TRANSPORT OF MICROSPHERES IN THE GENITAL TRACT OF THE FEMALE RABBIT

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Experimental analysis of the influence of endogenous hormones on egg transport in the oviduct requires groups of animals in which the endocrine environment differs from that prevailing during the early postovulatory period. In order to measure transport in these animals, a substitute for the egg must be introduced. Eggs in cumulus obtained from donor rabbits (Noyes, Adams & Walton, 1959) or radioactive microspheres (Harper, Bennett, Boursnell & Rowson, 1960) have been used as substitutes, but both require surgical techniques and neither substitute is readily available.

The present study was undertaken to see if dextran microspheres injected into the peritoneal cavity would act as useful substitutes for the egg in rabbits. Their uptake and transport by the genital tract was assessed under various endocrine conditions and the influence of their size on the speed of transport was also investigated.

Female rabbits obtained at the local market were isolated and given 25 or 50 i.u. HCG intravenously to ensure good development of their genital tract. Thirty days later, they were either mated, ovariectomized or used in oestrus. The does were injected intraperitoneally with 2 ml of a sterile suspension of Sephadex G-100 (Pharmacia, Sweden) in Tyrode solution containing approximately 360,000 microspheres. Mated rabbits were injected 10 hr after coitus, ovariectomized rabbits 2 months after ovariectomy, and oestrous rabbits 30 days after HCG.

Animals were killed 24, 48 or 72 hr after injection of Sephadex, and the entire genital tract was removed and dissected free of surrounding tissues. The oviducts were divided in three equal segments. These segments, the uterine horns and the vagina were individually flushed with Tyrode solution to determine the presence and number of microspheres. The distribution of the eggs was also examined in the mated group. The microspheres in the flushings were counted under the low power of a stereomicroscope. In a few instances, all the microspheres obtained from individual segments were washed with Tyrode solution and photographed to measure their diameter from enlarged prints. The diameter of these beads in Tyrode solution (298 mosmol) was 130±60 µm (mean±S.D.).

At all the times studied, the beads found in the peritoneal cavity were...
Table 1. Number of microspheres found in the genital tract at various times after intraperitoneal injection in rabbits

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (hr)</th>
<th>No. of animals</th>
<th>No. of spheres</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mated</td>
<td>24</td>
<td>5</td>
<td>2021</td>
<td>1506</td>
<td>849 to 2261</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>10</td>
<td>1506</td>
<td>585</td>
<td>10 to 1094</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>10</td>
<td>733</td>
<td>1034</td>
<td>12 to 1588</td>
</tr>
<tr>
<td>Oestrous</td>
<td>24</td>
<td>6</td>
<td>1034</td>
<td>407</td>
<td>35 to 3461</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>8</td>
<td>733</td>
<td>1034</td>
<td>36 to 2321</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>8</td>
<td>407</td>
<td>1034</td>
<td>12 to 1588</td>
</tr>
<tr>
<td>Ovariectomized</td>
<td>24</td>
<td>4</td>
<td>277</td>
<td>671</td>
<td>50 to 678</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>5</td>
<td>671</td>
<td>120</td>
<td>2 to 1573</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>7</td>
<td>120</td>
<td>120</td>
<td>0 to 469</td>
</tr>
</tbody>
</table>

Text-fig. 1. Distribution of microspheres throughout the genital tract of mated oestrous and ovariectomized rabbits. Segments: 1—oviduct, upper third; 2—oviduct, middle third; 3—oviduct, lower third; 4—uterus; 5—vagina.
Transport of microspheres by the rabbit oviduct

trapped within a fibrin network, suggesting that free microspheres were only available for uptake for a short time after injection.

The mean number and range of microspheres found in the genital tract of each group at various times is shown in Table 1. Only 2% of the beads injected were taken up and there was great variability between animals in each group. At 72 hr, there were smaller numbers than at previous times in all three groups. Since no evidence was obtained that the spheres were destroyed in the genital tract, it is likely that those missing were expelled through the vagina.

The total number of spheres found in the genital tract of each animal was taken as 100% and their distribution in the five segments analysed was expressed as a percentage of that figure. The mean for each group is shown in Text-fig. 1.

In mated rabbits, the majority of the spheres were found in the middle third of the oviduct at 24 hr, the lower third at 48 hr, and the uterus at 72 hr. In this group, the spheres, like the eggs, became surrounded by a mucin coat, a process which was already apparent in the spheres contained in the lower third of the oviduct of animals killed at 48 hr.

The eggs were usually located in the same segment where most of the spheres were found and their distribution resembled that of eggs in untreated mated rabbits (Greenwald, 1961).

In oestrous rabbits, the distribution up to 24 hr was similar to that of the mated group. At 48 hr, however, the spheres were found throughout the genital tract rather than in a single segment and 63% of them had passed beyond the isthmo-uterine junction. This distribution is similar to that found by Chang (1966) who reported that about 52% of eggs had passed into the uterus 49 hr after transfer in oestrous rabbits.

In contrast to the previous groups, the microspheres in ovariectomized rabbits were spread throughout the genital tract rather than concentrated in a single segment. At 24 hr, 28% of them had passed beyond the isthmo-uterine junction. This distribution is in agreement with previous findings of Noyes, Adams & Walton (1959), who found that 38% of the eggs had passed into the uterus and vagina 13 to 23 hr after transfer in ovariectomized rabbits. They are also in agreement with the distribution of artificial eggs found by Harper (1964) in ovariectomized rabbits. At 72 hr, 74% of the spheres were in the uterus and vagina, proving that even 2 months after ovariectomy the oviducts retain their ability to effect transport in this species. de Mattos & Coutinho (1971) have shown that tubal motility is also maintained 60 days after ovariectomy in the rabbit.

At 24 hr, most of the spheres were in the middle third of the oviduct, in mated and oestrous rabbits. This segment contained the ampullary–isthmic junction and, since it has been established that transport of natural or artificial eggs from the fimbria to this junction takes only a few minutes (Harper et al., 1960; Harper, 1966; Boling & Blandau, 1971), it can be concluded that progress beyond this point was prevented for nearly 24 hr. The nature of the obstacle seems to be oestrogen-dependent because it was present in mated and oestrous rabbits but not in ovariectomized animals. In the first two groups, the obstacle was overcome between 24 and 48 hr. This could be ascribed to
changes in the hormonal environment in the mated group but not in the oestrous rabbits. Under physiological conditions, release of the obstacle may be triggered by the physical stimulus provided by the particles themselves.

A comparison of the distribution between the three groups at 48 hr showed that in mated animals, there was a second obstacle that prevented passage of the spheres into the uterus until the 3rd day. Delayed passage into the uterus in mated rabbits as compared to oestrous animals could result from transient narrowing of the isthmo-uterine junction and/or slower transport through the isthmus and, since it was observed only in mated animals, it can be inferred that it is dependent on the specific hormonal changes that follow coitus in this species.

The mechanisms that delay transport into the uterus are likely to be more effective in mated rabbits because in these, the eggs and microspheres increase in size by deposition of a mucin coat while they pass through the isthmus. The spheres that had progressed down to the vagina at 72 hr were found to be the smallest ones, whereas those retained at the ampullary-isthmic junction were

| Table 2. Size of the microspheres obtained from the genital tract 48 hr after injection into a mated rabbit |
|--------------------------------------------------|--------------------------------------------------|----------------------------------|
| **Segment**                               | **No. of spheres** | **Diameter of spheres* (μm) (mean ± S.E.)** |
| Oviduct (middle third)                  | 125                 | 6400 ± 100, P < 0.001 |
| Uterus                                   | 29                  | 5300 ± 200, P < 0.01  |
| Vagina                                   | 25                  | 4300 ± 200            |

* Measured from enlarged prints.

the largest. The size of the spheres obtained from various segments was measured in three experiments involving mated rabbits. The results of a representative experiment are shown in Table 2. The average diameters of the spheres at 48 hr in a mated rabbit were 108 μm in the vagina, 133 μm in the uterus and 160 μm in the middle third of the oviduct. Statistical analysis by the Student t test showed that these differences were highly significant.

This observation is in keeping with the concept that changes in the width of the lumen (El Banna & Hafez, 1970) might be one of the physical factors that regulate the passage of eggs into the next segment and it also suggests that one of the functional rôles of the mucin coat might be to delay transport through the isthmus.

The use of artificial microspheres seems to be a practical method for studying ovum transport in the oviduct. Extension of this technique to species in which eggs are not easily available, such as the guinea-pig (H. B. Croxatto, unpublished observations) and the human (Avendaño, Pelaez & Croxatto, 1971) is proving to be a useful tool for comparative studies.

The present observations suggest that more clear-cut patterns of distribution should be obtained by the use of spheres of an even size.
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