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Allergic histological changes in the pregnant
rabbit organs induced by injection of the
globulin fractions from human placenta 1.
Immuno-serological study on the glo-bulin
fractions extracted from human placenta

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Allergic histological changes in the pregnant rabbit organs induced by injection of the globulin fractions from human placenta 1. Immuno-serological study on the globulin fractions extracted from human placenta*

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Abstract

From the immunological study of the soluble protein fractions extracted from the normal human placenta as well as from the placenta in pregnancy toxemia, the author has clearly shown the presence of specific antigenic protein in these protein fractions which can not be found in the serum proteins of non-pregnant woman, and that this specific protein is contained only in the globulin fractions of placenta but not in the albumin fractions.

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**ALLERGIC HISTOLOGICAL CHANGES IN THE PREGNANT
RABBIT ORGANS INDUCED BY INJECTION OF THE
GLOBULIN FRACTIONS FROM HUMAN
PLACENTA**

**1. IMMUNO-SEROLOGICAL STUDY ON THE GLOBULIN
FRACTIONS EXTRACTED FROM HUMAN PLACENTA**

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In the immunological study of fetal serum HIRASE¹ reported that the fetal serum contains specific protein which can not be found in adult human serum. KUSUMOTO, OTSUJI, TORIMARU, ITASAKA and MIYOSHI^{2,3,4,5,6} demonstrated that these specific proteins are present in human placenta, amniotic fluid, pregnant woman's serum, urine and meconium, and they attributed the production of these proteins to placenta. On the other hand, OKAMURA⁷ recognized that certain allergic changes are brought about in the viscera of pregnant rabbits when the serum protein fractions of fetus and placenta are injected to these animals and reported further that these pathological changes in the liver and kidney are similar to those changes in respective organs of pregnancy toxemia. In our study⁸ on antigenicity of the serum protein fractions of fetus it has been confirmed that the specific proteins mentioned above exist in the serum globulin fraction but not in the albumin fraction. On the basis of these facts the author has observed antigenicity of the globulin fractions extracted from human placenta and also investigated the histological changes occurring in the pregnant rabbits injected with the globulin fraction. The results obtained by these immunological studies are described in the present paper.

MATERIALS AND METHODS

The placentas from normal full-term pregnant women and those with toxemia are obtained at delivery. The techniques employed in the extraction of soluble protein fractions from the materials are as follows: The

fresh placenta is perfused with physiological saline solution through the umbilical veins and the blood components are washed away as soon as possible. Next, the umbilical cord, amnion and portion of the larger vessels are removed and only placental villous tissues are left for the use. The villous tissues are cut into small pieces with scissors and dried on filter paper and emulsified in a homogenizer. This is weighed accurately and double portions of 0.05 M sodium citrate, and then sodium chloride is added to it until the latter becomes 1.0 M, stirring constantly. This is adjusted to pH 7.8 with 0.2 M phosphate-buffer solution and then the total volume is measured again and kept in a refrigerator for 48 hours. It is then centrifuged at 3,000 r. p. m. and the supernate is separated. This supernate is considered as the soluble protein fraction. A portion of the soluble protein fraction is dialyzed in running water for 24 hours and then made into powder by lyophilic method. This dried powder is made into 1 % solution with physiological saline solution and is used for injection. To another portion of the soluble protein fraction an equal volume of saturated sodium sulfate solution is added and the mixture is kept in a incubator at 37°C for 24 hours. After centrifugation at 3,000 r. p. m, the supernate and sediment are separated. The sediment portion is dialyzed in running water for 24 hours and lyophilized into powder form. The powder is made into a 1.0 % globulin fraction solution with physiological saline solution. pH of the superate is adjusted to 4.8 with dilute sulfuric acid and sodium sulfate is added in such an amount as above until saturated. This solution is kept in an incubator at 37°C for 24 hours and centrifuged at 3,000 r.p.m. The sediment is dialyzed in running water for 24 hours and then lyophilized into powder form. This powder is made into 1.0 % albumin fraction with physiological saline solution.

For the observations of the antigenicity of these protein fractions, adult rabbits of both sexes are used. All the rabbit sera have been previously proven to show no precipitin reaction to the fractions extracted from human serum, fetal serum and the placenta. Animals are divided into 3 groups, each of which is consisted 5 animals, and the placental soluble protein fraction is injected to one group; the globulin fraction to the second group; and the albumin fraction to the last group. The injections are made into auricular vein 10 times at intervals of 2 days with 2 cc fraction each, and the whole blood is drawn from the animals 10 days after the last injection. The separated serum is inactivated for 30 minutes at 56°C and then made into antiserum by adding 0.5 % phenol and preserved in a refrigerator. For precipitin reaction, the double layer method of Uhlenhuth in the capillary vessels, is employed. Those proving positive 15 minutes after the

Table. 1. Precipitin tests with soluble

Kind of antiserum		Antiserum with placental pro- tein of normal pregnancy										Antiserum					
No. of antiserum		No. 74. 10-month normal pregnancy										No. 142. edema of					
Kind of antigen	dilution of antibody dilution of antigen	50	100	500	1,000	2,000	4,000	8,000	10,000	20,000	40,000	50	100	500	1,000	2,000	4,000
Non-pregnant woman's serum	1	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	10	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	20	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	40	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	80	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pregnant woman's serum	1	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	20	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	40	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	80	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fetal serum	1	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	10	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	20	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	40	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	80	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Placental extract	1	++	+	-	-	-	-	-	-	-	-	++	+	-	-	-	-
Amniotic fluid	1	++	+	+	-	-	-	-	-	-	-	++	+	-	-	-	-
Human hemoglobin	1	+	+	-	-	-	-	-	-	-	-	++	+	-	-	-	-
Fetal blood hemoglobin	1	+	+	-	-	-	-	-	-	-	-	++	+	-	-	-	-

protein extracted from human placenta

with placental protein of pregnancy toxemia																							
pregnancy				No. 154. kidney of pregnancy										No. 135. preeclampsia									
8,000	10,000	20,000	40,000	50	100	500	1,000	2,000	4,000	8,000	10,000	20,000	40,000	50	100	500	1,000	2,000	4,000	8,000	10,000	20,000	40,000
##	+	+	-	##	##	##	##	##	##	##	+	+	+	##	##	##	##	##	##	##	+	+	+
+	+	+	-	##	##	##	##	##	##	##	+	-	-	##	##	##	##	##	##	##	##	+	-
+	+	-	-	##	##	##	##	##	##	##	+	-	-	##	##	##	##	##	##	##	+	+	-
-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	-	-
-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
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-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
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-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
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-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
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-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
-	-	-	-	##	##	##	##	##	##	##	-	-	-	##	##	##	##	##	##	##	+	+	-
-	-	-	-	##	##	##	##	##	##														

formation of antigen double layer are classified as (+++); those positive 30 minutes later as (++) and those positive one hour afterwards as (+), all taken as respective one hour value. Negative after two hours are indicated as (-). For the antibody absorption a proper quantity of (non-pregnant woman's serum) is added to antiserum and the mixture is kept in an incubator at 37°C for 60 minutes, shaking occasionally. Then the mixture is placed in a refrigerator for 24 hours and centrifuged, and the antibody titers (precipitin values) of the supernate are determined.

RESULTS

The antibody titer of the soluble protein fraction of the normal human placental extract against non-pregnant woman's serum has been found to be 10,000—20,000 $\times$ 40—80; while that against pregnant woman's serum is 10,000—40,000 $\times$ 40—80; that against fetal serum 10,000—20,000 $\times$ 40—80; that against the 1% soluble protein fraction of placenta (to be abbreviated as placental extract hereafter) 50—100; that against amniotic fluid 100—500; and that against human hemoglobin or against fetal blood hemoglobin (10%) proves to be 50—100. The antibody titer of antiserum of the placental extract of pregnancy toxemia against non-pregnant woman's serum has been found to be 10,000—40,000 $\times$ 40—160; while that against pregnant woman's serum is 10,000—40,000 $\times$ 80—160; that against fetal serum 10,000—20,000 $\times$ 40—160; that against the placental extract 50—100; that against amniotic fluid 100—500; and that against human hemoglobin or against fetal blood hemoglobin proves to be 50—100 (Table 1).

Next, the results obtained in the antibody absorption tests with various sera against a proper quantity of non-pregnant woman's serum are as follows: In the case of the antiserum of normal placental soluble protein fraction against the serum of non-pregnant woman all antibody is absorbed and disappears completely, but in the case against the placental extract it reacts with 10—20 antibody titer; against the fetal serum with 80—160; against the pregnant woman's serum with 5—10; against the amniotic fluid with 10 antibody titer; and against the fetal blood hemoglobin with 5—10; respectively. However, no reaction can be recognized against the human hemoglobin. Even in the case of antiserum of the placental extract of pregnancy toxemia the antibody reacting against non-pregnant woman's serum is completely absorbed and disappears, but the same reacting against the placental extract shows 20—40 antibody titer; that against the fetal serum 160—320; that against the pregnant woman's serum 10: that

Table. 2. Specific protein in the soluble protein extracted from human placenta

Kind of antiserum	Antiserum with placental protein of normal pregnancy							Antiserum with placental protein of pregnancy toxemia																											
No. of antiserum	No. 32. 10-month normal pregnancy							No. 122. Pregnancy edema							No. 137. Pregnancy kidney							No. 135. Preeclampsia													
Dilution of antigen Kind of antigen	5	10	20	40	80	160	320	5	10	20	40	80	160	320	5	10	20	40	80	160	320	5	10	20	40	80	160	320							
Non-pregnant woman's serum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-							
Pregnant woman's serum	+	+	-	-	-	-	-	+	+	-	-	-	-	-	++	+	-	-	-	-	-	++	+	-	-	-	-	-							
Fetal serum	+++	+++	+++	++	++	+	-	+++	+++	+++	++	++	+	-	+++	+++	++	++	+	+	+	+++	+++	+++	++	++	++	+							
Placental extract	++	+	+	-	-	-	-	+++	++	+	+	-	-	-	+++	++	+	+	-	-	-	+++	++	++	+	-	-	-							
Amniotic fluid	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	++	+	-	-	-	-	-							
Human hemoglobin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-								
Fetal blood hemoglobin	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-							

Antigenicity of Placental Protein

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Table. 3. Precipitin production immunized with

Kind of antiserum		Antiserum with normal placental globulin fraction										Antiserum					
No. of antiserum		No. 84. 10-month normal pregnancy										No. 153. Pregnancy					
Kind of antigen	Dilution of antibody Dilution of antigen	50	100	500	1,000	2,000	4,000	8,000	10,000	20,000	40,000	50	100	500	1,000	2,000	4,000
Non-pregnant woman's serum	1	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	++	-	++	++	++	++	++	++
	10	++	++	++	++	++	++	+	-	-	-	++	++	++	++	++	++
	20	++	++	++	++	++	++	+	-	-	-	++	++	++	++	++	++
	40	++	++	++	++	++	++	+	-	-	-	++	++	++	++	++	++
	80	++	++	+	+	-	-	-	-	-	-	++	+	+	-	-	-
160	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pregnant woman's serum	1	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	++	-	++	++	++	++	++	++
	10	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	20	++	++	++	++	++	++	++	+	-	-	++	++	++	++	++	++
	40	++	++	++	++	++	++	++	+	-	-	++	++	++	++	++	++
	80	++	+	+	+	-	-	-	-	-	-	++	+	+	+	+	-
160	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	
Fetal serum	1	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	++	+	++	++	++	++	++	++
	10	++	++	++	++	++	++	++	++	+	+	++	++	++	++	++	++
	20	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	40	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	80	++	+	+	+	-	-	-	-	-	-	++	+	+	+	+	-
160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Placental extract	1	++	+	-	-	-	-	-	-	-	-	++	++	-	-	-	-
Amniotic fluid	1	++	++	++	-	-	-	-	-	-	-	++	++	+	-	-	-
Human hemoglobin	1	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Fetal blood hemoglobin	1	++	+	-	-	-	-	-	-	-	-	++	+	-	-	-	-

Antigenicity of Placental Protein

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globulin fractions extracted from human placenta

with placental globulin fraction of pregnancy toxemia		
edema	No. 167. Pregnancy kidney	No. 148. Preeclampsia
8,000 10,000 20,000 40,000	50 100 500 1,000 2,000 4,000 8,000 10,000 20,000 40,000	50 100 500 1,000 2,000 4,000 8,000 10,000 20,000 40,000
+++ ++ + -	+++ +++ +++ +++ +++ +++ +++ ++ +	+++ +++ +++ +++ +++ +++ +++ ++ +
++ + - -	+++ +++ +++ +++ +++ +++ ++ ++ -	+++ +++ +++ +++ +++ +++ +++ ++ - -
+ - - -	+++ +++ +++ +++ +++ ++ ++ - -	+++ +++ +++ +++ +++ +++ ++ + - -
- - - -	+++ +++ +++ ++ ++ + - - - -	+++ +++ +++ +++ ++ - - - - -
- - - -	+++ ++ + + - - - - -	+++ ++ + + - - - - -
- - - -	++ + - - - - - - - -	++ + - - - - - - - -
- - - -	+ - - - - - - - -	+ - - - - - - - -
+++ ++ + -	+++ +++ +++ +++ +++ +++ +++ ++ +	+++ +++ +++ +++ +++ +++ +++ ++ +
++ + - -	+++ +++ +++ +++ +++ ++ ++ + -	+++ +++ +++ +++ +++ +++ ++ ++ - -
+ - - -	+++ +++ +++ +++ ++ ++ + - -	+++ +++ +++ +++ ++ + + - -
- - - -	+++ +++ ++ + - - - - -	+++ +++ ++ ++ ++ + - - -
- - - -	+++ ++ + - - - - - -	+++ ++ + + - - - - -
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- - - -	- - - - - - - - -	+ - - - - - - - -
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- - - -	++ + - - - - - - - -	+ + - - - - - - -
- - - -	+ + - - - - - - -	++ + - - - - - - -

Table. 4. Precipitin tests with albumin

Kind of antiserum		Antiserum with normal placental albumin fraction										Antiserum					
No. of antiserum		No. 32. 10-month normal pregnancy										No. 122. Pregnancy					
Kind of antigen	dilution of antibody dilution of antigen	50	100	500	1,000	2,000	4,000	8,000	10,000	20,000	40,000	50	100	500	1,000	2,000	4,000
Non-pregnant woman's serum	1	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	+	-	++	++	++	++	++	++
	10	++	++	++	++	++	++	-	-	-	-	++	++	++	++	++	++
	20	++	++	+	+	+	-	-	-	-	-	++	++	++	+	-	-
	40	++	+	+	-	-	-	-	-	-	-	++	++	-	-	-	-
	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pregnant woman's serum	1	++	++	++	++	++	++	++	++	-	-	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	++	-	-	++	++	++	++	++	++
	10	++	++	++	++	++	++	-	-	-	-	++	++	++	++	++	++
	20	++	++	+	+	-	-	-	-	-	-	++	++	+	+	-	-
	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fetal serum	1	++	++	++	++	++	++	++	++	-	-	++	++	++	++	++	++
	5	++	++	++	++	++	++	++	+	-	-	++	++	++	++	++	++
	10	++	++	++	++	++	++	-	-	-	-	++	++	++	++	++	++
	20	++	++	+	-	-	-	-	-	-	-	++	++	+	+	+	-
	40	+	+	-	-	-	-	-	-	-	-	++	+	-	-	-	-
	80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	160	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Placental extract	1	+	+	-	-	-	-	-	-	-	-	++	+	-	-	-	-
Amniotic fluid	1	++	++	-	-	-	-	-	-	-	-	++	++	-	-	-	-
Human hemoglobin	1	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Fetal blood hemoglobin	1	+	-	-	-	-	-	-	-	-	-	++	-	-	-	-	-

Antigenicity of Placental Protein

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fractions extracted from human placenta

with placental albumin fraction of pregnancy toxemia												
edema	No. 115. Pregnancy kidney										No. 107. Preeclampsia	
8,000 10,000 20,000 40,000	50 100 500 1,000 2,000 4,000 8,000 10,000 20,000 40,000										50 100 500 1,000 2,000 4,000 8,000 10,000 20,000 40,000	
++ ++ + -	++ ++ ++ ++ ++ ++ ++ ++ ++ -										++ ++ ++ ++ ++ ++ ++ ++ ++ -	
++ + - - -	++ ++ ++ ++ ++ ++ ++ + - - -										++ ++ ++ ++ ++ ++ ++ + - - -	
+ - - - -	++ ++ ++ ++ ++ + + - - - -										++ ++ ++ ++ ++ + - - - -	
- - - - -	++ ++ ++ + + - - - - -										++ ++ ++ + - - - - -	
- - - - -	++ + - - - - - - - - -										- - - - - - - - - -	
- - - - -	- - - - - - - - - - -										- - - - - - - - - -	
- - - - -	- - - - - - - - - - -										- - - - - - - - - -	
++ + - - -	++ ++ ++ ++ ++ ++ ++ + -										++ ++ ++ ++ ++ ++ ++ + -	
++ - - - -	++ ++ ++ ++ ++ ++ ++ + - - -										++ ++ ++ ++ ++ ++ ++ + - - -	
- - - - -	++ ++ ++ ++ ++ + - - - -										++ ++ ++ ++ ++ + - - - -	
- - - - -	++ ++ ++ + + - - - - -										++ ++ ++ + + - - - - -	
- - - - -	+ + - - - - - - - - -										- - - - - - - - - -	
- - - - -	- - - - - - - - - - -										- - - - - - - - - -	
- - - - -	- - - - - - - - - - -										- - - - - - - - - -	
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- - - - -	++ + - - - - - - - - -										++ + - - - - - - - - -	
- - - - -	+ - - - - - - - - - -										+ - - - - - - - - - -	
- - - - -	+ - - - - - - - - - -										++ - - - - - - - - - -	

against the amniotic fluid 10 antibody titer; and that against the fetal blood hemoglobin 10 antibody titer; respectively. And also in the case against the human hemoglobin no reaction can be recognized (Table 2).

Now, in the observations carried out in the antibody used against the globulin fractions of human placental extract, the antibody titer of the antiserum of normal placental globulin against non-pregnant woman's serum is $10,000-40,000 \times 80-160$; that against pregnant woman's serum is $10,000-40,000 \times 80-160$; that against the placental extract 50—100; that against amniotic fluid 100—500; and that against human hemoglobin or against fetal blood hemoglobin is 100, respectively. The reaction of the antiserum produced by the placental globulin in toxemia against non-pregnant woman's serum gives the value of $10,000-40,000 \times 40-160$; the same against pregnant woman's serum $10,000-40,000 \times 40-160$; that against the fetal serum $10,000-40,000 \times 80-160$; that against the placental extract 50—100; against the amniotic fluid 100—500; and against the human hemoglobin or against the fetal blood hemoglobin the value is 100, respectively (Table 3).

In the observations conducted with the antibody used against the albumin fractions of human placental extract, the antiserum of normal placental albumin reacting against non-pregnant woman's serum gives the antibody titer of $10,000-20,000 \times 20-40$; that against the pregnant woman's serum $8,000-10,000 \times 20-40$; that against the fetal serum $8,000-10,000 \times 20-40$; that against the placental extract 50—100; that against the amniotic fluid 100—500; and against the human hemoglobin or against the fetal blood hemoglobin, each shows antibody titer of 50. Whereas, in the case using the antiserum of the placental albumin in toxemia, the antibody titer when reacting against non-pregnant woman's serum is $10,000-40,000 \times 20-40$; that against the pregnant woman's serum is $10,000-20,000 \times 20-40$; against the fetal serum $10,000-20,000 \times 20-40$; against the placental extract 50—100; against the amniotic fluid 100; and against the human hemoglobin or against the fetal blood hemoglobin, it is 50 in each (Table 4).

In the absorption tests conducted on various antisera above mentioned with the proper quantity of non-pregnant woman's serum the results are as follows (Tables 5, 6). In the case of the antiserum of normal placental globulin as shown in Table 5, when reacting against non-pregnant woman's serum all antibody is absorbed and disappears completely, but against the placental extract it reacts with antibody titer of 20—40; against fetal serum with 160—320; against pregnant woman's serum with 5—10; against amniotic fluid with 10; against fetal blood hemoglobin with 5—10,

Table. 5. Specific protein of human placental globulin fractions

Kind of antiserum	Antiserum with normal placental globulin fraction							Antiserum with placental globulin fraction of pregnancy toxemia																				
No. of antiserum	No. 74. 10-month normal pregnancy							No. 142. Pregnancy edema							No. 154. Pregnancy kidney							No. 122. Preeclampsia						
Dilution of antigen Kind of antigen	5	10	20	40	80	160	320	5	10	20	40	80	160	320	5	10	20	40	80	160	320	5	10	20	40	80	160	320
Non-pregnant woman's serum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pregnant woman's serum	+	+	-	-	-	-	-	+	+	-	-	-	-	-	‡	+	-	-	-	-	-	‡	+	-	-	-	-	
Fetal serum	‡‡	‡‡	‡‡	‡	+	+	-	‡‡	‡‡	‡‡	‡	‡	+	-	‡‡	‡‡	‡‡	‡	‡	+	+	‡‡	‡‡	‡‡	‡	‡	‡	+
Placental extract	‡	+	+	+	-	-	-	‡	+	+	+	+	-	-	‡‡	‡	+	+	-	-	-	‡‡	‡	+	+	-	-	-
Amniotic fluid	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	‡	+	-	-	-	-	-
Human hemoglobin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Fetal blood hemoglobin	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-

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Table. 6. Specific protein of human placental extractive albumin fractions

Kind of antiserum	Antiserum with normal placental albumin fraction	Antiserum with placental albumin fraction of pregnancy toxemia															
No. of antiserum	No. 80. 10-month normal pregnancy	No. 84. Pregnancy edema				No. 97. Pregnancy kidney				No. 104. Preeclampsia							
Kind fo antigen Dilution of antigen	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320	5 10 20 40 80 160 320		
Non-pregnant woman's serum	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
Pregnant woman's serum	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
Fetal serum	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
Placental extract	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
Amniotic fluid	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
Human hemoglobin	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		
Fetal blood hemoglobin	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -		

respectively. Likewise in the case of the antiserum of placental globulin in toxemia reacting against non-gravida's antiserum, all antibody is absorbed and disappears completely, but when reacting against the placental extract it reacts with antibody titer of 40; against the fetal serum with 160—320; against pregnant woman's serum with 5—10; against amniotic fluid with 5—10; and against fetal blood hemoglobin with 10. In the case with the antisera of normal placental and toxemic placental albumin no reaction at all occurs against non-pregnant woman's serum nor against any other antigens as shown in Table 6.

COMMENT

It is obvious from this study that the specific antigenic protein in the soluble protein extract of placenta is contained in the globulin fractions. Furthermore, it may be pointed out that the specific antigenic protein detected in the globulin fractions of the placenta exists in the sera of fetus and pregnant woman. The facts seem to support the studies of HIRASE, KUSUMOTO and TORIMARU^{1, 2, 4}, that the specific antigenic protein is produced in the placenta and then is secreted into the maternal and fetal sera. OGUCHI and TOKUNAGA⁹ have recognized the presence of a specific protein, which resembles the serum globulin, in the placenta, serum of puerpera, fetal and umbilical serum, as well as in the sera of newborn and infants, but this seems to be the same substance that the author has detected in present experiments.

CONCLUSION

From the immunological study of the soluble protein fractions extracted from the normal human placenta as well as from the placenta in pregnancy toxemia, the author has clearly shown the presence of specific antigenic protein in these protein fractions which can not be found in the serum proteins of non-pregnant woman, and that this specific protein is contained only in the globulin fractions of placenta but not in the albumin fractions.

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