

THE EFFECT OF PROLONGED APPLICATION OF LARGE
DOSES OF FOLLICULAR HORMONE ON THE UTERUS OF
RABBITS*

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Up to the present, research on the follicular hormone has chiefly been concerned with the effect of a single application of hormone on the uterus. It was first of all necessary to investigate the effect of such quantities of hormone as are produced physiologically in the body. We now know that folliculin stimulates oestrus in a castrated mouse when given in a dose of 0.1 γ and that 200,000 to 300,000 mouse units, equalling 0.02 to 0.03 gm., are required to produce proliferation in man. When the application to the organism of large amounts, exceeding the quantities which are produced physiologically, is continued over prolonged periods, we are dealing with the effect of hormones as drugs. In this way we are able to demonstrate secondary effects, which may be desirable or undesirable from a therapeutical point of view. We have thus been able to demonstrate lately that folliculin application may influence the physiological monthly cycle of women by delaying menstrual onset.¹ By application of 70,000 m. u. folliculin, *i.e.*, a third of the quantity of hormone necessary for proliferation, an amenorrhoea may be produced through a delay in the menstrual onset, an effect which, therapeutically speaking, may be desired at times. This clinical observation led us to ask, what effect might be produced by prolonged application of follicular hormone. In the present paper the effects on the rabbit's uterus only are to be reported. A record of further results will be given later.

*The experiments have been carried out with the aid of the Rockefeller Foundation.

¹Zondek, B., *Wien. klin. Woch.*, 1936, in press.

EXPERIMENTAL

Immature rabbits, weighing 1,400 gm., and sexually mature animals, weighing 2,000 gm., received follicular hormone subcutaneously twice a week, either hormone alone (Folliculin-Menformon)² or the benzoic ester of the dihydrofollicular hormone (Dimenformon). The doses varied from 2,000 to 25,000 m.u. per week. The animals were laparotomised under ether anesthesia several times at intervals of a few weeks in order to ensure continuous observation of the effect of the treatment. On each occasion, a larger or smaller specimen of the uterus was extirpated, in order to confirm the progress of anatomical changes. As it might take too much space to report all the experiments in detail, we shall only give a record of each variety of experiment. The result may be anticipated. Following application of large doses of follicular hormone during the course of weeks or months, we observed the following effects on the rabbit uterus.

1. *Hyperaemia*.—The vessels in the myometrium may show lacunar dilatation, occupying nearly half of the muscular layer. The hyperaemia may also extend to the endometrium. Extravasal haemorrhages occur at times. Thus we observed haemorrhages between the serous and the muscular layers and, very rarely, bleeding in proliferated villi. Special attention should be called to the fact that extravasal haemorrhages very rarely occurred in spite of the application of large doses of folliculin. We shall demonstrate elsewhere how haemorrhages from the mucous membrane may be regularly produced.

2. *Glandular-Cystic Hyperplasia of the Uterine Mucous Membrane*.—(Fig. 1.) There is an increased number of glands; the dilatation of their lumina amounts to several times their usual size; the epithelial lining shows marked flattening. The larger glands rupture, and the leucocytes which are found in their lumina, extravasate in the tissues. The cystic hyperplasia of the uterine mucous membrane, experimentally produced in rabbits, represents a condition, analogous to that of human beings, in which persistence of the follicle associated with the resulting increase in the production of folliculin produces glandular hyperplasia. As we were able to demonstrate some time ago (1933),³ a glandular-cystic hyperplasia of the uterine mucous membrane is also to be found in primary amenorrhoea caused by the persistence of the follicle.

3. *Aseptic Suppuration in the Uterine Cavity*.—(Fig. 2.) The uterus shows tremendous enlargement, being the thickness of the thumb, and is dough-like in consistence. When cut, large amounts of friable cheesy material are discharged. The muscular layer of the uterus is dilated and thinned out by the cheesy material. The endometrium is transformed to a honeycomb-like structure in the meshes of which the cheesy pulp is to be found. This consists mainly of eosinophil leucocytes and necrotic masses, in which bacteria could not be found. It would

² We are indebted to the Organon (Oss) for kindly supplying large amounts of Folliculin-Menformon and Dimenformon.

³ Zondek, B., *Acta Gynec. Skand.*, 1934, **12**, 309.

appear that an aseptic suppuration may occur after continued administration of a hormone.

4. *Necrosis of the Muscular Layer.*—(Fig. 3.) At the beginning only a whitish discoloration of the uterine surface is to be seen. Later there is a small solid white spot, and finally a white infiltration of the uterus, showing on cross-section a wedge-shaped area like hard cheese in appearance, with normal uterine tissue in its vicinity, towards the mesometrium. The aspect thus produced is so peculiar and striking, that, on the first occasion, we thought it the result of a faulty technique. We believed that pregnant animals had been used for the experiment, the fetus having died and necrosed during the course of treatment with folliculin which lasted for weeks. But when the same effect was produced on immature animals, it became evident that this was a reaction caused by the application of folliculin. The microscopic examination shows that the white spots represent infarct-like necrosis of the myometrium. The process starts with a pronounced hyperaemia, in which the vessels are highly distended by blood. Later, thrombosis of the vessels occurs and, as a sequel, wedge-shaped necrosis is produced in the muscular layer. The mucous membrane takes little part in this necrosis. The necrotising process usually starts in the uterine areas opposite the mesometrium, apparently preferring those places where placentation takes place following fertilisation. The white areas are disseminated throughout the uterus and are continuous with intact blue-red ones. The resulting contrast in colours produces a striking appearance.

Stress should be laid upon the fact that the four processes described above do not occur one after the other in each experiment, but may coexist in the same uterus. Thus a cystic hyperplasia of the endometrium, for instance, may be encountered at the top of one of the horns of the uterus, while 2 cm. lower down extreme hyperaemia is seen, and necrosis of the muscular wall is to be found elsewhere in the same horn.

The fact that the reactions just described can only be produced in the rabbit, not in the rat, appears important to me. Immature and sexually mature rats received large amounts of hormone (Folliculin-Menformon or Dimenformon) for weeks or months. The genital organs had grown markedly (hyperplasia); the uterine cornua showed livid discoloration, but extreme hyperaemia, thrombosis or necrosis was not to be observed. Following prolonged application of hormone the uterine mucous membrane of the rat showed marked proliferation, and the glands became more numerous; they also penetrated almost to the muscular layer, but a cystic degeneration of the glands was not observed. We thus see that different animals (rabbits, rats) react in different ways to prolonged application of folliculin. It is well known that the hormonal reactions in the various species of animals

differ widely.⁴ That hormone effects vary even among rodents, is also known. It need only be mentioned that we are regularly able to produce hematomas of the follicle in the rabbit and the mouse by way of prolan or the urine of pregnant women, but very rarely so in the rat.

Some individual protocols follow.

1. Immature Rabbit, 1,300 Gm. Weight.—

Exploratory laparotomy shows infantile ovaries and uteri. From the 4th of April to the 24th of June, 1935, the animal receives subcutaneous injections of 1,000 m.u. Folliculin-Menformon twice a week, altogether 18,000 m.u.

Laparotomy on June 24, 1935.—Uterus markedly hyperplastic, dark red, cystic at one spot, having the appearance of an early pregnancy. A specimen of the uterus is extirpated. Microscopic examination⁵ shows a cystic-hyperplastic mucosa (Fig. 1). The ovaries are completely infantile. Further treatment: Twice a week 1,000 m.u. Folliculin-Menformon.

Laparotomy on July 16, 1935.—(The animal received 27,000 m.u. Folliculin-Menformon in all.) The uterus looks quite different now. The left horn contains several glossy infiltrations (necrosis) which form a lively contrast to the neighbouring dark red uterine tissue. The right horn, on the contrary, does not show any necrosis, but its redness is still darker than that of the left one. Diffuse hemorrhages are to be found between the serous and the external muscular layer.

2. Immature Rabbit, 1,400 Gm. Weight.—

Exploratory laparotomy shows the genital organs to be infantile. The animal receives 10,000 m.u. Dimenformon subcutaneously twice a week from Apr. 4 to June 6, 180,000 m.u. in all.

Laparotomy on June 24, 1935.—The genital organs actually give the impression of being monstrous. The vagina has grown enormously, is as thick as a thumb. The cornua of the uterus are dark red; above the cervix, at a distance of 1 cm. each, are two white areas which infiltrate the uterus (necrosis) (Fig. 3). Above these, two stripes looking like white coatings extend almost to the top of the uterus. The genital organs (uterus and vagina) weigh 10 gm. together, in the control animal 2.5 gm. The ovaries are infantile.

The microscopic aspect of different parts of the uterus varies considerably: (a) A clearly differentiated necrosis of the myometrium was found above the cervix (at the white spots). In this case the necrotising process takes place only

⁴ For details see: Zondek, B., *Hormone des Ovariums und des Hypophysenvorderlappens*, Vienna, Julius Springer, 1935, Chapter 37.

⁵ I am indebted to Professor Sophia Getzova, chief of the Pathological Department of the Hadassah Hospital, who has kindly given her opinion on all the preparations.

in the external longitudinal part of the muscular layer. In the endometrium are hugely dilated vessels, which are hardly filled. (b) At another part of the uterus the longitudinal section of the muscular wall shows extreme hyperaemia with highly distended vessels, in addition thrombi with commencing organisation and incipient necrosis (white stripes). (c) In a third part the mucous membrane shows cystic hyperplasia.

3. *Immature Rabbit, 1,450 Gm. Weight.*—

Exploratory laparotomy shows the genital organs to be infantile. The animal receives 5,000 m.u. Dimenformon subcutaneously, twice a week, from Apr. 4 to June 12, 1935, 90,000 m.u. in all.

Laparotomy on June 12, 1935.—Uterine horns are much enlarged, dark red, of the thickness of a little finger. There is at the right uterine cornu a circumscribed white infiltration. The histological examination of the latter shows incipient necrosis of the muscular wall and cystic hyperplasia of the endometrium. Further treatment: The animal receives 5,000 m.u. Dimenformon twice a week up to July 16, 1935, 135,000 m.u. in all. Then it is killed.

The macroscopic aspect appears to be quite different from that of the first laparotomy (June 12, 1935). The livid discoloration has disappeared, the uteri look pale, but are immensely enlarged, being as thick as a thumb, and are filled with a doughy fluid. When cut, white masses, looking like friable white cheese, are evacuated.

Microscopic examination: Thin muscular layer; instead of the normal endometrium, cystic spaces of various sizes, most of which are already to be seen with the naked eye. Size of the largest spaces, 2 to 3 mm. The walls of the cystic spaces are lined by pavement epithelium. These are small glands with cyst-like dilatation. Small glands with a somewhat higher epithelial lining are very scanty. A loose connective tissue is to be found in the septa, separating the spaces from each other. Aggregations of eosinophil leucocytes are scattered throughout these septa. The leucocytes are frequently migrating through the epithelial lining. The uterine cavity is highly charged with eosinophil leucocytes. In addition, desquamated epithelial cells, frequently invaded by eosinophil leucocytes, are sometimes to be found. Muscular layer: Eosinophil leucocytes are scattered all round. Pronounced hyperaemia, some haemorrhages and oedema are to be observed. Bacteria could not be found. There was aseptic suppuration in the uterine cavity.

4. *Sexually Mature Rabbit, 1,800 Gm. Weight.*—

The animal receives 12,500 m.u. Dimenformon twice a week from Jan. 23 to Mar. 26, 1935, 250,000 m.u. in all. On Mar. 26 the animal is killed.

The uterus shows a mottled appearance. At a distance of $1\frac{1}{2}$ cm. from each other, the uterus shows white, clearly differentiated infiltrations (necrosis), alternating with blue-red areas. Anybody seeing this for the first time, will at once think of dead feti with secondary necrosis.

Microscopic examination: Wedge-shaped necrosis of the muscular layer. The endometrium is spongy and oedematous. A villus of the mucous membrane has been preserved and shows extravasal mucosal bleeding, in addition to hugely distended vessels. Examination of the blue-red areas shows cystic hyperplasia of the mucous membrane and a perfect muscular layer. Weight of the uterus, 4 gm.

SUMMARY

Follicular hormone under physiological conditions produced hyperplasia of the muscular wall and proliferation of the mucous membrane of the uterus of rabbits.

The following pathological changes were brought about by prolonged application of large doses of the hormone: (*a*) hyperaemia of the myometrium and the endometrium, with occasional scanty extravasal haemorrhages; (*b*) glandular-cystic hyperplasia of the endometrium; (*c*) infarct-like necrosis of the myometrium; (*d*) aseptic suppuration in the uterine cavity.

These four processes can sometimes be found simultaneously in the same uterus, but they occur more frequently in sequence. While follicular hormone, applied in physiological doses, has a stimulating effect, prolonged application of large doses destroys the uterus.

The effects described above were only to be observed in the rabbit, not in the rat. This illustrates the fact that hormone reactions may vary in different species.

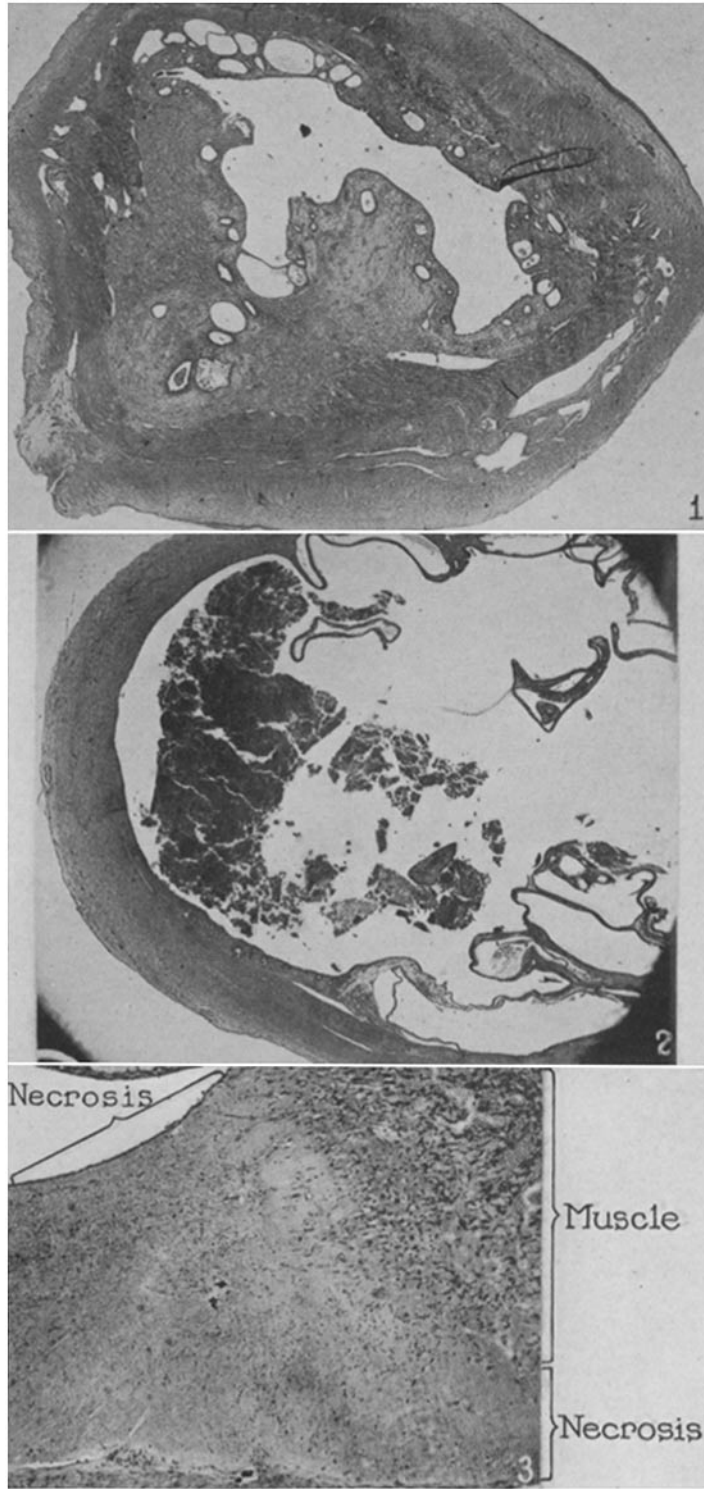
EXPLANATION OF PLATE 47

Hematoxylin-eosin stain. Zeiss microscope, ocular 10 ×, objective 8 ×.

FIG. 1. Glandular-cystic hyperplasia of the uterine mucous membrane.

FIG. 2. Suppuration in the uterine cavity.

FIG. 3. Wedge-shaped necrosis of the muscle.



(Zondek: Effect of follicular hormone on uterus)