinants can be attributed to the fact that they lacked proper outlets for their secretions, and hence lactocoeles developed. Depending on the extent to which these may have inhibited secretion at the time of sampling, variability in enzyme levels might be expected. The degree of success in achieving good recombination of epithelial and mesenchymal components is undoubtedly another factor affecting the uniformity of results.

These findings reinforce previous observations (*2*, *4*, *5*) showing that mesenchymal factors largely govern the form acquired by glands during morphogenetic processes, while biosynthetic function is qualitatively predetermined by the developmental history (that is, the organ type of origin) of the epithelial component. They do not exclude the possibility that qualitative or quantitative biochemical abnormalities may result from heterotypic recombinations of epithelium and mesenchyme. As yet there is no evidence available as to whether estrogen or prolactin receptors (or both) in mammary epithelium combined with heterotypic mesenchyme may differ qualitatively or quantitatively from hormone receptors of normal mammary glands. Whether or not heterotypic mesenchyme can affect the response of mammary epithelium to mammary tumor virus is also unknown. The work reported here was undertaken with questions such as these in mind, and with the object of developing further experimental methods to analyze the influence of stroma on the development of epithelial neoplasms in endocrine target organs.

Table 2. Lactose synthetase B protein activity in glands developing from 16-day mammary epithelium combined with 14-day salivary mesenchyme under the kidney capsule, and in mammary glands in lactating host mice.

Case No.	B protein activity (pmole/mg wet tissue/30 min)	
	Mammary glands in situ	Mammary epithelium plus salivary mesenchyme grafts
1	1918	290
2	2370	213
3	1716	2024
4	1176	752

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Melatonin: Effects on the Circadian Locomotor Rhythm of Sparrows

Abstract. *The continuous administration of low levels of melatonin via intraperitoneally placed Silastic capsules either (i) shortened the free-running period of activity or (ii) induced continuous activity in house sparrows (*Passer domesticus*) maintained in constant darkness. After the melatonin-filled capsules were removed, the period of the circadian rhythm of activity lengthened in rhythmic birds and normal rhythmicity was restored in continuously active birds. The results suggest that melatonin is involved in the physiological control of circadian rhythmicity in sparrows.*

There is now abundant evidence indicating that the pineal gland is involved in the control of circadian rhythmicity in house sparrows (*1*, *2*). Removal of the pineal gland abolishes the free-running circadian rhythm of activity in constant darkness, and transplantation of a pineal gland to the anterior chamber of the eye restores rhythmicity to pinealectomized arrhythmic birds. This latter result, and the fact that denervation of the pineal in situ does not abolish the free-running cir-

Table 1. Effect of melatonin-filled capsules on the circadian rhythm of locomotor activity in house sparrows. The period of the activity rhythm was considered to have shortened if it decreased by at least 5 minutes.

Melatonin capsule (mm)	Birds tested (No.)	No effect	Period shortened	Continuous activity
Five (empty)	7	7	0	0
Two (filled)	8	2	3	3
Five (filled)	19	2	6	11
Ten (filled)	3	0	1	2