ther attempts at isolation of virus or viral DNA from A-12 induced tumors.

The morphology, rapid growth rate and transplantability of HT-1 are consistent with findings by others with tumor cells grown *in vitro*. The apparent resistance of HT-1 cells to overt infection with A-12 appears similar to the response of the normal cells of the Syrian hamster in culture.

The essential findings contained herein were presented at the 14th Annual Meeting of the Tissue Culture Association(17). Since the preparation of this manuscript, a report has appeared by Strohl *et al*(17) indicating results comparable to some of those described herein.

*Summary.* Characteristics of a human adenovirus type 12 induced hamster tumor serially propagated *in vitro* are described. These include small cell size, epithelioid appearance, rapid growth rate, resistance to superinfection with A-12, and transplantability to weanling hamsters. These cells grew either as monolayers or as balls of aggregated cells detached from the glass, depending on whether calf serum or horse serum was added to the Eagle's medium. Attempts to demonstrate virus activity by subculture of supernatant fluids and lysed cells into HeLa cells, mixed culture with human and hamster cells, electron microscopy, and inoculation of newborn hamsters with irradiated tumor cells were negative.

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## Interchangeability of Adrenocortical Hormones in Initiating Mammary Secretion *in vitro*.* (29308)

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Adrenocortical hormones have been studied in a variety of experiments for their influence on mammary gland growth and function. Studies in mice, particularly with regard to induction of milk secretion, have involved the use of the glucocorticoid, cortisol(1,2). Cortisol has also been the corticoid employed for various mouse mammary gland studies *in*

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*vitro*(3-7). On the other hand, attempts to induce milk secretion in mice with the mineralocorticoid, deoxycorticosterone, have met with little success(8).

Inasmuch as neither cortisol nor deoxycorticosterone appears to be produced by the adult mouse adrenal(9,10), some attempts have been made to determine the lactogenic potencies of the naturally-occurring corticoids of the mouse, corticosterone and aldosterone. However, systemic injections of these corticoids have been considerably less effective than treatment with cortisol. The amount of corticosterone required in lactogenic hormone combinations was approximately 8 times that required of cortisol; simultaneous administration of aldosterone had little or no influence on the activity of corticosterone(11).

These findings need not necessarily imply that the naturally-occurring corticoids of mice are less active than cortisol. As suggested by Nandi and Bern(11), the requirement for large amounts of corticosterone for lactogenesis may reflect an endogenous increase in glucocorticoid production at time of parturition. However, it is also possible that corticosterone and aldosterone are absorbed more slowly than cortisol following systemic administration; alternatively, the former steroids may be absorbed, inactivated and excreted more rapidly. The end result of either of these situations would be the failure of the injected steroid to reach the mammary site of action in the concentration needed.

Because the organ-culture method permits observation of the direct effects of hormones, this method was used in the present study to compare the secretory effects of several adrenal corticoids on mouse mammary lobules. It had been previously shown that a combination of cortisol + insulin + prolactin + somatotropin was capable of inducing secretion in organ culture(6). In the present investigation, the corticoids characteristic of the mouse (corticosterone and aldosterone), as well as cortisol and deoxycorticosterone, were examined for their ability to fulfill the adrenal corticoid requirement for mammary secretion *in vitro*.

*Materials and methods. Mammary tissues.* Mammary lobules (Fig. 1) for explantation were taken from 10-13 day pregnant female C3H/Crgl mice. The procedure for removal of tissues for explantation has been described (6).

*Culture method and media.* A modification (6) of the organ-culture watchglass technic described by Chen(12) was used. The nonesterified steroid hormones: cortisol, corticosterone, aldosterone, and deoxycorticosterone, and the protein hormones: insulin (20 i.u./mg), prolactin (25-30 i.u./mg, C.H. Li), and somatotropin (25-30 i.u./mg, C.H. Li) were prepared as previously described(6,13). The hormones were employed in the range found minimally effective in a recent investigation(14): corticoid (1 and 5 $\mu$g/ml), insulin (5 $\mu$g/ml), prolactin (1 $\mu$g/ml), and somatotropin (1 $\mu$g/ml).

The basal culture medium was chemically-defined synthetic medium "199"(15). The medium was changed on the third day of cultivation and the cultures were terminated on the fifth day. Before fixation, the explants were individually examined with the aid of a dissecting microscope for the occurrence of "white" lobules (groups of milky alveoli). Histologic examinations were made of the serial sections of each explant (fixed in Tellyesniczky's fluid, embedded in paraffin, and stained by a modified Masson's method(16)).

The following symbols are used in the table to describe the secretory appearance of the alveoli:

— nonsecretory: no change over initial appearance; cell vacuolation and secretion in alveolar lumina minimal or absent; alveoli small

\+ submaximal secretion: few cells with vacuoles; scanty secretion in alveolar lumina; alveoli moderately distended

++ maximal secretion: most cells with vacuoles; abundant secretion in alveolar lumina; alveoli greatly distended

*Results and discussion.* Although some secretory responses were obtained with adrenal steroid-free medium, the alveolar structure was not so well-preserved (Fig. 2) as when corticoid was also present. Variable numbers of necrotic cells were seen in all explants. Previously, the omission of adrenal corticoid from medium containing high levels

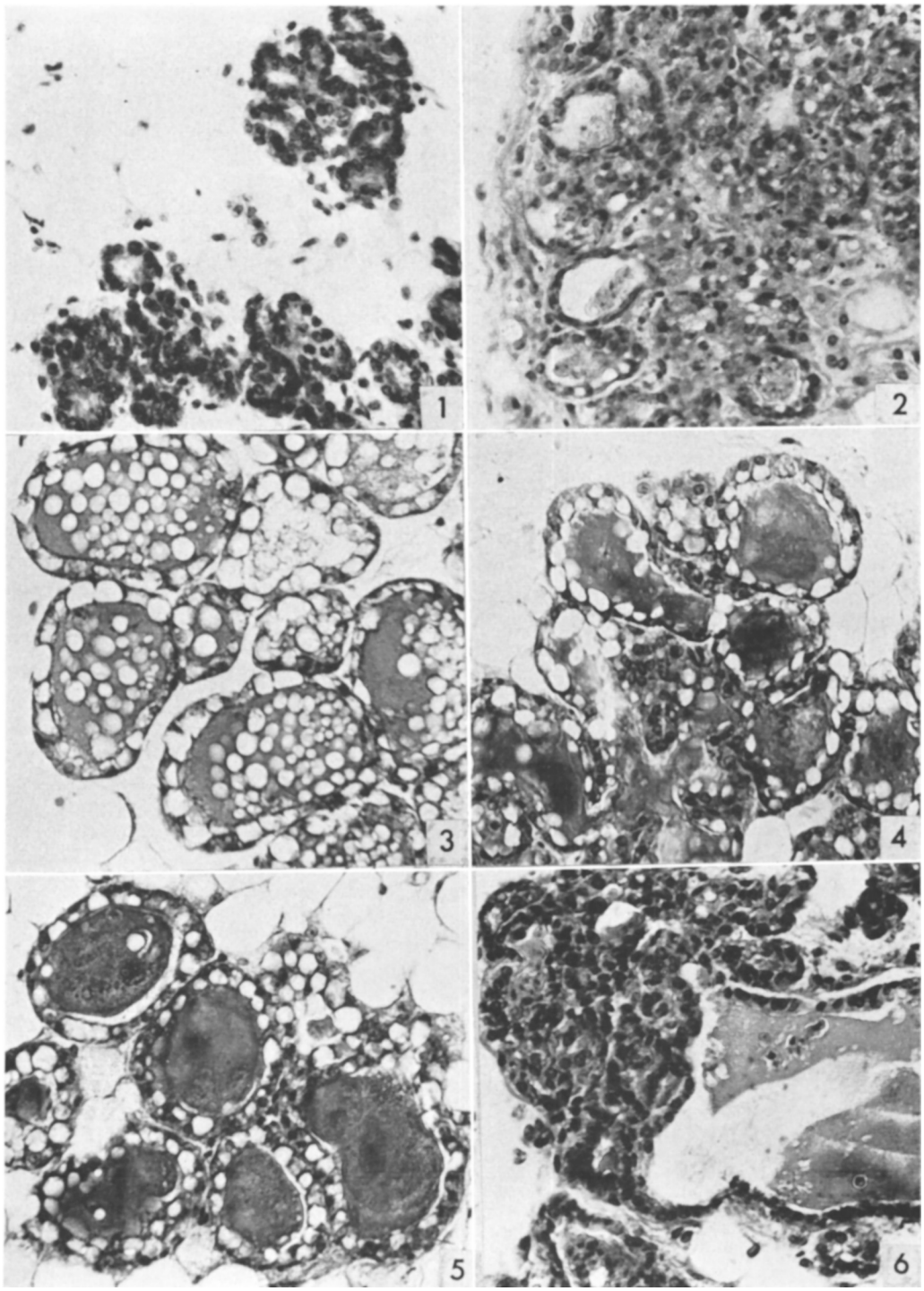


FIG. 1. Portion of mammary lobule from a 10-13 day pregnant C3H mouse, showing appearance before cultivation. Note small nondistended alveoli, absence of cell vacuoles and of secretion in alveolar lumina. × 270.

FIG. 2. Portion of lobular explant after cultivation with adrenal steroid-free medium containing

TABLE I. Response of C3H/Crgl Mouse Mammary Lobules to Adrenocortical Hormones, in Presence of Insulin + Prolactin + Somatotropin in Organ Culture.

| Adrenal corticoid, μg/ml* | No. of explants† | Alveolar maintenance | No. of explants showing response: Secretory appearance — | + | ++ | Milky alveoli |
|---|---|---|---|---|---|---|
| None | 8 | fair | | 6 | 2 | 0 |
| Cortisol, 1 | 8 | good | | | 8 | 3 |
| " 5 | 8 | " | | | 8 | 1 |
| Corticosterone, 1 | 8 | " | | | 8 | 3 |
| " 5 | 8 | " | | | 8 | 2 |
| Aldosterone, 1 | 8 | " | | | 8 | 2 |
| " 5 | 8 | " | | | 8 | 4 |
| Deoxycorticosterone, 1 | 8 | fair | 5 | 1 | 2 | 0 |
| " 5 | 8 | " | | 7 | 1 | 0 |

* All media contain insulin (5 μg/ml), prolactin (1 μg/ml), and somatotropin (1 μg/ml).
† Four mice provided explants.

of protein hormones resulted in complete tissue necrosis(6). Although the present observation suggests that the adrenal hormone requirement is less absolute with low protein hormone levels, its presence is necessary for maximal maintenance of alveolar structure.

Cortisol, or corticosterone, or aldosterone at the two levels used (1 and 5 μg/ml) were each capable of initiating maximal secretory responses in the presence of insulin, prolactin, and somatotropin (Table I; Fig. 3-5). Explants were all well-maintained, and a significant number contained milky-appearing alveoli in the living state (Table I).

In contrast, secretory responses were much less pronounced or absent in lobules cultivated with deoxycorticosterone, and explants were usually not so well-maintained as with the other corticoids. Milky alveoli were not seen in any of the explants (Table I). In those explants showing a moderate degree of survival the terminal ducts appeared more responsive to deoxycorticosterone than the alveoli (Fig. 6). The ducts were greatly dilated and contained appreciable amounts of secretion, whereas the alveoli were dilated only moderately, if at all, and contained little secretory material. Neither explant survival nor the moderate secretory response obtained in adrenal steroid-free medium were improved by deoxycorticosterone (Table I).

Although studies *in vivo* show that much higher levels of corticosterone than cortisol were required for lactogenesis(11), the two corticoids appeared equally effective in inducing mammary secretion in organ culture. This finding suggests that the need for larger amounts of corticosterone than cortisol *in vivo* is probably not due to inherent differences in lactogenic potency but rather to differences in rates of absorption or degradation in the animal. It might be argued that the levels of corticosterone and cortisol used in this study (1 and 5 μg/ml) were maximal concentrations and could account for the failure to observe differences in potency in organ culture. However, this seems unlikely, since it has been observed generally that effects *in vitro* usually require higher steroid hormone levels than *in vivo*(17).

insulin, prolactin, and somatotropin. Explant as a whole undergoing degeneration with a few partially-maintained alveoli. × 270.

FIG. 3. Portion of lobular explant after cultivation with cortisol, insulin, prolactin, and somatotropin: maximal secretion. × 270.

FIG. 4. Portion of lobular explant after cultivation with corticosterone, insulin, prolactin, and somatotropin: maximal secretion. × 270.

FIG. 5. Portion of lobular explant after cultivation with aldosterone, insulin, prolactin, and somatotropin: maximal secretion. × 270.

FIG. 6. Portion of lobular explant showing partial maintenance after cultivation with deoxycorticosterone, insulin, prolactin, and somatotropin: intralobular duct dilated and containing stainable secretion; absence of secretory responses in most alveoli. × 270.

Aldosterone has not proved to be lactogenic in mice *in vivo* and produced no additive effects when administered together with corticosterone(11). However, the highest dose used was 25 times less than the effective high level of corticosterone(11). As was the case with corticosterone, perhaps large amounts of aldosterone are required for initiating mammary secretion *in vivo*.

That the interchangeability of cortisol, corticosterone, and aldosterone in organ culture reflects specific tissue responses is strongly supported by the ineffectiveness of deoxycorticosterone in mice both *in vivo* and in this study *in vitro*.

The roles of the several adrenocortical hormones in mammary development are still incompletely defined. On the basis of results obtained in rats and guinea pigs, various investigators(18-20) postulate that mammary development and milk secretion are primarily dependent upon glucocorticoids. Others(21-23) have reported that mineralocorticoids are capable of maintaining milk secretion in rats to some degree and that in goats, deoxycorticosterone was more effective than cortisone. Although the *in vitro* study reported herein does not resolve the question, it does demonstrate that murine adrenal steroids, primarily glucocorticoid or primarily mineralocorticoid in activity, are equally capable of *initiating* mammary secretion. Perhaps some distinction between *mammogenic, lactogenic,* and *galactopoietic* effects of corticoid hormones might clarify the conflicting interpretations based on the use of various strains and species.

*Summary.* The naturally-occurring corticoids of the mouse adrenal, corticosterone and aldosterone, were interchangeable with cortisol in hormone combinations capable of inducing secretion in mammary lobules *in vitro*. The ability of the 3 corticoids to act similarly in organ culture suggests (1) that their differential activities *in vivo* are probably the result of systemic factors affecting the corticoids differently and (2) that corticoids with either primary mineralocorticoid or primary glucocorticoid activity are capable of initiating mammary secretion in organ culture. The ineffectiveness of deoxycorticosterone in this study was in accord with observations *in vivo*.

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