The Induction of Deciduomata in the Rat

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One of the main steps in the elucidation of the mechanism of ovum implantation is to determine how the blastocyst stimulates the uterus to undergo a decidual reaction. The extent of this reaction varies between species but is particularly marked in rodents, and normal ovum implantation does not occur without it. It has been known for several years, however, that the uterus of a suitably primed rodent can be stimulated to produce decidual cells in the absence of a blastocyst simply by traumatization of the uterus. The so-called deciduoma which results is thought to be analogous to the decidual reaction of normal implantation because (a) it is histologically similar (Krehbiel, 1937), and (b) the hormonal requirements for its formation are similar to those for implantation. Thus the deciduoma is a useful model on which to investigate factors which may be important in the initiation of the decidual response. It is also of interest as an example of extremely rapid cellular proliferation (the uterus may increase its weight four-fold in 72 hours) and so may provide information about cellular growth in general. If the deciduoma is analogous to the decidual reaction it is a reasonable assumption that the mechanisms by which the two responses are initiated have at least a final stage in common.

Shelesnyak (1952) found that histamine instilled into the lumen of the uterus initiated the formation of a deciduoma in rats and, on this and other evidence, postulated that histamine liberation was an important factor in the initiation of the decidual cell response both by the blastocyst and by trauma. However, the injection of any substance into the uterus is attended by sufficient trauma to cause a small deciduoma and thus one can only be sure that a substance is affecting deciduoma formation if a carefully controlled experiment has shown that the substance causes a response significantly different from that given by an injection of vehicle alone. To do this it is essential to have an objective and quantitative measure of the response and to design the experiments so that unconscious bias is avoided.

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In some earlier work we repeated Shelesnyak's experiments under carefully controlled conditions (Finn & Keen, 1962a) but were unable to obtain a greater decidual response to histamine than to the control injections of physiological saline, using both horns of the uterus to give a within-animal comparison. Similar results have been obtained by de Feo (1962).

The response to the injection of saline was less than that to the more intentional trauma such as crushing of the uterine horn. Chambon (1960) explained this by postulating that saline injection also released histamine but less than was released by trauma. If this were so, the addition of histamine should have given a response as large as the response to trauma, but this did not occur. A further finding that casts doubt on the histamine theory is that the reaction is not blocked by systemic anti-histamines (Finn & Keen, 1962b).

The purpose of the present work was to determine whether the technique of intra-uterine injection would yield information about the physical or chemical nature of substances whose presence in the uterus might initiate deciduoma, in the hope that this information would help to elucidate the mechanism of ovum implantation.

MATERIALS AND METHODS

Virgin albino rats weighing 180–210 g. were made pseudo-pregnant by electrical stimulation of the cervix during oestrus. The day of stimulation is counted as day 1 and stimulation was always carried out between 12 noon and 12.30 p.m. Unfortunately not all animals responded by becoming pseudo-pregnant, a few showing cornified vaginal smears on day 5. These were rejected from the experiment. On the afternoon of day 5, 100 hr. after stimulation, the pseudo-pregnant rats were anaesthetized with Avertin and each uterine horn was injected in turn through a small incision in the flank. An earlier paper (Finn & Keen, 1962a) showed that trans-cornual migration of fluids was unlikely and that each horn can be treated separately. Similarly trans-cornual migration of blastocysts in rodents occurs very rarely if at all (Skreb & Levak, 1957).

A 30 SWG needle (0.3 mm. outside diameter) was passed through the wall of the uterus at the utero-tubal junction and into the uterus for a distance of approximately 1/2 in. The standard volume of fluid used throughout was 0.05 ml. During the injection the bottom of the oviduct was held between fine forceps so that the only trauma to the uterus was that caused by insertion of the needle. To avoid any unconscious bias the operator himself did not, at the time of injection, know which solution he was injecting. In some cases a deciduoma was produced by crushing the uterine horn transversely in three places with artery forceps. The rats were killed on day 8 (72 hr. after operation), and the uterine horns dissected out and weighed separately. Wet uterine weight was used as a measure of the response because of its simplicity and objectivity. Further, there is good evidence that it is a fair measure of cellular proliferation;
for instance Berswordt-Wallrabe & Turner (1961) found a close correlation between the weight of uterine horns containing deciduomata and their DNA content. The same authors also showed an increase of weight with increasing dosage of progesterone in deciduoma experiments on spayed hormone-treated animals. The cellular proliferation can, of course, be seen histologically but histological sections were not taken as a routine as it would be extremely difficult to assess the responses quantitatively in this way.

RESULTS

pH and tonicity

We first attempted to determine why Shelesnyak had been able to produce deciduomata with solutions of histamine whilst we had failed to do so. It seemed possible that this might be due to a difference in the physical properties of the solutions. To test this we injected solutions of varying pH and tonicity. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Tonicity</th>
<th>N</th>
<th>Mean (mg.)</th>
<th>±</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>14</td>
<td>241.1</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>0.45% NaCl</td>
<td>12</td>
<td>245.8</td>
<td>20.4</td>
<td></td>
</tr>
<tr>
<td>1.00% KCl</td>
<td>3</td>
<td>206.3</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>0.58% KCl</td>
<td>5</td>
<td>227.2</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>5.15 pH Phosphate buffer</td>
<td>6</td>
<td>263.5</td>
<td>27.8</td>
<td></td>
</tr>
<tr>
<td>7.0 pH Phosphate buffer</td>
<td>6</td>
<td>231.0</td>
<td>31.8</td>
<td></td>
</tr>
<tr>
<td>7.8 pH Phosphate buffer</td>
<td>8</td>
<td>230.1</td>
<td>18.6</td>
<td></td>
</tr>
</tbody>
</table>

It will be seen that all the solutions used gave a similar response, none of the mean weights differing significantly from the response to physiological saline. In analysing the results to follow we have taken this response to physiological saline ('saline response' = 241.1 ± 20.1) as a standard with which to compare the response to other solutions.

Fat solvents

We planned to investigate the effect of several substances which were not water-soluble, and as a preliminary we injected into the uterus each of five organic solvents: ether, benzene, glycerol, polyethylene glycol and cetrimide. When these substances were injected no deciduomata could be seen macroscopically, even at the point of insertion of the needle. As small visible deciduomata are nearly always formed when electrolyte solutions are injected or
when a needle is passed into the uterus with no instillation of fluid, it appears that these organic solvents must be depressing decidual formation. To test this we injected the solutions into the uterus as before but at the same time crushed the uterus in three places. Although in all cases large deciduomata were formed in the control horns, which had been simply crushed, none was formed in the horns which had also been injected with a fat solvent, indicating clearly that these substances were suppressing the decidual cell response. Apparently the only factor they have in common is that they are all fat solvents.

<table>
<thead>
<tr>
<th>Weight</th>
<th>Substance</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td>S.E.</td>
<td></td>
</tr>
<tr>
<td>632.2 ± 34.8</td>
<td>Olive oil</td>
<td>30</td>
</tr>
<tr>
<td>617.5 ± 48.9</td>
<td>Arachis oil</td>
<td>8</td>
</tr>
<tr>
<td>514.5 ± 54.2</td>
<td>Liquid paraffin</td>
<td>4</td>
</tr>
<tr>
<td>241.1 ± 20.1</td>
<td>Saline</td>
<td>14</td>
</tr>
</tbody>
</table>

**Text-fig. 1.** Histogram showing the mean wet weight of rat uteri 72 hr. after intra-uterine injection of a series of ‘oils’. The response to saline is given for comparison.

**Oils**

We next tested the effect of a number of oils which might also be used as solvents and found (Text-fig. 1) that olive oil, arachis oil or liquid paraffin all produced massive deciduomata. We confirmed histologically that the increase in size was due to formation of decidual cells.

The diverse chemical nature of these substances suggests that their effect is a physical one due to their ‘oiliness’. It was, however, thought possible that the oils might be more effective than saline simply because they remained in the uterine lumen for a longer time. To test this we injected three substances which we thought would tend to be absorbed slowly from the uterus: serum, plasma and dextran. Each of these produced only a ‘saline response’ (Text-fig. 2).
Thus it seems unlikely that the effectiveness of the oils is due simply to their persistence.

**Carrageenin**

Oil injected subcutaneously stimulates the production of granulation tissue and, as it also stimulates the formation of deciduoma when injected into the uterus, it seemed possible that the decidual cell reaction might be in some way analogous to the granulation tissue reaction. Obviously, therefore, the next step was to determine whether other substances which stimulate granulation tissue would also stimulate deciduoma formation. It has been shown that the sulphated polysaccharide carrageenin, when injected subcutaneously, stimulates the rapid formation of granulation tissue (Robertson & Schwartz, 1953).

<table>
<thead>
<tr>
<th>Weight (mg)</th>
<th>Substance</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>220.4 ± 14.3</td>
<td>Serum</td>
<td>18</td>
</tr>
<tr>
<td>300.8 ± 38.1</td>
<td>Plasma</td>
<td>13</td>
</tr>
<tr>
<td>224.7 ± 10.8</td>
<td>Intradex</td>
<td>4</td>
</tr>
</tbody>
</table>

**TEXT-FIG. 2.** Histogram showing the mean wet weight of rat uteri 72 hr. after intra-uterine injection of serum, plasma and intradex (dextran). The broken line indicates the response to saline.

We therefore injected a solution of carrageenin into the uterus. It will be seen from Text-fig. 3 that carrageenin did induce massive deciduoma.

**Other polysaccharides**

To determine whether this ability to form deciduoma was possessed by other polysaccharides we tested the effect of intra-uterine injection of pectin, agar, dextran, glycogen and acacia. Of these, only agar produced a significantly greater response than the ‘saline response’ (Text-fig. 3).

This is very interesting in that, like carrageenin but unlike the others, agar is sulphated. From this it appears that there may be some connection between
sulphated polysaccharides and deciduoma formation. The sulphate ion alone is not effective as 1 per cent. sodium sulphate caused only a 'saline response'.

<table>
<thead>
<tr>
<th>Weight (mg.)</th>
<th>Substance</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>230.6 ± 21.0</td>
<td>Pectin 1%</td>
<td>9</td>
</tr>
<tr>
<td>595.7 ± 55.2</td>
<td>Agar 0.12%</td>
<td>11</td>
</tr>
<tr>
<td>604.7 ± 78.9</td>
<td>Carrageenin 1%</td>
<td>8</td>
</tr>
<tr>
<td>224.7 ± 10.8</td>
<td>Dextran 1%</td>
<td>4</td>
</tr>
<tr>
<td>346.2 ± 33.6</td>
<td>Glycogen 1%</td>
<td>7</td>
</tr>
<tr>
<td>293.4 ± 20.3</td>
<td>Acacia 1%</td>
<td>6</td>
</tr>
<tr>
<td>248.3 ± 18.0</td>
<td>Sodium sulphate 1%</td>
<td>8</td>
</tr>
</tbody>
</table>

**TEXT-FIG. 3.** Histogram showing the mean wet weight of rat uteri 72 hr. after intra-uterine injection of a number of polysaccharides and also sodium sulphate. The broken line indicates the response to saline.

**Heparin and chondroitin sulphate**

Next we tested the effect of three polysaccharides which occur naturally in the body; two sulphated polysaccharides, heparin and chondroitin sulphate, and one which contains no sulphate groups, hyaluronic acid.

Of these, only heparin gave a significant response. Large doses of heparin given intraperitoneally or intravenously, however, did not induce deciduomata.
This, presumably, means either that (a) the heparin molecule has to act in the lumen of the uterus and after systemic injection does not reach adequate concentration there, or (b) heparin is not itself initiating the deciduoma but merely augmenting the deciduoma caused by the trauma of injection.

Other naturally-occurring substances

Two other naturally-occurring substances were tested, 5-hydroxytryptamine and acetylcholine. Each caused only a saline response (Text-fig. 4).

<table>
<thead>
<tr>
<th>Weight (mg.)</th>
<th>Substance</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>209.2 ± 18.1</td>
<td>Hyaluronic acid (1%)</td>
<td>10</td>
</tr>
<tr>
<td>205.3 ± 25.4</td>
<td>Chondroitin sulphate (1%)</td>
<td>6</td>
</tr>
<tr>
<td>486.1 ± 31.3</td>
<td>Heparin (400 I.U./ml.)</td>
<td>14</td>
</tr>
<tr>
<td>195.6 ± 20.7</td>
<td>5 Hydroxytryptamine (1%)</td>
<td>5</td>
</tr>
<tr>
<td>199.4 ± 16.4</td>
<td>Acetylcholine (0.1%)</td>
<td>5</td>
</tr>
</tbody>
</table>

TEXT-FIG. 4. Histogram showing the mean wet weight of rat uteri 72 hr. after intra-uterine injection of a number of polysaccharides, 5-hydroxytryptamine and acetylcholine. The broken line indicates the response to saline.

DISCUSSION

The deciduoma is produced by the massive development of decidual cells in the stroma of the uterus. Although the exact origin of these cells is not decided, it seems fairly certain that they are connective tissue in origin, the most likely source, according to Amoroso (1958), being undifferentiated perivascular
mesenchymal cells. The very rapid multiplication of these cells in response to
the presence of the blastocyst or to trauma, poses the question of the nature of
the stimulus for their sudden development. This is effective only when the
uterus is under the overall dominance of progesterone, with probably some
need for oestrogen, which distinguishes the reaction from other growth responses
of connective tissue cells. However, it should be noted that the decidual cell
response is a connective tissue reaction, however specialized. It is significant
that corticoids, which inhibit granulation tissue formation in connective tissue,
inhibit deciduoma formation. Possibly, as suggested long ago by Turner (1876),
the decidual cell reaction ‘represents a reaction against the advance of the para-
sitic ovum’ in the same way that a foreign body is encased in a mass of granu-
lation cells. There might therefore be some similarity between the stimulus
for granulation tissue formation and for deciduoma formation. Certainly the
egg can be likened to a foreign body and it is interesting that in cases of ectopic
implantation a granulation tissue reaction has been found (Wade & Watson,
1908). Unfortunately, the stimulus for granulation tissue or wound healing
generally has not been elucidated. Abercrombie (1957) favours the view that
reparative growth, for example in wounds, is due to a wound hormone. No
such hormone, however, has been found and others (Bullough & Laurence,
1960) favour the view that the stimulus is supplied by removal of inhibitory
substances. Nevertheless, it is very interesting that carrageenin, a sulphated
polysaccharide, which stimulates granulation tissue, should also cause decidu-
oma formation. Whether it is active due to its similarity to the natural
stimulator or whether it stimulates the reaction in some other way is unknown.
Riley (1959) has postulated that heparin, a sulphated polysaccharide and potent
stimulator of deciduoma formation, is liberated on injury to tissues and plays
an important part in the laying down of new tissue. Shelesnyak (1959) has
shown that there is a breakdown of mast cells of the uterus at the time of
implantation, which may indicate that one of their constituents plays a part in
deciduoma formation. He considers this to be evidence for the rôle of histamine
as the specific stimulator. We, however, have been unable to obtain any
experimental evidence for such a rôle and, as heparin is also a constituent of
mast cells, being in fact responsible for the metachromatic staining of their
granules, we think that this may indicate a rôle for heparin in deciduoma for-
mation. Further, there is suggestive evidence from other sources that sulphated
substances may be important in uterine physiology at the time of implantation.
Thus, Gothie (1960), using radioactive S^35 has shown large accumulation of
S^35 containing substances, probably mucopolysaccharides, in the mucosa,
especially at the site of implantation. Zachariae (1958) has demonstrated the
excretion of acid sulphopolysaccharides into the uterus under the influence of
oestrogens. This may give a lead to the elucidation of why a small surge of
oestrogen can precipitate implantation in lactating rodents or ovariectomized
pregnant rats given injections of progesterone (Psychoyos, 1961). Shelesnyak
(1959) considers that a small surge of oestrogen normally occurs just prior to implantation, and this he has shown causes breakup of mast cells.

SUMMARY

In an attempt to investigate the nature of the stimulus for the decidual cell reaction, small quantities of various substances were injected into the lumen of the uterus of pseudopregnant rats. The decidual cell response, as measured by wet weight of the uterus, was compared with the response to an equivalent injection of physiological saline. The only substances producing a significantly greater response than saline were oils and three sulphated polysaccharides, agar, carrageenin and heparin. The significance of these findings is discussed.

RÉSUMÉ

Induction de déciduomes chez le rat

Pour essayer de rechercher la nature du stimulus provoquant la réaction des cellules déciduales, on a injecté de petites quantités de diverses substances dans la lumière de l'utérus de râtes en pseudo-gestation. La réaction des cellules déciduales, mesurée par le poids frais de l'utérus, a été comparée à celle que provoque une injection équivalente de liquide physiologique. Les seules substances provoquant une réaction significativement plus forte que ce dernier ont été les huiles et trois polysaccharides sulfatés, l'agar, la carragénine et l'héparine.

On discute la signification de ces résultats.

REFERENCES


(Manuscript received 29th April 1963)