Experiments on the Maternal-Foetal Barrier in the Mouse

II. A Test for the Transmission of Maternal Serum Albumin into the Foetal Circulation following X-irradiation

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INTRODUCTION

Lengerová (1957) obtained indirect evidence that placental permeability to transplantation antigens could be induced in the rat by delivering 200 r. of X-irradiation to the pregnant uterus on the 15th day of gestation. The young were found subsequently to be immunologically tolerant of grafts of maternal skin. The passage of transplantation antigens into the foetus in sufficient quantity to induce tolerance strongly suggests transplacental leakage of leucocytes (since erythrocytes are ineffective in inducing tolerance to grafted skin).

Finegold & Michie (1961) made a direct test for mother-to-foetus transmission of labelled erythrocytes in the mouse following X-irradiation at various stages of pregnancy, but obtained negative results. In order to characterize further the limits to permeation from mother to foetus, we have tested for the transmission, both with and without X-ray treatment, of albumin-bound Evans blue and of any free dye which may, under the conditions employed, be present in the circulation of the mother.

MATERIALS AND METHODS

Pregnant mice were exposed to X-rays at a dose of 300 r. Evans blue (T-1824) in standard concentration was injected into irradiated and non-irradiated females on day 19 of pregnancy. After a half-hour interval the transmission density of the maternal and foetal blood was compared.

The experimental mice were female F₁ hybrids between the C57BL and A inbred strains. Pregnancies were obtained by mating them with males of the same genetic constitution as themselves. The dates of mating were established by daily examination of the females for copulation plugs. For a given test, two

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females were used at the same stage of pregnancy, one receiving the X-ray treatment and the other serving as control. In each case both mice were injected subcutaneously with 2·5 mg. progesterone (‘Lutocyclin’, CIBA Laboratories) on day 17 and day 18 of pregnancy, counting the day on which the copulation plug was found as day 0. This was done to ensure maintenance of pregnancy until day 19, on which the measurements on maternal and foetal blood were made. The full-term foetuses were delivered on day 19 by Caesarian section, since new-born mice can ingest the dye from the mother’s milk.

The pregnant females received whole-body X-irradiation at various stages of gestation ranging from day 13 to day 19. A Westinghouse radiotherapy unit was operated at 230 kV, 15 mA., at a tube-to-floor distance of 50 cm. At this distance the field diameter is 8·0 cm., and the effective radiation dose, allowing for back-scatter, 150 r./min.

Typically, injections of dye were made into the tail vein, the standard dose being 0·3 ml. of a 2 per cent. aqueous solution of Evans blue. All solutions were filtered before use.

Blood samples were taken from the adult females with a specially calibrated pipette previously flushed through with heparin (‘Liquemin’ Roche), and from foetuses with the same pipette following decapitation. The blood of the females was taken in most cases from the retro-orbital venous plexus, but in some cases from the abdominal aorta immediately post mortem. In many cases blood from two or three foetuses was pooled to make up the sample for measurement. All samples were of 0·05 ml. volume, immediately diluted in 4·95 ml. of 3·8 per cent. sodium citrate. After centrifugation at 2,000 rev./min. to remove the erythrocytes, the supernatant was taken for spectrophotometric examination.

The transmission density of the samples of diluted plasma obtained as described above was determined with a Unicam spectrophotometer operated at a wavelength of 6250 Å. This wavelength was selected as giving a favourable discrimination between Evans blue and any traces of haemoglobin which might be present as contaminant.

RESULTS

Thirteen pregnant mice were examined of which seven were irradiated in a single standard dose of 300 r. Except in the case of day 15, every irradiated mouse had a non-irradiated control.

Blood samples were taken from 97 foetuses in all, 55 coming from the irradiated mothers and 42 from the control mothers.

A summary of the results obtained is shown in Table 1.

In none of the young examined could any significant increase in transmission density be found, although a satisfactory high level of dye was obtained in every female injected. The median estimate for new-born blood was 17 μg./l. in the offspring of irradiated females and 23 μg./l. in the control young. These values
are scarcely distinguishable from experimental and instrument error, having in mind that the average concentration in the blood of the mothers was 3,501 µg./l. and 3,400 µg./l. respectively.

Table 1

Summary of results

Numbers in brackets denote the number of foetuses examined. The number following the < sign in each case represents the highest reading obtained in the litter.

<table>
<thead>
<tr>
<th>Day of pregnancy on which irradiation was administered</th>
<th>Treated mice</th>
<th>Control mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal blood</td>
<td>Foetal blood</td>
</tr>
<tr>
<td></td>
<td>At start of 1/4-hour period</td>
<td>At end of 1/4-hour period</td>
</tr>
<tr>
<td>13</td>
<td>4,308</td>
<td>3,846</td>
</tr>
<tr>
<td>14</td>
<td>4,692</td>
<td>3,923</td>
</tr>
<tr>
<td>15</td>
<td>4,138</td>
<td>3,969</td>
</tr>
<tr>
<td>16</td>
<td>4,277</td>
<td>4,000</td>
</tr>
<tr>
<td>17</td>
<td>*</td>
<td>3,723</td>
</tr>
<tr>
<td>18</td>
<td>3,538</td>
<td>3,277</td>
</tr>
<tr>
<td>19</td>
<td>2,646</td>
<td>1,769</td>
</tr>
</tbody>
</table>

* Dye injected intraperitoneally: see text.

In the case of day 17 of pregnancy the intravenous injection was unsuccessful in both the irradiated and control mothers and an intraperitoneal injection of 0.6 ml. of the 2 per cent. solution of Evans blue had to be given. A 3-hour period was given (instead of the usual half-hour as in the others) for the dye to be absorbed into the blood from the peritoneal cavity. The blood sample taken at Caesarian section showed that a satisfactory level of Evans blue in the blood was obtained (see Table 1). On removal of the foetuses it was observed that the peritoneal cavity including the uterus and its appendages was deeply stained with dye. In spite of this, however, no trace of dye could be detected in the blood of the foetuses.

Discussion

Our results strongly suggest that serum albumin does not cross the mouse placenta or associated membranes. An alternative possibility must, however, be accorded formal recognition, namely dissociation of the dye-protein complex in the placenta, with passage of the larger molecule but not the smaller.

Opposing views are held concerning the permeability of the placenta to various substances in various species. Hagerman & Villee (1960) state that the discrepancies are due to the different dosages of the test substance given, the different species of animals and their respective types of placenta, and to the difficulties of chemical or physiological measurement.

The transfer of serum-proteins from maternal to foetal blood has been
studied in many animals including man. In some cases the evidence for trans-
mission to the foetus has been based on immunological studies comparing the
titre of specific antibodies in maternal blood with their titre in foetal or cord
blood. Hagerman & Villee, however, still entertain some doubt as to whether
these antibodies (mostly gamma-globulins) cross the placenta at all.

The work of Brambell and his school (Brambell, Hemmings, & Rowlands,
1947; Brambell, Hemmings, Henderson, & Oakley, 1952; Brambell, 1954;
Brambell, Halliday, Brierley, & Hemmings, 1954; Hemmings & Oakley, 1957)
indicates that the yolk-sac splanchnopleur rather than the placenta is the major
pathway of antibody transfer to the rat and rabbit embryo. A selection accord-
ing to the character of the protein occurs during this process. Brambell, Halliday,
Brierley, & Hemmings (1954) state that there is no experimental evidence that
maternal antibodies cross the human placenta directly. Brown, McGandy,
Gillie, & Doyle (1959), however, do not support this generalization.

There is also some disagreement with regard to the permeability of the
placenta to other proteins.

Whipple, Hill, Terry, Lucas, & Yuile (1955), studying the transfer of iodine-
labelled serum albumin in rabbits and dogs, found labelled albumin in the
rabbit foetuses but not in the dog foetuses. On the other hand, Shmerling's
experiments cited by Hagerman & Villee (1960) with 14C-labelled proteins in rats
detected no transfer across the placenta. The same method was also used by
Whipple and his colleagues with the same negative results.

Bangham, Hobbs, & Terry (1958) in their experiments on transfer of serum-
proteins in the rhesus monkey demonstrated the direct transmission of homo-
logous maternal albumin and gamma-globulin to the foetal circulation. They
concluded that it was selective, in that gamma-globulin was transmitted 15–20
times more easily than albumin. They also believe that the transfer is not via the
amniotic fluid but is transplacental.

The present results indicate that mice resemble rats and dogs rather than
monkeys and rabbits in respect of mother-to-foetus transmission of serum
albumin. It should be noted that they exclude not only the placental pathway of
transmission but also the alternative pathway via the uterine lumen and yolk-
sac splanchnopleur implicated by the above-mentioned studies of Brambell and
his colleagues on the transmission of γ-globulin.

Since only a short period at full term was allowed for transplacental trans-
mission of the label, the results have decisive application only to the terminal
stage of pregnancy. At this terminal stage it can be concluded, subject to the
formal qualification stated at the outset of the discussion, that mouse albumin,
which has a molecular weight of 70,000, is unable to pass. If substantial quanti-
ties of free dye were also present in the maternal blood, unattached to albumin,
then the upper limit which our results set to permeability would be greatly
lowered, since Evans blue has a molecular weight of 961 and, in our experience,
will not pass through a dialysis membrane of 25 Å pore diameter. We have not
undertaken the rather complex studies which would be needed to settle this question.

SUMMARY

Serum albumin labelled with Evans blue did not pass from the maternal into the foetal circulation in 13 pregnant mice, of which 7 had received 300 r. X-irradiation at stages ranging from day 13 to day 19, and 6 were untreated. The bearing of this result on studies of placental permeability in other species is considered.

RÉSUMÉ

Expériences sur la barrière placentaire chez la souris

II. Test pour la transmission du sérum albumin maternel dans la circulation foetale, après irradiation aux rayons X

Le sérum albumin marqué au bleu Evans ne passe pas de la circulation maternelle dans la circulation foetale chez 13 souris gestantes: 7 avaient reçu 300 r à des stades allant du 13e au 19e jour, et 6 n’avaient pas été traitées. La portée de ces résultats sur l’étude de la perméabilité placentaire chez d’autres espèces est discusée.

ACKNOWLEDGEMENTS

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REFERENCES


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