

RESULTS OBTAINED BY THE INJECTION OF PLACENTA INTO ANIMALS OF THE SAME AND OF DIFFERENT SPECIES.

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Our anatomical knowledge of the various stages of pregnancy, except in its very earliest period, appears to be nearly complete. The clinical data dealing with the physiology and pathology of pregnancy and the puerperium are also numerous and well understood; but when we seek to obtain information about the agencies which govern the organism during the many changes incident to gestation, we meet with nothing but conjecture and hypotheses. The numerous, but often disconnected facts, upon which these various hypotheses have been founded, are based upon observations obtained at the bed side, the autopsy table, from chemical researches and animal experimentation. In view of these widely differing sources, it is not surprising that contradictions and gaps are numerous.

The object of this study has been to investigate experimentally whether actual proof can be found, that the chorionepithelium (the placenta) exerts a specific influence, whether trophic or other, upon the maternal organism. Two ways of demonstrating such an influence are at our disposal. The one consists of the injection of placental tissue into females of the same species, followed by examination of their generative organs, in order to ascertain whether any anatomical changes, particularly such as are found during gestation, have been produced. The other method is the injection of placental tissue into animals of the same and of other species with the object of producing a specific biological reaction, which can be demonstrated by various methods. In order to acquaint the reader with the current ideas entertained on this subject it will be necessary to refer to some of the literature.

It is generally conceded that the ovary instigates and regulates the periodical menstrual cycle, which is physiologically interrupted only during pregnancy and lactation until the woman reaches her menopause. According to the Born-Fränkell theory (1) which, however, from recent investigations must be accepted with some reserve, the corpus luteum exerts a powerful influence upon the nidation of the ovum. Halban (2) thinks it likely that the ovary and the corpus luteum elaborate products which are antagonistic to one another. When in the right proportion (this would be during gestation), each directly neutralizes the actions of the other. During this time, therefore, some other influence would have to bring about the numerous changes observed. Halban ascribes to the placenta this temporary governing or trophic function. Many observations have definitely shown that nervous influences play no rôle (section of the cord, lactation in transplanted breast tissue, etc.).¹ Halban has accumulated numerous clinical observations upon which he has built up an ingenious working theory. The placenta, according to this author, causes the hypertrophy and hyperemia of the uterus and of the neighboring organs. In response to the same stimulus the decidual reaction occurs in the uterine mucosa and sometimes in other tissues, and likewise occur the hyperemia and hypertrophy of the breasts. When the placental influence is removed, as after labor or abortion, the pelvic organs undergo a marked involution, whereas on the other hand the previously hypertrophied but inactive breasts begin to secrete. The so-called "menstruation" of the newborn, and the "milk" in the breasts of infants are also ascribed, by the same author, to the withdrawal of the placental influence. Many facts and interesting observations are adduced in this paper, but on the whole Halban's theory has not met with general acceptance. The sole experimental confirmation is that of Miss Lane-Clayton and Starling (3) who

¹Eckhard, "Beiträge zur Anatomie und Physiologie," *Eckhard's Beiträge*, 1858, i, 1, sectioned the nerves leading to the breast in goats, and found that the secretion of the milk went on unchanged.

Ribbert, *Archiv für Entwicklungsmechanik*, 1898, vii, 688, transplanted the breasts of a young guinea-pig into subcutaneous pockets in the ears. During a subsequent pregnancy the transplanted organs were found to contain functioning acini.

found that the extract derived from a rabbit's placenta, or fetus, repeatedly injected into virgin rabbits produced an hypertrophy of the breasts.² The incomplete experiments I have performed along somewhat similar lines will be referred to later.

Another group of investigators have sought for the true explanation by means of anatomical investigation. Chief among them is Schmorl (4) who has with great regularity found trophoblastic elements in the maternal organs. Rarely in normal women, almost regularly in eclamptics, he has discovered emboli, composed of syncytium, in the blood vessels of the lung. Kollman (5) has shown that syncytium undergoes dissolution when subjected to the maternal tissue fluids.

A third group of investigations are based purely upon experimental work, seeking to apply the Ehrlich side-chain theory to solve the problem. The activity in this line of research is so great that new observations are constantly cropping up, and consequently older conceptions will require frequent revision or rejection. Some of the older work has been made doubtful by more recently acquired knowledge. Much of this work unfortunately is still quoted and used in support of theories and far reaching conclusions.

Basing their ideas on the observations of Schmorl and Kollmann, Scholten and Veit (6) set out to prove experimentally, that fetal syncytium is dissolved by the maternal organism. It had been known that the organism reacts to the injection of foreign cells by elaborating a cytolyisin which brings about the disintegration of the invader. Animals (rabbits) were injected with human placenta; their serum dissolved placental cells in vitro. Liepmann, who will be referred to immediately, was unable to obtain similar results.

Kawasoye (7) found that human placental cells when placed in the serum of gravid women produced a slight cloudy precipitate and underwent a partial solution. His controls which were kept in sodium chloride solution were, however, quite inadequate.³

²Lane-Clayton and Starling believe that the placenta and the fetus elaborate an *hormone* which produces the breast changes. They passed the fetal or placental maceration through a Berckfeld filter and injected the fluid.

³Kawasoye's experiments may be divided into three groups:

1. A placental antiserum was obtained by injecting human placenta into rabbits. Large placental injections caused death, small ones albuminuria; a

When it first became known that the injection of foreign (art-fremdes) proteid produced a reaction in the living organism and could be demonstrated by a visible precipitate, many investigations along these lines were undertaken. If proteid (serum, organs, red blood cells) of a certain species are repeatedly injected into an animal of another species, the serum of the immunized animal becomes cloudy when the serum or the organ extract of the animal used to produce the immunization is added in the proper proportion. The value of this reaction is generally accepted and it has great forensic importance [Uhlenhuth (8)]. It was not only thought, at first, that the reaction applied to the proteid of a given species, but it was believed that a given organ produced an even more specific serum. This refinement has, however, not stood the test of further research, and reaction only for species can in most instances be looked for [Rostoski (9), Sata (10)]. Liepmann (11) announced that rabbits injected with human placenta produced a serum, which contained precipitins for placenta or placental blood. If either of these tissues were added to the placental immune serum, a precipitate was formed. In a later communication (11) he acknowledged that the washed placenta used for immunization necessarily contained general body proteids (connective tissue, red blood cells) as well as the special placental cells, and that, to exclude a general "human reaction" a previous partial precipitation with the serum of a non-gravid subject and clearing by centrifugalizing was required, to exclude this source of error. Several other investigators [Weichardt (12), Opitz (13), Wormser (14)] have obtained negative results in repeating Liepmann's work; while Kawasoye and R. Freund (15) agree with his findings.

Veit (16) in an interesting monograph has based an elaborate theory which seeks to account for all the phenomena of pregnancy, tolerance could be obtained. Precipitine reaction was positive with the blood of grvida, with retroplacental and cord blood, and with placental extract but negative with blood from the non-gravid and males.

2. The blood of gravid women dissolved placenta in vitro.

3. Albuminous urine of gravid women also produced an antiserum when injected into rabbits. The action of this serum was weaker and less specific than that of placental antiserum.

both those in the mother as well as those of the fetus, largely upon the above quoted works of Scholten, Kawasoye, Liepmann, Kollmann, and Schmorl. The main conclusions, germane to this branch of the subject, are of such general interest that they cannot be omitted.

In broad outline Veit's explanation is as follows: Schmorl has demonstrated syncytium in the lungs of the mother. Liepmann has furnished the biological proof that syncytial constituents are present in the blood of pregnant women, and this fact substantiates Veit's assumption that the placental proteid follows the laws of antitoxin formation and conforms to the side chain theory. Kollman has shown anatomically that syncytium is dissolved by the maternal blood; this solution presupposes a solvent chemical substance in the blood—a lysin. Veit bases all that is to follow upon the assumption "*that there is a continued entrance of syncytium from the intervillous space into the veins of the mother, and a subsequent lysis of the syncytium*" from the very beginning of pregnancy to its termination. Veit's own investigations (17) have convinced him that purely physico-chemical processes (differences in osmotic pressure) do not account for the absorption of salts and proteids by the fetus. Absorption is attended to by the villi, which dip into the maternal blood and show a selective affinity for certain substances only. This selective activity has been demonstrated by Ascoli (18) who proved that egg albumen though present in the blood of the mother did not appear in the circulation of the fetus, and by Schenk (19) who noted quantitative differences in the hemolysins and agglutinins of mother and child.

A possible explanation, according to Veit, is that the nucleus of the syncytium may control the function of assimilation, and the cell protoplasm that of excretion. When the syncytial cell is filled with excretory substances it is cast off from the villus and falls a prey to solution in the maternal blood.

The absorption of iron, which is not in solution, but is an integral part of the hemoglobin molecule and is enclosed by the red blood cell, is brought about by a similar reaction. The lysin acts as an intermediary between the red blood cell and the syncytium, and when this action occurs in the intervillous space the erythro-

cyte is bound to the functioning syncytium of the villus. Thus hemoglobin is removed without producing general hemolysis. Hemoglobin has actually been demonstrated in the villi by Hofbauer (20).

Referring to the pathological phenomena of pregnancy Veit offers the following explanation: The albumin found in the urine of patients suffering from albuminuria of pregnancy, is a true heterologous albumen, such as Liepmann pronounced it to be; it is due to dissolved syncytium which has entered the maternal blood stream in large amounts. If the quantity is very great, the syncytium which is then only partly neutralized acts as a toxin on the kidneys, and produces an actual nephritis. The proof of the nature of this albumin lies in the fact that the specific precipitine reaction is obtained with such urines (Kawasoye). When the syncytium is in greatly excessive amount it no longer acts as a merely local poison, a renal irritant, but produces systemic symptoms which manifest themselves in the eclamptic seizure. Possibly other morbid conditions of pregnancy, such as hyperemesis, icterus, morbus maculatus, etc., are due to this same cause.

The views of Veit, as set forth in the article which has been mentioned, cover every branch of the subject, and in building up his hypothesis he has made use of practically all the work, which has been done along these lines. In the first place he has accepted the anatomical findings of Schmorl and Kollmann.⁴ The discoveries of Schmorl may be considered conclusive.

The biological experiments, which are believed to show that the syncytium not only can, but does enter the maternal circulation, acts as a toxine on the tissues of the mother, and stimulates receptor formation, have not been convincing. In opposition to the positive findings of Veit and Scholten, Liepmann, and Kawasoye, Weichardt, Opitz and Wormser obtained negative results, as already stated. Veit has apparently not attached sufficient weight

⁴The work of Kollmann, as far as it bears upon this point, is not convincing. The material was obtained from a fetus three weeks old; some specimens were from pregnant monkeys. He interprets the vacuolization and disintegration noted in the syncytium, as due to lytic processes. It may be said, that changes, such as he pictures, are to be found in almost any rapidly multiplying tissue, and certainly do not necessarily imply any specific solvent process.

to the negative findings, particularly if we take into consideration the fact that some of the positive results were not constant or strictly specific.

Without entering into the merits of Veit's interesting hypothesis, which may yet prove to contain the true explanation of the phenomena of pregnancy, I have sought to repeat some of the work performed, and have used in part the methods already employed by others, in part newer or different methods.

1. To investigate Halban's placental theory three rabbits were repeatedly injected with fresh placenta of pregnant rabbits removed by operation just before term. These animals were used: (*a*) anatomically to study the uterus, ovary and tubes; (*b*) biologically to test the serum for precipitines. The difficulty in obtaining sufficient material for injection made it necessary to cut short this series of experiments somewhat summarily.

2. To control the work performed by Liepmann, etc., three groups of rabbits, each group composed of two animals, a male and a female, were injected repeatedly with the following materials: (*a*) A solution of nucleo-proteid obtained from the human placenta. This was tested for precipitines, and for deflection of complement by the method of Neisser and Sachs. (*b*) A maceration of human placenta made blood-free by washing in plain running water; it was tested by the same methods. (*c*) A maceration of human placenta made as bloodless as possible by washing with large quantities of normal saline solution; it was tested for precipitines, deflection, cytolysis, etc.

RABBITS INJECTED WITH PLACENTA OF RABBITS.⁵

A double purpose was kept in view in this series of experiments. Firstly, if placental tissue should prove to have an inherent specific action such as Halban ascribes to it, repeated intraperitoneal injections ought to produce distinct changes in the genital system. Such changes in the mammary glands were reported by Lane-Clayton and Starling a number of months after my experiments were

⁵This group of experiments was performed in the Pathological Laboratory of the College of Physicians and Surgeons during the months of April and May, 1906. I desire to express my obligations to Professor T. M. Prudden for the courtesies extended to me.

completed. The duration of pregnancy in the rabbit covers thirty days, therefore it would seem necessary to extend the injections over a period of about that length.

Secondly the use of the serum obtained, if potent, would avoid a disturbing factor not to be overcome if the injections were made into an animal of another species, namely hemolysins, and "a general species reaction," for the tissues of the animal used to produce immunization. The work of Kraus and Ludwig (21), and still more recently that of Schultz (22), shows that iso-hemolysins cannot be obtained in rabbits. It might be supposed that experimentally, at least, iso-syncytiolysins, would also not be formed. However the analogy is not quite exact, for in virgin animals (such as were used in this series) the placenta, if it differ biologically from other tissues of the body is a foreign substance. An article by Ed. Martin (23) has just appeared reporting attempts to prove the presence of iso-hemolysins and iso-agglutinins obtained in the following way. Twelve rabbits were operated upon, one half of the pregnant uterus (which in the rabbit is didelphous) being removed, and 0.5 grams of the mashed placenta at once injected into the ear vein of the same animal. In none of the experiments were either hemolysins or agglutinins obtained.

Technic.—Three young female rabbits were employed. A number of other rabbits were allowed to be impregnated at regular intervals, and the gravid uterus taken out at about the twenty-second to twenty-fifth day of gestation. The six to eight placentæ obtained at each operation were at once cut up and mashed in a mortar, the maceration thinned out with an equal volume of normal salt solution and ten cubic centimeters injected intraperitoneally through a large needle into the experimental animals. Throughout, aseptic precautions were employed and no infection occurred. After each injection the animals did not take food for a few hours, but showed no other ill effects; however, during the entire course of the experiments they lost some weight. Nine injections were given to each rabbit at three-day intervals. On the fifth day after the last injection the animals were bled to death and the clear serum obtained in the usual way. The pelvic organs were at once placed in five per cent. formalin and prepared for examination by embedding in celloidin.

The material for histological examination consisted of blocks taken from at least four levels of the tubes and uterus, and longitudinal sections of each ovary. The controls consisted of material taken from normal animals killed in the laboratory during the time in which these experiments were performed. The result of

the examination of these organs can be summed up by stating that there was not the slightest difference between the organs of the injected and of the control animals. No decidual reaction, not even an increased vascularity, was found. Macroscopically no difference in size or appearance could be noted.

The serum tests were performed as follows:

Fresh serum from the injected rabbits was taken in increasing dilution (see Table), placed in small test tubes, and to this serum was added extract of rabbit's placenta (allowed to stand forty-eight hours and prepared in the cold) of various strengths. In another series a loop full of maceration of washed rabbit's placenta was added to the immune serum. Both series were at once brought to a uniform volume of two cubic centimeters by adding normal salt solution. They were placed in the thermostat for one hour and then in the ice-chest for from twelve to twenty-four hours. In none of these series did a precipitate appear.

Anti-serum.	Placental Extract or Placental Tissue		
	0.1 c.c.	0.05 c.c.	0.01 c.c.
0.2 c.c.	0.1 c.c.	0.05 c.c.	0.01 c.c.
0.1	"	"	"
0.05	"	"	"
0.2	—	—	—
—	"	"	"

RABBITS INJECTED WITH HUMAN PLACENTA.⁶

Placental Nucleo-Proteid.—The work of Bierry and Meyer (24) and that of Beebe (25) and others lead one to expect that the nucleo-proteid, of a tissue, yields a more specific immune serum and gives less "general species reaction" than the serum obtained from injecting the washed organ. Levene (26) however says, that extracts made from various constituents of red blood cells and used for immunization, either give no hemolytic sera or sera far less potent than those obtained from the entire red blood cells. Pearce and Jackson (27) in a paper published recently, claim that a repetition of Beebe's work has given negative results. These investigators have made a very complete series of experiments from

* Almost all the human material used for these and the succeeding experiments was obtained through the kindness of Dr. George Ryder, resident obstetrician of the Sloane Maternity Hospital.

which they conclude that the nucleo-proteids act as mild toxic agents, affecting chiefly the excretory organs, and that they do not produce specific anti-sera. They say that Ehrlich and Morgenroth have shown that anti-sera are produced not by specific cells, but by specific free receptors. Specificity in the morphological sense cannot be demonstrated. The sole criticism that can be applied to the work of Pearce and Jackson is that they have prepared the nucleo-proteid by the "hot" method, which necessitates bringing the mashed organ to the boiling point! Such a method is inadvisable if biological reactions are to be employed. These authors did not attempt to make precipitine tests, but studied the effect of the injections upon the organs *in vivo* and also histologically.

Technic.—Many (ten to twelve) placentæ were passed through a meat machine and washed in large quantities of 0.9 per cent. saline solution. The blood-free tissue, to which a 0.5 per cent. sodium carbonate solution in the proportion of 1:3 had been added, was placed in the ice chest for twelve hours. Microscopical examination of the last wash water still showed the presence of red blood cells in small number, about one hundred being present in the ordinary low power field. The extract was next filtered through several thicknesses of gauze and the nucleo-proteid precipitated by means of a slight excess of acetic acid. The precipitate was now washed by decantation and attempts made to redissolve it with sodium carbonate solution. It was found that unless the process was hastened, the carbonate solution did not act as a solvent, but a decinormal sodium hydrate caused solution. Reprecipitation and redissolving completed the process.

No exact analysis of the amount by weight represented by each unit of solution was determined, but as from twenty-five to thirty cubic centimeters of very concentrated solution were given, at each injection, a considerable quantity of the nucleo-proteid was used.⁷ Dr. P. Levene of the Rockefeller Institute had the kindness to make a Phosphorus determination on the nucleo-proteid used in these experiments. The phosphorus percentage proved to be 0.35.

The solution was injected into two rabbits, a male and a female, intraperitoneally at intervals of about five days, seven injections in all being given to each animal. Serum was taken after the fifth injection and again after two additional injections. The animals flourished during the course of the experiments and gained in weight.

The serum of these rabbits was tested, while fresh, with placental extract (human placenta) and with bits of washed placenta, just as in the previous series. No precipitin reaction was obtained, nor did human blood-serum produce any result.

⁷ The first set of sera obtained from these animals were inactivated at 55° C. Noguchi's paper (*Journal of Experimental Medicine*, 1906, viii, 726) refers to the substances which he calls "protectines," or antilynsines, formed when sera are heated above 56° C. To avoid all possibility of error from this source the subsequently obtained sera were all inactivated at 52° C.

Another portion of each serum was inactivated at 55° C., for fifteen minutes and then tested by the method of Neisser and Sachs (28) for deflection of complement. This method is regarded as even more delicate than the precipitin reaction, although it is more troublesome and is somewhat capricious.

The rationale of the reaction is as follows: An indicator consisting of an independent hemolytic system is used. In my experiments rabbit's serum made lytic to hen's corpuscles by repeated injections of the washed corpuscles of hen's blood was employed. This anti-hen's serum was inactivated by heat. The other component of this series was hen's corpuscles diluted to a strength of five per cent. If fresh guinea-pig's serum (complement) is added, hemolysis is complete within one hour at a temperature of 37° C.

The second or main components of the reaction are the following: The placenta anti-serum (inactivated) is supposed to contain the amboceptor or specific binding-body if such is elaborated. The organ extract used for immunization must contain corresponding receptors; it is called the antigen. If to these two bodies combined in the proper proportion (see Table) a sufficient but not too great quantity of complement is added, the amboceptor should serve to bind the complement to the receptors. As the amboceptor is specific, it will serve only to bind complement to corresponding receptors. In other words, should an appropriate antiserum be obtained, the free complement is bound to the receptors, and when the hemolytic series (which contains no complement) is added, no hemolysis results. As actually performed, the amboceptor, antigen and complement are added in varying proportion, placed in the thermostat for one hour to allow deflection of complement to take place, and then the second series (hen's antiserum and hen's corpuscles) are added. The complete mixture is then again placed in the thermostat for two hours more. The result, *i. e.*, hemolysis complete, incomplete or absent, is read. The tubes are put in the ice chest for from twelve to twenty-four hours and the final reading taken.

In my experiments various quantities of the components were tried. The following table shows the proportion which was most economical of anti-serum and yet gave the greatest number of proportions of amboceptor and antigen. Throughout, just sufficient complement was used to assure complete hemolysis. The necessary quantity of complement was determined anew each time fresh complement was used.

To these and to all succeeding reactions enough normal salt solution was added to bring the total volume to four cubic centimeters.

When negative results were obtained in reactions similar to those described in the above tables, other experiments were performed, using as much as 1.0 c.c. of anti-serum, and 0.5 c.c. of placental extract. Only after repeated trials of these various proportions, was a negative result accepted.

Anti-serum (Amboceptor).	Placental Extract (Antigen).	G. P. Serum (Complement).	Hen's Anti-serum.	Hen's Corpuscles 5%.
0.2 c.c.	0.01 c.c.	0.04-0.025 c.c.	0.01 c.c.	1.0 c.c.
0.1	"	"	"	"
0.05	"	"	"	"
0.01	"	"	"	"
0.005	"	"	"	"
0.001	"	"	"	"
0.0005	"	"	"	"
0.2	—	"	"	"
0.2	—	"	—	"
—	0.01	"	0.01	"
—	—	"	"	"
—	—	"	—	"
—	—	—	0.01	"
—	—	—	"	"
Placental Extract.	Anti-serum.	G. P. Serum.	Hen's Anti-serum.	Hen's Corpuscles.
0.1 c.c.	0.1 c.c.	0.04-0.025 c.c.	0.01 c.c.	1.0 c.c.
0.05	"	"	"	"
0.01	"	"	"	"
0.005	"	"	"	"
0.001	"	"	"	"
0.0005	"	"	"	"
—	"	"	"	"
—	"	"	—	"
0.1	"	"	0.01	"
0.1	—	—	0.01	"
—	—	0.04-0.025	"	"
—	—	—	"	"
—	—	—	"	"

Before proceeding to the complete reactions, which the tables show, it was necessary to determine in each case that the given anti-serum was inactivated and was neither in itself hemolytic or anti-hemolytic. Two of the anti-sera (those obtained from human placenta washed with normal salt solution) proved to contain a small trace of *amboceptor for hen's corpuscles*. This disturbing factor was removed by treating these sera, for one hour at 37° C., or over night at 0° C., with a great excess of hen's corpuscles and using the supernatant clear fluid after centrifuging. Various prepared extracts were tried. One set was obtained by placing the macerated washed placenta, diluted with normal salt solution 1:3 for forty-eight hours in a shaker, the other in the thermostat for forty-eight hours. A second set of extracts were made using plain distilled water. As no great differences were found, the extract finally used was the one prepared with salt solution in the thermostat. Toluol was added to prevent bacterial growth, and

this antiseptic was later removed by evaporation. The extract was always tested for its hemolytic action and a quantity far below this point used in the reactions.

The four nucleo-proteid sera obtained from the two injected animals showed no deflection of complement. In other terms, the presence of a specific antibody could not be demonstrated either by the precipitin or by the reaction for deflection of complement.

Placenta Washed in Running Water.—As not all the red blood cells could be removed by washing the placenta on a filter with normal salt solution, and it was not feasible to obtain the placentae so early that they could be exsanguinated by washing through the umbilical cord, the finely chopped placenta was placed in a large gauze bag and attached to the cold water faucet. By these means the tissue became practically blood free in a short time, the last wash water showing very few red blood cells, or rather "shadows." The drawbacks attached to this method were fully realized, but it was hoped to control the results by experiments which follow.⁸ The placental tissue was then ground in a mortar with sand, and a thick, yet finely divided placental suspension prepared with sterile salt solution. Cultures of each batch of suspensions either showed no growth, or in the case in which growth occurred, an apparently non-pathogenic saprophyte was found.

Three rabbits, two males and one female, were used. One male died after the second injection, autopsy showing a peritonitis due to accidental perforation of the intestine. The other two animals received, respectively, six and eight injections intraperitoneally. The one which received eight injections was given 15 c.c. each time; the other was given from 25 to 30 c.c. The first animal gained over 200 grams in weight, the second lost nearly 400 grams.

The two sera obtained were tested both by the precipitin reaction and by the method of deflection of complement. *The results were absolutely negative.*

Placenta Washed in Normal Salt Solution.—The previous ex-

⁸Washing the tissue with a solution which was not isotonic made it not unlikely that at least some of the antigen would be carried away. Yet by this method a more complete removal of the red blood cells was obtained, and the microscopical examination of the tissue showed that enormous quantities of well preserved placental cells remained for injection.

periments, in which the placenta was rendered practically blood free, at the cost of losing at least some of its active constituents, resulted negatively. As a check and control, the placentae in the series now to be detailed, were first chopped fine with a meat machine, and then each placenta was washed on a filter with ten liters of salt solution, with constant stirring. A careful lookout was kept for all visible blood clots and these were removed. The last wash water appeared clear macroscopically, but under the microscope, from twenty-five to one hundred red blood cells to the low power field were noted. I have been unable to find any reference, in the literature, to the minimum quantity of blood necessary to produce an immune serum. The further treatment of the material in no way differed from that employed in the previous series.

Technic.—Three rabbits were used at the outset, two males and one female; but one male was so seriously injured in a fight with the others that the injections had to be discontinued.

Both the remaining rabbits received nine injections each, at intervals of about five days, from twenty-five to thirty cubic centimeters being introduced intraperitoneally. The animals were bled after the seventh and again after the ninth injection. Both gained markedly in weight during the treatment. In spite of the injections, the *female conceived and bore four normal young* during the course of the experiment. When bled to death she was found to be again pregnant (about twelve days gravid).

Of the four sera, the one serum obtained from the female (after the seventh injection) was rendered useless by being overheated, due to a faulty thermometer. The three other sera were tested for:

1. Precipitin reactions.
 - a. Precipitin reactions with placental extract.
 - b. Precipitin reactions with cord blood serum.
 - c. Precipitin reactions with retro-placental blood serum.
 - d. Precipitin reactions with normal blood serum from a male.
 - e. Precipitin reactions with placental extract after saturation with male blood serum and with human red corpuscles.
 - f. Precipitin reactions with urine (non-albuminous) of gravidæ.
 - g. Precipitin reactions with urine (albuminous) of gravidæ.
 - h. Precipitin reactions with urine of a male, containing albumin.
2. Reactions for deflection of complement.
 - a. Reaction for deflection of complement with placental extract.
 - b. Reaction for deflection of complement with normal human blood serum (male).
3. Agglutination reaction with human corpuscles.
4. Hemolysis reaction with human corpuscles.
5. Cytolytic reactions.

- a. Cytolytic reactions with emulsions of placental cells.
- b. Cytolytic reactions with small pieces of placental tissue.

The results obtained by the various tests follow, and as the three sera have given the same results, in approximately the same dilution, only one set of records will be described.

I. PRECIPITIN TESTS.

- a. A faint but positive reaction was obtained with washed placental extract (0.05 c. c.) and the immune sera in dilutions of from 1:10 to 1:20 corresponding to 0.2 to 0.1 c.c. *Normal rabbit serum gave no precipitate to placental extract or to the other substances tested in Experiments e to h.*
- b. The cord blood, from two cases, gave similarly positive results.
- c. Retro-placental blood serum, from two cases, gave a distinct but weaker reaction.
- d. Normal human blood serum (from a male) gave a precipitate, in the same dilutions as in Experiment a.
- e. Partial precipitates were sought for. To the immune serum was added an excess of normal human serum. After the precipitate had formed, the fluid was cleared by centrifuging, and then placental extract added as in Experiment a. No precipitate appeared in the tubes to which placental extract had been added, nor in those into which more human serum was placed, although even stronger solutions than in Experiment a were used. After it was observed that the antisera were hemolytic, another method for obtaining a partial reaction was tried. An excess of a five per cent. solution of human corpuscles was added to the antisera and kept in the ice chest for twenty-four hours. To the clear solution was added placental extract and human serum, in the same proportions as in Experiment a. No precipitate was obtained. The control with more human blood cells showed no further hemolysis.
- f. The urines of six gravid women (containing no albumin) were tested, using 0.1 to 0.05 c.c. of urine, to the same quantity of antiserum as in the other experiments. No precipitate resulted.
- g. The urines of two gravid women (containing much albumin, one in the pre-eclamptic stage, the other toxemic) were tested. The one containing the most albumin gave a negative, the other a faint but positive reaction.
- h. The urine of a man containing much albumin gave a faint but positive reaction.

The precipitin reaction in all these experiments showed a faint but unmistakable *human reaction, but no specific placental reaction.*

2. TESTS FOR DEFLECTION OF COMPLEMENT.

For experimental details see heading "Nucleo-Proteid Tests."

- a. For all three sera it was found that with from 0.004 to 0.001 c.c. of antiserum, and 0.01 c.c. of placental extract, using slightly more than the minimum amount of complement required (0.03 to 0.025 c.c.), hemolysis was incomplete or absent.

- b. With human serum (male) a positive reaction was obtained in much greater dilutions (down to 0.001 c.c. of human serum with 0.003 to 0.001 c.c. of antiserum).

Deflection of complement was more strongly marked in the case of normal blood serum than in that of placental extract.

3. **AGGLUTINATION TESTS.** Agglutination of a five per cent. solution of normal human blood corpuscles was obtained in dilutions of the antisera between 1:6 and 1:12, 0.5 c.c. of corpuscles being used. Normal rabbits' serum failed to agglutinate.
4. **HEMOLYSIS TESTS.** Hemolysis of 0.5 c.c. of a five per cent. solution of human corpuscles was obtained in dilutions of from 1:5 to 1:12 of the antisera, the controls with normal rabbits' serum again proving negative.
5. **CYTOLYSIS TESTS.**
 - a. Suspensions of placental cells, obtained by the method used by Flexner and Noguchi (29) in their cytolytic experiments, were added to 0.5 c.c. of the undiluted antisera, to normal rabbits' serum and to normal sodium chloride solution, kept in the incubator at body temperature for one hour, and then in the ice chest for twelve hours. Unstained and stained specimens of the cells were examined. No very marked differences were noted. On the whole the specimens kept in normal rabbit serum showed the best preservation. In all the others the chromatin network was found more coarsely granular and the nuclear outline less distinct; while in the unincubated specimens, made for control, immediately after preparing the cell emulsion, there were numerous Langhans' cells in addition to the syncytium; in the incubated specimens the syncytial cell complexes greatly preponderated.
 - b. A similar set of experiments to the foregoing were performed, using small pieces of placental tissue instead of isolated cells. All the specimens were transferred to a five per cent. solution of formalin embedded by the celloidin method, cut, and stained with hematoxylin and eosin. No differences were noted except that as in the previous series the specimens preserved in sodium chloride solution showed less perfect staining qualities. Otherwise the placental tissue was perfectly normal.

CONCLUSIONS.

The injection of rabbits' placenta into rabbits produces no iso-precipitins.

From the incomplete experiments performed it would appear that placental injections into animals of the same species cause no changes in the generative organs. Further research into this question will be pursued.

The injection of human placental nucleo-proteid, prepared from placental tissue made nearly blood free, does not produce an anti-serum. This result confirms the conclusions of Pearce and Jack-

son, that nucleo-proteids act merely as mild toxic agents, without specific qualities.

The injection, into rabbits, of human placental tissue, rendered practically blood-free, fails to produce any specific reaction. This confirms the view that the serum reaction following the injection of cells into a foreign organism is largely due to the blood contained in the injected tissues.

The injection into rabbits, of the human placenta, made nearly blood-free, produces a weak "human reaction" which can be demonstrated by the reactions for precipitin, deflection of complement, agglutinin, and hemolysis. *No specific placental reaction can be shown.* This is in strict accord with the view that cytotoxines are not specific; that there is no morphological specificity.

The anti-sera obtained showed no cytolytic action; therefore no specific syncytiolytic action could be demonstrated.

If the information obtained in this investigation is applied to the theory of Halban, it will be noted that no experimental proof of the specific action of placental tissue upon the female generative organs could be demonstrated. The number of experiments performed, bearing upon this one point, were however too few to permit of a definite and final opinion.

The work dealing with Veit's ingenious hypothesis was more complete and carried out by many methods, which would necessarily act as a check upon one another. As the results of all these experiments were in complete harmony, I feel justified in making a positive statement that *no experimental proof of a specific placental immune reaction can be demonstrated by our present biological methods.* Whether Veit's hypothesis, thus deprived of its biological proof, must in consequence be discarded, is a question which I do not consider myself competent to answer.

In conclusion I desire to thank Dr. Simon Flexner, Director of the Rockefeller Institute for Medical Research, for extending to me the privileges of his laboratories. I also wish to acknowledge my indebtedness to Drs. Jobling, Noguchi and Levene for the frequent advice and assistance I have received from them.

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