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ACTIVE IODIDE TRANSPORT ACROSS THE PLACENTA OF THE GUINEA-PIG, RABBIT AND RAT

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Jost, Morel & Marois (1952) provided evidence that 1 hr after an intravenous injection of ^{131}I into pregnant rabbits the foetal plasma radioactivity was 2–2·5 times higher than that of the maternal plasma. They advocated two possible mechanisms for the gradient: either a placental transport mechanism for iodide or the formation of organic compounds of ^{131}I in the foetus.

The following experiments demonstrate that the inorganic iodide in the guinea-pig, rabbit and rat is higher in the foetal than in the maternal plasma, and that the placental transport mechanism responsible for the gradient is inhibited by thiocyanate.

In principle, the method was to label the maternal iodide space by a subcutaneous injection of carrier-free ^{131}I, allow time for equilibrium across the placental barrier, and then measure the concentration of radioactivity in the foetal and maternal circulations. In order to eliminate the effect of organic binding of ^{131}I by the foetal and maternal thyroids on the establishment of an equilibrium of ^{131}I across the placenta, propylthiouracil was injected into the mother.

The (Foetal/Maternal) plasma ratio (F/M) ^{131}I was presumed to approximate to the true chemical ratio of iodide provided that: (*a*) a steady level of radioactivity was maintained in the maternal circulation; (*b*) there was sufficient time for establishment of equilibrium of specific activities of iodide across the placental membrane; and (*c*) the ^{131}I in the foetal blood was in the same chemical and physical state as in the maternal blood.

A short preliminary report has already been published (Logothetopoulos & Scott, 1955).

METHODS

Young adult female guinea-pigs, rabbits and albino rats of mixed origin were used. The day after mating was taken as the first day of the gestation period. Rabbits and guinea-pigs were fed on 'Research pellets' supplemented for guinea-pigs with green vegetables daily. Rats were fed on 'Research rat cubes' and given bread twice weekly.

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Guinea-pigs. The guinea-pigs used were between the 36th and 65th day of their gestational period. Organic binding of ^{131}I by the maternal and foetal thyroids was prevented by subcutaneous injections of an alkaline solution of crystalline propylthiouracil (4 mg/100 g body weight); half the dose 40 min before and the remainder at the time of ^{131}I injection. 100 μc of carrier-free ^{131}I in 2 ml. of water were injected deep into the subcutaneous tissue of the right axilla. Sixty to 70 min later the animals were anaesthetized by an intraperitoneal injection of sodium pentobarbitone and by ether when necessary. The abdomen was opened in the mid-line and a sample of blood was obtained by cardiac puncture. The uterine wall over each foetus was incised along the surface opposite to the placenta, the foetal membranes were ruptured, and a sample of amniotic fluid taken for measurement of radioactivity. The exposed foetus was dried with filter-paper, and blood was collected in a heparinized centrifuge tube either by cutting the umbilical cord, or by opening the chest wall and puncturing the heart. A second blood sample was withdrawn from the mother after removal of the last foetus. The time interval between the first and second maternal blood samples varied between 15 and 25 min, depending on the number of foetuses.

Rabbits. Essentially the same procedure was followed in experiments on pregnant rabbits, all of which were between the 23rd and 30th day of gestation: 3 mg of propylthiouracil per 100 g body weight being injected. Pentobarbitone (approximately 0·5 ml./kg) was given intravenously 70–80 min after the ^{131}I injection (120–150 μc carrier-free ^{131}I in 3 ml. water subcutaneously). An additional maternal blood sample was taken from the ear vein 40 min after the ^{131}I injection.

Rats. Pregnant rats were used at the 20th and 21st day of their gestational period. A dose of 20 μc carrier-free ^{131}I in 1 ml. distilled water was injected into the right axilla 30 min after the injection of 20 mg propylthiouracil. Removal of the foetuses was commenced in one group 45 min and in another group 75 min after the ^{131}I injection. Samples of foetal blood collected by cardiac puncture were pooled. A maternal blood sample was taken only after the removal of the last foetus.

Animals without propylthiouracil. In five pregnant guinea-pigs and three pregnant rabbits ^{131}I was injected without any previous injection of propylthiouracil.

Thiocyanate-treated animals. The following doses were used per animal: 75 mg NaSCN for guinea-pigs, 150 mg for rabbits and 25 mg for rats. A freshly prepared watery solution was injected subcutaneously at the same time as the propylthiouracil injections.

Measurement of radioactivity. Maternal and foetal bloods collected in heparinized tubes were centrifuged, and 0·1–1 ml. samples of plasma transferred to standard 12 ml. vials and made up to 10 ml. with distilled water. Plasma from animals which had not been injected with propylthiouracil was diluted to 10 ml. with a carrier solution of 0·1% potassium iodide and 0·2% bovine albumen. Five ml. of 10% (w/v) trichloracetic acid was added and the precipitate separated by centrifuging. Only the radioactivity of the supernatant fluid was used for estimation of the (F/M) ^{131}I ratio. A multi-tube gamma Geiger Müller ring counter (Veall & Baptista, 1954) or a ring-type scintillation counter was used for counting the radioactivity, depending on the amount of plasma obtained.

Calculation of (F/M) ^{131}I *ratio.* In guinea-pigs and rabbits the mean of the radioactivity concentrations of maternal plasma before and after the removal of the foetuses was compared with that in the plasma of individual foetuses. In rats the radioactivity concentration of the maternal sample taken after the removal of the last foetus was compared with that of the pooled foetal blood.

Chromatography of plasma. Untreated plasma samples were applied to Whatman No. 1 paper. They were developed with butanol-acetic solvent. After drying, chromatograms were brought into contact with X-ray films and these were then developed after an adequate exposure.

RESULTS

Guinea-pigs. Fig. 1 summarizes in graphical form the results in guinea-pigs. The (F/M) ^{131}I ratios were consistently above unity and attained values of 4–5 in a few animals. The variation among foetuses of the same litter was smaller than the variation between litters from different animals. The radioactive concentrations of the maternal blood samples before and after removal

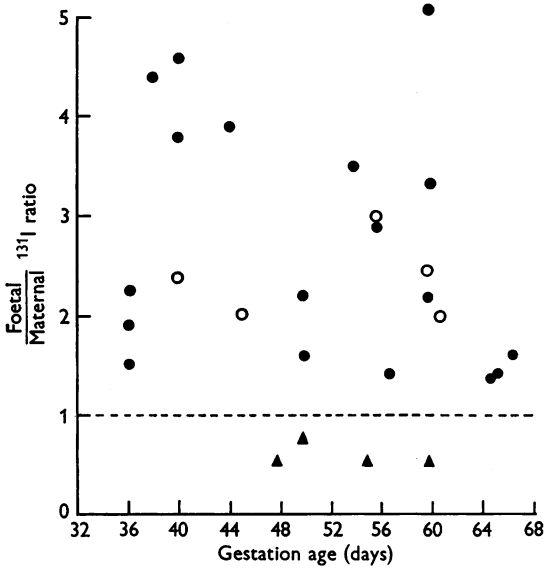

Fig. 1. (F/M) ^{131}I ratios in pregnant guinea-pigs of different gestation age. ●, represent the mean value for the plasma of several foetuses of the same litter, the animals having been injected with propylthiouracil. ○, represent the mean value in untreated animals. ▲, represent the ratio in litters from animals injected with propylthiouracil and thiocyanate.

of the foetuses were of about the same order in most experiments. A raised (F/M) ^{131}I plasma ratio was found as early as the 36th day of gestation. The raised (F/M) ^{131}I ratio was established in the five guinea-pigs in which no propylthiouracil was injected. In the four guinea-pigs in which NaCNS was injected the (F/M) ^{131}I ratio failed to reach unity. In all animals the ^{131}I concentration in the amniotic fluid was only a small fraction of the ^{131}I concentration in the maternal plasma.

Rabbits. A similar pattern of results was found for rabbits (Fig. 2). However, in two rabbits (F/M) ^{131}I ratios were found which did not exceed unity, although no thiocyanate injection was given.

Rats. In rats the (F/M) ^{131}I ratio is below unity by 45 min and between 1 and 1·5 by 75 min (Table 1). The (F/M) ^{131}I ratios found in pregnant rats injected with 25 mg NaCNS were less than one-third of those of their control group (Table 1).

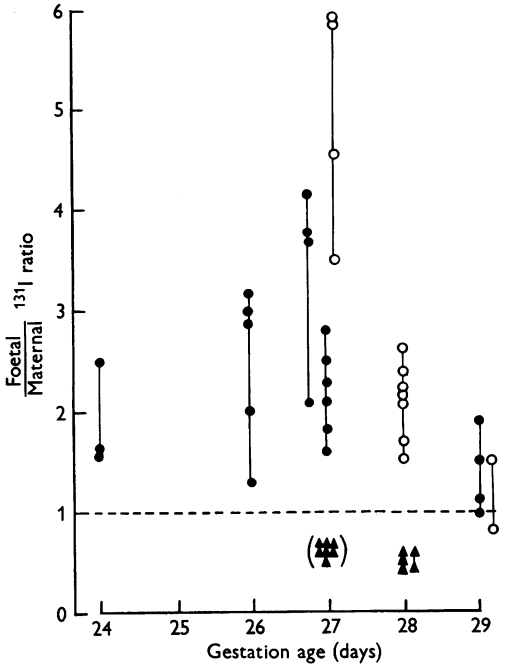

Fig. 2. (F/M) ^{131}I ratios in pregnant rabbits of different gestation age. ●, represent ratio for individual foetuses in litters from rabbits injected with propylthiouracil; ○, represent ratios in untreated animals; ▲, represent the ratio in animals injected with propylthiouracil and thiocyanate.

Chemical state of the ^{131}I *in the foetal blood*

The radioautographs of chromatograms from foetal and maternal plasmas of guinea-pigs and rabbits with a high (F/M) ^{131}I ratio showed single spots of the same R_F. These corresponded to spots obtained from non-radioactive guinea-pig plasma and a water solution of potassium iodide, both labelled *in vitro* with ^{131}I.

Foetal plasmas were placed in collodion bags and dialysed against a multifold volume of distilled water at 4° C. Complete passage of the radioactivity through the membrane was observed. After trichloracetic acid protein precipitation and centrifugation the radioactivity of the foetal plasma was recovered quantitatively in the supernatant fluid.

Carrier potassium iodide was added to foetal plasma dialysates and to the supernatant fluid of the trichloracetic acid precipitates, and oxidized to I_2 with excess iodate at an acid pH. The radioactivity was quantitatively extractable into CCl_4. This meant that there was no organically bound ^{131}I present (Taurog, Chaikoff & Feller, 1947).

Foetal plasma from guinea-pigs and rabbits was sealed in a small collodion bag and allowed to equilibrate, with continuous mixing, against an equal volume of its corresponding maternal plasma for 15 hr at 4° C. The ^{131}I concentration gradient established *in vivo* was abolished and the same ^{131}I concentration was found in both plasmas after the dialysis-equilibrium.

TABLE 1. (F/M) ^{131}I ratios in rats

No. of rats	Treatment	Time (min) between ^{131}I injection and the beginning of operation	No. of foetuses	(F/M) ^{131}I ratios
6	Propylthiouracil	45	12	0·37
			8	0·41
			7	0·69
			8	0·87
			8	0·99
			10	1·00
5	Propylthiouracil + thiocyanate	45	12	0·15
			8	0·17
			8	0·18
			11	0·24
			11	0·22
5	Propylthiouracil	75	10	1·17
			8	1·24
			12	1·35
			9	1·36
			11	1·46
6	Propylthiouracil + thiocyanate	75	12	0·27
			10	0·30
			3	0·31
			12	0·33
			10	0·36
			8	0·42

DISCUSSION

The results show that the (F/M) ^{131}I ratio in guinea-pigs and rabbits can be considered to represent a true chemical inorganic iodide ratio for the following reasons. The raised (F/M) ^{131}I ratio was found in most pregnant animals when the maternal plasma radioactivity concentrations were steady or on a slightly rising curve. The chromatographic findings of foetal and maternal plasmas and the tests for identification of the form of ^{131}I in the foetal plasma indicate that the foetal ^{131}I was of the same nature as that in the maternal plasma, namely in the form of inorganic iodide (I^-). Equilibration of foetal plasma with its corresponding maternal plasma showed no evidence of any loose physico-chemical linkage to a specific foetal plasma protein and implied that foetal iodide was osmotically active.

Active iodide transport by the placental membrane is responsible for the concentration gradient. In guinea-pigs the concentrating mechanism is manifested as early as the 36th day of gestation. We cannot make a comparison of its efficiency at different gestational periods because of the small

number of animals used and the fixed time at which foetuses were removed after ^{131}I injection, irrespective of their size.

The blocking of thyroid binding of iodide proved unnecessary for establishment of the (F/M) ^{131}I ratio. Ratios obtained from the guinea-pigs and rabbits in which thyroid binding of iodide proceeded during the equilibration of ^{131}I across the placenta are of about the same order as ratios obtained from animals injected with propylthiouracil.

Thiocyanate has a striking effect on the (F/M) ^{131}I ratio, either by inhibiting or competing with iodide for the placental transport mechanism. After its injection the iodide concentrations in the foetal and maternal circulations would tend to equalize by simple diffusion. Under these conditions a (F/M) ^{131}I ratio of about unity would have been expected at equilibrium. However, presumably due to a fall in the flux rates of iodide across the placenta, the (F/M) ^{131}I ratios found were significantly below unity 75–80 min after the ^{131}I injection.

The results in rats suggest that a marked difference in iodide concentrations between foetal and maternal blood may not exist at the 20th or 21st day of their gestational period. Attempts to collect blood from foetuses at an earlier age were not successful. However, the (F/M) ^{131}I ratios found in pregnant rats after the injection of NaCNS reached only one-third of the values found in the control groups. This would exclude simple diffusion as the process underlying the exchange and transfer of iodide across the placenta in the rat.

If the iodide concentrating mechanism proves to exist in the placenta of bigger mammals, a more accurate study of its kinetics should be technically possible. The effects of a low iodine diet and various hormones would be of interest.

SUMMARY

1. ^{131}I was injected subcutaneously into pregnant guinea-pigs and pregnant rabbits, with and without a preceding propylthiouracil injection.

2. Radioactivity concentrations were measured in the foetal and maternal plasmas 70–80 min later, and expressed as a (Foetal/Maternal) plasma (F/M) ^{131}I ratio. These ratios were found to be 1·5–5 at a time when the ^{131}I concentrations in the maternal circulation were on a rising or steady level.

3. After injection of sodium thiocyanate (NaCNS) the (F/M) ^{131}I ratios found were less than unity.

4. In pregnant rats the (F/M) ^{131}I ratios are not significantly higher than unity, but ratios of 0·3–0·4 only were found in animals injected with NaCNS.

5. The ^{131}I in foetal and maternal plasma was shown to be present as the free iodide ion.

6. A placental transport mechanism for iodide, inhibited by thiocyanate, is postulated.

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