

tolerance was notably low, also. This change may have been due to the stimulation and sensitization of the adrenal mechanism.

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### Hyperplasia of Uterine Muscle, as Studied by the Colchicine Method.

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Distention of the uterus is undoubtedly a factor in stimulating its growth during pregnancy.<sup>1, 2, 3</sup> The scarcity of mitotic figures in muscle during the enlargement of the pregnant uterus has led to the usual textbook statement that increase in size is due to hypertrophy rather than to hyperplasia of the muscle fibers. It is usually difficult to find dividing cells in uterine muscle even when the endometrium is undergoing marked hyperplasia in response to stimulation by ovarian follicular hormone.

The use of the drug, colchicine, which arrests mitoses in metaphase and thus accentuates hyperplasia<sup>4, 5, 6</sup> has made the search for cell division much more effective.

In a first series of short experiments (48 hours) with theelin and colchicine in adult ovariectomized mice, mitotic figures were abundant in the surface epithelium and glands of the uterus, but very scarce in the uterine muscle.<sup>7, 8</sup>

In later experiments, where injections of theelin, prephysin and pregnant mares' serum were made for longer periods in conjunction with colchicine (0.1 to 0.3 mg.) into puberal or young adult mice

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<sup>1</sup> van Dyke, H. B., and Gustavson, R. G., *J. Pharm. and Exp. Therap.*, 1929, **37**, 379.

<sup>2</sup> Markee, J. E., and Hinsey, J. C., *Anat. Rec.*, 1935, **61**, 311.

<sup>3</sup> Markee, J. E., Wells, W. M., and Hinsey, J. C., *Anat. Rec.*, 1936, **64**, 221; Reynolds, S. R. M., *Am. J. Obstet. and Gynec.*, 1937, **33**, 968.

<sup>4</sup> Dustin, A. P., *Jour. des Sci. Med. de Lille*, 1934, No. 49, 561.

<sup>5</sup> Lits, F., *C. R. Seance de Biol. Belge*, 1934, **115**, 1421.

<sup>6</sup> Ludford, R. J., *Arch. f. Exp. Zellforsch.*, 1936, **18**, 411.

<sup>7</sup> Allen, E., Smith, G. M., and Gardner, W. U., *Anat. Rec.*, 1936, **67**, (Suppl. 1), 49.

<sup>8</sup> Allen, E., Smith, G. M., Gardner, W. U., *Am. J. Anat.*, in press.

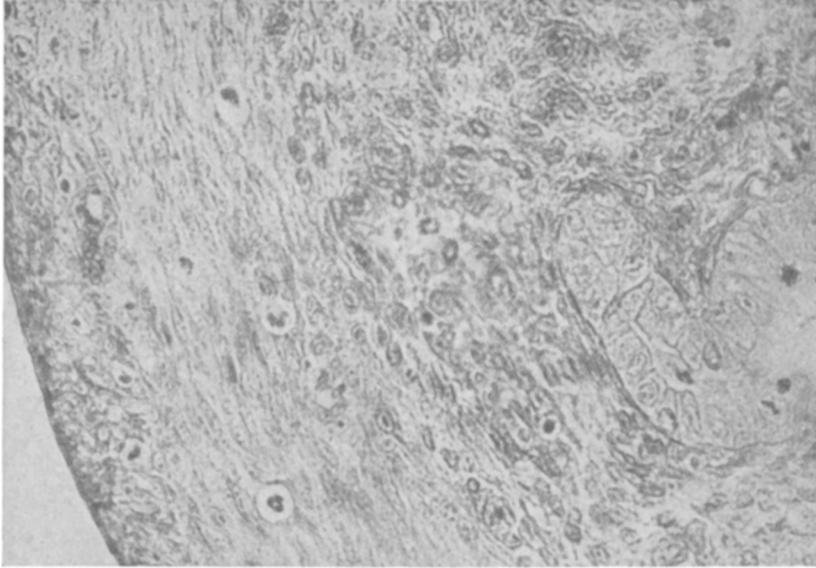


FIG. 1.

Frequent mitoses in circular and longitudinal muscle of the uterus (at left) of the mouse injected with prephysin and colchicine. Prephysin stimulates development of ovarian follicles which secrete folliculin (estrone) causing growth of the uterus. Colchicine arrests mitosis in metaphase, thus accentuating the mitotic index.

and rats,<sup>9</sup> mitoses were frequent in both circular and longitudinal muscle (Fig. 1). It was noted that the maximum incidence of mitosis occurred when there was distention of the uterus with secretion as occurs in both mouse and rat at estrus. This brought up the old question, "How much of this hyperplasia is due to hormonal stimulation and how much to the stimulus of distention?" The endometrium, both epithelial structures and stroma, contained frequent mitoses and the vagina, tubes, and mammary glands were also very hyperplastic. Since most of these tissues are not subjected to distention the endocrine factor must be partly responsible.

At this point Dr. Reynolds visited our laboratory and we discussed the above results and his recent findings that distention of the rabbit's uterus with paraffin pellets, even in ovariectomized animals, causes an increase in size as measured with the planimeter.<sup>10</sup> This led to the following preliminary experiment, with the colchicine method, to find out how much of the increased size of the distended uterus is due to hyperplasia.

<sup>9</sup> Allen, E., Smith, G. M., and Gardner, W. U., *Anat. Rec.*, 1937, **67** (Suppl. 3), 3.

<sup>10</sup> Reynolds, S. R. M., and Kaminester, S., *Am. J. Physiol.*, 1936, **116**, 510.

Four rabbits were ovariectomized on March 11th. On March 17th, several moulded pellets of 2 sizes were inserted in one uterine horn of each animal as described previously.<sup>10</sup> The animals were killed at 27, 50, 73, and 98 hours after insertion of the pellets. Approximately 18 and 9 hours before sacrificing, 2 injections of 0.3 mg. each of colchicine were made to hold in metaphase all cells dividing during the last 18 hours of life. This method accumulates evidence of hyperplasia and makes mitotic indices much more convincing.

A preliminary study of sections shows definite hyperplasia was at a maximum at 73 hours in the uterine segments distended with the larger sized pellets. There were a few mitoses in the 98-hour stage. There were very few mitoses in the endometrium—occasional ones being located in the glands. The endometrium was infiltrated with leucocytes. This and some edema might account for most of the increase in size of this part of the uterus but this point requires further study. At the same time a study of the effect of trauma incident to vigorous smearing of ovariectomized rats has shown (by means of the colchicine technique) that this also stimulates partial estrous growth.<sup>11</sup>

This work must be extended and the problem studied much more thoroughly. The necessity of postponement of further attack made this preliminary report seem worthwhile.

*Summary and Conclusions.* (1) The muscles of the uterus of the ovariectomized mouse and rat undergo hyperplasia in response to ovarian follicular hormone (theelin) and a similar response is obtained in normal animals injected with prephysin and pregnant mares' serum. (2) The mitotic index, accentuated by arrest of mitosis in metaphase with colchicine, makes this new evidence especially convincing. (3) The highest index is obtained after 2 or more days of hormone action when the uterus is distended by secretion, as occurs at estrus in the mouse and rat. (4) The uterus of the ovariectomized rabbit, distended with paraffin pellets, but not treated with sex hormones, also shows hyperplasia at the sites of distention. As many as 50 mitoses may be counted in the muscle in one cross section of the uterus after 73 hours of distention. The endometrium in the same section contains few mitoses, is infiltrated with leucocytes and shows other signs of castrate atrophy. (5) Apparently both (a) endocrine stimulation and (b) distention are factors in hyperplasia of uterine muscles. The second factor alone induces hyperplasia in muscle tissue in the ovariectomized rabbit.

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<sup>11</sup> Rogers, P. V., and Allen, E., *Endocrinology*, in press.