although the effect was temporary (Fig. 1). The concentration of hemoglobin ran in all experiments parallel to the red cell count.

According to these experiments cobalt polycythemia seems to be due to the inhibition by cobalt of the respiratory function of immature red cells. Once these cells have lost their ability to respire they are thrown into the general circulation as mature non-respiring cells, being replaced in the bone marrow by new cells. The function of ascorbic acid as one of the regulators of the level of red cells in the circulating blood seems probable in the light of these experiments.

8999 C

Participation of Ovarian Factors Other than "Estrin" in the Estrus Phenomenon.

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We have recently shown that the evaluation of the potency of estrogenic substances cannot be based solely on the ability of these substances to cause vaginal cornification in test animals. Other physiologic effects, perhaps more important from a therapeutic standpoint, must also be considered.[1] However, even if the various activities of the known estrogenic substances were available to a high degree in a single "estrin", it would still be doubtful whether this estrin could serve therapeutically as a perfect substitute for the normal ovary, in bringing about the estrus phenomenon.

Aside from teleological reasoning based on its site of origin, the estrogenic substance elaborated by the ovarian follicle† merits consideration as being the true estrus hormone chiefly because its administration to castrate animals results in a state which resembles spontaneous estrus in the intact animal. With neither this nor any other estrogenic substance, however, has it been possible to reproduce in castrate animals a certain phase of estrus which has been

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Freed, S. C., and Soskin, Samuel, *Endocrinology*, 1936, **20**, 863.

† Recently identified as dihydroxyestrin.[3]

3 MacCorquodale, D. W., Thayer, S., and Doisy, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1182.

observed in intact animals. We refer to the tremendously enlarged uteri in parabiotic rats, where one partner has been castrated.[2] In such an experiment the constant elaboration of follicle stimulating hormone by the pituitary of the castrate animal influences the ovaries of its partner to bring about a continuous estrus. Since there is no evidence that any pituitary substance can produce the above uterine response without the intermediation of the ovary, and since it has been impossible to evoke this response with the known estrogenic substances in the absence of the ovary, it seems necessary to postulate an ovarian factor other than or complementary to estrin, as being the responsible agent.

In view of the above, we were interested in comparing the results of prolonged estrin administration in normal and in castrated adult female animals. Our results indicate the existence of ovarian factors other than estrin, which affect the uterus in both an augmentative and inhibitory manner.

Methods and results. 25 International Units of dihydroxyestrin benzoate‡ were administered daily to a number of normal and castrated adult female rats. After periods of 4, 20, 30 and 60 days respectively of this daily treatment, about 10 of the normal and a similar number of the castrate animals were sacrificed for examination. The results are summarized in Table I.

Although vaginal cornification was present throughout the course of injections in both normal and castrate animals, examination of the uteri showed marked differences between the 2 types of animal, and in each type of animal at different times. Thus the uterus in the castrate animals reached its maximum weight after about 20 days of estrin treatment. Continued injections gave no further response, but rather some retrogression. The normal uterus, on the other hand, was not affected by the estrin treatment up to the 20th day. It then rapidly increased in weight and continued to do so throughout the time that the injections were maintained.

On closer examination the uteri showed even more marked differences. After 4 days of estrin administration, the uteri in the castrate animals were hyperemic and distended with fluid; the uterine epithelium was very high columnar, of the secretory type—the typical estrus reaction. After the same period of estrin treatment, the endometrium in the normal rats varied from the low cuboidal to high columnar. Only 2 out of the 10 normal animals presented

2 Martins, T., and Rocha, A., *Endocrinology*, 1931, **15**, 421.

‡ We are indebted to Dr. Gregory Stragnell of the Schering Corporation for a supply of this material.

TABLE I.

Effect of Daily Estrin Administration on the Uterus of Normal and of Castrated Adult Female Rats.

Days of Estrin Administration	Normals			Castrate		
	No. of Animals	Aver. Wt. of Uterus,* mg.	Description	No. of Animals	Aver. Wt. of Uterus,* mg.	Description
0	12	382	Different stages of estrous cycle	9	122	Atrophic
4	10	386	As above	14	316	Thin-walled; distended with fluid; advanced endometrial proliferation
20	8	390	" "	8	420	Thick-walled; moderate endometrial proliferation
30	8	496	(a) 6 animals—thick-walled, moderate and advanced endometrial proliferation. (b) 2 animals—hypertrophied wall; distended with fluid; advanced endometrial proliferation.	8	384	As above
60	9	570	(a) 6 animals—as in (a) above (b) 3 animals as in (b) above	6	360	" "

*Where distension is noted, uteri were weighed after removal of fluid.

uteri typical of estrus, others being in various stages of diestrus. The distribution of results in these normal animals differed in no way from that found in examining a similar number of untreated control rats.

After 20 days of estrin administration, the uterus in the castrate animals was pale and thick-walled. The uterine epithelium was not so tall as in the preceding stage. At this time the uterus in the normal animals appeared to be still unaffected by the estrin injections, various stages of endometrial development being found, as before. The ovaries, however, had undergone considerable atrophy.

At the end of 30 and 60 days of treatment, the uterus in the castrate animals was similar to that found at the 20-day interval. In the normal animals, however, the uterus had undergone a significant hypertrophy. Its diameter averaged twice that of the treated castrate uterus at the corresponding time. Five out of the 17 normal animals which comprised the 30- and 60-day groups had greatly enlarged uteri due to distension with fluid as well as thickening of the wall. The uterine endometrium in all the normal animals was now definitely proliferative, to a degree equal or surpassing that of the castrate uterus at this stage. By the 30th day, the ovaries had become extremely atrophic.

Our results indicate that the animals retaining their ovaries differ from castrate animals, in their uterine response to prolonged estrin administration, in at least 3 respects. The ovaries, therefore, presumably elaborate a factor or factors concerned with the following reactions (in order of their appearance in our results):

1. Preliminary inhibition of the uterine response to estrin.
2. Rhythmic activity of the endometrium.
3. Augmentation of the uterine response to estrin.

On first thought the remarkable resistance of the normal uterus for the first 20 days of estrin administration does not seem difficult of explanation. Since infantile animals resemble the castrate adults in their response to estrin, it is apparent that the refractoriness of the uterus in the normal adults must be due to some element in the mature ovary. It might seem reasonable to assume that the administered estrin stimulates the hypophysis to liberate its luteinizing hormone[4]; the corpora lutea are activated, giving rise to progestin, which antagonizes the action of the estrin. On closer examination, however, this explanation is not satisfactory. The simultaneous administration of progestin with estrin has been shown to prevent cornification of the vagina and to inhibit estrus in the

[4] Fevold, H. L., Hisaw, F. L., and Greep, J. R., *Am. J. Physiol.*, 1936, **114**, 508.

uterus, although it does not prevent the enlargement and maturation of the uterus.[5] In our work, on the contrary, the vaginal cornification was not inhibited and the uterus failed to develop.

The cyclic activity of the uterine endometrium in the normal rats throughout the 60 days of estrin administration, stands in sharp contrast to the relatively stable condition of the endometrium in the castrate animals under the same conditions. Since both types of animals were receiving the same ample supplies of estrin, it seems evident that the ovary must supply the factor responsible for the endometrial rhythm. And since, after the 20th day at least, the follicular apparatus of the ovary is extensively damaged, it seems likely on anatomical grounds that the rhythm factor originates in the interstitial tissue. In this connection, it is of interest that an extract of interstitial tissue recently has been shown to possess peculiar estrogenic properties[6] which are, as yet, difficult to correlate with our observations. But whether the rhythm factor is an estrogenic substance or a supplementary material having no estrogenic properties by itself, it is apparent that it is not included in the estrin of follicular origin.

The greater hypertrophy of the uterus in the normal animals during the later stages of our experiments, like the endometrial rhythm, can hardly be ascribed to the atrophic follicular apparatus. It is, of course, impossible to judge at present as to whether this augmenting factor is identical with the rhythm factor, whether it is an estrogenic substance more potent than those hitherto observed, or whether it is a substance which supplements the activity of the follicular estrin. In this regard, it may be noted that a very potent new, crystalline estrogenic substance which differs chemically from the known estrins, has recently been isolated from the hog's ovaries.[7]

Our results offer ample reasons to explain the failure of the castrate animal which is receiving estrin to reproduce the gigantic uterus of the parabiotic animal in continuous estrus. We have ascribed this failure to the lack in the castrate of ovarian factors which are available to the parabiotic animal. It may be that the lack of these same factors is responsible for the poor therapeutic results in certain cases, when estrin is administered as substitutional therapy to the human female. We are well aware of the possibility that the removal of the ovaries may result in a secondary disturbance in function of other organs associated with sex activity, and

[5] Selye, H., Browne, J. S. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 198.

[6] Marlow, H. W., *Endocrinology*, 1936, **20**, 339.

[7] Andrew, R. H., and Fenger, F., *Endocrinology*, 1936, **20**, 563.

that our results may be due to these secondary disturbances rather than the lack of our hypothetical factors. Nevertheless the evidence for the latter seems to us to be sufficiently strong to warrant a careful search, in the hope of adding some useful agents to our endocrine armamentarium.

Conclusion. The effects of prolonged daily administration of estrogenic substance to normal and to castrated adult female rats are compared. It is concluded that, in the rat, "estrin" alone cannot completely replace the ovary in the induction of the uterine changes of the estrus phenomenon. Our results indicate the existence of ovarian factors other than estrin (or progestin) which influence the estrus phenomenon in at least 3 respects: 1. Preliminary inhibition of the uterine response to estrin. 2. Rhythmic activity of the endometrium. 3. Augmentation of the uterine response to estrin.

9000 C

Effect of Administration of Parathyroid Extract on Serum Calcium Level in the Nephrectomized Rat.*

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Collip, Pugsley, Selye and Thomson[1] have observed resorption of bone in bilaterally nephrectomized rats which had been injected with massive doses of parathyroid extract. They are of the opinion that the primary action of parathyroid hormone is to cause an increased proliferation of osteoclasts, which actively function to release calcium from the bones to the blood. Since the observations of these workers were not supplemented by serum calcium determinations, which should yield evidence of the passage of calcium into the blood, we have carried out similar experiments† in which calcium analyses have been made.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1 Collip, J. B., Pugsley, L. I., Selye, H., and Thomson, D. L., *Brit. J. Exp. Path.*, 1934, **15**, 335.

† The incisors of the animals will be examined by Dr. Isaac Schour, and the femurs will be studied by Dr. F. A. McJunkin. Their findings will be reported in later communications.