THE ACTION OF OESTRADIOL AND HISTAMINE ON THE SITE OF NIDATION IN THE RABBIT

A Histological and Histochemical Study

By

Albrecht Pfleiderer jr.

ABSTRACT

Administration of oestradiol causes interruption of pregnancy in the rabbit, but not in man. The morphological changes after injection of oestradiol-benzoate have been investigated in 84 rabbits. Exogenous doses of oestradiol cause necroses and haemorrhages in the endometrium and even in the myometrium of the rabbit. Preceding activity of gestagen, however, is indispensable. Whereas in pseudopregnancy, doses greater than 10 µg/kg bring about a visible effect, 2 µg/kg is sufficient during pregnancy. Necroses are found in the decidua basalis, followed by a disturbance of blood circulation in the placenta. 1 µg/kg oestradiol-benzoate only destroys the decidua marginalis and the marginal parts of the placenta. Similar changes are physiological. They can also be demonstrated in the decidua of women and are probably caused by the action of endogenous oestrogens. Prevailing disturbance of the decidua basalis suggests a production of gestagen in the placenta not only in man but also in the rabbit. Morphologically corresponding transformations are also observed without the administration of oestrogens after laparotomy and particularly after injections into the aorta, as well as in relation to a generalized Shwartzman-Sanarelli-reaction. However, a disturbance of blood coagulation does not occur. Histamine alone does not seem to cause similar transformations. Antihistamines used in this way do not prevent the disturbance caused by oestradiol. The possibility that other vaso-active substances or a local effect of oestradiol can cause interruption of pregnancy is discussed.

The importance of oestradiol for reproduction in man and animals is well established. The chemical and biological properties of this hormone have been
extensively investigated. Its action and its site of action in the organism, however, are still not completely known.

Formerly the main interest was the action of oestrogen in blood vessels, whereas the investigations of Mueller et al. (1958) and Villee (1959, 1961) have more and more drawn attention to the site of activity in the enzyme systems.

Treatment of imminent and habitual abortion with oestrogens (Karnaky 1950; Smith et al. 1946; Smith 1948; Smith & Smith 1949 a, b) was not successful but showed that human pregnancy cannot be interrupted by the administration of oestrogen after the second month of pregnancy. In the guinea-pig, cat, dog, cow, and goat, pregnancy can regularly be interrupted within the first few days, in the rabbit, rat, and mouse this is possible at any time (References see: Pfleiderer 1958).

The reason for this interruption is not known. Apart from a hormonal antagonism between oestrogens and gestagens (Courrier 1945; Courrier & Sakiz 1955) or a direct effect of oestrogen on the ovary (Allen & Wu 1959), induction of labour (Druckrey & Bachmann 1937; Marrian & Newton 1935), and a toxic effect on the foetus (Cagianut 1949) have been discussed as causative factors. A direct disturbance of the placenta or decidua have been assumed (Augustin & Rothe 1956; Augustin 1959; Pfleiderer 1958, 1962; Schofield 1962). Allen & Wu (1959) observed in rabbits, after the administration of ethynyl-19-nortestosterone, a premature separation of the placenta and death of the does.

Shelesnyak (1957), Shelesnyak & Kraicer (1960), Shelesnyak et al. (1963), Kraicer et al. (1963), Marcus et al. (1963), Spaziani & Szego (1958, 1959), Spaziani (1963) and Szego & Lawson (1964) pointed out in the recent years that oestradiol has an effect on the uterus, especially during the development of the decidua, by releasing free histamine. This concept has recently been questioned by Finn & Keen (1962), Cecil et al. (1962, 1964) and Wrenn et al. (1964).

According to the classical experiments of Dale & Laidlaw (1910), histamine causes contraction of the uterus. In the guinea-pig it brings about dilatation of the vessels at the site of nidation and separation of the placenta (Hofbauer 1926). As is well-known, moreover, histaminase is considerably increased in the serum of the pregnant women. The decidua is believed to be responsible for this. In the animal, the increase of histamine is essentially smaller, and the decidual part of the placenta also contains histaminase (Swanberg 1950). According to the investigations of Lindsay et al. (1963), Poulson et al. (1960) and Robson & Sullivan (1963), 5-hydroxytryptamine regularly causes interruption of pregnancy in the rabbit and mouse. This substance is believed to have a damaging effect on the placental vessels.

In order to get further information on the activity of oestrogen, we have investigated the morphologically obvious changes at the site of nidation after administration of oestradiol and histamine.
MATERIALS AND METHODS

The experiments were performed on 84 mature does of different breeds weighing between 2500 and 5500 g. Oestradiol was administered as oestradiol-benzoate (Progynon B ol., Schering) in 0.1 ml sesame-oil. Imido-Roche (histamine-dihydrochloride) was used as the histamine-preparation. Table 1 gives a survey of the scheme of the experiments.

As a rule, the animals were killed by a blow on the neck, 24 hours after the last injection of oestradiol. The uterus was removed after the ligation of all the vessels, fixed in formalin or in Bouin solution and imbedded in paraffin. All sites of implantation (generally 8-9 per animal) were investigated histologically. As staining, haematoxyline-eosine and Masson trichromie staining were used. In 27 animals, at least one implantation site per animal was deepfrozen immediately after the operation and cut in the cryostate (model Dittes & Duspiva). These sections were investigated by histochemical and enzyme-histochemical methods (cf. Table 2). (Details of methods according to Pearse 1960). Sections of different sites of human female pregnant uterus which had to be removed for medical reasons, were used for comparison.

RESULTS

1. Effects of Oestradiol-benzoate

Investigations of the hormonal action were first made on the uterus of the nonpregnant animals. After the injection of 10-20 μg/kg oestradiol-benzoate for 4 days, the endometrium of the rabbit shows oedema or a marked hyper-

Table 1.
Plan of the experiments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Moment of sacrifice</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>none pregnancy</td>
<td>none</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>oestradiol</td>
<td>2-20 μg/kg</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>on 4 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pseudo-pregnancy</td>
<td>none</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>oestradiol</td>
<td>7-42 μg/kg</td>
<td>10th, 12th, 14th</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>on 4 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pregnancy</td>
<td>none</td>
<td>–</td>
<td>11</td>
</tr>
<tr>
<td>oestradiol</td>
<td>0.8-280 μg/kg</td>
<td>10th-16th</td>
<td>36</td>
</tr>
<tr>
<td>and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>medicaments</td>
<td>0.1-8 mg</td>
<td>15th and 16th</td>
<td>13</td>
</tr>
<tr>
<td>histamine</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Details of these investigations will be published elsewhere (cf. also Pfleiderer 1958).
Table 2.
Results of the histochemical investigations at the site of nidation of the rabbit.

<table>
<thead>
<tr>
<th>Histochemical reaction</th>
<th>Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trophoblast</td>
</tr>
<tr>
<td>PAS (diastase acetylisation)</td>
<td>Ø</td>
</tr>
<tr>
<td>BEST-glykogen</td>
<td>Ø</td>
</tr>
<tr>
<td>Sudanblack B</td>
<td>+</td>
</tr>
<tr>
<td>Scharlachrot</td>
<td>(+)</td>
</tr>
<tr>
<td>unspecific esterase</td>
<td>(+)</td>
</tr>
<tr>
<td>alkaline phosphatase</td>
<td>++</td>
</tr>
<tr>
<td>acid phosphatase</td>
<td>++</td>
</tr>
<tr>
<td>leucine-amino-peptidase</td>
<td>Ø</td>
</tr>
<tr>
<td>DPN-diaphorase</td>
<td>+</td>
</tr>
<tr>
<td>endogenous dehydrogenase</td>
<td>+</td>
</tr>
<tr>
<td>succino-dehydrogenase</td>
<td>(+)</td>
</tr>
<tr>
<td>lactate-dehydrogenase</td>
<td>++</td>
</tr>
<tr>
<td>a-glycerophosphate-dehydrogenase</td>
<td>Ø</td>
</tr>
</tbody>
</table>

aemia (Fig. 1). If the same dose of hormone is administered during pseudopregnancy, the internal layer of the endometrium breaks down (Fig. 2). The necroses are extended to the mucous membrane and to the musculature. The antimesometral regions with a poor blood supply are more markedly affected. Parts of the tissue near the large vessels are preserved. The necrosis is characterized by a capillary hyperaemia. If the daily dose of oestradiol is not more than 10 µg/kg, no change is seen (Fig. 3). The uterus is considerably smaller, and there are no mesometral swellings. The exogenous amount of oestradiol is not harmful for the pseudopregnant uterus in amounts up to 10–12 µg/kg.

On the other hand, the pregnant uterus is a great deal more sensitive. During implantation 4–5 µg/kg are sufficient to interrupt pregnancy. The internal layer of the endometrium and trophoblast are necrotic. The disturbance, however, is limited to the implantation site.

After vascularisation of the placenta, which is completed after the 12th day of pregnancy, the situation is different. More than 10 µg/kg oestradiol-benzoate produce complete necrosis of the internal layer of the uterus, similar to that in
1. Normal uterus not in a progestational stage. $4 \times 20 \mu g/kg$ oestradiol-benzoate. Oedema and hyperaemia of endometrium. (Masson 13 $\times$).

2. Pseudopregnancy, 14th day. $4 \times 20 \mu g/kg$ oestradiol-benzoate. Oedema of the endometrium. Necrosis of the internal layer. Least mesometrally (below). Necrosis of antimesometral musculature (clear regions) thin subserous zone excepted. (HE 13 $\times$).

Fig. 3.

Pseudopregnancy, 14th day. No treatment. Regular progestational proliferation of the uterus. (HE 13 $\times$).
Survey of the extent of necroses (black) in uterus of rabbit (cross-section) after administration of oestradiol.

pseudopregnancy (cf. Fig. 4). From the endometrium only parts of the mesometral side are preserved.

Antimesometrally, the necrosis extends to the musculature. The uterine lining is destroyed except for a subserous band and the surroundings of larger vessels (Fig. 5). The placental changes should be specially examined. Treatment with oestrogens brings about a hyperaemia and dilatation of maternal blood spaces. All degrees of these phenomena are to be found right up to extreme dilatation. After a short time, this results in necrosis of the trophoblast and destruction of the foetal mesenchymo.

4–5 µg/kg still cause a radical necrosis and a disturbance of the antimesometral side (Fig. 4). When the same hormonal dose is administered to pseudopregnant rabbits, the uterus shows no morphological change. During implanta-
tion, only the site of implantation is damaged. After vascularisation of the placenta, however, the whole site of nidation is destroyed.

If the dose of oestradiol is decreased to 2–3 μg/kg (Fig. 4), only the site of implantation itself and especially the multinuclear decidua is affected. Necrosis and haemorrhage are extensive. The trophoblast tubes are dilated and filled with blood, yet not necrotic in many cases (Fig. 6). Often, parts of the subplacental zone are still intact with this dose.

A still smaller extent of necroses is observed after 1–2 μg/kg (Fig. 4). In many cases, only the marginal parts of the subplacental zone are destroyed. Accordingly, the hyperaemia is limited to the marginal parts of the placenta.

If the dose of oestradiol is further decreased to less than 1 μg/kg, only a weakly increased content of the trophoblast tubes is to be seen in the marginal parts of the placenta. The decidual necroses are adjacent to the contact of placenta (Fig. 4).

2. Changes in the Decidua without Administration of Oestradiol

At the site of nidation of untreated rabbits, parts of the decidua also regularly disappear after the 12th to 14th day. They are situated in the so-called

![Fig. 6.](image1)

6. Base of foetal placenta on 12th day of pregnancy. 2 × 1.7 μg/kg oestradiol. Haemorrhage in subplacental decidua (below). Dilatation of trophoblast tubes (dark): Trophoblast not yet necrotic. (HE 210 ×).

![Fig. 7.](image2)

7. Decidua compacta of human female mens V. Necrosis of internal layer (above). (Lactate – dehydrogenase 45 ×).
periplacental bolsters and the blood-sinuses are immediately adjacent to these necroses. By extending they prepare for the physiological separation of the placenta in rabbits during birth. These necroses correspond to those caused by low dose of oestradiol (< 1 µg/kg) (Fig. 4). These observations suggest a comparison with parallel changes in the human decidua (Fig. 7). In the course of pregnancy, regression phenomena and necroses in the decidua are constantly increasing. Details of these investigations obtained by histochemical methods will be published separately.

3. Histochemical Investigations of the Site of Nidation of the Rabbit

Oestrogen mainly has an effect on the subplacental zone, especially on the vessels and the multinuclear decidua. These facts led to a study of the characteristic metabolism of these tissues, by means of histochemical methods (Table 2).

The decidual cells mainly serve as a storage for glycogen and have only slight metabolic activity. On the other hand, the endothelium of the vessels show an extremely marked reaction indicating the presence of lactate-dehydrogenase and α-glycero-phosphate-dehydrogenase. DPN-diaphorase as well as succino-dehydrogenase are detectable in increased quantities in the walls of the vessels. These cells have high metabolic activity and are of particular importance in glycolysis. In the trophoblast as well as lipids and phosphatases, enzymes of citric acid cycle but no steroid-3β-ol-dehydrogenase are found.

After exogenous administration of oestradiol, there is, first of all, a decrease in the glycogen content and development of lipid-containing cells in the multinuclear decidua. The dehydrogenases become prematurely negative in the walls of the vessels of multinuclear decidua. Soon the decidual changes are followed by disappearance of these enzymes from the trophoblast.

4. Studies on Action of Histamine*

If the animal receives intravenous, subcutaneous, or intramuscular injections of histamine up to 3 mg/d, definite oedema, hyperaemia, haemorrhages and necroses of the endothelium of the vessels are found subplacentally (Table 3). Nevertheless, the multinuclear decidua is well preserved, as a rule, and neither hyperaemia of the placenta nor necrosis of the trophoblast can be seen (Fig. 8).

In order to administer a sufficient dose of histamine to the site of nidation, we laparotomized rabbits, prepared the abdominal aorta and injected histamine up to 4 mg into the aorta. The placenta was completely necrotic and the labyrinth was dilated. In the mesometral decidua, superficial necroses, hyperaemia and haemorrhages were observed. This seemed to demonstrate an effect

* The experiments were made in collaboration with H. Keck.

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Table 3.
The site of nidation of the rabbit after administration of histamine (15th and 16th day of pregnancy).

<table>
<thead>
<tr>
<th>Administration of histamine</th>
<th>decidua mesometral</th>
<th>trophoblast tubes of the placenta</th>
<th>autolytic foetus</th>
<th>decidua antimesometral</th>
</tr>
</thead>
<tbody>
<tr>
<td>control animals i. m. 0.1 mg/d</td>
<td>isolated necroses</td>
<td>intact</td>
<td>isolated</td>
<td>intact</td>
</tr>
<tr>
<td>s. c. to 3 mg/d i. m. i. v. 1–4 days</td>
<td>hyperaemia and haemorrhages</td>
<td>sometimes vaguely hyperaemical</td>
<td>isolated</td>
<td>intact</td>
</tr>
<tr>
<td>trapanalnarcosis laparotomy</td>
<td>increased necroses</td>
<td>sometimes strongly hyperaemical</td>
<td>isolated</td>
<td>intact</td>
</tr>
<tr>
<td>laparotomy 3 ml physiol. NaCl in the aorta</td>
<td>haemorrhages increased necroses</td>
<td>extensive hyperaemia</td>
<td>almost all</td>
<td></td>
</tr>
<tr>
<td>laparotomy 3–4 mg histamine in the aorta</td>
<td>haemorrhages extensive necroses</td>
<td>extensive hyperaemia necroses of trophoblast</td>
<td>almost all</td>
<td>sometimes</td>
</tr>
</tbody>
</table>

Fig. 8.
Section through implantation site of rabbit. 16th day of pregnancy. 4 × 1 mg histamine i. m. Oedema in the subplacental decidua (middle). (Lactate – dehydrogenase 18 ×).
Table 4.
Experiments to block effects of oestrogen.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Chemical name</th>
<th>Single dose</th>
<th>Method of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avil (Hoechst)</td>
<td>phenyldimethylaminopropane</td>
<td>50 mg</td>
<td>6 hourly i. m. or once local</td>
</tr>
<tr>
<td>Ilvin (Merck)</td>
<td>1-(2 pyridyl)-1-p-bromphenyl-3-dimethylamino-</td>
<td>2.5 mg</td>
<td>6 hourly i. m.</td>
</tr>
<tr>
<td>Dabylen (Schiwa)</td>
<td>dimethylaminoethyl-benzhydrylether</td>
<td>20 mg</td>
<td>hourly i. v.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg, 100 mg</td>
<td>6 hourly i. m.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 mg</td>
<td>i. v. permanent</td>
</tr>
<tr>
<td>Atosil (Bayer)</td>
<td>promethazine</td>
<td>2.5 mg</td>
<td>6 hourly i. m.</td>
</tr>
<tr>
<td>Proluton-Depot (Schering)</td>
<td>17-hydroxyprogesterone-caproate</td>
<td>125 mg</td>
<td>(24 h before oestrogen) i. m.</td>
</tr>
<tr>
<td>Lutocyclin (Ciba)</td>
<td>anhydro-oxyprogesterone</td>
<td>20 mg</td>
<td>intravenous</td>
</tr>
<tr>
<td>Trasylol (Bayer)</td>
<td>trypsin-kallikrein-inactivator</td>
<td>200 000 to 450 000 IU</td>
<td>i. v. permanent</td>
</tr>
<tr>
<td>Liquemin</td>
<td>heparin</td>
<td>250 IU</td>
<td>5 hourly i. v.</td>
</tr>
</tbody>
</table>

of histamine. Laparotomy, however, is not without effect in pregnant rabbits. Even after a simple laparotomy, there is hyperaemia in the placenta and haemorrhages and necroses in the multinuclear decidua. If the aorta is prepared and injected with 3 ml of physiological NaCl-solution, the placenta shows changes which, as regards their shape and extent, look like the changes caused by histamine given in the same way (unimportant differences excepted). The reaction at the site of nidation after manipulations of the abdominal aorta suggests that the changes are part of an unspecific vascular reaction. This is emphasized by the fact that rabbits showing a generalized Shwartzman-Sanarelli reaction* caused by the injection of coli endotoxin, show changes in the nidation sites which cannot be morphologically distinguished from those produced by large doses of oestradiol. As a matter of fact, this reaction is characterized by generalized intravascular blood clotting and the release of vasoactive substances.

* Sections were kindly given by Prof. Dr. A. Bohle.
5. *Experiments to Block Changes Caused by Oestrogen* **

The idea of an unspecific vascular reaction suggested the use of drugs to block the effect of oestradiol. For this purpose, the animals received a single dose of 3 or 6 μg/kg of oestradiol-benzoate. According to the well-known studies of Spaziani and Shelesnyak, the animals first received antihistamines. For our investigations which had to be limited to a few substances, we used Avil (Hoechst), Ilvin (Merck), Dabylen (Schiwa), and Atosil (Bayer). We were unable to influence the effect of oestradiol in any of the experiments.

Since antihistamines were not effective, we studied the activity of some other substances. Progesterone too was not effective.

As a generalized Shwartzman-Sanarelli-reaction can be blocked by heparinization, we gave liquemin to one animal. The reaction to oestradiol at the site of nidation was the same.

Finally, Trasylol is able to block anaphylactic shock, according to the experiments of Steichele & Herschlein (1961, 1964). Moreover, Trasylol (Bayer) has an important antiproteolytic activity and activates plasmin. Even after extremely high doses of Trasylol, however, the necroses at the site of nidation after administration of oestradiol-benzoate did not change. Thus, the action of oestradiol-benzoate could not be blocked by any of the methods used.

**DISCUSSION**

As shown by our investigations, exogenous doses of oestradiol cause necroses in the endometrium and even in the myometrium of the rabbit. Preceding activity of gestagen is indispensable. If high doses are administered, the necroses extend to the internal layer of the whole horn of the uterus. By administration of smaller doses of oestrogen, the necroses are limited to the immediate surroundings of the implantation site. Most sensitive was the endometrium, directly adjacent to the trophoblast tissue (1–2 μg/kg). This probably suggests that in rabbits, progesterone is also produced in the trophoblast. The result is contradictory to all preceding ideas (Bengtsson & Ejarque 1964). Mikhail et al. (1961) maintained that the rabbit’s placenta is not able to produce progesterone. A conclusion, which was drawn from the fact that after ovariectomy during pregnancy in the rabbit, interruption of pregnancy occurs, if progesterone is not given. Only Csapo & Lloyd-Jacobs (1962) were able to show for the first time that pregnancy continues in spite of ovariectomy after careful reduction of progesterone doses, at least within the last few days.

In undisturbed pregnancy too the decidual necroses appear and therefore must be considered as physiological. As for their form and localisation they

** The experiments were made in collaboration with A. Furche.
correspond to those observed after low doses of oestradiol. These necroses in untreated animals are probably the result of physiological endogenous hormone production. The regression of the human decidua (Deelman 1933; Kaiser 1960) could have a similar cause. In rabbits these decidual necroses are situated at the margin of the placental contact site and prepare for the physiological separation of the placenta during birth.

Extending of these necroses, following administration of 1–2 μg/kg oestradiol-benzoate for 2 days, brings about a premature disturbance of the blood circulation in the marginal parts of the placenta.

Considering the mechanism of implantation of the human ovum and the conditions of blood circulation in the placenta, necroses are not dangerous, as a rule. The small amount of oestradiol is comparatively high for the rabbit. Besides progesterone, only 0.8–1 μg/kg oestradiol are necessary for the maintenance of pregnancy in ovariectomized rabbits (Courrier 1941; Chambon & Lefrein 1952; Noyes et al. 1959). Further investigations, however, will be aimed at the question whether necroses could not be the reason for premature separation of the placenta. Utero-placental apoplexy (Kiss & Tarjan 1960, a. o.) with necroses and haemorrhages in the myometrium is analogous to the experiments on animals.

According to our experience, oestradiol effects the boundary zone between mother and child. The disturbance of the trophoblast is of secondary importance and the death of the foetus results from disturbed circulation in the placenta. As a matter of fact, the special character of blood circulation in the placenta of the rabbit (Mossman 1926) explains the extraordinary sensitivity of the rabbit to exogenous administration of oestrogens.

The extent of the necroses after large doses of hormones suggests an acute disturbance of vascularisation. This concept is underlined by the observation that generalized Shwartzman-Sanarelli-reaction implies the same phenomena. The disturbances of blood coagulation (Bohle & Krecke 1959; Kleinmaier et al. 1959; Mc Kay et al. 1959) which definitely cause the disturbance of vascularisation, cannot be expected to be involved in the action of oestrogen, as heparin or Trasylol (Bayer) are unable to block the damaging effect. In connection with a Shwartzman-Sanarelli-reaction, histamine is released (Hinshaw et al. 1960, 1962). Oestradiol is supposed to have the same effect (Chambon & Lefrein 1952; Shelesnyak 1957; Shelesnyak et al. 1963; Spaziani & Szego 1958, 1959; Spaziani 1963; Szego & Lawson 1964). Experiments to bring about the corresponding changes at the site of nidation by means of histamine have not been successful, however, in spite of the relatively high doses administered. The observations of a reaction on foetal capillaries, made by Fischer (1957), cannot be confirmed.

The action of oestrogen could not be blocked by antihistamines. This may result from the choice of preparations, dosage or method of administration.
Spaziani & Szego (1959) were able to block the oedema of oestradiol by the local administration of chlortrimeton. Bromtrimeton (Ilvin-Merck) or local injection of Avil (Hoechst), and intraarterial infusion of Dabylen (Sshiwa), however, were not effective in our experiments.

The wellknown high content of histaminase in the placenta (Swanberg 1950) does not support the view that histamine easily causes disturbances particularly here. The experiments of Keck (personal communication) using isonicotinacid-hydradize (INH) to block histaminase did not have any success.

Changes at the site of nidation after manipulations of the abdominal aorta and experiments with serotonin by Poulson et al. (1960), Robson & Sullivan (1963) and Lindsay et al. (1963) suggest the possibility that other vasoactive substances are released. Proteolysis, a wellknown reaction in the shock-syndrom, must equally be eliminated as result of oestrogen action. As a large dose of an effective trypsin-kallikrein-inactivator was used, this reaction should have been blocked.

Thus our experiments do not enable us to conclude which, if any, vasoactive substances are liberated. The idea of a direct, local effect of oestradiol seems, therefore, more probable. It is not impossible, however, that when high doses are used, vasoactive substances are released which cause the extensive disturbances.

Recent investigations reveal new clinical aspects of the necroses at the site of nidation and of the vascular changes. Berger & Boucek (1964) observed that after ischaemia of the rabbit placenta there is a pressor-like active substance which, in other animals produces a toxaemia. McKay (1962) in rats, fed on a diet of saturated fatty acids, found necroses in the giant cell layer at the placental base and the symptoms of toxaemia. In addition there were changes in all organs like those of a generalized Shwartzman-Sanarelli-reaction.

After administration of oestrogens Augustin & Rothe (1956) described exactly the same necroses in the giant cell layer at the placental base of rats. The surprising correspondence between the morphological substrates of these different phenomena will be the subject of further investigations.

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