Effect of Anti-mitotic Drugs on the *in Vitro* Secretory Activity of Mammotrophs and Somatotrophs and on Their Microtubules[1] (37569)

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A number of studies have demonstrated that vincristine and colchicine, substances which are known to induce disassembly of cytoplasmic microtubules, and cytochalasin B, which interferes with the preservation of microfilaments, impair hormonal secretion in certain endocrine tissues. It is thought that these drugs by inducing alterations in microtubules and microfilaments interfere with the role attributed to these structures in determining the pathways for the migration of secretory material or in generating the forces necessary for this event.

Recently, Kraicer and Milligan (1) showed that incubation of rat anterior pituitary glands with colchicine suppressed the augmented ACTH release induced by a high potassium concentration. Schofield (2) found that cytochalasin B inhibited growth hormone release in the presence of high potassium and blocked the stimulatory effect of PGE. Williams and Wolff (3) showed that the addition of cytochalasin B to prelabeled mouse thyroid glands interferes with iodine release.

We have investigated the effects of these plant alkaloids and cytochalasin B on prolactin and growth hormone production and release by the rat anterior pituitary gland. The functional studies of the effects of vincristine and colchicine have been integrated with electron microscopic observation of the microtubules. Additionally, we have studied the effect of vincristine treatment on the growth and function of a transplanted, hormone-secreting pituitary tumor.

Materials and Methods. Mature female (180–200 g) and male (200–230 g) Sprague–Dawley rats were obtained from Flow Research Animals, Dublin, VA.

Pituitary tumor 7315a was transplanted into mature female rats of the Buffalo strain, obtained from Simonsen Laboratories, Gilroy, CA, as previously described (4).

Incorporation of leucine-4-5-^{3}H (29.8 Ci/mmole) into prolactin and growth hormone was studied by incubating bisected anterior pituitary glands in 1 ml of tissue culture medium 199 (Microbiological Associates, Inc., Bethesda, MD) containing 10 μCi of the isotope (obtained from International Chemical and Nuclear, Irvine, CA). The glands were incubated for 6–7 hr at 37° in a Dubnoff shaker and gassed with 95% O_2–5% CO_2. Each incubation flask contained four hemipituitaries from four different rats and each group contained at least four flasks. Each experiment was repeated at least once. At the termination of the incubation, the tissue was homogenized in 1 ml 0.05 *M* phosphate buffer (pH 7.2) using a glass homogenizer fitted with a Teflon pestle. The homogenates were frozen and thawed three times to lyse all granules. Duplicate 50 μl aliquots of the homogenates were subjected to polyacrylamide gel electrophoresis as described by Jones *et al.* (5). Aliquots of the incubation media were "top-loaded" onto polyacrylamide gel columns as described by Reisfeld, Lewis and Wiliams (6). The protein bands were stained with amido black and their intensity was quantitated with a Canalco mi-

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crodensitometer equipped with an integrator. Our results are in excellent agreement with those of Jones *et al.* (5) who eluted these proteins from the polyacrylamide gels and established their identity by bioassay. In addition, the excellent separation of rat growth hormone and prolactin by this method has been described by other workers (7–10).

Serum prolactin was measured by double antibody radioimmunoassay using the protocol and reagents supplied by the Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases. The prolactin was iodinated with ^{125}I or ^{131}I, obtained from New England Nuclear, Boston, MA. Sheep anti-rabbit gamma globulin was obtained from Dr. Ann J. Johanson of the University of Virginia School of Medicine.

Colchicine was obtained from Fisher Scientific Co. The vincristine sulfate used in the *in vitro* experiments was a gift from Dr. Robert J. Hosley of Eli Lilly Co., Indianapolis, IN. The injected vincristine was Oncovin (Lilly). Perphenazine was a gift from Dr. P. L. Perlman, Schering Corp., Bloomfield, NJ. Cytochalasin B was purchased from Imperial Chemical Industries, Ltd., Macclesfield, Cheshire, England.

When appropriate, the data are presented as the means ± SEM. The significance of the differences between the means was determined by Student's *t* test.

For morphologic studies, experimental pituitary glands from female rats were incubated for 2 hr at 37° in medium 199 containing 10^{-5} *M* colchicine or 10^{-5} *M* vincristine. Glands incubated for 2 hr in medium 199 alone were used as controls. A difference in incubation time is noted between tissues for functional studies (6–7 hr) and tissues for morphologic studies (2 hr). The shorter incubation time was selected on the basis of preliminary observations which indicated that 6 hr incubation in medium 199 alone induced a remarkable degree of swelling and disruption of cell mebraneous components. Tissues were fixed in 3% glutaraldehyde in phosphate buffer (pH 7.3) followed by 1 hr in 1% OsO_4. Tissues were fixed in part at 4° and in part at room temperature and after dehydration in alcohols, and embedding in Epon, were cut in a Porter-Blum MT-1 (Sorvall) ultramicrotome. Sections were examined in a 300-Philips electron microscope after being stained with uranyl acetate and lead citrate.

Results. The effects of vincristine and of colchicine on the *in vitro* synthesis and release of prolactin and growth hormone are illustrated in Fig. 1. The open bars represent newly synthesized (radioactive) prolactin and growth hormone found in the incubation medium and therefore indicate "release." The solid bars represent newly synthesized prolactin and growth hormone found in the pituitary glands at the termination of the incubation. The sum of the open bars and the solid bars is termed "total synthesis." At 10^{-6} *M*, vincristine significantly ($p < 0.05$) reduced release of newly synthesized prolactin into the incubation medium. An even greater effect was observed at 10^{-5} *M* ($p < 0.02$). Vincristine had no effect on the amount of radioactive prolactin remaining in the gland. Growth hormone synthesis was slightly decreased at these concentrations but the alkaloid had no effect on the amount of newly synthesized growth hormone released into the medium. Although it was not as effective as vincristine, 10^{-5} *M* colchicine also decreased release of newly synthesized prolactin. Neither growth hormone synthesis nor release was changed by colchicine.

We have previously shown that injection of male rats with perphenazine greatly stimulates the *in vitro* synthesis and release of prolactin by the pituitary gland (11). When the pituitary glands of male rats treated with perphenazine were incubated with 10^{-5} *M* colchicine or vincristine, the perphenazine-induced increase in *in vitro* prolactin release was greatly reduced ($p < 0.01$), particularly by vincristine (Fig. 2). The drugs had no significant effect on the amount of newly synthesized prolactin in the gland. As previously reported, the stimulation of prolactin production caused by treatment with perphenazine is usually accompanied by a decrease in growth hormone production (12). Incubation with vincristine further suppressed growth hormone synthesis and its release into the medium ($p < 0.01$). Colchicine produced a smaller and statistically insignificant decrease in growth hormone release.

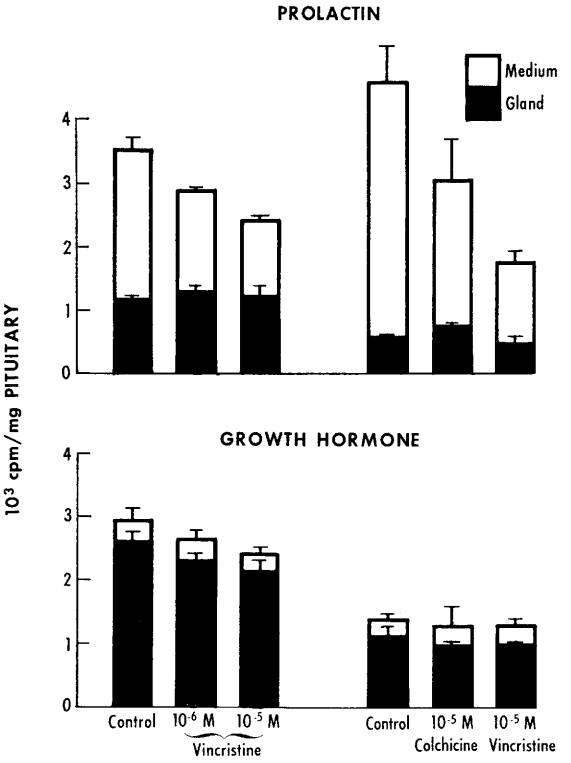

FIG. 1. Inhibition of radioactive prolactin and GH release by vincristine and colchicine. Anterior pituitary glands of mature female rats were bisected and incubated, 4 hemipituitaries/incubation flask, in TC medium 199 with 10 μCi leucine-4,5-^{3}H. The glands were incubated 6–7 hr at 37°. The prolactin and growth hormone in the media and in the tissue homogenates were separated by polyacrylamide gel electrophoresis. The solid portion and the open portion of the bars represent the radioactive hormone present in the gland and that released into the medium, respectively.

The effect of these alkaloids on growth hormone production by glands of normal male rats was next studied. As noted before, the effect of these drugs on growth hormone production by glands of female rats was very small (Fig. 1). When 10^{-5} *M* vincristine was incubated with the pituitary glands of male rats, however (Fig. 3), growth hormone synthesis was significantly reduced (5216 ± 96 vs 4057 ± 352 cpm/mg, $p < 0.05$). The effect on release (1159 ± 98 vs 824 ± 81) was not significant. Incubation of glands with dibutyryl cyclic AMP has been shown to stimulate release and, to a lesser extent, synthesis, of growth hormone (13). When the glands of male rats were incubated with the cyclic nucleotide at a concentration of 10^{-3} *M*, growth hormone synthesis and release were increased (Fig. 3). The addition of the cyclic nucleotide to glands preincubated for 30 min with 10^{-5} *M* vincristine completely overcame the suppressive effects of the alkaloid and significantly increased growth hormone release (824 ± 81 vs 2342 ± 117 cpm/mg, $p < 0.01$). At a concentration of 10^{-4} *M*, vincristine significantly inhibited both synthesis (6370 ± 254 vs 3893 ± 196, $p < 0.01$) and release (1942 ± 98 vs 1442 ± 144, $p < 0.05$) of growth hormone but, again, addition of dibutyryl cyclic AMP overcame the vincristine-induced suppression of growth hormone release (1442 ± 144 vs 3362 ± 208, $p < 0.01$).

A study of the *in vitro* binding of radioactive colchicine to pituitary glands was initiated. Despite repeated attempts, no binding of colchicine-^{14}C to any pituitary gland subcellular component was observed.

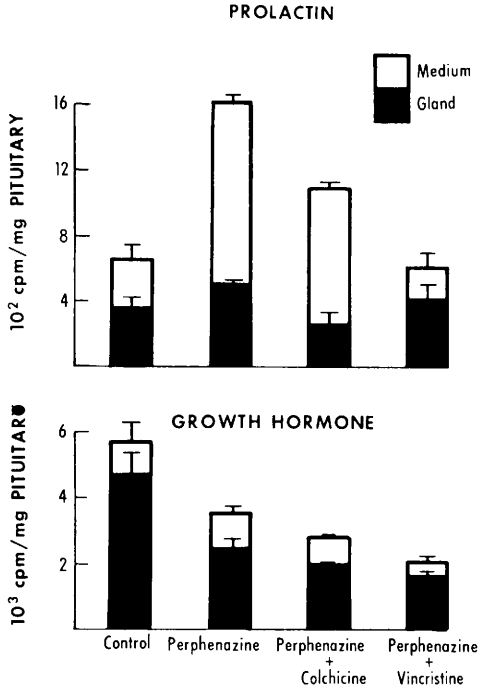

FIG. 2. Effect of anti-mitotic drugs on *in vitro* hormone release following injection of perphenazine. The pituitary glands of male rats were incubated as described under Fig. 1. Perphenazine-treated rats received 4 daily injections of 5 mg/kg perphenazine sc. Colchicine and vincristine were added to the incubation medium at a concentration of 10^{-5} *M*.

The effects of cytochalasin B on hormone secretion by the rat anterior pituitary gland are summarized in Fig. 4. The glands of male rats synthesize and release very small amounts of prolactin *in vitro* and incubation of these glands with cytochalasin B resulted in a decreased release of newly synthesized prolactin into the incubation medium (2733 ± 260 vs 1029 ± 107 cpm/mg, $p < 0.05$). Cytochalasin significantly decreased the *in vitro* release of growth hormone by glands of both male (1672 ± 96 vs 1123 ± 92, $p < 0.05$) and female (672 ± 14 vs 479 ± 23, $p < 0.02$) rats.

The purpose of the experiments described next was to determine if treatment of tumor-bearing animals with vincristine could suppress the growth and function of a prolactin-scecreting pituitary tumor and, subsequently, affect the function of the host's pituitary gland.

Animals bearing the prolactin- and ACTH-secreting pituitary tumor 7315a were given daily injections of 20 μg vincristine for 6 days. Because vincristine may cause considerable irritation if leakage into the surrounding tissue should occur, the drug was injected directly into the tumor. Tumor size was measured daily. Vincristine treatment dramatically suppressed tumor growth and at the time of autopsy the average tumor weight of the treated animals was 4.6 ± 1.2 g, compared with an untreated animal tumor weight of 11.9 ± 2.5 g (Fig. 5).

The presence of the prolactin-secreting tumor 7315a caused a 1600% increase in the serum prolactin level (Fig. 6). Tumor-bearing animals injected with vincristine had greatly reduced serum prolactin levels, although they remained considerably higher than the normal control value.

We have previously reported that these transplanted, hormone-secreting pituitary tumors cause atrophy of the host's pituitary gland and decrease hormone synthesis and release by the gland, presumably via a feedback mechanism. The effect of the high serum prolactin levels in the tumor animals on the *in vitro* production of prolactin by the hosts' glands is shown in Fig. 6. The amount of radioactive prolactin in the gland and that released into the medium by the glands of tumor-bearing animals were profoundly suppressed (Fig. 6). Although vincristine greatly decreased the serum prolactin levels in the tumor animals, the circulating prolactin was presumably still sufficiently high to prevent the function of the glands of the treated animals from recovering from their suppressed state. Isotope incorporation data and radioimmunoassay data on the incubation media revealed no difference in prolactin production between vincristine-treated and untreated animals (Fig. 6).

Cytoplasmic microtubules were not promptly found either in the mammotroph or in the somatotroph of control rats. Indeed, a large number of these cells do not exhibit microtubules at all. Differences in the number and other features of microtubules between tissues fixed at 4° and tissues fixed at room temperature have not been detected.

Figure 7 illustrates the highest number of

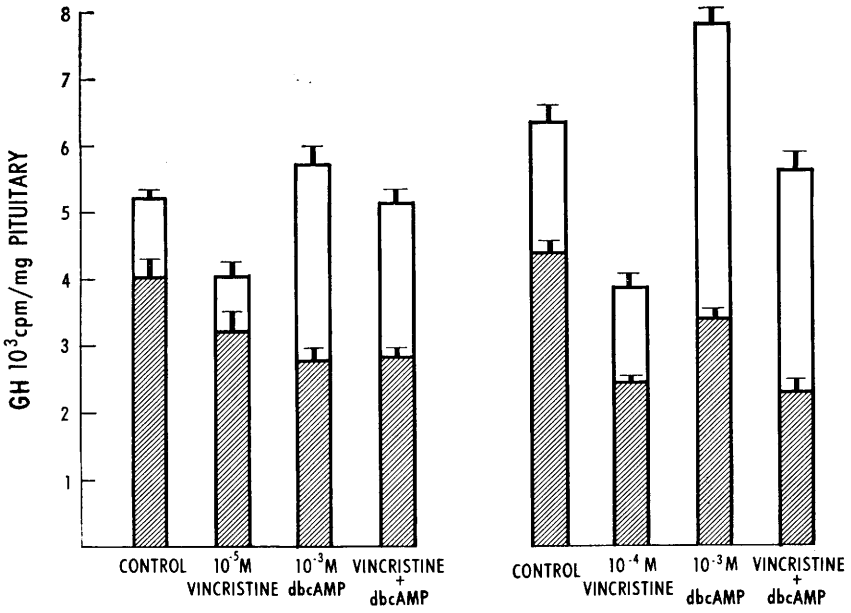

FIG. 3. Prevention by dibutyryl cyclic AMP of the vincristine-mediated decrease in growth hormone release. Anterior pituitary glands of male rats were incubated as described under Fig. 1. The shaded portion of the bars represents radioactive growth hormone in the gland. The open portion shows radioactive growth hormone released into the medium.

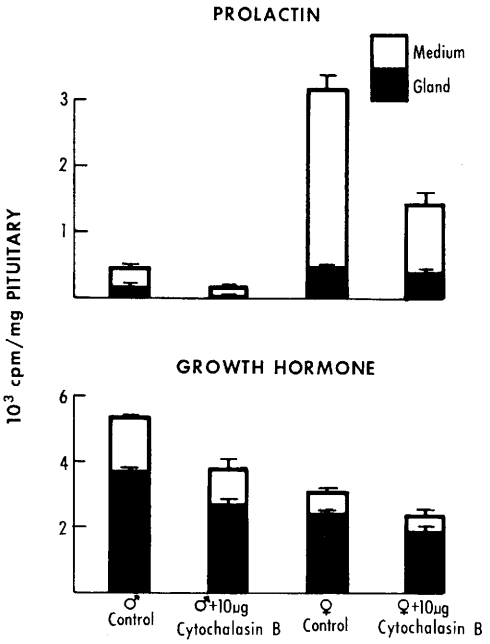

FIG. 4. Effect of cytochalasin B on the *in vitro* release of prolactin and growth hormone. Pituitary glands of male and female rats were incubated as described under Fig. 1. Cytochalasin B was added *in vitro*.

cytoplasmic microtubules in a section of a mammotroph. They are usually straight and can be followed for a short distance. Because of their uncommon presence, it is not possible to present additional remarks on certain general features such as their orientation, organization, and preferential site, which are significant in providing insight into their functional activities in other cell types.

Figure 8 represents a portion of a mammotroph obtained from a pituitary gland incubated for 2 hr with vincristine. The cytoplasm revealed microtubules which did not differ in any respect from microtubules of untreated tissue. It is difficult to observe cells with microtubules from either normal or vincristine-treated pituitary glands. For that reason no quantitative estimate of the number of microtubules was made. No obvious difference in the number of microtubules was observed in the two groups. Microtubules were also found in pituitary glands exposed to colchicine.

Discussion. The results of these experiments demonstrate that vincristine, colchicine and cytochalasin B impair the secretory activity

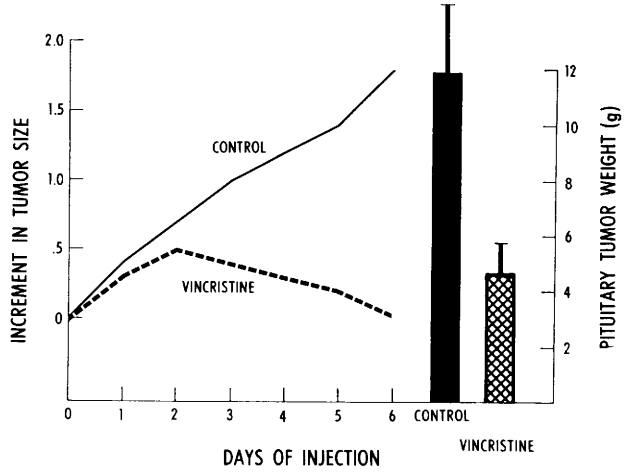

FIG. 5. Effect of vincristine treatment on tumor growth. Female rats bearing the prolactin- and ACTH-secreting tumor 7315a received 6 daily injections of 20 μg vincristine sulfate. Tumor size was measured daily and the tumors were weighed at sacrifice.

of the rat pituitary gland. In female rats they decrease primarily synthesis of prolactin, whereas in male rats they decrease growth hormone production. Moreover, these drugs inhibit the release of the prolactin which was newly synthesized by the gland *in vitro*, but do not affect the release of growth hormone.

At the morphologic level the present study indicates that cytoplasmic microtubules are normally an uncommon component of both the mammotroph and the somatotroph. It is, therefore, difficult to assign to the microtubules in the pituitary gland the active role which they have in directing the pathways for the translocation of the pigment granules in the melanophores (14, 15). In this cell type, microtubules are not only present in high quantity, but are also structurally parallel with the direction of granule migration. The infrequent observation of microtubules in the normal rat pituitary gland had been reported in several studies (16, 17).

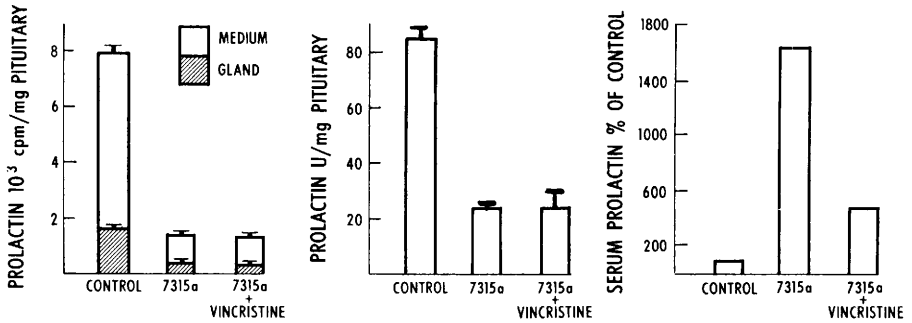

FIG. 6. Effect of vincristine treatment on the *in vitro* synthesis and release of prolactin and on serum prolactin levels of pituitary tumor-bearing rats. The animals were injected as described under Fig. 5. The shaded bars in the left figure represent radioactive prolactin in the glands and the open bars indicate radioactive prolactin released into the medium. The central figure illustrates total prolactin released into the incubation medium as quantitated by a microdensitometric scan of the gels on which the prolactin was separated. The data is expressed in terms of arbitrary units per milligram of pituitary. The right figure shows serum prolactin levels, expressed as a percentage of the control value. Serum prolactin was measured by means of the radioimmunoassay technique described under the Methods section.

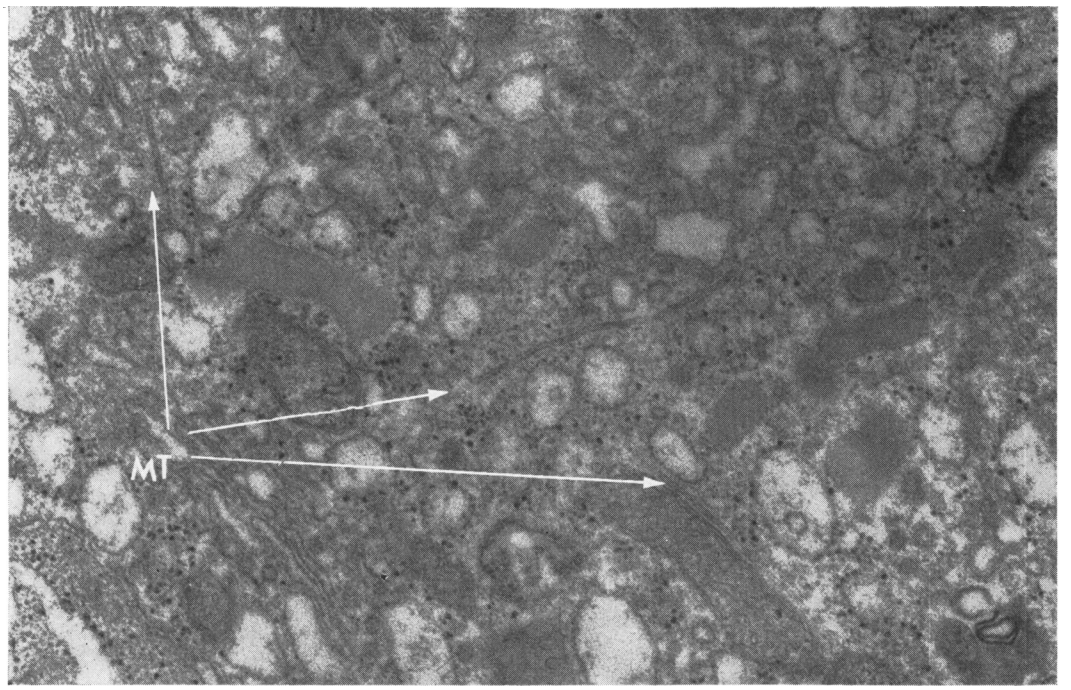

FIG. 7. Microtubules (MT) in the Golgi region of control mammotroph (×38,000). Anterior pituitary glands of female rats were incubated 2 hr at 37° in TC medium 199.

This is in agreement with the observation of the present investigation that no binding of radioactive colchicine to the pituitary gland occurred. A positive relationship between the quantity of microtubules, as seen in the electron microscope, and colchicine-binding activity of several cell types has been reported by Borisy and Taylor (18). We also note that, in contrast to the present study, Williams and Wolff (19) found, in the thyroid gland exposed to vincristine and colchicine, suppression of the release of thyroid hormone mediated by TSH or dibutyryl cyclic AMP in concomitance with binding of colchicine to the microtubular protein.

The present morphologic observations indicate also that microtubules are still present after cell exposure to a concentration of vincristine or colchicine which induces delay in prolactin release. This observation is as interesting as the observation, discussed earlier, that microtubules are uncommon in the mammotroph and suggests that microtubule deviations do not play a prominent role in the decreased release of prolactin induced by these antimitotic agents.

These present results confirm the observations of Ewart and Taylor (20) who also were unable to block growth hormone release with colchicine and suggested that microtubular proteins were unimportant in growth hormone secretion. Kraicer and Milligan (1) showed that preincubation of pituitary glands with colchicine blocked the increased release of ACTH caused by increasing the potassium concentration of the incubation medium. In other experiments, an extract of stalk median eminence caused an increase in ACTH release from pituitary glands in the presence and absence of colchicine. These authors concluded that release of ACTH, induced by physiological hypothalamic factors, does not occur by the microtubular system in the pituitary gland. McPherson and Schofield (21) have recently found that cytochalasin B inhibits the PGE-induced release of growth hormone in the presence of glucose alone but potentiates release if β-hydroxybutyrate and glutamate are also added. They suggested that this inhibition of release might be due to changes in pituitary metabolism rather than in microfilament activity (21).

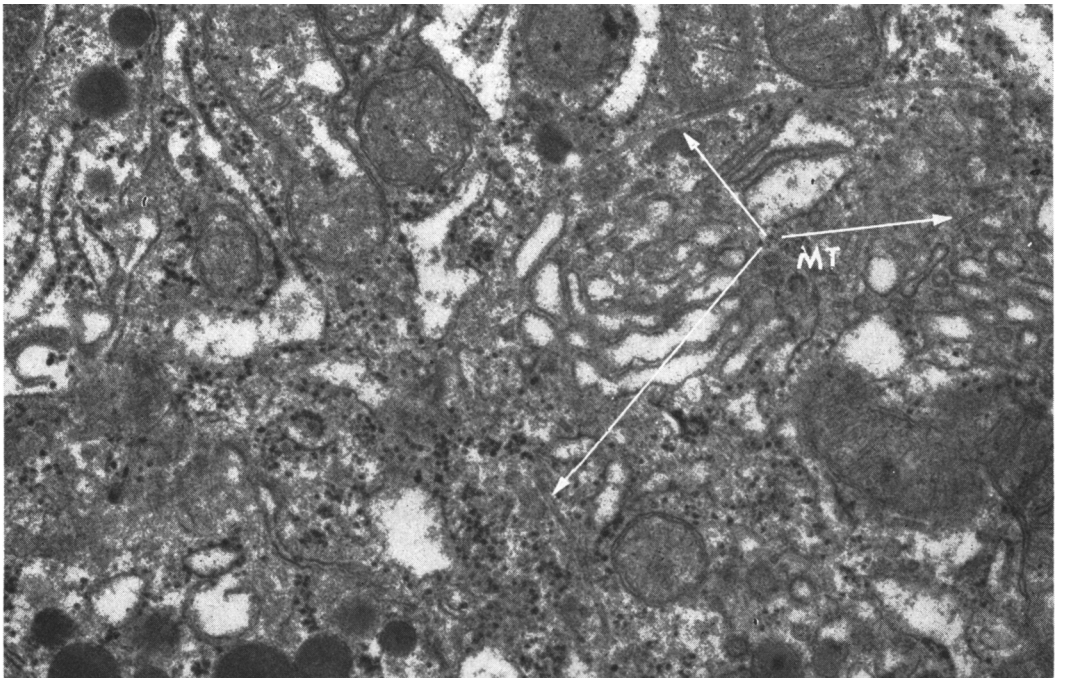

FIG. 8. Microtubules (MT) in mammotroph exposed to vincristine sulfate do not differ in number or in structure from control microtubules (×38,000).

The ability of vincristine to inhibit the growth of a transplanted pituitary tumor and to partially block the increased serum prolactin is of interest. Since this experiment was conducted following daily injections of vincristine, the tumorostatic action of the drug may be related to its potent antimitotic activity.

Summary. Incubation of anterior pituitary glands of female rats with 10^{-5} *M* vincristine or colchicine reduced both synthesis and release of prolactin. Incubation of glands of perphenazine-treated male rats with 10^{-5} *M* colchicine or vincristine blocked the perphenazine-induced increase in *in vitro* prolactin synthesis. At 10^{-4} *M*, vincristine inhibited synthesis and release of growth hormone by glands of untreated male rats but could not block the dibutyryl cyclic AMP-induced stimulation of growth hormone release. Incubation of glands of male rats with 10 μg/ml cytochalasin B resulted in decreased growth hormone synthesis. Incubation of glands of female rats with the drug significantly decreased synthesis and release of both prolactin and growth hormone. Concomitant electron microscopic observations indicated that cytoplasmic microtubules are uncommon in the pituitary gland and that they are still present after glands were treated with vincristine and colchicine at doses capable of inhibiting prolactin release. For this reason and because no binding of radioactive colchicine to pituitary glands occurred *in vitro,* it is suggested that microtubules are not significantly involved in prolactin and growth hormone secretion.

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