

## The Cyclic Changes of the Lymph Vessels in the Uterus of the Mouse which has been pregnant\*

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### Summary

When adapted to the natural possibility of reabsorption of the uterus, using the dye Patent Blue Violet and Japanese ink, the orthograde demonstration of the cyclical behaviour of the lymphatics during the oestrus cycle was successful even with parous mice.

As investigations have already shown in nulliparous mice (5), the characteristic variations in all five stages of the sexual cycle are also found here:

As a result of the pregnancies the lymphatics of the endometrium are more strongly developed, in contrast to the nulliparous mouse. However, in the stage of pre-oestrus and oestrus the marked or very marked widening, of varying calibre does not show in such a striking way. In the subsequent two stages of the sexual cycle prominent elevations are found in the lumen of the uterus, and these have lymphatic capillaries running in them. The further stage, a more or less reticulate arrangement of the lymphatic capillaries, with fine branching ramifications, is equally typical.

As a result of the pregnancies, the larger lymphatic vessels are often curled up like a cork-screw, and also show their typical beaded appearance.

In order to demonstrate the cyclical behaviour of the uterine lymphatics in parous animals, experiments were undertaken with Patent Blue Violet and with Japanese ink. The nature of the experiment was modified in order to adapt it to the normal possibilities of reabsorption in the uterus. The mouse, which has a haemo-endothelial type of placenta, is a particularly suitable experimental animal, as material easily identifiable in its different phases, can be obtained without any great expenditure of time and equipment.

### Material and Methods

As in earlier investigations in nulliparous mice (5), Patent Blue Violet was again chosen as it

possesses, as is well-known, an affinity for the lymphatic system (1, 10). Japanese ink shows a similar affinity (7).

The investigations were undertaken on healthy female mice at intervals of three weeks (for vaginal smears) to seven weeks (for dye applications), after their second pregnancy. As in nulliparous mice (5) the stain was placed in the cavity of the left cornu of the uterus, of the anaesthetised animals, and also post mortem.

In accordance with the comparative standardized terminology of *Preuss* (9), the parous mouse goes through a sexual cycle of five stages, as was shown in nulliparous mice by the investigations of *Fabian* (5) and *Nitschke* and *Fabian* (8). These stages are:

1. Oestrus
2. Early post-oestrus
3. Late post-oestrus
4. Inter-oestrus
5. Pre-oestrus.

### Results

The following appearances were shown in all five stages of the sexual cycle of the parous mouse:

The application of the dye to the uterine mucous membrane produced a black pattern on the surface, between the superficial epithelium and the sub-epithelial layers (Fig. 1), an appearance almost identical to that seen in the nulliparous mouse.

Later on, from the surface of the uterine mucous membrane, there develops a clearly outlined lymphatic capillary network. One repeatedly finds nodular thickenings, which in their turn carry a few very fine, brush-like

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Fig. 1: Application of dye to the endometrium. Black mark on the surface between the epithelia. Absorption of Japanese ink, cleared in Salicylsäuremethylester (oil of Wintergreen). Negative enlargement 400 x, positive enlargement 1320 x.



Fig. 2: Button-shaped thickenings and very fine brush-like ramifications in the endometrium. Absorption of Japanese ink, cleared in Salicylsäuremethylester. Negative enlargement 400 x, positive enlargement 1320 x.

branches, which are still visible with the light microscope (Fig. 2). However, it is not possible to see these in similar preparations in nulliparous mice.

Japanese ink gets into the orifices of the glands which are not filled with secretion and also extends for a short way from the glandular openings into the peri-acinar region. The removal of the dye from the base of the mucous membrane takes place through larger efferent lymphatics, which run through the circular muscle layer into the stratum vasculare, which is more strongly developed here. In the parous animals the lymphatic capillaries have a larger diameter and hence fill even more markedly, in comparison with the nulliparous animals.

In *oestrus* the mucous membrane is also "opened up" by the oedema.

The more or less typical picture of this phase of the cycle, seen in nulliparous animals (5), which consists here and there of variable and enormously developed lymph capillaries, is also found here, but not in such a strongly

marked way. On account of the previous pregnancies the meshwork pattern of the lymphatic capillaries is more uniformly distributed over the whole oedematous mucous membrane.

The very markedly oedematous stroma of the previous phase of the cycle is markedly compressed and condensed in this phase, the *early post-oestrus*, even in parous animals. At the beginning of the phase the lymphatic capillary network in the upper third of the mucous membrane is still wide meshed, but becomes very narrowed in the sub-epithelial portion up to the middle of the phase, which is in striking contrast to the nulliparous animals where, during the whole phase, a very wide-meshed network is found. Later on, very definite little prominences develop, which project into the lumen of the uterus and show a particularly dense lymphatic capillary network (Fig. 3).

In the *late post-oestrus* the stroma is markedly condensed as in the previous phase, as is also seen in nulliparous animals.

What is striking here, in contrast to the nulliparous animals, is the marked development of

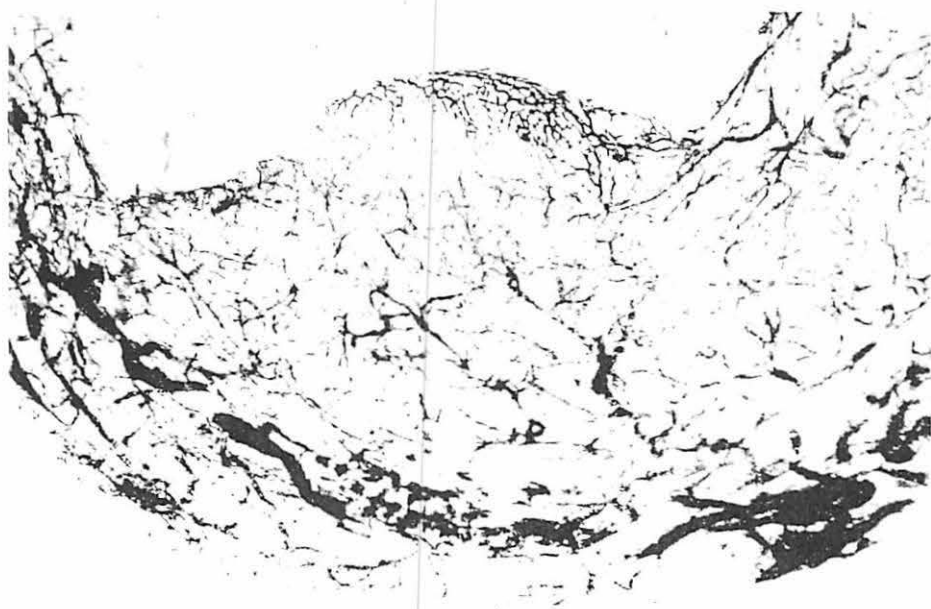


Fig. 3: Early post-oestrus. Protuberance projecting into the lumen of the uterus with a very dense lymph capillaries reticulum in the subepithelium. Absorption of Japanese ink, cleared in Salicylsäuremethylester. Negative enlargement 200 x, positive enlargement 666 x.

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the prominences directed towards the lumen, which appeared in the previous phase. These are also seen in carnivores (6), and in pigs (2). These prominences can be so markedly developed that they remind one of the villi in the small intestine (3, 4).

In suitable sections one can see in these structures, usually one, or even here and there several, very prominent axially running lymph capillaries (Fig. 4). The glandular ducts present there are surrounded by ring-like lymph capillaries. The mucous membrane between such formations shows a dense sub-epithelial lymphatic capillary network, resembling that in nulliparous animals, where condensation of the stroma exists between two adjoining circular vessels.

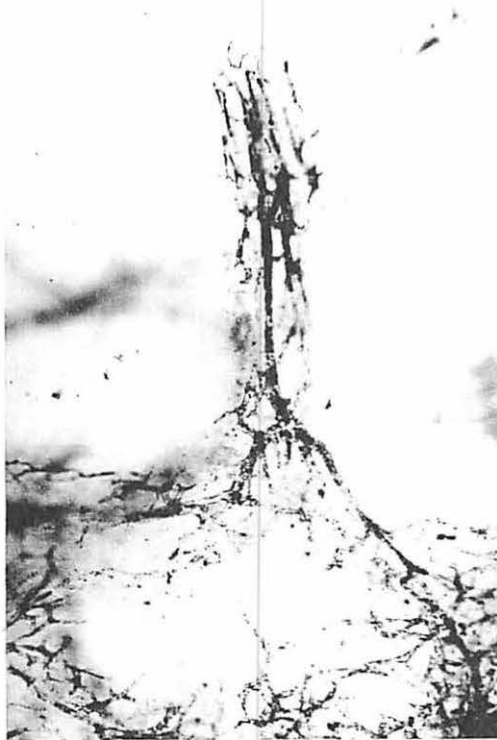


Fig. 4: Late post-oestrus. Great development of a protuberance into the lumen of the uterus. Lymph capillaries in the middle of the protuberance. Absorption of Japanese ink, cleared in Salicylsäure-methylester. Negative enlargement 250 x, positive enlargement 832 x.

The relatively long *inter-oestrus* likewise, continues to show a marked condensation of the stroma. The processes of the mucous membrane directed towards the lumen, which are seen in parous animals in the previous phase, have here almost completely undergone involution, towards the end of the interoestrus.

In comparison with the nulliparous animals it is not possible to demonstrate any further variations in the cyclical behaviour of the lymphatics of the uterine mucous membrane in this phase.

In the last phase of the cycle, the *pre-oestrus*, the mucous membrane is again oedematous and "porous". The lymphatic vessels show the same appearance as in the nulliparous mouse. Here, they also start sub-epithelially and show a variable size, even in some cases being enormous with numerous very fine efferent branches, measuring up to 2  $\mu\text{m}$ . The lymphatic capillary network in the central and basal parts of the mucous membrane is similarly arranged to that in nulliparous mice (5).

Here, in the parous mouse the removal of the dye also takes place rapidly, in a matter of seconds, in all stages of the cycle, through large lymphatics in the mesometrium, which run alongside the utero-tubal veins. These are here often curled up like a cork-screw, on account of the pregnancies (Fig. 5) and also have their typical beaded appearance.

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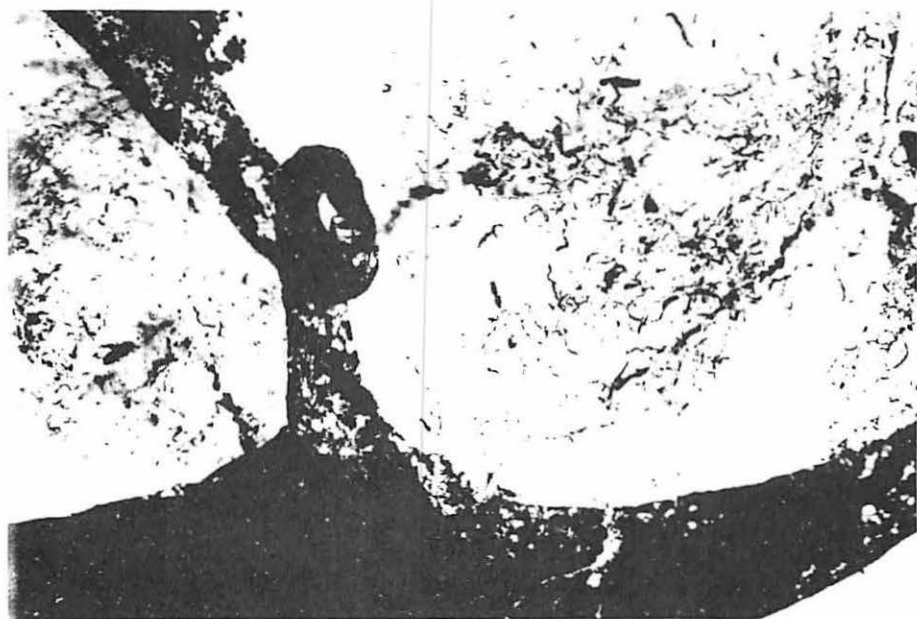


Fig. 5: Lymph vessel in the mesometrium in form of a coil like a corkscrew. Absorption of Japanese ink, cleared in Salicylsäuremethylester. Negative enlargement 250 x, positive enlargement 832 x.

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