ACTOMYOSIN FORMATION BY ESTROGEN ACTION

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CORNER and his associates have shown that uterine activity is under the control of the ovaries (1). Frank *et al.* observed that after administration of estrogen to ovariectomized rats, the excised uterus, which is ordinarily inactive as a result of castration, exhibits rhythmic contractility (2).

Reynolds observed the effect of estrogen on the uterine muscle *in vivo*, using unanesthetized rabbits, and pointed out that estrogen affects uterine muscle only *in vivo* and only after a definite latent period following administration. He also found that the relative inactivation of the myometrium following ovariectomy takes place far more rapidly than any of the regressive changes in the histological characteristics of the uterus (3, 4).

With this briefly mentioned background in mind one can ask, how does estrogen modify the nature of the uterine cell? Among the many possible ways of studying this question one in particular was suggested by the work of Szent-Györgyi and his associates (5–8) who discovered that actomyosin (AM) is the contractile 'skeleton' of the striated muscle fibril and so gave a better understanding of the AM-ATP system.

Starting in the laboratory of Prof. Szent-Györgyi in Budapest, the author observed the presence of AM in the uterus (9). With the author's method of uterine-AM extraction (9, 10) and with Straub's viscosimetric technique (5) for determination of the AM concentration it was found that uterine muscle has a lower AM concentration than that of skeletal muscle. Furthermore, an increase was found in the AM concentration of the uterus, in advancing pregnancy (9–11). The viscosimetric determinations were confirmed later by the use of the Svedberg ultracentrifuge and Tiselius electrophoresis technique in Uppsala, Sweden (12).

A number of experimentally established facts show that AM varies in amount in different tissues under various circumstances. We know that there is an increasing AM concentration in the uterus during pregnancy (9–11). We know too, that the active, working part of the uterus, the corpus uteri, during labor contains more AM than the passive, dilating cervix uteri (Naeslund, Snellman, Erdös, Csapo unpublished), and that a different AM concentration exists in the muscle of the left and right ventricles and the atria of the heart (decreasing in the mentioned order) (13). Finally, in one single case after X-ray castration, a lower AM concentration was found in the human uterus (9, 11). From all of these one may conclude that nature

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builds up a high AM concentration where an appreciable amount of work is to be expected and vice versa.

All these observations suggested the working hypothesis, that estrogen affects uterine motility partially through the AM-ATP system. If the above results are correctly interpreted, one can expect a decrease in the AM system after castration as well as in the ATP-ase activity, the first being the contractile skeleton of the uterine cell, the second a basic factor of the energy-supplying mechanism. After administration of estrogen on the other hand a restoration must occur.

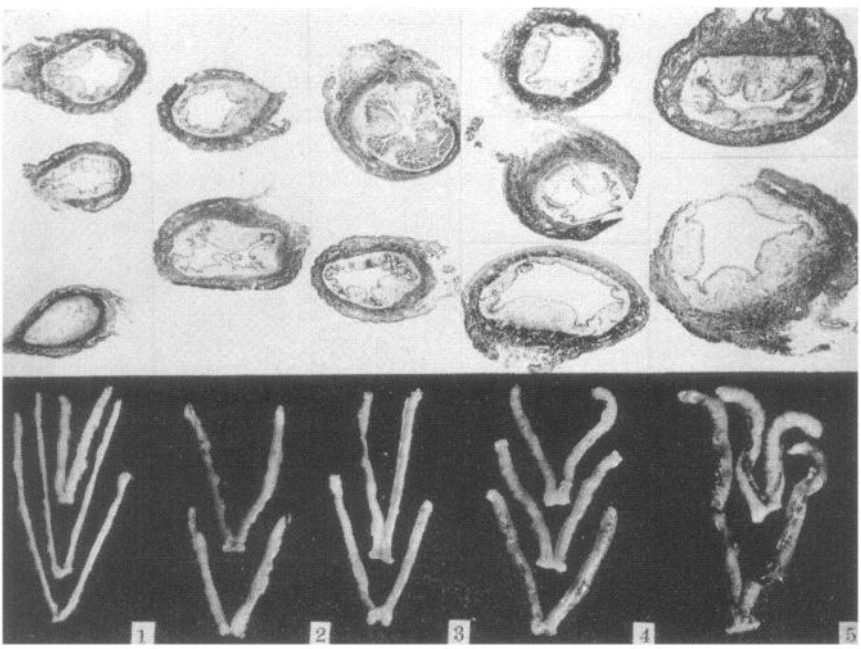

Fig. 1. EFFECT OF OVARIECTOMY and subsequent administration of estrogen in size and diameter changes of rabbit uteri. *Group 1:* after ovariectomy; *group 2:* 12 hours; *group 3:* 24 hours; *group 4:* 48 hours; and *group 5:* 96 hours after estrogen treatment. *Above:* cross section of uteri (magnification: 3.4); *below:* whole uteri (magnification: 0.25). See text for discussion.

RESULTS

In order to study the question a series of experiments was carried out on mature, virgin, female rabbits (3000–4000 gm.). The animals were isolated for 3 weeks to make certain they had no corpora lutea of pseudopregnancy. Ten mg. of gonadotrophic hormone (Squibb-Follutein, from human pregnancy urine = 24 rat units) was administered intravenously. In this way pseudopregnancy was caused, and after 3 weeks all the animals were in the same estrous condition. Three weeks after the gonadotrophin injection all animals were ovariectomized by laparotomy. Two months later the animals were divided into 5 groups, each containing 2 or 3 animals. *Group 1* was kept as a control group, without estrogen administration. *Groups 2* and *3* received one injection of estrogen; *group 2* was killed 12 hours later and *group 3* 24

hours later. The animals of *group 4* each received 2 injections one day apart and were killed 2 days after the first injection. Those of *group 5* had 4 injections, one every day, and were killed 4 days after the first administration of estrogen. One mg. of crystalline estrogen in one ml. oil (Squibb Amniotin, natural estrogen from pregnant mare's urine, equivalent to 10,000 IU) was used intramuscularly (in 2 divided doses 0.5 ml. each). The animals were killed by a blow, followed by decapitation. The uteri were prepared, photographed and measured very rapidly, keeping the uteri at 0° C. The AM system was then extracted (11, 14) and the concentration of AM and the concentration of M + AM were determined viscosimetrically (5). The total N content of the extracts were also measured by the Kjehldal technique. The ATP-ase activity was also measured using the author's method (14).

Results are summarized in figures 1 to 3. Figure 1 shows changes in size and diameter of the 5 different groups of uteri. *Groups 1* and *5* represent the extremes, the effect of ovariectomy *group 1* and the effect of 4 days estrogen treatment *group 5*. Both the size of the uterus and the diameter of the uterine wall increase after estrogen

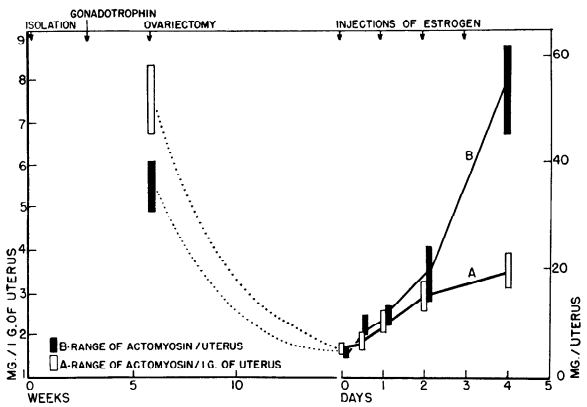

FIG. 2. EFFECT OF OVARIECTOMY and subsequent administration of estrogen on actomyosin content of rabbit uteri.

administration. Figure 2 shows changes in AM concentration of the uterus following ovariectomy, and after estrogen administration. Ovariectomy results in decrease in the AM concentration and 2 months after castration the concentration of AM is only 20 per cent of the original estrous value. Twelve hours after a single injection of estrogen the AM formation is marked, and up to the 4th day a gradual increase is observed in the AM concentration. This very early increase of AM concentration is remarkable, because we know from the work of Astwood that about 12 hours following estrogen injection the uterus is hyperemic and contains more water, so that the protein content is diluted. After 4 days of estrogen treatment a 120 per cent increase is found in the AM concentration, but the estrous value, however, is not reached. These data explain the changes in one gm. of muscle. But synchronously with the AM formation, the amount of uterine muscle also increases. If we wish to know how much AM is formed as a result of estrogen action we must multiply the concentration of AM by the weight of the uterus. This calculation results in *curve B*, which shows the change in the AM content per uterus. One can see that the ovariectomized animals contain only about 14 per cent of the uterine AM present in the

estrous animals. Four days of estrogen treatment result in 15-fold increase in the AM content per uterus exceeding the estrous level. Figure 3, *curve A*, shows the decrease due to the ovariectomy and the following increase after administration of estrogen in the whole M + AM complex. *Curve B* shows changes in the total N content of 4 ml. of extract (representing one gm. of uterus). The curve has a similar form to that representing the changes in the M + AM concentration shown in the previous figure, but the differences are not so extreme. Studying the relation of the N content of the *extract* to the N content of the whole *muscle*, it was found that about 85 to 90 per cent of the N-containing substances go into solution by extraction. On the other hand, it has been shown that about 65 per cent of the total N is protein N. With the help of these data one can calculate the protein concentration during the different conditions of the uterus.

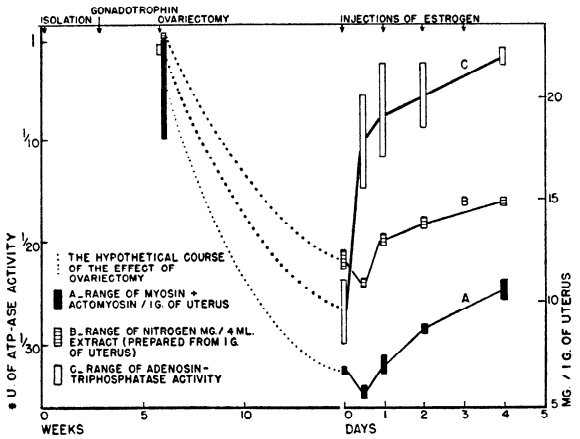

FIG. 3. EFFECT OF OVARIECTOMY and subsequent administration of estrogen on the myosin + actomyosin content, nitrogen content, and on the adenosine-triphosphatase activity of rabbit uteri.

* One U of ATP-ase activity = ATP-ase activity of one ml. original extract which splits 0.2 mg. ATP (potassium salt) at 25° C. during one minute.

Following ovariectomy a decrease in the ATP-ase activity occurs, *curve C*, and 2 months after, the activity is 15 times less. Twelve hours after the single injection of estrogen (when the AM concentration is low but increasing) a 10-fold increase takes place and after 4 days of treatment the ATP-ase activity practically reaches the original estrous level. The difference among the degree of change in protein, ATP-ase and AM following ovariectomy and substitution of estrogen is evidence that uterine muscle may be functionally as well as chemically different after castration and estrogen administration.

SUMMARY

Following ovariectomy the contractile actomyosin system of the uterus and the adenosine-triphosphatase activity decrease. The administration of estrogen initiates a recovery toward the situation existing in estrus by causing an increase in the actomyosin concentration and also an increase in the adenosine-triphosphatase activity. These data can be correlated with changes in uterine contractility observed by physiologists after ovariectomy and after *in vivo* action of estrogen in the ovariectomized animal.

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