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Maternal–Foetal Transfer of Human Immune Globulins and Fragments in Rabbits

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Summary. Studies of the ability of human 7S and 19S γ-globulins and some of their structural units to pass from the maternal to the foetal circulation in the rabbit have demonstrated that 7Sγ, 19Sγ, the '8S' units of the 19S γ-globulin and the artificially and naturally occurring F(B) fragments enter the foetal circulation readily. On the other hand, the slow (AC) papain fragment and Bence Jones proteins enter the foetal circulation poorly or not at all.

It would appear that some structure associated with the 'H chain' and found in the F(B) fragment of 7S γ-globulin is essential for the active process involved in the transfer of proteins across the rabbit splanchnopleure.

INTRODUCTION

Although each of the three major immune globulins (7Sγ, γ_{1A} and γ_{1M} globulins) possesses antibody activity, they differ from each other in a number of other biological properties, one of which is the ability to pass from the maternal to the foetal circulation (Hemmings, 1961). Studies with intact rabbit antibodies and antibody fragments in rabbits have clearly shown that transfer to the foetus is dependent on certain structural features of the molecules, rather than simply their size or shape (Brambell, Hemmings, Oakley and Porter, 1960). Similar studies of factors responsible for maternal–foetal transfer of proteins in humans are difficult to perform since they would require the use of radioactively labelled proteins in pregnant females. In spite of known differences in the routes of maternal–foetal transfer in the rabbit and human, and in the behaviour of certain proteins (19S γ-globulins pass to the foetus in rabbits, but not in human (Franklin and Kunkel, 1958)), it was thought that information on the structural requirements for this property could be obtained by examining the transfer of human proteins in the rabbit. Obviously, the results cannot be applied directly to man without additional studies in that species.

MATERIALS AND METHODS

Proteins Studied

1. Human 7S(γ_2) globulin free of 19S and γ_{1A} globulin. This was used as a reference protein in all studies. 2. A macroglobulin from a patient with macroglobulinaemia purified by precipitation in distilled water and repeated ultracentrifugation (Reisner and Franklin, 1961). This protein gave only a single precipitin line on immunoelectrophoresis with an antiserum to normal serum. 3. The '8S'monomer of this protein produced by treatment

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with mercaptoethanol followed by iodoacetamide (Reisner and Franklin, 1961). 4. Five Bence Jones proteins of the two major antigenic types. 5. Papain digests of human 7S γ-globulin containing a mixture of about 67 per cent slow (AC) fragment and 33 per cent fast (B) fragment. 6. A protein closely related to the F(B) fragment of 7S γ-globulin from a patient with so-called 'H chain disease' (Franklin, Lowenstein, Bigelow and Meltzer, 1964; Osserman and Takatsuki,1964).

Method of Assay of Proteins

The amount of protein in the foetus and in the amniotic fluid was determined by the quantitative double diffusion technique of Gell, thought to be accurate within ±15 per cent (Gell, 1955). Each foetal serum or amniotic fluid was set up against serial dilutions of the antiserum in standardized Ouchterlony plates. The precipitin patterns were compared to those obtained with known amounts of the reference proteins and similar dilutions of the same antiserum. For the 19S γ-globulins and the Bence Jones proteins, specific antisera were used. Once it was established that only trace amounts of the slow (AC) fragment entered the foetal circulation, the amounts of 7S γ-globulin, B(F) fragment and 'H chain' was estimated with an antiserum to 7S γ-globulin and the appropriate reference protein. The amount of F(B) fragment injected was assumed to be about one-third the amount of the total papain digest. The preparation and properties of these antisera have been described (Reisner and Franklin, 1961).

Experimental Design

The design was exactly as described by Brambell, Hemmings, Henderson and Rowlands (1950). On the 24th day of pregnancy, rabbits were subjected to nembutal and ether anaesthesia. A laparotomy was performed under sterile conditions. The uterus was exposed, 7S γ-globulin was injected into the distal end of the uterine cavity on one side, and the protein under study on the other. After closure of the wound, the pregnant females were returned to their cages for 24 hours at which time the abdomen was reopened and the foetuses were removed. The amniotic fluids were collected individually. The foetuses were then rinsed with normal saline, blotted dry, and blood was collected in heparinized tubes either by cutting the axillary artery or occasionally by cardiac puncture or decapitation. In all but four of the rabbits, foetuses were present in both horns of the uterus. Thus, most animals served as their own controls and allowed comparison of the protein under study with the reference 7S γ-globulin injected in the opposite horn. In view of differences in the amounts injected, transfer was expressed as the quotient

$$\frac{\text{mg. per cent in the foetus}}{\text{mg. injected per foetus}}.$$

Foetuses containing a lower concentration of protein than the amniotic fluid were excluded since this is due either to foetal death, non-selective transfer or injection in the amniotic fluid. It should be emphasized that in view of the large variations noted among different foetuses in a single uterine horn, and the limited number of animals studied, little significance can be placed on the quantitative variations noted among the different fractions studied. However, it would appear that the observation that some of the proteins entered the foetal circulation, while others failed to do so or did so only erratically, can be considered valid even in this small series of animals.

RESULTS

Tables 1–4 compare the entry into the foetal circulation of each of the proteins studied with that of 7S γ-globulin injected into the opposite uterine horn. The artificially prepared or naturally occurring F(B) fragments (molecular weight 55,000) passed to the foetal circulation readily, in each of the animals studied (Table 1a and b). In animals

TABLE 1

COMPARISON OF SPLANCHNOPLEURE TRANSFER OF 7S γ-GLOBULIN AND (a) PAPAIN FRAGMENT (F OR B); (b) THE PROTEIN FROM A PATIENT WITH 'H CHAIN' DISEASE

(a)

Rabbit No.	*7S γ-globulin*				*F(B) fragment*			
	No. foetuses	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$	*No. foetuses*	*Amount* injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$
1	1	39	60	1·54	5	2·5	0·95	0·44
2	—	—	—	—	5	2·1	0·95	0·45
3	1	30·9	24	0·78	1	5·6	24·0	4·30
Mean				1·16				1·7

(b)

Rabbit No.	*7S γ-globulin*				*'H chain'*			
	No. foetuses	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$	*No. foetuses*	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$
1	4	15·4	60	3·9	7	11·5	38	3·3
2	4	15·4	3·7	0·2	3	29	194	6·7
3	2	20·5	2·3	0·1	3	15	22	1·5
Mean				1·4				3·8

* Considered to be one third of total papain digest.

Ratios: (a) $\frac{\text{F(B) fragment}}{\text{7S γ}} = 1{\cdot}5$; (b) $\frac{\text{'H chain'}}{\text{7S γ}} = 2{\cdot}7$.

given the whole papain digest, only the F(B) fragment could be detected in the foetuses. Careful search for the slow (AC) fragment with potent antisera to this fragment revealed only trace amounts in a few of the animals, and consequently, no attempt was made to quantitate the amount transferred. 19S γ-globulin, with a molecular weight of about 1,000,000, appeared to pass erratically, but in appreciable amounts in those foetuses where passage occurred (Table 2). In contrast, the smaller '8S monomers' (molecular weight 160,000) passed somewhat less effectively than 7S γ-globulin in each of three animals studied (Table 3). Although there was significant variation from rabbit to rabbit and foetus to foetus in the passage of 7S γ-globulin, F(B) fragment and the monomer of 19S γ-globulin, significant amounts of protein were present in each of the foetuses considered acceptable for the study. In contrast, in the rabbits given the native macroglobulin, no protein was detected in 44 per cent of the foetuses, while the remainder contained amounts up to 25 mg. per cent. Because of this variability, the results in this group have

TABLE 2

COMPARISON OF SPLANCHNOPLEURE TRANSFER OF 19S γ-GLOBULIN AND 7S γ-GLOBULIN

Rabbit No.	*7S γ-globulin*				*19S γ-globulin*				
	No. foetuses	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)**	*Transfer coefficient* $\left(\frac{mg.\ \%\ plasma}{mg./foetus}\right)$	*No. foetuses*	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)†*	*Transfer coefficient (mg. % plasma/mg./foetuses)*	
								All 37† foetuses	*21 foetuses with 19S γ-globulin*
1	7	8·6	24	2·8	4	12·8	67	5·20	5·20
2	3	19·8	40	2·0	3	9·1	1·4	0·16	3·90
3	2	20·0	60	3·0	4	10·0	6	0·60	0·75
4	2	60·0	15	0·25	2	16·0	0	0	
5	2	69·0	24	0·35	3	31·0	12	0·39	0·39
6	5	10·0	9	0·9	6	8·5	6	0·07	0·50
7	—	—	—	—	4	23·0	3	0·13	0·52
8	—	—	—	—	6	20·0	9	0·45	0·90
9	—	—	—	—	5	28·0	10	0·36	0·50
Mean				1·54				0·82	1·72

* 1/21 had no 7S γ-globulin in foetus.

† 16/37 had no detectable 19S γ-globulin in foetus.

Ratio $\frac{19S\gamma}{7S\gamma}$; all foetuses 0·53; 21 (+) foetuses 1·12.

TABLE 3

COMPARISON OF SPLANCHNOPLEURE TRANSFER OF 19S γ-GLOBULIN MONOMERS AND 7S γ-GLOBULIN

Rabbit No.	*7S γ-globulin*				*19S (SH) monomers*			
	No. foetuses	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$	*No. foetuses*	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$
1	4	20·0	46	2·30	3	9·6	0·80	0·08
2	2	19·2	24	1·25	2	6·0	24·0	4·00
3	6	17·6	24	1·36	1	16·8	3·0	0·18
Mean				1·64				1·42

$$\text{Ratio } \frac{\text{19S monomers}}{\text{7S } \gamma} = 0{\cdot}87.$$

been expressed in two ways. One is the transfer coefficient for all the animals; the other is the transfer coefficient recalculated for those foetuses showing significant foetal transfer. In the latter group, transfer was equivalent to that in the control foetuses subjected to 7S γ-globulin. The marked variability in transfer of this protein remains to be explained, but suggests that conditions necessary for transfer of this protein are particularly stringent. In contrast to these proteins, the slow (AC) fragment and Bence Jones proteins (molecular weight ≏45,000) were present only in trace amounts in the foetuses after injection into the uterine cavity (Table 4). It is obvious from the data that significant individual variations

TABLE 4

COMPARISON OF SPLANCHNOPLEURE TRANSFER OF BENCE JONES PROTEINS AND 7S γ-GLOBULIN

Rabbit No.	*7S γ-globulin*				*Bence Jones proteins*			
	No. foetuses	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$	*No. foetuses*	*Amount injected (mg./foetus)*	*Mean plasma concentration (mg. %)*	*Transfer coefficient* $\left(\frac{\text{mg. \% plasma}}{\text{mg./foetus}}\right)$
1	2	19·7	3·7	0·19	2	12·3	0·14	0·01
2	4	9·85	1·3	0·13	6	5·6	0·04	0·01
3	3	19·8	60·0	3·03	2	11·2	0·00	0·0
4	4	15·0	9·9	0·66	3	8·3	0·08	0·09
5	5	11·9	60·0	5·04	3	10·5	0·25	0·02
Mean				1·8				0·03

$$\text{Ratio } \frac{\text{Bence Jones}}{\text{7S } \gamma} = 0{\cdot}001.$$

occur not only in different animals, but even in different embryos in the same animal. Consequently, the results are compared only in a gross qualitative fashion. Little weight can be attached to the more precise ratios which are listed in each table.

DISCUSSION

The results of previous studies with rabbit proteins in the rabbit (Hemmings, 1961; Brambell *et al.* 1960), and those of the present experiments with human fractions in the

same species allow certain generalizations to be made concerning factors responsible for transfer of antibodies from the mother to the foetus. It would appear that for the two classes of proteins studied (7S and 19S γ-globulins) there was some transfer of those fragments or proteins which contained either the intact heavy chain or the part found in Fragment III (F or B), regardless of their size. In contrast, those proteins containing only L chains (Bence Jones proteins) or the fragments of the molecule lacking the critical part of the H chain (slow (AC) fragments) crossed into the foetal circulation only poorly or not at all. The nature of the structure responsible is not known, but one possibility worthy of consideration is that it may be associated with the carbohydrate prosthetic group (Brambell, 1963). It is important to emphasize that these results cannot be directly applied to man since it is well-known from studies of the proteins present at birth that 19S γ-globulins do not cross the placenta in man (Franklin and Kunkel, 1958; Hemmings, 1961). This may be due in part to the fact that transfer in man occurs through the placental circulation. The marked variability with all proteins, and especially with the macroglobulin where transfer was present in only about half the foetuses, suggests that local factors may play a very important role in regulation of materno-foetal transfer.

From these and similar studies of skin fixation (Ovary and Karush, 1961) and complement fixation (Taranta and Franklin, 1961), it would appear that a number of important biological properties associated with 7S γ, and possibly with the other immune globulins are associated primarily with the part of the H chain found in Fragment III or F(B) in man.

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