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PASSAGE OF EXOGENOUS THYROXINE AND OF IODIDE BETWEEN MOTHER AND FOETUS IN PREGNANT RABBITS

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There is conflicting evidence about the permeability of the placenta to thyroid hormone. Courrier & Aron (1929) concluded that in dogs thyroxine does not cross the placenta from mother to foetus. Other workers, however, have concluded that thyroxine crosses the placenta in guinea-pigs (Peterson & Young, 1952) and rats (Theresa, 1939). In each case these conclusions were based on the histological appearance of the foetal thyroids after thyroid hormone had been given to the mother.

In the experiments reported here we have studied the passage of labelled thyroxine from mother to foetus and from foetus to mother in rabbits at different stages of pregnancy.

METHODS

Radioactive L-thyroxine or radioiodide was injected intravenously into pregnant rabbits. Half an hour or more after the injection, the rabbit was anaesthetized with pentobarbitone (40 mg/kg I.V.), the uterus opened, and a sample of foetal blood obtained. When the rabbit was more than 17 days pregnant, blood was taken from the umbilical vein with a needle and syringe. At earlier stages of pregnancy, several foetuses were removed, their chest walls opened, and the blood allowed to drip into a centrifuge tube, the blood from all the foetuses of each rabbit being pooled. Blood samples were taken from the mother by cardiac puncture. The maternal and foetal portions of the placenta were removed separately, as described by Huggett & Hammond (1952). By the 20th day of pregnancy the foetal rabbit's thyroid begins to make thyroxine from the plasma iodide (Jost, Morel & Marois, 1949). There is also evidence suggesting that towards the end of pregnancy the synthesis of thyroxine in the foetal thyroid may be more rapid than in the mother (Jost, Morel & Marois, 1952). Any ^{131}I released from labelled thyroxine in the mother or present as iodide in the injection might, therefore, cross the placenta and appear in the foetal serum as labelled thyroxine within a few hours of the injection. In order to exclude this source of error, foetal blood samples were not taken later than 3 hr after the injection if the foetus was more than 18 days old.

For the injection of thyroxine into the foetus, the uterus was opened and the injection made into the muscles along the back of the foetus, the needle being passed through the foetal membranes.

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The uterus was then closed with a silk suture, and the mother kept anaesthetized until the foetuses were removed at the end of the experiment.

Measurement of radioactivity. The samples of serum were weighed, made up to 10 ml. with water, and the total radioactivity measured in a M.R.C. sodium iodide scintillation counter. The ^{131}I was then extracted with water-saturated *n*-butanol, after acidification of the serum to pH 4, and fractionated by the method of Taurog & Chaikoff (1946). In this method, the inorganic iodine and any di-iodotyrosine present (alkali-soluble fraction) are extracted from the butanol into 5% Na_2CO_3 in 4N-NaOH, leaving the thyroxine in the butanol (butanol-soluble fraction). The proportion of the total ^{131}I in the serum recovered in the butanol extract varied from 83·1 to 94·3%. When radioiodide was added to fresh plasma *in vitro* and extracted by this procedure, less than 4·5% of the radioactivity remained in the butanol after extraction with alkali. The concentration of butanol- and alkali-soluble ^{131}I in the serum was calculated from the total ^{131}I concentration, measured before the butanol extraction, and the proportions of butanol- and alkali-soluble ^{131}I in the butanol extract.

The placentae were removed from the uterus, blotted, weighed, and homogenized in a Nelco homogenizer with 5 vol. of *n*-butanol saturated with 0·1N-HCl. The butanol was then separated by centrifuging and treated in the same way as the butanol extracts from serum. After removal of the butanol layer, the residue was digested in 2N-NaOH, made up to 10 ml. with water, and the radioactivity measured. The concentration of butanol-soluble ^{131}I in the placenta was calculated from the total (residue plus butanol extract) and the proportion of butanol-soluble ^{131}I in the butanol extract. The chief error in estimates of the foetal/maternal plasma ^{131}I concentration ratio was due to statistical errors of counting. When labelled thyroxine was injected into the mother the error on the ratio was about $\pm 15\%$ at the early stages of pregnancy, and fell to $\pm 5\%$ during the later stages of pregnancy, when the radioactivity in the foetal plasma was higher and larger samples could be obtained. The error on the ratio estimated from samples taken after injection into the foetus was between ± 5 and $\pm 20\%$.

Chromatography. In some experiments the untreated butanol extract was evaporated to dryness under reduced pressure. The residue was dissolved in a few drops of NH_4OH and put on strips of Whatman No. 3MM paper for ascending chromatography in one dimension. Thyroxine, tri-iodothyronine, and iodide were added to the residue as markers. The solvents were either butanol (4 vol.), redistilled dioxane (1 vol.) and ammonia (2N), or butanol and acetic acid (2N). Radioactivity on the chromatogram was measured along the paper with a continuously recording scanner, and the activity at each spot estimated from the area under the corresponding peak.

Materials. Radioactive L-thyroxine was prepared by the iodination of di-iodo-L-thyronine with radioactive ^{131}I, essentially according to the method of Gross & Leblond (1950), modified as described elsewhere (Myant, 1956). On the day of the injection a sample of the labelled material was analysed quantitatively by paper chromatography with butanol, dioxane and ammonia as solvents. In fresh preparations traces of radioactive iodide (5–10% of the total radioactivity) were always present on the chromatogram, but there was no detectable radioactive tri-iodothyronine. The amount of radioactive iodide increased if the thyroxine was kept in solution for a few days. For most of the experiments freshly prepared thyroxine was used. In a few cases, when the labelled thyroxine had been stored for several days, up to 20% of the total radioactivity was due to iodide. The specific activity of the labelled thyroxine varied from 1 to 10 $\mu c/\mu g$ and the amount of unlabelled L-thyroxine given with each injection varied from 5 to 100 μg.

RESULTS

Injection of radiothyroxine into mother

After single injections of radiothyroxine the concentration of butanol-soluble ^{131}I in the foetal serum was much less than that in the maternal serum at all stages of pregnancy (Table 1, rabbits 1–19). Until at least the 18th day the foetal/maternal (F/M) concentration ratio hardly exceeded 0·02 at 6 or more hours after the injection. After the 21st day there seemed to be an increase in the amount of butanol-soluble ^{131}I reaching the foetal serum (Fig. 1). A few observations were made at different sampling intervals on rabbits at the same stage of pregnancy (Table 1). These results (Fig. 2) also suggest that the rate of rise of ^{131}I concentration in the foetal plasma increases during the later stages of pregnancy.

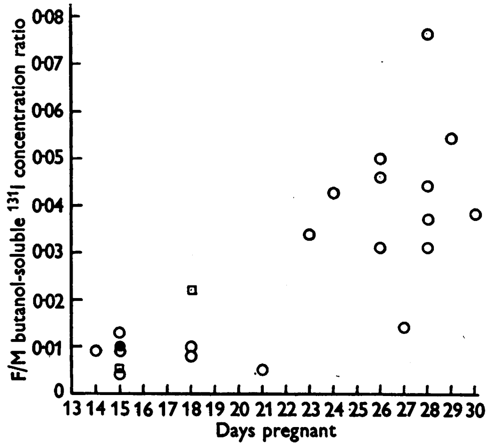

Fig. 1

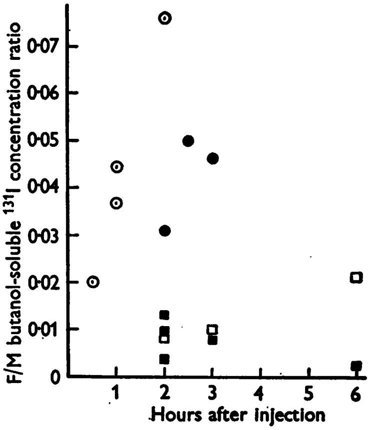

Fig. 2

Fig. 1. Ratio of foetal to maternal serum concentration of butanol-soluble ^{131}I after injection of radiothyroxine into mother between 14th and 30th days of pregnancy. ○, 1–3 hr after injection; □, 6 hr after injection; ●, 24 hr after injection.

Fig. 2. Ratio of foetal to maternal serum concentration of butanol-soluble ^{131}I after injection of radiothyroxine into mother. Ratios observed at different times after injection at 15th day (■), 18th day (□), 26th day (●) and 28th day (⊙) of pregnancy.

In order to test whether more radiothyroxine appears in the foetal circulation in young foetuses when several days are allowed for equilibration between maternal and foetal plasma thyroxine, the concentration of radiothyroxine in the maternal blood was maintained for 5–8 days by injections given twice daily. Maternal and foetal blood samples were then taken 3 hr. after the last injection. After these intervals (Table 1, rabbits 20–23) the concentration of ^{131}I in the butanol-soluble fraction from the foetal serum was still only a small proportion of that in the corresponding fraction from the maternal serum. For reasons given above (see Methods) the injections in these experiments were not continued into the later stages of pregnancy.

The low concentration of butanol-soluble ^{131}I observed in the foetal serum could be explained if radiothyroxine reaches the foetal circulation, but is rapidly removed and stored in the extravascular tissues of the foetus. This explanation was excluded by measuring the concentration of butanol-soluble ^{131}I in the liver and the remainder of the foetus, after injections given at two stages of pregnancy (rabbits 8 and 11). Six foetuses from each rabbit were removed after the blood samples had been taken. The liver and remainder of each foetus were then weighed separately, homogenized with butanol, and the

TABLE 1. Concentration of butanol- and alkali-soluble ^{131}I in maternal and foetal serum, and of butanol-soluble ^{131}I in maternal and foetal placenta after injection of radiothyroxine into mother

			Foetal/Maternal serum ^{131}I concn.		Placenta/Maternal serum butanol-soluble ^{131}I	
Rabbit no.	Duration of pregnancy (days)	Time after injection (hr)	Butanol-soluble	Alkali-soluble	Maternal placenta	Foetal placenta
1	30	3	0·038	5·20	—	—
2	29	3	0·054	2·30	—	—
3*	28	½	0·020	1·50	0·115	0·300
3*	28	1	0·037	1·09	0·050	0·222
3*	28	1½	0·031	1·32	0·034	0·201
4	28	1	0·044	3·00	—	—
5	28	2	0·076	3·10	—	—
6*	27	½	0·011	2·30	0·146	0·291
6*	27	1	0·014	1·80	0·114	0·360
7*	26	1	0·031	3·40	0·237	0·251
7*	26	2½	0·050	—	—	—
7*	26	3	0·046	0·82	—	—
8	24	3	0·043	—	0·230	0·331
9	23	2	0·034	6·90	—	—
10	21	2	0·005	1·30	—	—
11	18	6	0·022†	—	0·316	0·194
12	18	2	0·008	0·15	0·129	0·187
13	18	3	0·010	0·77	0·436	0·537
14	15	2	0·009	0·39	—	—
15	15	6	0·005	0·09	0·185	0·381
16	15	24	0·010	0·37	—	—
17	15	2	0·013	0·04	0·340	0·654
18	15	2	0·004	0·11	—	—
19	14	3	0·009	0·06	—	—
20‡	9–15	3	0·010	0·61	0·248	0·237
21‡	9–16	3	0·021	2·00	1·120	1·270
22‡	9–15	3	0·050	0·64	1·040	1·190
23‡	11–16	3	0·022	0·37	1·120	0·942

* ^{131}I measured in more than one foetus from the same rabbit.

† Average value from six foetuses.

‡ Time after injection refers to interval after last of the series of injections. Placental ^{131}I was not fractionated. Values in last two columns for these four rabbits give: concentration of total ^{131}I in placenta/concentration of butanol-soluble ^{131}I in maternal serum.

^{131}I fractionated by extraction with alkali. The concentration of butanol-soluble ^{131}I in the six foetal carcasses of each rabbit (nos. 8 and 11) averaged $47{\cdot}0 \pm 19{\cdot}1$ and $15{\cdot}3 \pm 2{\cdot}1\,\%$ of the concentration in the foetal serum. In the foetal livers the concentration averaged $74{\cdot}9 \pm 6{\cdot}2$ and $36{\cdot}7 \pm 5{\cdot}3\,\%$ of that in the foetal serum. There is, therefore, no evidence that radiothyroxine is selectively concentrated in any of the foetal organs.

In most experiments the concentration of ^{131}I in the butanol-soluble fraction of the foetal serum was too great to be attributable to radioiodide remaining in the butanol after extraction with alkali. Without further evidence, however, the ^{131}I in this fraction could not be assumed to be in the form of radiothyroxine derived from the mother. In only one rabbit (no. 3) was there enough radioactivity in the foetal serum for satisfactory identification of the chemical form of the ^{131}I. A portion of the butanol extract from each of the three foetal sera was set aside for extraction with alkali, and the remainder evaporated to

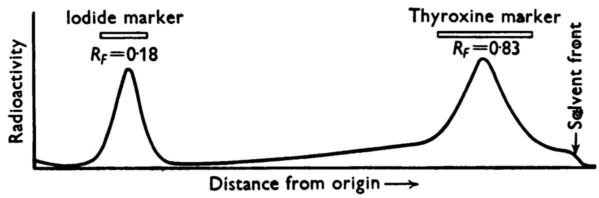

Fig. 3. Ascending chromatogram of butanol extract of serum from foetus 1 (rabbit no. 3), showing peaks of radioactivity at positions of iodide and thyroxine spots. Solvents: *n*-butanol and acetic acid; spots stained by diazotized sulphanilic acid.

TABLE 2. Percentage of ^{131}I in foetal serum (rabbit no. 3) remaining in butanol after alkali extraction, or behaving as thyroxine on chromatogram

Foetus	Percentage butanol-soluble	Percentage at thyroxine spot
1	64·3	55·7
2	45·4	39·8
3	52·0	42·1

dryness and chromatographed with butanol and acetic acid as solvents. On each chromatogram there was a clearly marked peak at the thyroxine spot (Fig. 3) and the proportion of the total radioactivity on the chromatogram, present at this spot, was in fair agreement with the proportion of the total ^{131}I remaining in the butanol after extraction with alkali (Table 2). From this result it seems justifiable to conclude that the butanol-soluble ^{131}I in the foetal serum from other rabbits was also present as thyroxine.

In some rabbits the concentration of butanol-soluble ^{131}I was measured separately in the maternal and foetal portions of the placenta removed immediately after the foetal blood sample was taken. The concentration in both portions of the placenta, at all intervals after injection and at all stages of pregnancy, was lower than the concentration in the maternal serum and higher than that in the foetal serum (Table 1). The proportion of alkali-soluble ^{131}I in the butanol extracts from both foetal and maternal placenta varied from 3·7 to 23·0 %. In most cases this was not significantly greater than the proportion of ^{131}I present as iodide in the material injected. It seems unlikely, therefore, that thyroxine is deiodinated to any extent in the placenta. After repeated injections of labelled thyroxine the concentration of total ^{131}I in both parts of the placenta from 3 rabbits (nos. 21–23) was about equal to that in the butanol-soluble fraction in the maternal serum.

Injection of radiothyroxine into foetus

When labelled thyroxine was injected intramuscularly into foetuses, high concentrations of ^{131}I were found in the foetal serum at intervals varying from 1 to 4 hr after the injection. In almost all the samples of foetal serum more than 90% of the radioactivity was butanol-soluble or behaved as thyroxine when analysed by paper chromatography. During the same intervals after the injection very little butanol-soluble radioiodine appeared in the mother's circulation (Table 3), except in one rabbit (no. 29). In this experiment contamination of the sample of maternal serum by radiothyroxine outside the body could not be excluded. The concentration of ^{131}I in the foetal placenta was always lower than that in the foetal serum, and higher than that in the maternal placenta.

TABLE 3. Concentration of butanol-soluble ^{131}I in foetal and maternal serum, and of total ^{131}I in foetal and maternal placenta, after injection of radiothyroxine into foetus

Rabbit no.	Duration of pregnancy (days)	Time after injection (hr)	Maternal/Foetal butanol-soluble ^{131}I concn. in serum	Concn. of total ^{131}I in placenta/concn. of butanol-soluble ^{131}I in foetal serum	
				Foetal placenta	Maternal placenta
24	28	2	0·017	0·65	0·11
25	26	3	0·012*	—	—
26	26	4	0·028*	—	—
27	24	3	0·016	0·15	0·09
28	23	1	0·019	0·15	0·09
29	23	2	0·230	0·62	0·43
30	21	1	0·002	—	—
31	21	4	0·052	—	—
32	20	4	0·030*	—	—
33	19	1	0·012	0·76	0·34
34	18	1	0·013	0·26	0·17
35	18	1½	0·010	—	—
36	18	2	0·071	—	—
37	16	1	0·022	0·29	0·11

* Average of three values.

The results obtained when labelled thyroxine was injected into the mother suggest that the passage of thyroxine from mother to foetus is very slow at all stages of pregnancy. However, the low concentrations of labelled thyroxine observed in the foetal serum (Table 1) could be explained if thyroxine crosses rapidly into the foetal circulation, but is equally rapidly deiodinated by the foetus. In order to test this possibility, known quantities of labelled thyroxine were injected intramuscularly into foetuses, and the amount of labelled thyroxine left in the foetus and placenta estimated at 2 or more hours after the injection. Three foetuses in each of three rabbits (nos. 25, 26 and 32) were injected. At the intervals shown (Table 4) one foetus and both portions of the corresponding placenta were removed. The foetus and the maternal and foetal portions of the placenta were extracted separately with butanol, and the total radioactivity measured in the butanol extracts and in the residues of foetal and placental tissue after extraction. The ^{131}I in the butanol extracts was then analysed either by paper chromatography or by further extraction with alkali. The amounts of ^{131}I recovered in the foetus and placenta (including that in the butanol extracts and the residues) are shown in Table 4. Except for one anomalous result (rabbit no. 32, foetus 2), the total amount of ^{131}I recovered varied from 86 to 99 %, more than 90 % of the ^{131}I extracted into butanol

TABLE 4. Recovery of radiothyroxine from foetus and placenta after injection of radiothyroxine into foetus

Rabbit no.	Foetus	^{131}I recovered (percentage dose injected) Foetus	Placenta	Total
25	1	91·4 (94·3)*	2·0 (90·2)*	93·4
	2	90·3 —	3·7 (90·4)	94·0
	3	93·1 (97·1)	4·0 (92·7)	97·1
26	1	92·0 (96·3)	4·2 (95·3)	96·2
	2	79·6 (93·4)	6·1 —	85·7
	3	94·6 (91·6)	4·7 —	99·3
32	1	92·3 (96·6)	3·8 (94·1)	96·1
	2	116·7 (96·8)	3·9 —	120·6
	3	88·0 (97·1)	6·1 —	94·1

* Values in parentheses give percentage of ^{131}I in butanol extract remaining in butanol after extraction with alkali.

remaining in the butanol after extraction with alkali. The thyroid was dissected from the neck of two foetuses (rabbit no. 26, foetuses 2 and 3) and the ^{131}I content estimated separately. Both thyroids contained less than 1 % of the injected dose. In two rabbits (nos. 25 and 26) a foetus that had not had an injection of ^{131}I was removed from the uterus, together with the corresponding placenta, at the end of the experiment. In each case the total amount of ^{131}I recovered from the foetus and placenta was less than 0·1 % of the amount injected into one other foetus of the same rabbit, showing that radiothyroxine does not pass from one foetus to another.

Injection of radioiodide into mother

The values for alkali-soluble ^{131}I in the 5th column of Table 1 suggest that radioiodide, injected into the mother during the latter half of pregnancy, reaches a higher concentration in the foetal serum than in the maternal serum. In order to study this in greater detail, carrier-free radioiodide was injected into pregnant rabbits at various stages of pregnancy. The concentration of alkali- and butanol-soluble ^{131}I was then measured 2–3 hr later in the maternal and foetal serum. In some cases the concentration of total ^{131}I was measured in the placenta. In all the samples of maternal and foetal serum, the amount of butanol-soluble ^{131}I was no greater than the error due to radioiodide remaining in a butanol extract after extraction with alkali. Nor were significant amounts of radiothyroxine present on any of the chromatograms made from foetal or maternal serum at 3 hr after the injection of radioiodide (Fig. 4). It may be concluded, therefore, that no radiothyroxine was secreted into the blood stream by either the maternal or the foetal thyroid during the period of these

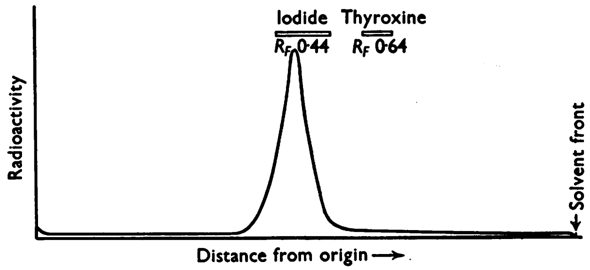

Fig. 4. Ascending chromatogram of butanol extract of serum from foetus of rabbit no. 39 at 3 hr after injection of radioiodide into mother. Solvents: *n*-butanol, dioxane, ammonia. A similar chromatogram was obtained from the maternal serum taken at the same time.

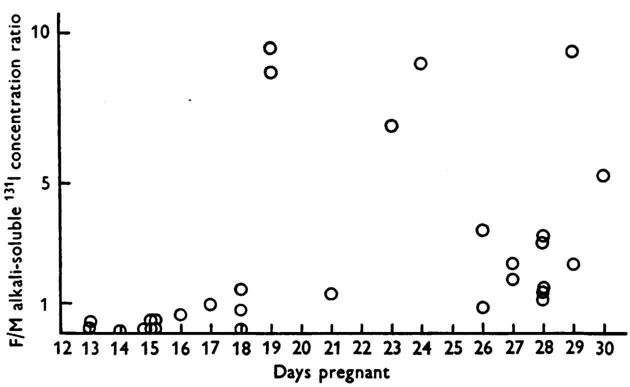

Fig. 5. Ratio of foetal to maternal serum ^{131}I concentration in alkali-soluble fraction, after single injections of radiothyroxine or radioiodide into mother. Observations collected from Tables 1 and 5.

observations. The results from these experiments (Table 5) and from Table 1 show that by the end of pregnancy radioiodide may be concentrated in the foetal serum to between 5 and 9 times the concentration in the maternal serum. This effect appears to begin at some time between the 16th and 20th days of pregnancy (Fig. 5). After the 17th day of pregnancy, the concentration of ^{131}I in both maternal and foetal placenta exceeded that in the maternal serum, but was usually less than the concentration in the foetal serum.

Injection of radioiodide into foetus

When carrier-free radioiodide was injected into the foetus, radioactivity was readily detectable in the maternal serum at 1 hr after the injection (Table 6). At the four stages of pregnancy studied between the 14th and 24th days, the concentration of ^{131}I in the foetal serum exceeded that in the maternal serum. At the later stages of pregnancy this relationship is to be expected, since the iodide-concentrating mechanism has been shown to begin to act by about the

TABLE 5. Concentration of alkali-soluble ^{131}I in maternal and foetal serum and of total ^{131}I in placenta after injection of carrier-free radioiodide into mother

Rabbit no.	Duration of pregnancy (days)	Time after injection (hr)	Foetal/Maternal alkali-soluble ^{131}I concn. in serum	Foetal alkali-soluble/Placental total ^{131}I concn.	
				Maternal placenta	Foetal placenta
38	29	2	9·30	1·37	1·60
39	24	3	8·96	4·30	8·01
40	19	3	8·67	3·01	1·87
41	19	2	9·36	—	—
42	18	2	1·64	2·09	2·07
43	17	2	0·92	1·12	0·92
44	16	2	0·62	0·42	0·27
45	15	3	0·09	—	—
46	13	2	0·18	—	—
47	13	2	0·21	0·94	0·49

TABLE 6. Concentration of alkali-soluble ^{131}I in maternal and foetal serum after injection of carrier-free radioiodide into foetus

Rabbit no.	Duration of pregnancy (days)	Time after injection (hr)	Maternal/Foetal alkali-soluble ^{131}I concn. in serum
48	24	1	0·20
49	19	1	0·32
50	17	1	0·50
51	14	1	0·48

17th day (Fig. 2). In the experiment done on the 14th day of pregnancy, the low concentration of ^{131}I in the maternal serum might, on the other hand, be explained if equilibrium had not been reached when the blood samples were taken. In all four rabbits the concentration of ^{131}I in both parts of the placenta was lower than that in the foetal serum.

DISCUSSION

Passage of exogenous thyroxine between mother and foetus

Reasons have already been given (Table 2) for concluding that the butanol-soluble fraction of the ^{131}I in the serum represents radiothyroxine, and in the following discussion we shall refer to this fraction as radiothyroxine. All experiments were done either before the stage at which the foetal thyroid makes thyroxine, or with so short a post-injection interval that any labelled thyroxine made in the foetal thyroid would not have had time to appear in the foetal serum. The radiothyroxine found in the foetal serum after an injection into the mother may therefore be assumed to have passed from the mother to the foetus as thyroxine.

The low F/M radiothyroxine concentration ratio observed after injection into the mother at all stages of pregnancy (Table 1) might be explained in various ways. It cannot be due to rapid deiodination of thyroxine in the foetus, because a high proportion of a dose injected into a foetus can be recovered as free thyroxine 4 hr later from the same foetus and placenta (Table 4). Nor can it be explained by storage of radiothyroxine in the extravascular tissues of the foetus, since this does not occur. If the placenta were freely permeable to thyroxine, a high M/F concentration gradient might, nevertheless, be maintained if the concentration of thyroxine-binding protein is much greater in the maternal than in the foetal serum, or if the placenta continually pumps thyroxine from the foetus to the mother. In either case, equilibrium between maternal and foetal circulating radiothyroxine should be established at a high M/F concentration ratio, whether the injection is made into the mother or into the foetus. But these observations suggest that very little radiothyroxine reaches the maternal circulation after an injection into the foetus (Table 3), most of the thyroxine remaining in the foetus for at least 4 hr (Table 4). However, it should be pointed out that even if the placenta were freely permeable to thyroxine in the foetal circulation, the rate at which the maternal and foetal plasma concentrations approach equilibrium would necessarily be slow, since the volume of foetal plasma is many times smaller than the volume of maternal plasma.

It seems more likely that the tissues lying between the maternal and foetal circulations act as a barrier to the passage of thyroxine. If so, the barrier appears to be almost complete from the 9th to the 20th day or later, as judged by the low F/M concentration ratios observed when several days have been allowed for the passage of radiothyroxine from mother to foetus; thereafter it becomes less complete (Figs. 1, 2). A quantitative expression for the permeability of the placenta to thyroxine cannot be derived from these observations but it seems to be much less than the permeability to iodide (Table 5), and to sodium, even at the later stages of pregnancy. Flexner & Pohl (1941), for example, have shown that when radioactive sodium is injected into pregnant rabbits from the 19th to the 30th day, the concentration in the foetus reaches 20% of the concentration at equilibrium within half an hour of the injection.

The observations on foetuses less than 19 days old (that is, before the foetal thyroid begins to make thyroxine) suggest that, during this stage of pregnancy, the concentration of the natural hormone in the foetus is much lower than the concentration in the maternal circulation. This, however, rests on the assumptions that the foetus does not make thyroxine in tissues other than its thyroid, and that the thyroid hormone normally circulating in the maternal plasma behaves in the same way as exogenous labelled thyroxine. It is not known whether the foetus can make thyroxine outside the thyroid at any stage of its development, but the possibility cannot be excluded, since there is some evidence that adult rats can make thyroxine in the absence of the thyroid (Morton, Chaikoff, Reinhardt & Anderson, 1943). The amount of non-radioactive thyroxine injected into the mother must, in some cases, have greatly exceeded the daily output of hormone from the animal's own thyroid. If the barrier to thyroxine depends on some mechanism which only acts when the maternal plasma concentration exceeds a certain value, such as storage within the placenta, it could be argued that thyroxine at physiological concentrations may cross the barrier more easily than the labelled thyroxine used in these experiments. Against this, there was no obvious relationship between the M/F radiothyroxine concentration ratio and the amount of non-radioactive thyroxine given with the injection. Owing to the difficulty of obtaining sufficient quantities of blood from young foetuses, it has not yet been possible to estimate directly the concentration of protein-bound iodine in foetal serum before the 19th day of pregnancy. Nor have we been able to find any references to estimations of protein-bound iodine in rabbit foetuses, or in foetuses of other species at comparable stages of development.

The observations of Peterson & Young (1952), showing that thyroxine crosses from the mother to the foetus in guinea-pigs, do not necessarily conflict with the conclusions reached here. In their experiments, thyroxine, given to the mother continuously during the last 30 days of pregnancy, was shown to prevent the formation of thiouracil-induced goitres in foetuses delivered at full term. This, however, only shows that thyroxine is able to reach the foetus when its thyroid is capable of responding to thyroxine deficiency by becoming hyperplastic. It does not prove that thyroxine reaches the foetus before the foetal thyroid begins to function.

The barrier to the passage of thyroxine from the mother to the foetus may depend on the presence of the thyroxine-binding protein in the maternal serum. If the maternal blood-vessels in the placenta are more or less impermeable to this protein, very little thyroxine would be expected to reach the foetus. Except for three experiments in which the ^{131}I was not fractionated (rabbits nos. 20–23, Table 1), the observations on the radiothyroxine concentration in the placenta are consistent with this explanation. After injection into the mother, the concentration of radiothyroxine in both parts of the placenta averaged less than 20% of the concentration in the maternal serum. It is, therefore, possible to account for all the radiothyroxine in the placenta by the presence of maternal blood. However, the possibility of some leakage of radiothyroxine into the extravascular tissues of the placenta cannot be excluded, since no information is available about the volume of maternal blood in the placenta.

Passage of radioiodide from mother to foetus

The high F/M concentration ratio observed after injection of radioiodide into the mother is in agreement with the results obtained in rabbits and guinea-pigs by Logothetopoulos & Scott (1955). A comparable F/M radioiodide concentration gradient in pregnant rabbits is suggested by the earlier results of Jost *et al.* (1952), although these workers did not show that the ^{131}I in the foetal serum was in the form of iodide. Gorbman, Lissitzky, Michel, Michel & Roche (1951) have also shown that the concentration of non-precipitable ^{131}I is greater in the serum of the newborn calf than in that of the mother, when radioiodide is injected into the mother 24 hr before delivery of the foetus. The finding that the F/M concentration ratio does not exceed unity until after the 15th day (Fig. 5) suggests that the iodide-concentrating mechanism is in the placenta since the placenta becomes fully developed at about this time in rabbits. However, these results do not exclude the possibility that the high concentration of radioiodide in the foetus is brought about by the foetal membranes.

SUMMARY

1. The passage of ^{131}I-labelled thyroxine from mother to foetus in pregnant rabbits was studied by measuring the foetal/maternal serum concentration ratio after injections of radiothyroxine into the mother.

2. Up to the 19th day of pregnancy the F/M ratio did not exceed 0·022 when the samples were taken from 1 to 24 hours after a single injection, and did not exceed 0·05 after injections given twice daily for several days. Slightly higher concentration ratios were observed after injections at later stages of pregnancy.

3. The low concentration of radiothyroxine in the foetal serum is not due to deiodination or storage by the foetus.

4. After an injection of radiothyroxine into the foetus, the concentration in the maternal serum did not exceed 7·1 % of that in the foetal serum at 1–4 hours.

5. These results suggest that the tissues between the mother and foetus are almost completely impermeable to exogenous thyroxine up to about the 19th day, but that they become more permeable thereafter.

6. Since the foetal thyroid does not make thyroxine until after the 19th day these results suggest that the serum concentration of thyroid hormone in early foetuses is lower than that in the mother.

7. In confirmation of the results of other workers, the concentration of radioiodide in the serum of the foetus exceeded that in the mother, when radioiodide was injected into the mother after the 17th day of pregnancy.

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