Table 1. Effect of divalent cations on the activity of phosphodiesterase.

Cations added	Concentration (mM)	Activity (%)
		48
$MgCl_2$	4	82
	10	100
$MnCl_2$	4	92
	10	98
$CaCl_2$	4	43
	10	43

by several other methods with a more purified form of the enzyme.

The enzyme was stable at room temperature for at least 5 hours and could be stored at −20°C for at least 6 weeks without loss of activity. Boiling the enzyme alone for 5 minutes completely destroyed the activity; however, the enzyme was partially protected from heat inactivation by the mixture of cyclic 3′,5′-AMP, $MgCl_2$, and tris buffer in the standard assay.

The fraction precipitated by ammonium sulfate does not contain nucleotidase activities, since it did not hydrolyze 5′-AMP or 2′(3′)-AMP.

In general, the properties of cyclic 3′,5′-AMP phosphodiesterase of slime molds resemble those of the phosphodiesterases of animal tissues. The one major difference is that phosphodiesterase of *D. discoideum* is relatively insensitive to the methyl xanthines. In this respect, it is similar to the phosphodiesterase of *E. coli* which is also not inhibited by caffeine (*21*). A minor difference is the fact that the K_m of the *D. discoideum* phosphodiesterase is higher than the value obtained from other sources.

One important problem for the future is the role this enzyme plays in the normal development of the cellular slime mold. In addition, there is another aspect of this study which may have very general significance. This particular *D. discoideum* phosphodiesterase is liberated into the medium in large quantities and is remarkably stable. For these reasons this enzyme should be exceptionally useful in the study of the chemistry of cyclic 3′,5′-AMP.

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Rat Mammary Gland Differentiation in vitro in the Absence of Steroids

Abstract. *Mammary glands from several strains of immature female rats will form small lobules of alveoli when cultured in chemically defined media lacking steroid hormones but containing insulin and prolactin. The degree of lobulo-alveolar differentiation is increased if estradiol, progesterone, and aldosterone are also included in the culture media.*

Estradiol, progesterone, and prolactin previously have been considered to be the minimum hormone combination necessary for lobulo-alveolar differentiation in the mammary glands of rats (*1*) and mice (*2*). Organ culture experiments with both mouse (*3*) and rat (*4*) mammary glands have indicated a steroidal requirement for lobulo-alveolar development. However, lobulo-alveolar development has been reported in ovariectomized-adrenalectomized-hypophysectomized rats injected with large doses of prolactin and somatotropin (*5*). In recent experiments (*6*), steroids were found to be unnecessary for lobulo-alveolar differentiation in vitro of mammary glands from immature Long-Evans rats. The present report extends these results to other strains of rats.

The entire middle thoracic mammary glands from 3- to 4-week-old female Fischer, Long-Evans, Sprague-Dawley, and Wistar rats were removed and cultured in Waymouth's MB752/1 media containing penicillin G (35 μg/ml) and supplemented with the hormones insulin, or insulin + prolactin, or insulin + prolactin + estradiol-17β + progesterone + aldosterone. The culture method used was the same as that described by Ichinose and Nandi (*3*). Hormones were used at the following doses: crystalline bovine insulin, 0.124 unit/ml; prolactin (NIH P-S-6 and P-S-8), 0.124 unit/ml; estra-

Table 1. Effect of hormones on lobulo-alveolar development in vitro of mammary glands from immature rats of strains indicated.

Hormones	Number of explants with indicated grade				
	0	0.5 to 1	1.5 to 2	2.5 to 3	3.5 to 4
		Fischer			
I	4				
I-MH	2	4	3		
I-MH-E-P-A		1	7	1	
		Long-Evans			
I	6	4			
I-MH	1	2	8	3	
I-MH-E-P-A				10	4
		Sprague-Dawley			
I	4				
I-MH	2	1	1	4	1
I-MH-E-P-A	1	3		4	1
		Wistar			
I	3		1		
I-MH	1	2	2	2	2
I-MH-E-P-A		1	2	2	4

diol-17β, 0.001 μg/ml; progesterone, 1 μg/ml; aldosterone, 1 μg/ml.

It is not necessary to pretreat rats with injections of estradiol-17β and progesterone before culture, as is necessary in mice, in order to achieve lobulo-alveolar differentiation (*7*). Following the 5-day culture period, alum-carmine–stained whole-mounts were prepared from the explants and graded blindly on a scale 0 to 4 according to the degree of lobulo-alveolar differentiation.

The results, summarized in Table 1, indicate that insulin alone will not support lobulo-alveolar differentiation although it does provide for good maintenance of the mammary tissues. Both media containing prolactin promoted lobulo-alveolar differentiation in the mammary glands of all strains tested. Glands cultured in the presence of insulin + prolactin + estradiol-17β + progesterone + aldosterone were slightly more stimulated than those cultured in the presence of insulin + prolactin (Fig. 1). When results from all strains were pooled, this difference was statistically significant ($P < 0.001$) by the Mann-Whitney U test (*8*). The Long-Evans strain reacted the most vigorously and Fischer the poorest, but the general pattern of response to hormones was the same in all the strains.

The discrepancy between these results with those of Mishkinsky *et al.* (*4*), who found that the steroids estradiol-17β, progesterone, and aldosterone were necessary for lobulo-aveolar development of rat mammary tissue in vitro, may be due to the strain or age differences of the rats used in their experiments. Immature rat mammary tissue may have a lower hormonal requirement for differentiation than that of adult tissue. A difference of this type was observed by Voytovich and Topper (*9*), who reported that immature mouse mammary glands require less insulin than do adult glands for DNA synthesis in vitro. In contrast, Ichinose and Nandi (*3*) and Prop (*10*) observed that mammary glands from immature mice require the steroid(s) aldosterone or progesterone and cortisol for lobulo-alveolar differentiation in vitro.

In the present experiments, although lobulo-alveolar differentiation was obtained without adding steroids to the media, it is possible that they were carried into the cultures within the mammary fat pads. Alternatively, endogenous steroids may have had a priming action on the mammary epithelium prior to culture. However, these suggestions might equally apply to mouse tissues, which fail to develop in the absence of added steroids. Although further investigations are needed to demonstrate conclusively that immature rat mammary tissues have no steroid requirement for lobulo-alveolar development, there appears to be a distinct difference between rats and mice in this respect.

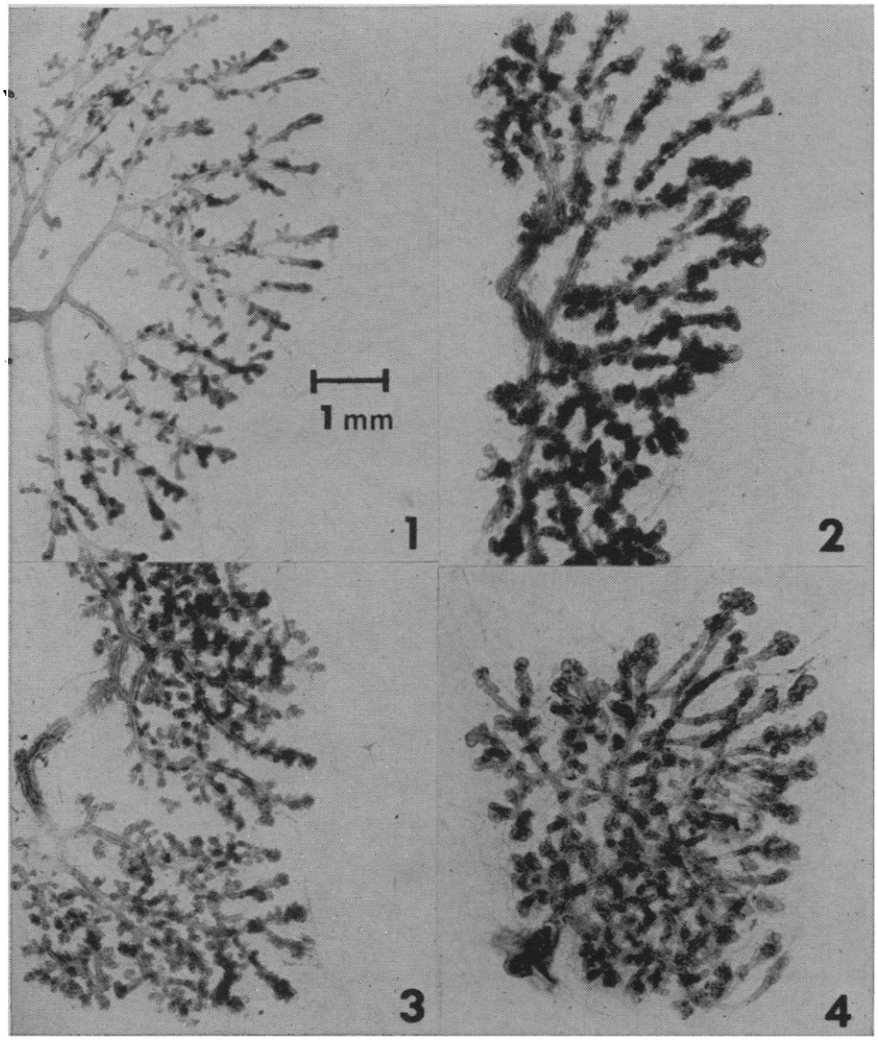

Fig. 1. All photographs show alum-carmine–stained whole-mounts of mammary glands from 3-week-old female rats. All glands had been cultured for 5 days. (1) Explant from Wistar rat cultured in media containing insulin. Terminal club-ends have been maintained. (2) Explant from Wistar rat cultured with insulin and prolactin. All terminal club-ends have differentiated into small lobules of alveoli. (3) Explant from Wistar rat cultured with insulin, prolactin, estradiol-17β, progesterone, and aldosterone. Alveoli are smaller but more numerous. (4) Explant from Long-Evans rat cultured with insulin and prolactin. Some of the alveoli at the periphery are not perfectly differentiated.

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