Lymphology 11 (1978) 123–127

Investigations on the Uterine Lymphatics of Juvenile Mice, up to the Onset of Sexual Maturity

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Summary

By means of orthograde and retrograde application of dyestuffs (using patent blue violet and Japan ink) one can succesfully demonstrate the uterine lymphatics in juvenile mice, up to the onset of sexual maturity. However, the three subgroups used here do not show the typical features of the cyclical events of nulliparous and parous mice. However, the morphological changes already present, allow one even now to recognize, that with the increasing sequence of individual sexual cycles, cyclical changes in the uterine lymphatics of the mouse were identified.

The fundamental researches of *Hitschmann* and *Adler* (9) whose views are still valid today, on the nature of ovulation and menstruation, as well as their functional and temporal interrelationship, were expanded from various points of view in numerous publications, which concerned the processes in the ovary and uterus during the human sexual cycle. In the years that followed there were similar investigations concerning the cyclical changes in the sex organs of domestic mammals and also laboratory animals. There were also investigations on the cyclic behaviour of the uterine blood vessels in man, domestic mammals and laboratory animals.

So far little notice has been taken of the lymph vessels in these investigations on the sex organs, although *Nuck* (12) had already made his first observations in the year 1692. However, in recent years authors have repeatedly investigated the uterine lymphatics, as a result of which, observations were made particularly about the lymph circulation of the human uterus by *Kubik* and *Várady* (10, 11) and by *Várady* (12). Up to the present, investigations on the uterine lymphatics in the mouse have only been done by *Fabian* (5, 6, 7) who, in this connexion, investigated the cyclical changes during the sexual cycle in nulliparous (5) and in parous animals (6).

In order to obtain information about the uterine lymphatics of juvenile mice, up to the onset of sexual maturity, investigations ought likewise to be undertaken, on which evidence the previous findings should be endorsed (*Fabian* 5, 6, 7).

Material and Methods

As in the previous investigations in nulliparous mice (5) and in parous mice (6) patent blue violet* was again chosen because as is well known, it has an affinity for the lymphatic system (1, 4, 13) as also has Japan ink (8).

The investigations were carried out on sexty healthy white mice divided into three subgroups of twenty each, from the age of 22 days up to the time of the first oestrus the onset of which, however, is very variable in individual animals, usually from the 33rd to the 45th day, with an arithmetical mean of the 39th day.

The time of oestrus was determined on the basis of vaginal smears (3). Between the two dates mentioned – the 22nd and the 39th days, it was possible at about halfway, the 30th day, to identify a very early preoestrus; set against this the 22nd day presiously mentioned, in the juvenile mouse corresponds comparatively to the interoestrus and the onset of sexual maturity (2).

Hence, we find in

Subgroup 1: Mice of 22 day old in comparative interoestrus.

Subgroup 2: Mice of about 30 days showing very early pre-oestrus, and in

Subgroup 3: Mice in the first oestrus and also with possibly completion of the first ovulation.

* Makers: Byk-Gulden, Constance

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The application of dyestuff is made in the left cornu of the uterine cavity of anaesthetized animals, and also post mortem. Retrograde demonstration of the lymphatics was also undertaken with Japan ink.

Findings

With the orthograde demonstration of the lymphatics after application of dye to the uterine mucous membrane, the findings in the three subgroups mentioned here, are the same as in the earlier investigations (5, 6, 7).

The mucous membrane is outlined in black on the surface, and also between the epithelial cells and subepithelially. The latter shows the formation of a fine network (Fig. 1) whose ramifications then pass through at a right angle in the depths of the endometrium and whose lymph capillaries at their base empty into larger efferent lymphatics. The further transport of the dye takes place through the circular muscle layer into the stratum vasculare, which is already developed in the first two subgroups.

In the first two subgroups of juvenile animals the uterine cornu is relatively small in diameter compared with the adult and parous mice. Because of this it was necessary after the orthograde application of patent blue violet, to undertake the retrograde filling of the lymphatics with Japan ink in order to secure a better delineation of the lymphatic system.

Subgroup 1

The uterine mucous membrane is markedly compact and very low. By retrograde injection with Japan ink, parallel coursing lymph capillaries are displayed in the upper third of the mucous membrane, closely packed together under the epithelium (Fig. 2). Their significance becomes apparent in the next subgroup. The uniformly arranged capillary network in the middle and lower thirds of the mucous membrane, as seen in nulliparous and parous animals, is no longer apparent here. However, the more distally running lymph capillaries and the lymphatics are very distensible, often up to six times in the case of the latter.

Subgroup 2

In this subgroup, compared with the former, the mucous membrane is rather spongy and higher, but not yet oedematous, as in the nulliparous and parous animals. After retrograde injection with Japan ink, the lymphatics are very large in calibre. The parallel coursing lymph capillaries, seen in the upper third of the mucous membrane in the previous subgroup, are here spread out (Fig. 3) and give

Fig. 1. Lymph capillaries coursing in the subepithelial layer, with commencing plexus formation and fine ramifications, after the application of dyestuff to the endometrium. Absorption of Japan ink, cleared in Salicylsäuremethylester (oil of wintergreen). Negative enlargement 400 x, positive enlargement 1320 x.

Fig. 2. Parallel coursing subepithelial lymph capillaries in the endometrium of a 22-day-old mouse. Absorption of Japan ink, cleared in Salicylsäuremethylester. Negative enlargement 200 x, positive enlargement 666 x.

off the vessels already leaving at a right angle into the depths of the endometrium. These also can measure, although very sporadically, up to 2 μm, as in the nulliparous and parous animals.

Subgroup 3

The mucous membrane in this subgroup already shows a more or less typical picture. It is slightly oedematous and spongy, although it does not attain in the first oestrus the corresponding development of many oestruses seen in nulliparous and parous animals. Also here, there are in some cases very fine capillaries which run subepithelially in the upper third of the mucous membrane. These then turn downward at a right angle and become enlarged in the middle third of the mucous membrane (Fig. 4). Also with retrograde injection of Japan ink lymph vessels of large calibre are shown, which have here rather a reticulate appearance. The removal of the dyestuff, just as in the nulliparous and parous animals, also takes place here with great speed through lymphatics, in the mesometrium adjacent to the vena utero-tubalis, which also have a beaded appearance.

However, one must bear in mind, that these mice are only at the beginning of their development. Although they are already sexually mature, that is not to say that they are also ready for breeding. This is shown here very clearly in these three subgroups. Although the uterine lymphatics are very well developed,

Fig 3. Very wide-meshed lymph capillary plexus in the endometrium of a 30-day-old mouse.
Absorption of Japan ink, cleared in Salicylsäuremethylester. Negative enlargement 200 x, positive enlargement 666 x.

Fig. 4. Slightly oedematous, spongy endometrium of a mouse in the first oestrus, showing subepithelially in the upper third very fine lymph capillaries which widen in their further course in the middle third. Absorption of Japan ink, cleared in Salicylsäuremethylester. Negative enlargement 200 x, positive enlargement 666 x.

they do not show at all the typical features of the cyclical activity of nulliparous and parous mice. The changes in the uterine lymphatics of juvenile mice with commencing sexual maturity are however shown at the onset, and with an increasing number of individual oestruses would also show the changes determined by the sexual cycles, which have already been described in nulliparous (5) and parous mice (6).

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