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Maternal inhibition of physiologic trophoblastic metastasis

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MATERNAL INHIBITION OF PHYSIOLOGIC TROPHOBLASTIC METASTASIS

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INTRODUCTION

Because of the major problem that neoplasms present in medicine today, it would perhaps be interesting and possibly enlightening to review the literature on trophoblastic growth and what is known of its control. As far as is known today they are the only normal cells which invade and destroy other normal tissue. Trophoblasts are also unique in that they run the gamut from physiological in-vasion, to benign hypertrophy to malignant invasion with the destruction of life itself. This normal gradation of tissue activity may provide new avenues of approach to the over all problem of neoplastic growth.

HISTORY AND FREQUENCY

G. Schmorl in 1893 contended that fragments of trophoblasts are tranported by blood stream frequently, if not physiologically, from the placental site to the lungs, He found structures closely re-sembling trophoblast in the lungs of 17 females who had died of eclampsia. Later he stated he had examined the lungs and only the lungs of 158 females who had died at various stages of pregnancy and the pueperum, embolic displacement of placental fregments occurred frequently in 83 eclamptic females while such displacement was not present, or present to less degree in female dying of other causes. He concluded that a mechanical factor, i.e., parturition, uterine contractions or manual removal of the placenta was not necessary for the dislodgement of cells, since they could be found in the first few months of pregnancy. He emphasized he had found evidence of active cell growth in only 3. Kossjanow found emboli of syncytium like cells in the lungs of 11 pregnant females who had not had eclampsia and Schmorl's obser-vation were also confirmed. Vict examined the site of a tubal preg-nancy in which the fetus was alive at removal. He found the villi could be followed into the maternal veins for a distance of 1 cm. Poten considered that the displacement of torn off villi and their epithelium into the maternal circulation occurred frequently and probably in every pregnancy. He frequently found syncytial fragments and sometimes whole villi in the myometrial veins. He regarded these appearances as the cross section at death of a continuous showering of such fragments from the placental site to the lungs. (68)

In 1936, S. Warrens (77) found that in order to get 100% takes of Walker Carcinoma 256 in Sloniker strain of white rats, it was necessary to inject a total number of about 11,600,000 neoplastic cells intravenously. The Sloniker rat is considered readily susceptible to the Walker Carcinoma 256. If we can tranfer these results only in part to humans the number of embolic trophoblastic cells bombarding the maternal host must indeed be huge.

Since nothing else could be found in the literature about trophoblastic metastasis in pregnancy, it was thought that a review of the normal Histoanatomy and Histochemistry at the maternal-fetal junction would be the next step.

EMBRYOLOGY OF TROPHOBLASTS

Trophoblasts are first identifiable lining the blastocyst. At this time the egg is about four days old.

Early implanted ova 8 to 8½ days old are characterized by superficial implantation with about one-half to one-third of the unaltered blastocyst above the endometrium. The maternal epithelium has been digested; the solid trophoblastic plate is in intimate contact with the edematous, non-deciduous endometrial stroma. The latter is beginning to show moderate vascular responses but no congestion of its arterioles, capillaries or venules.

The solid trophoblastic plate is composed of primitive admixed, syncytio and cytotrophoblasts. The latter is beginning to delaminate into the primitive amnion over the dorsum of the embryo and into mesoblastic tissue lining the chorionic cavity. The embryo is a

primitive bilaminar mass of cells consisting of ectoderm and entoderm.

By 9 days the specimen has sunk into the endometrium and is nearly flush with the surface. It is still small with little congestion of the enlarging endometrial capillaries at the implantation site. The trophoblastic plate has now become more like a thick shell but it is still essentially solid. The syncytiotrophoblastic elements are more prominent; are extending into the maternal stroma and partially surrounding the enlarging endometrial sinusoids. There is no essential change in the chorionic cavity or embryonic disc.

By 10 days the specimens average about .4 to .6 mm. The endometrium is now congested. In general it is wrinkled and being in the 24-25the day of the cycle, begins to resemble early decidua grossly.

The trophoblastic shell completely covers the ovum at this time. The trophoblast at the embryonic pole (implantation pole), however, is nore fully developed than that at the abembryonic pole. This is a function of the length of time each area has been in contact with the endometrial stroma.

The trophoblast has changed in two significant ways. The syncytiotrophoblast has tended to assume a periphral location whereas the cytotrophoblasts tend to lie just around the chorionic cavity; and the syncytiotrophoblast has formed a series of cytoplasmic lacunae which soon coalesce to form an irregular sponge-like shell surrounding the ovum. The growing invading syncytiotrophoblast, meanwhile, is surrounding and eroding the enlarging vascular sinusoids, thereby allowing the maternal blood to seep into the lacunar spaces. This is in essence the beginning of the utero-placental circulation al-

though the blood still small in amount is generally stagnant. It is their largely ingested and used as food by the growing trophoblast.

The 11th, 12th, and 13th days, ovum's trophoblast are characterized by the following: The size of the specimen is about .75 to 1.7 mm. The implantation site shows prominent sinusoidal response, congestion, edema, and diapedesis of maternal red blood cells through purpose-fully imperfect sinusoidal walls. The entire source of the blood supply for the ovum at this stage is a pair of vessels, an arteriole and a venule. The enlarging capillary sinusoidal network is tapped by the growing trophoblastic shell thereby allowing the blood to flow in increasing amounts into the lacunar space. This by definition will be intervillous space as soon as the chorionic villi are formed.

The chorion is now composed of a well defined outer syncytiotrophoblastic cortex (containing maternal blood) and an inner cytotrophoblastic layer lining the chorionic cavity. Primordial villi are now forming rapidly from the cytotrophoblast and are pushing outward through the syncytiotrophoblastic cortex. These primordial villi, using the solid portion of this lacunar-riddled shelf as sort of a guide, become invested by the syncytiotrophoblast. The latter thus becomes by definition, the syncytium of the villus. As the primordial villi extend peripherally and coalesce to form the placental floor or plate the excess syncytiotrophoblast is "desquamated" and remains in the placental site as nest of multinucleated giant cells.

The stage is thus set for a relatively active influx of maternal blood into the intervillous spaces as the villi are forming. This coincides in time with the missed menstrual period. Should the

influx of blood be too great for the imperfectly formed placental floor to contain, the blood seeps out beneath the edges of the "Schluss-coaglium" at the abembryonic pole and thereby escapes into the uterine cavity.

Early villous formation occurs from the 14th day of ovular life and gradually merges into the stage of placental formation. The specimens vary from 2.5 to 3.75 mm. in their greatest diameter. It is characterized by a rapidly metamorphosing endometrium resulting in phosing endametrium resulting in

decidual formation. The still unhealed implant site often bleeds, though slightly into the endometrial cavity. Bleeding through the floor of the placenta into the underlying placental site is also a usual finding at this time. The entire surface of the ovum is, at the beginning of the phase covered by simple unbranced chorionic villi. These consist mainly of solid cytotrophoblastic core covered by syncytiotrophoblasts which conversely lines the intervillous space. The periphral tips of the villi coalesce to form the cytotrophoblastic placental floor or plate, the latter perforated by capillary sinusoids which supply the intervillous space with blood. As the villi assume there definitive form mesoblastic and angioblastic cores form within their bases by in situ delamination of cytotrophoblast. The latter as the villi develop become by definition, Langhans epithelium, and lies beneath the syncytium. Even in the maturing placenta the tips of the anchoring primordial villi possess undifferentiated cytotrophoblast. These masses become known as the cell columns and are the means together with the cytotrophoblastic floor of the placenta by which this organ remains anchored to the endometrium of the placental site. (Hertig & Rock 79)
the endometrium of the placental site. {Hartig & Rock 79)

HISTOCHEMISTRY OF TROPHOBLASTS

Production of Chorionic Gonadotrophin:

Gey, Jones and Hellman, (1938) and Jones, Gey and Gey, (1943) have demonstrated that tissue cultures containing actively growing trophoblasts produce appreciable quantities of chorionic gonadotrophin even after repeated transplantations over a period of several months. Furthermore, their observation as previous ones by Friedham, (1929) and Singrepta, (1935) indicate that the cytotrophoblasts rather than the syncytiotrophoblasts are the source of the hormone.

Teuney and Parker, (1939) remarked that the amount of this hormone excreted in hydatiform mole or chorioepithelioma corresponds roughly to the amount of trophoblastic cells and that a mole with cystic villi and slight trophoblastic proliferation gives a low titer. Their findings suggest that actively proliferating cytotrophoblasts are more important in the production of chorionic gonadotrophin hormone than the syncytiotrophoblasts derived from it. Furthermore, Wislocki and Bennett, (1943) have emphasized that the curve of excretion of gonadotrophin hormone in the pregnant female parallels approximately the rise and decline of the cytrophoblast rather than simulating the slow, steady increase in the amount of syncytiotrophoblasts.

Production of Placental Steroid Hormones:

No single method is available for identifying such compounds in tissue section but by use of a combination of reactions, the sum of which characterizes ketosteroids but no other known compound; they can be identified with some assurance. Bennett.

Frozen Section of Formalin Fixed Material are Prepared:

1. Since all Ketosteroids are soluble in acetone at room temperature, all of the subsequent methods will be negative following extraction of the sections in this solvent.

2. The steroids are sudoanophilic (as with other lipids) and hence will be stained with sudan dyes.

3. Because of their ketone groups, the steroid hormones (but not cholesterol) will react with phenylhydrazine to produce yellow hydrazines and will recolor leucofuchsin (Schiff's Reagent) to produce a purple color.

4. The unsaturated steroids exhibit autoflourescence when illuminated by ultra violet light. This characteristically of this class of compound is greenish or yellow. Consequently if an unstained section is viewed with ultra violet light, the steroid droplets will appear yellow green on a bluish background.

5. Because of the ionization of their terminal radicals, steroids orient themselves as birefringent spherocrystals within the lipid droplets of the cells that secrete or store them.

Birefringent sudanophilic lipid droplets are abundantly present in the trophoblastic syncytium throughout gestation. They are acetone soluble, react with phenylhydrazine, give a positive Schiff's reaction and exhibit yellowish green autoflourescence. (Dempsey & Wislocki) The cytoplasm including the Laughans cells is devoid of lipid droplets, while the sudanophilic droplets in the decidual cells and in the epithelium of the uterine glands are neither birefringent nor floures-cent. From this the conclusion is drawn that the syncytium is very

probably the site of origin of placental steroids.

The Occurrence of Cytoplasmic Nucleoprotein in the Trophoblast:

Two types of nucleoprotein are present in the body cells, either ribose or desoxyribose. Nucleoprotein being acids in character have an affinity for basic dyes and hence are basophilic. Desoxyribose nucleoprotein is present exclusively in the chromatin of nuclei whereas ribose nucleoprotein occurs in nucleoli as well as frequently in the cytoplasm of cells. This form can be identified histochemically by the Feulgen reaction and the latter by its solubility in ribonuclease. These two reactions serve also to distinguish nucleoprotein from other basophilic constituents of cells for example acid mucopolysaccharides. These substances can also be distinguished from one another by means of a photometric method based upon the relative affinities of basophilic constituents of cells for methylene blue. (Dempsey & Singer '46)

The cytotrophoblast and syncytiotrophoblast are very rich in cytoplasmic basophilia attributable to the presence of ribose nucleoprotein. The cells of cytotrophoblastic columns and cell islands contain conspicuous dark clumps of basophilic material in their cytoplasm, and these are readily digested by ribonuclease. The cytoplasm of the syncytium is characterized by a zone of deeply staining basophilic material surrounding the nuclei and this is also removed by exposure to ribonuclease. The basophilic zone characterizing the syncytium stains deeply in the early months of gestation but staining diminishes slowly as the placenta ages. It is noteworthy that as the basophilia of the syncytium decre ses with advancing gestation both acid and alkaline phosphatase increase greatly. In contrast to the

other elements of the trophoblast, the Langhans cell of the chorionic villi possesses relatively little cytoplasmic basophilia.

Nucleoprotein is generally concentrated in cells in which the synthesis of protein is actively taking place, where they are thought to act as enzymes in the formation of other proteins. (Dempsey & Wislocki 1946) In the placenta cytoplasmic ribose nucleoprotein is especially plentiful in the cytotrophoblast of the trophoblastic shell and cell columns and in the trophoblastic syncytium. Wislocki interprets its presence in these regions as being indicative of active protein synthesis. In the case of the cytotrophoblastic shell and cell columns it might conceivable be related to the synthesis there of chorionic gonadotrophin hormone. In the syncytium on the other hand where it is present particularly in the first months of gestation it might well represent the primary site of synthesis of the proteins of the fetal blood plasma, a function presumably taken over by hepatic cells when the fetal liver becomes sufficiently differentiated.

Glycogen in the Trophoblast:

Glycogen occurs widely in the placenta. It is especially abundant in the cytotrophoblast of the cell columns, and islands of the trophoblastic shell. Little occurs normally in the Langhans cell or syncytium clothing the chorionic villi. Some is present in the stroma of chorionic villi. It occurs in large quantities in the glandular epithelium and decidual cells of the endometrium. Dempsey and Wislocki have expressed the opinion that it occurs especially in the regions of the placenta that are relatively avascular and therefore poorly supplied with oxygen and that under anaerobic conditions it

may provide a readily available source of energy. In cytotrophoblast of hydatiform moles unusually large amounts of glycogen are encountered in keeping with the relatively avascularity apparent in these pathological formations. Similarly in two placentae of the 3rd month removed therapeutically because of maternal cardiac disorders, the impression was gained that the syncytium and Langhans cells covering the villi contained increased amounts of glycogen.

The Occurrence of Mitochondria and Golgi Apparatus in Trophoblast:

Mitochondria are extremely abundant in syncytiotrophoblast in the early part of gestation and diminish as the placenta ages. In the Langhans cells and cytotrophoblast in general there are fewer of them. Recent investigation indicate that in part these bodies are phospholipids. The Golgi Apparatus is demonstrable in both the cytotrophoblast and the syncytiotrophoblast; in the latter it occurs in the form of threads extending rather diffusely through the cytoplasm in the nuclear zone. These cell structures are also partially phospholipid.

The Occurrence of Indophenol Oxidase and Reduction:

Oxidation Reaction in the Trophoblast.

The Indophenol Oxidase reaction was carried out with the nadi reagent on fresh teased villi and unfixed sections of the placenta of the sixth week of a pregnancy. By this procedure indophenol blue was produced in trophoblastic syncytium, a reaction which is believed to reveal the presence of cytochrome-oxidase, cytochrome C system. This system because of its almost universal occurrence, and because of the marked decline in metabolism after cytochrome inhibition, is

thought to provide the principle energy transfer in animal tissues. The stroma of the villi in contrast to the syncytium did not stain with indophenol. Similarly a series of reduction oxidation indicators were applied to fresh teased villi and fresh frozen sections. The Syncytiotrophoblast concentrated all of their dyes in the oxidized form, whereas the stroma of the villi react far less intensly. These results indicate that the syncytial layer is maintained in air at a more positive reduction oxidation potential than are the deeper part of the villi and are in keeping with similar distribution of indophenol. (Wislocki: 61)

HISTOLOGY OF THE INVADED UTERUS

In pregnancy the human endometrium contains enlarged actively secreting glands and characteristic decidual cells. The uterine glandular epithelium and the decidual cells are laden with glycogen and lipids. Acid phosphatase is abundantly present in the glandular epithelium and the decidual cells. While alkaline phosphatase is especially plentiful in the glands as well as in the endothelium of the edometrial blood vessels, the decidual cells are surrounded by an argyrophil reticulum and a ground substance which is intensely metachromatic. This metachromasia is attributable to the accumulation of acid mucopolysaccharides in the stroma with the onset of gestation. Metachromatic mucoprotein is also demonstrable in the lumen of the uterine glands. The histological appearance at the margin of the growing trophoblastic shell of the human placenta suggest that the elements of the decidua are attached and slowly destroyed by the

action of the cytotrophoblast. (Wislocki & Bennett '43) In sections which have been impregnated with silver, the dissolution of the reticulum fibers can be observed. Immediately adjacent to the border of the trophoblast, the fibers become broken up and the individual bits dissolve apparently in the outermost part of the matrix of the trophoblastic shell. (Wislocki and Dempsey '46) Similar fragments of collogen fibers in the vicinity of the trophoblast has been noted in the pregnancy of rodents.

Apparently the metachromatic acid mucopolysaccharide forming the ground substance between the decidual cells is also destroyed. Like the reticulum it ceases quite abruptly at the margin of the trophoblastic shell, no metachromasia whatsoever being noticable in the matrix of the shell. The multiplicity of the substances dissolved by the growing cytotrophoblast suggests the release of powerful proteolytic enzymes.

It is interesting to speculate on the role of the mucopolysaccharide present in the decidua. The metachromatic ground substance extends with undiminished intensity of staining right up to the border of the trophoblastic shell. This observation indicates that the trophoblast does not release an enzymatic "spreading factor" because if it did one would expect the decidual ground substance to be hydrolyzed with consequent diminution of its metachromasia in the immediate vicinity of the trophoblastic shell. This acid mucopolysaccharide may, therefore represent a viscous barrier capable of retarding the activity and progress of the trophoblast. The ground substance of the human decidua is still intensely metachromatic in the second half of

pregnancy at a time when, according to Caffier (1929), the trophoblast has completely lost its proteolytic activity. For this and other reasons, it appears as though the changes occurring in the fetal-maternal junctional zone of the placenta during the course of gestation are probably the outcome of both mutually supportive and mutually antagonistic forces resident in the trophoblast and in the dicidual barrier. (Wislocki: 61)

Dias (37) pointed out that early placentae show cells situated in the depth of the maternal tissue close to the covering of decidual cells and in proximity to the large vessels. In the subchorial layer, at the junction of uterine and placental tissues, these are chorionic hypertrophic giant cells with enormous nuclei, irregular contours, and dentate membrane against which the chromatin is condensed in large nodules. They have phagocytic power and do not show mitosis; except for the last mentioned characteristic, they resemble neoplastic cells.

GROWTH OF TROPHOBLAST IN TISSUE CULTURE

Maximow (52) in 1924 was experimenting with rabbit embryo's in tissue culture when he observed the following. Even though the embryo may be excised out of the wall of the blastodermic vesicle very carefully, there still remains, as a rule, small particles of ectoplacental trophoblastic areas attached to the explant. These areas remain latent for a while and the embryo proper has usually time to attain a certain degree of development. Usually after about two to four days the trophoblastic elements became active and change completely

the conditions of the growing explant.

The cells of the deeper layer of the trophoblast ectoderm (Langhans) begin to increase in size, to hypertrophy and to proliferate. They are of unequal size and sometimes form distinct intercellular bridges, and contain large irregular nuclei with one or several nucleoli. A very peculiar attribute of these elements, in some cases at least, is the presence of a varying, sometimes very considerable, quanity of needle-like crystals in the protoplasm. Mitoses are numerous and large multipolar figures are quite common. Some of these cells hypertrophy excessively; their nuclei increase enormously in size and their distinctly basophilic protoplasm accumulates, besides the crystals numerous fat droplets occur. Such elements are easily noticed in the living culture in its peripheral parts and show a very active ameboid movement. In many places cells of this type fuse together and form still larger giant cells with nuclei of very different, occasionally enormous, size. As the membranes of such nuclei often show deep, straight inward folds, it is quite possible that the number of nuclei in such trophoblastic giant cells may increase partly through amitosis.

In the normal development (Maximow, 1900) the trophoblastic giant cells penetrate into the mucous membrane in the region of the placenta, usually each contain one or two nuclei of enormous size, whereas in the ectoplacental area a regular continous trophoblastic syncytium without cell limits at all and with innumerable nuclei is formed. This destroys the uterine epithelium, penetrates into the maternal tissue and forms the ectoplacenta. In the explants the trophoblastic elements are still more polymorphous and show a more or

less typical structure. As the number of the largest cells with one or several enormous nuclei gradually increase, their fusion and the amitotic fragmentation of their nuclei goes on to a certain extent, but, instead of a continuous plasmodium or syncytium, large number of separate huge multinucleated giant cells are formed. Their form is very variable, often fantastic, round oval spindle-shaped with rounded ends or enormously elongated with flat, thin, shovel-like, sometimes branched enlargements are at the ends. Their contour always shows large lobulated bulgings, which are everywhere beset with tranparent, motile coral shape or fingershaped secondary pseudopodia. In vivo they show a beautiful glass-like, absolutely homogenous ectoplasm, forming a thick layer on the periphery of the cell body and sending out the pseudopodia just described. The endoplasm contains numerous granules and brilliant fat droplets, whereas the crystals are not clearly seen. The nuclei in the granular endoplasm appear as clear oval or round spots with distinct nucleoli. The ameboid movements are very active. The ectoplasmic pseudopodia are constantly changing their outline; the cell bodies as a whole also rapidly change their form and move in different directions like huge amoebae.

After fixation and staining, the large irregular nuclei, with their hypertrophied dark nucleoli are very conspicuous. The endoplasm surrounding the nuclei now shows besides the fat vacuoles, numerous granular inclusions, originating principally from engulfed red blood cells, and in many cases at least the crystals already mentioned. The ectoplasm and pseudopodia are very transparent and

clear and show long, straight or slightly curved, parallel or diverging fibrils, such as often found in different types of homogenous protoplasm after fixation.

The described giant trophoblastic elements show a distinct tendency to unite and to form, through close adhesion to one another, large thick sheets, in which however, the outlines of each individual cell are still well preserved. In many places on the surface of the cover slip they form net-like syncytial framework with large irregular meshes and long, thick strands of protoplasm. They may cover large areas 6 to 8 mm. in diameter. In this condition the trophoblast has a striking resemblance to mxomycete plasmodium. The arrangement of these sheets or nets is constantly changing in life and often I have seen large thick protoplasmic trabeculae suddenly rupture and both ends rapidly contract and draw away.

The trophoblastic protoplasm liquefies the fibrin and doubtless secretes enzymes, dissolving and destroying all other tissue and substance in its neighborhood. The areas covered with the described trophoblast are always easily discernible with the naked eye; they show a delicate white network with large empty transparent meshes. From the very first moment of their formation in vitro the trophoblastic element whose function under normal conditions is to destroy, resorb, and penetrate into the uterine mucous membrane, attach the growing embryonic tissue. At first single large, uninucleated or multinucleated trophoblastic elements push their way into the embryonic tissues. They glide between the cells through

the intercellular spaces along blood vessels, gnaw large holes in epithelial sheets, and gather in crowds at the surface of various developing structures for instance the medullary tube. Where ever they appear they dissolve, destroy and resorb everything surrounding them; sometimes engulfing small particles. Gradually the number of invading trophoblastic giant cells increases, and the embryonic tissue degenerates and is quickly destroyed, liguefied and resorbed. The picture sometimes shows a striking resemblance to chorioepithelioma. After six days of life in vitro only a vast trophoblastic syncytium may remain of the whole culture.

These tissue cultures show that the trophoblasts can develop independently in complete absence of maternal tissue. This development is determined by inherent qualities of its cells, apparently without being influenced by external factors. Cooperation of the maternal tissue is evidently not needed for its production, but only for its subsequent nutrition and implantation of the embryo. As there is no maternal tissue the destructive tendencies of the trophoblasts are towards the next and only avialable tissue. This is rapidly destroyed and used up for the nutrition and growth of the trophoblasts. Similiar occurrence have been observed by Leo Loeb (1923) in guinea pig ovary where embryo like structure were destroyed by overgrowth of their trophoblastic elements.

TROPHOBLASTIC TRANSPLANTS

In 1943, Jones, Gey, and Gey (60) presented the first direct evidence for the formation of gonadotrophin by the Langhans cells.

Growth of Langhan cells from early placental tissue in vitro confirmed these findings. These studies failed to establish the placenta as the origin of estrogen because gonadotrophin secretion is more abundant early in pregnancy while estrogen and progesterone curves of secretion reach a maximum peak late. Hence ability of mature placenta to grow would be desirable in vitro but was unobtainable. However, explants of term placenta will grow in rabbit eyes. From the author's experiments synctial cells were grown from term placenta and evidence of estrogen production observed.

Indirect evidence that the placenta is an organ of internal secretion during pregnancy is:

1. Extracts of human placenta implanted into infantile mice and rats produce positive gonadotrophin responses. When implanted into castrated mice and rats estrogenic response resulted.

2. Although the pituitary is enlarged during pregnancy, implantation of pituitary into infantile mice failed to show any increase in gonadotrophin potency as would be expected if it were the source of the large amounts present in pregnancy.

E.F. Hirsch (66) was unable to grow adult placenta of 8 and 9 month duration in tissue culture. There was a overgrowth of fibroblastic tissue, but a complete absence of Langhans and syncytial elements. Endocrine assays were negative for chorionic gonadotrophins and estrogens. Hirsch then concluded that a direct relationship existed between the growth of Langhans cells and chorionic gonadotrophin concentration. The Langhans cells are probably responsible for gonadotrophic during pregnancy. It is reasonable

to believe that the relative lower level of the blood and urinary gonadotrophin in later pregnancy falls in direct correlation with the fewer number of Langhans cells.

Most authorities agree that chorionic gonadotrophin is produced by the cytotrophoblast. However, Bonilia (11) after studying chorionic gonadotrophin levels in hydatiform moles and their histology doubted this. In most of his cases few cytotrophoblastic cells were observed where as the syncytium was well developed. However, the histological level was not always correlatable with the hormonal level since identical histological pictures frequently had different chorionic gonadotrophin levels. Cases with high chorionic gonadotrophin levels showed histochemical immaturity of the syncytium with predominance of protoplasmic basophilia. Curves of estrogen, however, show a gradual rise from the third month to the highest peak just prior to parturition.

E. Howarka (39) observed the tranformation of placental fragments in hanging drop preparations. 826 Fragments were observed from two to three month old placentae. The fibrinolytic activity and the liquifying of serum clot in the proximity of the villi were seen to occur.

Clifford Grobstein (49) noted that introduction into mouse eye, of tubal eggs, whole blastocyst, or isolated ectoplacental trophoblasts of the mouse will produce a vigorous hemorrhagic reaction in mature or immature mice of either sex. The occurrence of this reaction in immature or adult animals indicates that it does not need the same hormonal requirements as occur with endometrial implanta-

tion. To delineate the hypophyseal part in implantation, the ectoplacental regions of 8 day CXC3H mice embryos were implanted into the right eye of 18 hypophysectomized and 10 normal CXC3H males. Hypophysectomy was preformed at eight weeks of age and implantations were made 6, 21, and 35 days later into groups of 8, 5, and 5 hypophysectomized and 5, 3, and 2 control animals respectively. The reaction of all groups was indistinguishable. In all groups intraocular hemorrhage appeared by 48 hours. As the reaction continued; the whole eye became dark red and protuberant, frequently rupturing at the incision site. In animals held longer than 7-10 days the reaction appreared to regress. From this work Grobstein concluded that the intraocular hemorrhage due to trophoblasts in the mouse eye is independent not only of the particular hormonal condition that occur in females at the time of implantation, but since it occurs in full strength in animals 35 days after hypophysectomy; is independent of all hormonal background from the hypophysis.

C. Gurchot (54), believes from his studies of trophoblast explants in rabbit eyes that chorioepitheliomas are a simple overgrowth of normal trophoblasts. Since in the trophoblastic portion of cultured rabbit conceptus practically all cell types known to be malignant were present. One of the criterion for malignancy is the growth of heterologous tumors in the anterior chamber of the eye. Term placenta fulfills this since they grow readily in female or male rabbit eyes and are serially transplantable. These transplants produce chorionic gonadotrophin as demonstrated by the ovary of the

Friedman doe. Steriods are also produced as demonstrated by inhibition of spermatogenesis on the host.

D. W. Fawcett; et al. (50) also worked with ova transplants to eye and abdomen in mice. They showed that:

1. Ova in the eye are retarded in implantation by about 48 hours.

2. In multiple ova transplants only one grew. (Ova are capable of developing in close proximity only until the most precocious among them begins to implant then it sets up a sphere of influence around it which results in degeneration of all ova in the vicinity.

3. Marked proteolytic activity of cytotrophoblasts growing in tissue cultures prepared with bits of chorionic villi obtained from human placenta of the first month of pregnancy were observed.

4. The inner cell mass inplanting in the eye is not wholly surpressed, but it is invariably greatly retarded and abnormal in its development.

5. In early stages of gestation in primates, hemorrhage in the decidua coincides with the erosion of the uterine mucosa by the advancing trophoblast. It has generally been assumed that the extravasated blood originates from maternal vessels whose walls are actually invaded by the trophoblast. There is reason to believe that a chemical substance is elaborated by the trophoblast which can initiate such changes in decidua even though the two are not yet in physical contact. In a previllous human ovum of 11 days ovulation age an area of congestion and hemorrhage was found on

the opposite endometrial surface which had merely been in close proximity to the implantation site. (Hertig and Rock 1941) A similar process occurs in the posterior chamber with implanation in the anterior chamber of the mouse eye. Apparently interstitial hemorrhage and edema in the early hours of nidation are the result of diffuse damage to vessels by cytolytic enzymes emanating from the trophoblast.

6. The uterus apparently helps establish placental circulation, this not occurring in the eye causes early death of mouse embryos.

7. Formation of maternal placenta can be induced by mechanical or electrical stimulation of uterus in pregnancy, pseudo-pregnancy, and lactation. These deciduoma exactly duplicate the deciduoma of pregnancy in vascular pattern and histology. Bleeding may also occur from sub-epithelium vessels. Prehaps extravasation of blood is a normal feature of early maternal placental development and doesn't depend on trophoblast in mice.

MECHANISM OF TROPHOBLASTIC INVASION

In the first month of pregnancy the cytotrophoblastic shell appears to produce proteolytic and cytolytic substances capable of attaching the endometrium. In early stages of gestation in primates, hemorrhage in the decidua coincides with the erosion of the uterine mucosa by the advancing trophoblasts. It has generally been assumed that the extravasted blood originates from maternal vessels whose walls are actually invaded by the trophoblast. On the other hand there is reason to believe that a chemicle substance

is elaborated by the trophoblast which can initiate such changes in decidual tissue which is not yet in contact with the egg. In the macague, evidence of such a chemical factor is found in the fact that the epithelium at the secondary implantation site begins to proliferate before erosion of the uterine surface has taken place.

In a previllous human ovum of 11 days ovulation age an area of congestion and hemorrhage was found on the opposite endometrial surface which had merely been in close proximity to the implantation site. (Hertig & Rock '41)

Similar proteolytic activity has been demonstrated experimentally in the presence of fertilized mouse ova transplanted to various extrauterine sites including the anterior chamber of the eye. (Fawcett; et al 64) In the eye of a mouse containing a proliferating ovum, leakage of blood from enlarged vessels in the iris and cornea appear to take place before the blastocyst has actually become attached to the wall of the anterior chamber. In as much as these vessels are not in direct contact with the blastocyst and hence are not apt to have been disrupted by actual invasion, the most satisfactory way to account for bleeding in the posterior chamber is to attribute it to a chemical product of the trophoblast.

Although the trophoblast does invade maternal vessels in later stages of implantation, direct observation and study of histological section of ova transplanted to the eye suggest that interstial hemorrhage and edema in the early hours of nidation are the result of diffuse damage to vessels by cytolytic enzymes emanating from the

trophoblast. Further evidence of a cytolytic factor is demonstrated by the observation that ova placed in the eye are capable of developing in close proximity only until the most precocious one among them begins to implant. Thereafter the others quickly degenerate. Finally bits of chorionic villi obtained from human placenta of the first months of pregnancy and grown on plasma clots, liquefy the medium. (Grafenberg '09) (Wislock 61)

Blandou (53) in 1947, was able to demonstrate the role of the decidua in the process of implantation. Glass and paraffin beads, ova size were introduced into the uterus of guinea pig and rat uteri luminae between the 2nd and 6th day after the onset of behavioriial estrus. They were killed on the 8th to 12th day. In rats decidual reaction and implantation occur with beads at the same rate as with ova. In guinea pig, decidual reaction was minimal. This seems to suggest that no chemical factor is needed for rat ova to implant.

CHORIONIC GONADOTROPHIN RELATIONSHIP TO TROPHOBLASTIC PROLIFERATION

The part chorionic gonadotrophin plays in implantation other than lutenization can only be guessed, but biologic confirmation of pregnancy occurs with greatest frequency five days prior to first expected menstrual period after fertilization. By serial assay of chorionic gonadotrophin a plateau of values of 1000 to 5000 I.U./24 hrs. specimen of urine and 10 to 50 I.U./cc of blood serum was established between day 24 and day 40 after the first day of the last normal menstrual period. Between day 40 and day 90, an elevation

of values for excretion is noted with the peaks excretion accurring at day 70 in both the urine and blood serum. After day 90 values for chorionic gonadotrophin in the urine range between 2,000 and 15,000 I.U./24 hr. specimen and between 10 and 100 I.U./cc of serum.

The fact that chorionic gonadotrophin indirectly stimulates the corpus luteum causing decidual reaction is pretty well substantiated by the following observations and experiments:

Seagaloff (56) gave six post ovulatory, non pregnant females daily doses of 10,000 I.U. of chorionic gonadotrophin. Delay in menstrual onset occurred in all until chorionic gonadotrophin was stopped and was accompanied by decidual changes in all. In the four who came to laparotomy large corpus lutea of pregnancy were found. In none of the patients did antihormones develop. There was no proof in any of the six that chorionic gonadotrophin was anything but lutenizing.

The authors were able to maintain six day pregnant hypophysectomized rats by injecting 12 day placentae. Pregnancy was not maintained if they were in addition oophorectomized. Averill (76) Nulliparous rats were castrated on the 6th day and pregnancy was maintained with injected placentae. The daily injection of 5 to 8 placentae maintained the pregnancy in 8 out of 10 hypophysectomized rats. But only one placenta was necessary if at the same time .5 micrograms of estrogen were given. Pregnancy was not maintained on the same regime if they were also oophorized. (Averill, S.C. 19) Apparently the chorionic gonadotrophin is produced solely for the

maternal host with the placenta acting as a semipermeable membrane since Bedoya (10) demonstrated that in fifty full term infants 2.5 to 3 ml. of cord blood failed to elicited a positive pregnancy reaction in males of Rana esculenta where as .5 ml. of maternal serum would.

J.A. Bruner (74) reports that chorionic gonadotrophin is found in two widely separated taxonomic groups the equidae and the primates. In the mare production is limited to the first one half of pregnancy and is highest between the 45th and 80th day. The rhesus monkey gives positive reaction to chorionic gonadotrophin during only one week; between the 18th and the 25th day of gestation. In the chimpanzee chorionic gonadotrophin becomes positive about the 25th day and peaks on the 42nd day and dissappears between the 120th and 130th day of gestation.

In view of the fact that in the majority of mammalian species pregnancy takes a normal course without similar hormone production one must admit that its significance in man and horse is questionable. It is well known that removal of the corpus luteum early in pregnancy is incompatible with implantation and maintenance of the pregnancy. In man removal of the corpus luteum before the 2nd month of pregnancy results in abortion. Hisa found that in monkey chorionic gonadotrophin prolongs the functional phase of the corpus luteum for as long as 15 days. Brown and Bradburg reported injections of chorionic gonadotrophin prolong the activity of the corpus luteum in man for at least 10 days. These results indicate that chorionic gonadotrophin hormone of man may be

instrumental in maintaining and prolonging the active life of the corpus luteum. Therefore it is an interesting fact that the species which produce large amounts of pregnancy hormone, i.e., man and horse have a low content of Lutenizing Hormone in the hypophysis as compared with Follicle Stimulating Hormone. Apparently the chorionic gonadotrophin in man is primarily lutenizing in character. It is produced by the fetus to create favorable enviormental conditions within the maternal host.

The successful growth of trophoblasts in the endometrium depends greatly upon the nutritive state of it at implantation time. This nutritive state is brought about by estrogen and progesterone (from the corpus luteum) acting on the endometrium. After the trophoblasts are implanted chorionic gonadotrophin produced by the Langhans cells maintains the corpus luteum which in turn maintains the secretive state of the endometrium. The authors felt that carbohydrates must constitute the source of nutrition for the invading trophoblasts.

The prospect of ovular survival are good when the endometrium had ample amounts of available food. The trophoblasts grew rapidly after implantation becoming differentiated into cytotrophoblasts and syncytiotrophoblasts. This not only provide for adequate nutrition of the ovum but also insured by chorionic gonadotrophin that there be adequate nutritive factors available to be absorbed.

The authors studied endometrium of 324 patients who gave histories of sterility, abortion or both. Endometrium was studied for carbohydrate metabolism and enzyme production. They found 58%

of there patients did not have normal glycogenesis in the endometrium. With abnormal glycogenesis poor trophoblastic development occurred and chorionic gonadotrophin titers were below normal levels. But some pregnancies even with low chorionic gonadotrophin proceeded to viability if the progesterone secretion was normal or abnormally high.

E.C. Hughes; et al, (69) concluded that the development of the trophoblast depends upon normal germ plasm, adequate secretion from endometrium and enough chorionic gonadotrophin to stimulate the decidua to put forth adequate amounts of food for growth of the embryo.

Apparently, however, chorionic gonadotrophin is not necessary for trophoblastic growth since there are several cases of fatal chorioepithelioma which early gave a negative Friedman test. One case in point is reported by Thompson which terminated fatally and the test for chorionic gonadotrophin was not positive until three months after metastasis had been identified hitologically. Some attempt to explain the negative Friedman by saying chorionic elements are not in contact with maternal tissue, but this is unlikely since metastasis were widespread at this time. (65)

TROPHOBLASTIC GROWTH IN CHORIOEPITHELIOMA AND HYDATIFORM MOLES

In most carcinoma there is a microscopic picture of polymorphism of cells, hyperchromatism, mitotic figures, etc., which gives the impression that the property of the carcinoma lies in the cells themselves. In chorioepithelioma we find a trophoblastic tissue

which looks the same as physilogic trophoblast and apparently is the same in the wide continum of trophoblastic behavier stretching from benign, self extruding moles to fulminating chorioepithelioma. This and occasional, but a significant regression and the constancy of hormonal production combine to give an impression that the behavior of chorioepithelioma is conditioned by a balance of forces in a way that the behavior of other tumors is not.

In fact that in general the course of the disease is limited to six months or so also suggest an acute dysfunction initiated only during the state of pregnancy and lapsing as the changes of pregnancy disappear. The constancy with which the results of the biological test are positive and the frequency of luteal cysts suggest strongly that this hormone production, even if the hormone is only a by product, is a manifestation of the mechanism which results in the proliferation of the trophoblast. In chorioepithelioma the proliferation of the trophoblast is constantly associated with the production of a hormone which is an integral part of the endocrine set up of pregnancy. In the conventioal concept of chorioepithelioma this production of gonadotrophin is a necessary, but apart from the purpose of diagnosis, unimportant by product and the essential nature of chorioepithelioma is that of a neoplasm which grows, metastasizes and kills as do other cancerous neoplasms. This is correct as far as present knowledge goes. One might well suspect, however, that all this production of hormone would have endocrine causes and effects with interrelationships; indeed the peculiarities of the chorioepithelioma apart from its fetal nature also point vaguely

in this direction, namely that there is an endocrine background to the causation and behavior of trophoblastic proliferation which is still to be worked out, although there is plenty of hints that this is so. There is as yet little real evidence. (Venning 62)

A.T. Hertig; et al, (55) has observed that chorionic villi in human placenta develop spontaneously with their intrinsic blood supply. However, if the blood supply fails to develop, fluid accumulates within the villi which secondarily stimulates epithelial growth. That it explains the genesis of hydatiform mole is substantiated by the fact that hydatiform degeneration is present in 66.9% of pathological ova.

The authors can not distinguish chorioepithelioma histologically from other types of benign trophoblastic growth. Trophoblastic growth is maximal in the first two months, but chorioepithelioma are evenly distributed over entire pregnancy. (Park, W.W.; Lee, J.C., 40)

Differences Between Pathological and Physiological Trophoblastic Growth:

1. Destructiveness of trophoblast is what makes implantation possible. "Its contact with the endometrium is the kiss of death."

2. When the trophoblast exhibits destructive properties just as marked as the chorioepithelioma there are none of the histological cell changes which pathologists ordinarily look upon as the structural evidence of these destructive propensities. The trophoblast in the early phase of pregnancy possesses in a quantitatively lesser degree practically all the properties of a chorioepithelioma. It actually destroys tissue just as the latter though this property appears

to be lost as soon as the villous apparatus is established. But the capacity to invade tissue is not lost, being seen throughout pregnancy. This is evidenced not only by trophoblastic outgrowth from the villous surface but also by the invasion of the decidua and uterine musculature by so caled chorionic or trophoblastic wondering cells. The exact nature of these cells, whether they are syncitial or Langhans, is not yet clear. Histologically it would seem possible that both layers may be involved in this invasion. In some cases the infiltrating cells seem to be of intermediate type. This would not be surprising since the syncytium itself is a derivative of the cytotrophoblast. Many believe that the trophoblastic invasion is due to ameboid properties of the cells. The degree of trophoblastic cell invasion is very variable, but it is often very pronounced in the implantation area.

There are two chief points of difference between the normal trophoblastic invasion of the uterine wall and that seen in chorio epithelioma. In the former the cells infiltrate along the tissue space singly, and they exert none of the destructive, lytic effect upon the uterine muscle which the earlier trophoblast or the cell masses of choriocarcinoma inflict upon the maternal structure. Not only do the infiltrating cells stain sharply and clearly but so does the uterine muscle in which they are scattered. By contrast chorioepithelioma invades uterine wall in large columns or masses of trophoblasts which advance into muscle always causing coagulation necrosis and hemorrhage. Decidua may also show normal trophoblastic

invasion. Still another pseudo-malignant attribute of the trophoblast depending upon its destructiveness is its capacity to break through the walls of blood vessels so that trophoblastic cells can be carried to distant organs (lungs) and set up metastasis. These tranplanted cells undergo rapid regression if they ever actually grow in the lung; as indicated by negative pregnancy test soon after delivery. Benign hydatiform trophoblastic tissue may also be transported to the lungs. These likewise appear to regress quickly and completely. Nor do they give clinical or X-ray evidence of their short lived existence. While spontaneous and rapid regression of such tranported trophoblastic tissue must be the rule their are apparently rare exceptions in which it may become neoplastic giving rise to ectopic chorioepithelioma. However, in cases of this sort it is impossible to exclude a primary chorioepithelioma in the uterus since it may have disappeared completely.

Even in cases of unquestioned chorioepithelioma with extensive metastasis to the lung as well as other organs, examination of the pulmonary metastasis on a histological basis often shows sign of regression.

Chorioepithelioma in females is a tumor of one individual invading the tissue of another. A fetal tumor growing in the uterus. The invaded organ is by its nature endowed with the capacity of resisting the encroachment of the trophoblast. This resistance is not ordinarily sufficient to hold in check the heightened invasiveness of malignant trophoblast. A considerable number of cases are reported in which the uterus has completely thrown off the

chorioepithelioma, the genuinely malignant nature of the original uterine growth being attested by the fact that the patient has died from extensive matastasis. (E. Novak 48)

TROPHOBLASTIC RELATIONSHIP TO CANCER IN GENERAL

E.T. Krebbs (51) confirmed the findings of Roffo (1944) that, when injected into white rats, an extract of the blood or urine of cancerous patient, causes enlargement of the uterus and formation of corpora lutea in the female animals. The authors have obtained from cancerous patients of both sexes, by urinary extraction proparation having pronounced estrogenic as well as gonadotrophin properties. Non malignant, non pregnacy controls were negative. Authors conclude that the estrogenic factor termed arising from definitive malignant elements is identical with steroids produced by the syncytial trophoblast of pregnancy. Heretofore, it was impossible to distinguish between anterior pituitary and chorionic prolans. However, by using chromatrographic absorption technique and the African clawed toad (Xenopus laevis) these obstacles were overcome.

With these techniques the authors have obtained egg extrusion in Xenopus laevis with injection of lcc of concentrate of as little as 800cc of urine from non genital carcinoma in the human male. Controls of the same age were negative. Not enough work has been done to conclude that specific steroids and, or cytotrophoblastic prolans are present in all cases of carcinoma, but this is suggested.

It is significant that the most malignant carcinoma are known to yield a readily detectable quantity of gonadotrophin duplicated

only by that produced by the trophoblastic cells, and now tumors of lesser malignancy are found to yield this same gonadotrophin. Finally that the only cell never observed in the benign state in the male or aside from pregnancy in the female is the trophoblast. This would seem further to substantiate the unitarian nature of all types of carcinoma and to suggest trophlblastic elements, (however, masked morphologically) as the constant malignant component.

ENZYMES OF THE PLACENTA

Monamine oxidase in placenta probably functions here in the detoxification of amines as it does in other organs. (Luschinsky 80)

Alkaline Phasphatase in placentae of rats is found in large quantities from the 11th day until term. It is thought to be important during cell proliferation and differentiation when concentrated in nucleous. When concentrated in cytoplasm it is important in transference. It is least active in areas of glycogen storage. (Pritchard 35)

Glutaminase activity of human placenta is comparable to that of human kidney and decreases throughout the intra uterine period. It may take the place of glutaminase in the kidney until term. (Luschinsky 2)

Thirty milligrams of free acetylcholine per 100 grams of incubated placenta tissue, can be formed in 30 to 60 minutes. (Berkovitsj 4)

The growth of buckwheat and oat seeds was accelerated by placental extracts. Demonstrating that some type of growth factor is produced by placentae. (Moskow 24)

MATERNAL INHIBITION

About 1900, Frankel demonstrated a certain lytic substance in the serum of normal women during pregnancy which was capable of destroying placental tissue. He further demonstrated that this substance was not present in women suffering from chorioepithelioma. No other work was found in the literature which would substantiate or refute this claim. (Johnson 57)

The histological examination of the maternal-fetal junction reveals the so called "Layer of Nitabuch" which is fibrinoid and coagulative in nature. Some investigators, Williams, et al (68), and Novak (48) believe that this is a defensive reaction and that it denies nutrition to and physically compresses the trophoblasts. Aquero from Madrid (44) states that the "Layer of Nitabuch" need not be considered a defensive reaction of the mother. He believes the "Layer of Nitabuch" originates from mixed maternal parts of decidua and chorion tissues which have undergone coagulative necrosis as a result of the trophoblastic action. Physiologically only lytic-fermentative and necrotic processes occur here. It is traversed by many prolongations of trophoblasts and these chorionic elements are seen lying along side of well preserved maternal elements.

Since the trophoblast as is commonly accepted by most authorities is a derivative of chorionic epithelium it would therefore have a different genetic background than cells of the maternal host. If this is true, the maternal organism may react to trophoblastic invasion in much the same manner as a recipient reacts to a heterologous graft. Why the graft fails to grow is not known, though it is assumed by some that it is on a antigen-antibody basis. Schuster (43) believes

that chorioepithelioma run a course similar to that of inoculation tumor in which antibodies are formed against the foreign protein of the tumor so that healing of the lesion and immunity to a further innoculation are produced. Gurchot (45) believes that in man trophoblasts or its anlage is separated during very early clevage from the zygote. From then on the trophoblast and its sister cell, the totipotent cell go their separate ways. As Streeter expresses it, one can never become the other. If an embryo is to develop it will come from only the totipotent cell since the trophoblast has no somatic potentialities.

Why is it that in the majority of pregnancies, an invasive trophoblast which has the ability to invade blood vessels, enter the circulation and set up metastasis in the lung has by the second month lost to a great degree most of these properties? Trophoblasts in the 8 day embryo are considered by many to be identical histologically to those seen in fatal chorioepithelioma. Two possibilities could occur:

1. The trophoblasts could have a predetermine growth potential much as embryonic organs have and undergo maturation and spontaneously cease growing. (The high production of placental estrogen, which peaks at about the 38th week, may represent attempts of the placenta to inhibit its own growth.)

2. There may be a dynamic state of equilibrium between the chorionic epithelium and the maternal cells with a hormonal, enzymatic or antibody system of checks and counter checks. Most investigators feel that there must be some type of antitrophoblastic substance in

the blood of the pregnant female. A very powerful antitrophoblastic substance apparently is present in an implanting embryo. As observed by Fawcett (64) three or four embryos in a mouse eye all continue to segment until the most precocious one begans to implant then disintergration occur to all the remaining zygotes in this area. This indeed must be a powerful lytic substance for it is at this time that the trophoblasts are the most invasive. Maximow (52) in describing the preparation of culture media gave explicit instructions never to use the blood of the pregnant doe when trying to get trophoblasts to grow. Also in Maximow's, embryo cultures after a lag of about three or four days the trophoblasts began to invade the young embryo and soon had totally destroyed it. These cells histologically appeared as those of chorioepithelioma. Antitrophoblastic substances in the mother may protect the fetus as well as the mother. Some European writers have advocated transfusion in treatment of chorioepithelium with blood from normal pregnant mothers. Adding fuel to the fire is Park and Lee's (40) analysis of the world literature in which approximately twenty cases of chorioepithelioma's with pulmonary lesions have regressed. There are about four cases which have been proven without a doubt to have been chorioepithelioma which have regressed. All of the metastasis which occur in pregnancy also are in some way held in check by the pregnant female. Metastasis after a hydatiform mole (Savage 46) are also resolved. Some of the cases of resolving chorioepithelioma and hydatiform mole with metastasis to lungs have demonstrated cure by going through a normal pregnancy. Apparently they have now developed a permanent immunity to trophoblastic invasion but

this immunity is such that it allows local invasion to occur.

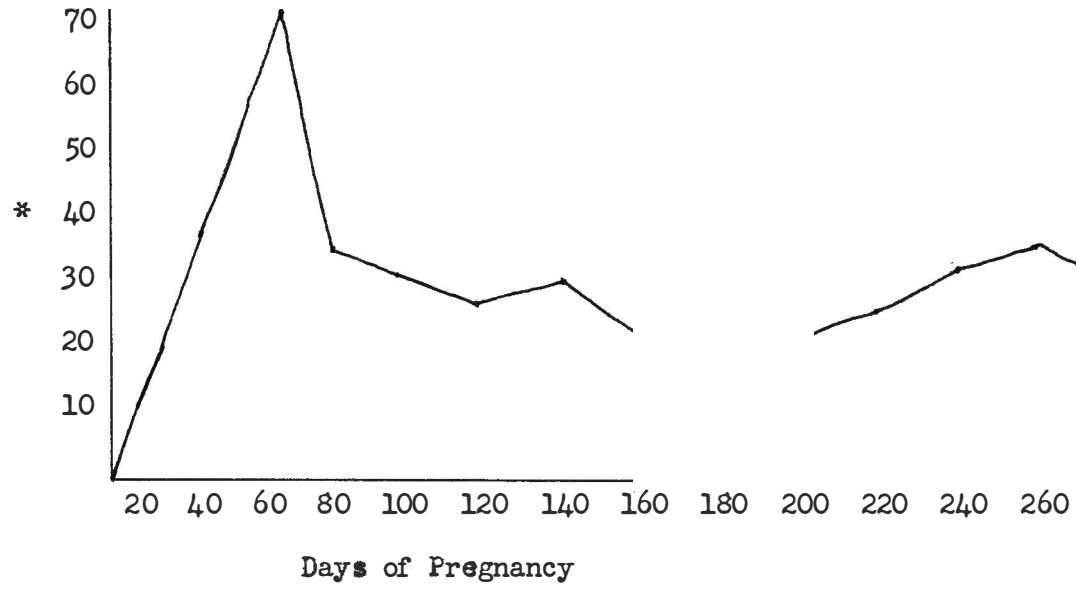
In analyzing pregnancy there are two things that are very striking:

1. The massive hormone production by the placenta of chorionic gonadotrophin, estrogen and progesterone.

2. The generalized decidual reation which occurs in some females.

Chorionic Gonadotrophin:

Its origin and what is know of its function has been previously discussed. Its presence in the urine is unchallengable evidence of proliferating trophoblast somewhere in the body. The ability of the female to excrete or detoxify this hormone may be a measure of a pregnant females ability to cope with trophoblastic invasion. Savage (46) noted in a case of benign hydatiform mole with pulmonary metastasis that a negative biological test was the first indication of regression of lung lesions. (Wilson 72) J.S. Brown and G.E. Jones have studied quite extensively the concentration of the chorionic gonadotrophin in blood and its urinary excretion in normal pregnancy. A rather characteristic picture is present; consisting of a rise in both serum concentration and urinary excretion between the fiftieth and seventieth day of pregnancy and a fall to a lower rather constant level for the rest of pregnancy. The hormone disappears from both urine and blood within a few days after delivery. The authors grafted results from 112 normal pregnant females.



* Chorionic Gonadotrophin I.U./cc of Serum.

The levels in the blood and urine may be the result of many factors. Brown, Henry and Venning in 1936 thought that peak excretion reflected the physiological necessity that the function of corpus luteum in early pregnancy be maintained. Wislocki, Bennett and Dempsey also hold that there is a direct porportionality between the chorionic gonadotrophin excreted in urine and the number of Langhan cells present on histological section. Wilson determined renal clearance of chorionic gonadotrophin in normal pregnant females and found it to remain at .38 cc/min. throughout pregnancy. Since renal excretion can not account for the changing urinary concentration, there are only two other variables; changing production or changing detoxification. One of these must hold the answer. There are no means by which a direct determination of rate of production can be done. However, an indirect estimation of production could be obtained if it were possible to determine how much hormone was disposed

of by all processes other than renal elemination. Such processes would represent the total of endogenous destruction, utilization or excretion by other routes than the kidney. An approach to the problem was accomplished by Johnson, Albert and Wilson. The concentration of the hormone in serum was determined at the time of delivery in 15 normal pregnant females and also in all of the urine excreted after pregnancy. From these values the total amount in the circulating blood stream can be estimated as a product of the concentration of the hormone in serum and the total volume of serum. The total amount of hormone excreted in the urine was determined directly. Results of this study indicated that only 6% of the hormone is excreted in the urine, the remaining 94% must be destroyed, utilized, or disposed of by other means. This is in excellent agreement with results obtained by Zondek and Sulman in 1945 in animals. They found that only 5-10% of injected hormones appeared in the urine. From this, Wilson concluded that probably fluctuating levels of the hormone were due to different rates of production. I am unable to understand from what was presented in the articles how the author ruled out hormone destruction as the cause of the fluctuating hormone levels.

Role of Estrogen and Progesterone:

The role of estrogen and progesterone is well known in preparing the uterus for implantation. Namely that estrogen stimulates the regeneration of endometrium and glands whereas progesterone causes the endometrium to go into the secretory phase. The roles they play in pregnancy has not as yet been clearly defined. Forbes (16) found

that the progesterone level in pregnant humans was; free about 2 micrograms/ml. and bound about .6 micrograms/ml. of plasma. The estrogen level as reported by Smith and Smith reaches a maximum level during the 38th week and then falls.

It is interesting to compare the excretion of chorionic gonadotrophin, estrogen and progesterone as done by E.H. Venning (62). Venning found that chorionic gonadotrophin peaks between the 50th and 70th day after the last menstrual period at levels as high as 40,000 to 200,000 R.U. (this is comparable to chorioepithelioma peaks) The pregnanediol complex (progesterone) is excreted at the same level or slightly higher levels than during the luteal phase. Estrogens are also somewhat increased. Between the 60th and 90th day a definite rise occurs in the excretion of pregnanediol and estrogens. This is associated with increasing activity of the placenta in production of these hormones. In the meanwhile the gonadotrophin hormone is decreasing reaching levels between 5,000 and 10,000 R.U./24 hrs. by the 100th to 120th day. It continues to be excreted at this level up to term although a slight rise is occassionally seen in the last trimester. Estrogen and pregnanediol continue to be excreted in increasing amounts up to parturition. The peak of pregnanediol levels occurred at 220-240 day and was in the order of 50-100 mgm./24 hr. urine volume. Estrogen peaks at 230 days and was in the order of 40,000 to 50,000 micrograms/24 hrs. urine volume. In two of authors cases oophorectomy was done on 54th and 98th day respectively, however, estrogen and progesterone rose in the usual manner. Their origin is probably placental. Corticoids

were also increasing in the first and last trimester of pregnancy. They rose as high as 300 glycogenic units. This is surprising in that it is in the range seen in Cushings desease.

Experimental Work on Progesterone, and Estrogen:

Kehl (27) found that in rabbits after treatment, for four days with dehydrofolliculin (10 micrograms daily) and three days later with pure progesterone in variable amounts and the setting of a thread through the uterus on the fifth day he was unable to obtain any traumatic deciduoma. In other animals the fallopian tube was ligated 24 hours after fecundation, and the thread was placed on the fifth day. Ovariectomy was performed and progesterone treatment followed. No decidual action was provoked before the 8th day which is the normal delay of nidation. This suggests that deciduomata in rabbits requires more than progesterone for formation. Chambon (30) showed that oophorectomized pregnant rabbits with large litters could be maintained from the sixteenth day on with small doses of estrogen or by progesterone alone whereas abortion occurred in smaller litters. This is compatible with earlier work where a oophorectomized rabbit with several fetuses could be maintained by progesterone alone. In a similar situation, however, with only one fetus abortion occurred. This suggests that estrogen of trophoblastic origin is synergistic in action with exogenous progesterone. Perhaps to further demonstrate this, Peckman (32) was unable to produce the deciduomata in rats given daily 2, 4 and 8 micrograms daily of progesterone. These levels of progesterone also did not interfere with normal implantation and development of embryos. Chambon (18) found that oestradiol

(estrogen) applied locally to progesterone uteri failed to cause a decidual reaction though large amounts inevitably produced abortion. In castrated rabbits if progestational uteri were developed by sensitization by estrogen followed by progesterone, local application of estrogen produced the following effects; large doses of estrogen produced abortive like effect but no deciduoma, minute amounts of estrogen produced no changes but if accompanied by trauma resulted in a marked decidua reaction. Trauma alone has no effect.

Hisaw (15) in attempting to inhibit deciduoma with substances chemically related to progesterone found that in castrated pseudo pregnant rat decidua induced by progesterone was completely inhibited by .25 micrograms of pregnanediol, 1.5 milligrams of testosterone, 3 milligrams of 11 desoxycorticosterone acetate and 4.5 milligrams of cortisone. A.c.t.h. in daily doses of 1.5 milligrams caused complete inhibition probably as a result of adrenal corticosteroids. Peckman (22) experimented with eight unilaterally pregnant rats who had remaining ovary removed on fifth day of pregnancy after which 2 milligrams of progesterone were given through day eleven. On day 9, the non-pregnant horn was puncture stimulated. Three days later, 2 of 8 rats had deciduomata, suggesting that in removal of the ovary there was a reduction of inhibitor. On repeating the experiment with removal of ovary and oviduct on day 4 (thus excluding the products of conception) deciduomata developed in all, suggesting fetal origin of some of the inhibitor factor. A third group of six rats were treated similarly, except that 1 microgram of estrogen was

added to the 2 mgm. of progesterone. None of the estrogen group developed deciduomata and the vaginal epithelium showed a higher degree of mucification than vagenae with embryo's in situ. This suggests that the equivalent of estrogen produced by the fetal trophoblast is less than 1 microgram of estrogen. The observation in the implantation and maintenance of pregnancy in the castrate rat with 2 mgm. progesterone daily was repeated. Kelsey (21) judging from the maintenance of pregnancy or lack of it after removal of corpus lutea was able to conclude that the rat placenta at 8 days was not a supplementary source of progesterone whereas by the 15th day it was. Artunkal (28) was able to prevent abortion in oophorectomized guinea pig on the 15th day if progesterone was given. Courier (29) after doing oophorectomies on rats noted the compression exerted by the uterine muscle and the subsequent echymosis and cephalic deformities. Following this up in rabbits, where death of the fetus constantly occurs after oophorectomy, he was able to prevent this if an extra uterine gestation was produced by a slight rupture of the uterus. From this evidence he concluded that progesterone adapts the uterine musculature to its enlarging contents.

Bedoya (10) tried to determine the effect female hormones had on the production of chorionic gonadotrophin. Chorionic gonadotrophin was determined in thirty-nine pregnancies before and after treatment with estrogen. An increase was found in thirty-one cases, no changes in seven and a decrease in one. In fifteen normal pregnancies gonadotrophin of serum was assayed before and after 10 mgm. of progesterone per day. It was decreased in eight, slightly increased

in two and unchanged in five. From this Bedoya concluded that progesterone probably regulated chorionic gonadotrophin of blood by restraining placental production, while estrogen stimulated this production indirectly via an increased destruction of progesterone. It would be interesting to know the doses of estrogen given by Bedoya. It may very well be that estrogen in small doses causes increased production of chorionic gonadotrophin while large doses would cause just the opposite.

Bonilia (14) was able to demonstrate in 33 of 47 cases of threatened abortions a fall in urinary excretion of chorionic gonadotrophin and for 16 cases of habitual aborters a fall occurred in 65%. By the administration of Stilbene they were able to bring excretion of chorionic gonadotrophin back to normal in 9 of 14 cases. In 8 other cases no increase could be brought about by corpus luteum hormone, but subsequent estrogen treatment caused a rise in gonadotrophin excretion. Paelone (26) believes that 200 mgm. of di-ethyl-stilbestrol daily will prevent abortion in many threatened abortions.

Treatment of Chorioepithelioma with Estrogen and Para-Oxy-propiophenone:

Kullander (31) reports the treatment of two cases of chorioepithelioma with stilbesterol. The first case was treated by panhysterectomy and then radiation to pulmonary metastasis. The patients condition deteriorated and so she was started on 30 mgm. of stilbestrol daily. There was marked improvement, however, a vaginal metastasis regressed. Death resulted from cachexia following hemmorrhage and infection of the vaginal metastasis. The second case

showed no response to stilbestrol in spite of 15,820 mgm. in 20 days. The authors had previously reasoned that since estrogen was known to counter act the increase production of gonadotrophin hormones after castration or during menopause perhaps estrogen would also decrease the gonadotrophin of a chorioepithelioma and thus inhibit its growth. Perrault (25) from Paris reports a case of chorioepithelioma treated with a synthetic hypophseal inhibitor (para-oxypropiophenone) for six months. The patient had a tubal pregnancy for which she was operated on. Metastasis had occurred in the lungs and the diagnosis was verified by histological section. Para-oxypropiophenone (H-365) was given. The patient showed rapid improvement. The temperature fell, the anemia improved and the appetite and body weight increased. The hormonal change disappeared more slowly, pulmonary metastasis also receded. Six months later the patient was still doing well and had received 120 grams of the substance.

Substance in Sera of Pregnant Female:

Anti enzymes;

Malagamba (1) was unable to demonstrate any significant hyaluronidase inhibitor in pregnant female sera.

Vignes (7) demonstrated that antiproteolytic activity of the serum in pregnancy was raised from a normal of 19.3 to a mean value of 53 units.

West (63), who primarily was interested in antichymotroypsin and antirennin in malignant deseases assayed the sera of normal pregnant parous woman (all of whom delivered normally) for these

inhibitors. He found a large increase in both during pregnancy. Antichymotrypsin was already significantly increased in two cases by the 10th week. Considerable fluctuation in antichymotrypsin occurred thereafter reaching maximum levels of 10 to 20 units; antirennin in one case reached approximately 90 units. Large peaks appeared in the antirennin curves which were not correlated with the stage of pregnancy nor consistant from one case to another. By the 6th post partum week both antienzymes had returned to normal or near normal. Normals for adults are antichymotrypsin 5 units. antirennin 7 to 12 units. Pregnancy differs from malignancy in that in the former antirennin is in the excess while in malignancy the opposite is true.

Butler (75) observed a progressive rise in trypsin inhibitor in pregnant females. The levels vary from 360 to 630 inhibitor units with a gradual rise as pregnancy advances. The authors assumed that antitrypsin and antifibrinolysin systems are probably similar. The rise is probably due to cellular degeneration in placenta with release of cytofibrinokinase into the maternal system.

Decidua:

The term decidua is applied to altered endometrium following pregnancy. Decidual changes are described in textbooks of Pathology as a continuation and completion of endometrial alterations which occur normally during the intramenstrual cycle. Endometrial glands become dilated, with marked increase in transverse diameter; their epithelial lining cells proliferate and pile up as intraglandular projections of epithelial cells without a stromal core. Also the

stromal cytogenic cells become enlarged, the nuclei whowing a lesser relative degree of enlargement than the cytoplasm. The nuclei are pale, ovoid or round and appear relatively small because of the marked increase in the cytoplasm. The cells assume a spindle shape, and are in close contact so that they have an epithelium like appearance. Decidual changes involve the whole endometrial lining and are more pronounced on the wall where the ovum imbeds itself; but direct mechanical effect of the imbedded ovum and its proliferating chorion are not the cause of the decidual changes. Ectopic decidual changes may develop outside the uterine cavity in the presence of a normal intrauterine pregnancy. Such changes are very frequent in certain sites such as the albigenia of the ovary, the subperitoneal tissue of the Pouch of Douglas and the posterior wall of the uterus. More unusual location are appendix, lymph nodes, inferior aspect of diaphragm, omentum, vagnia, in granulation tissue of cervical erosions and cervical polyps. Peritoneal nodes of ectopic decidua appear as small flat or slightly elevated grayish patches. Cells which show decidual change are sharply demarcated from adjacent cells without transition. Ectopic areas of endometriosis also undergo decidual changes. (108)

Almost all authorities agree that the function of the decidua is to make invasion of the maternal host possible and maintain this invasion so a normal fetus is produced approximately 280 days later. Some believe that in addition it plays an antagonistic role to trophoblasts.

Even though the trophoblast is probably the most invasive of all

known tissue, its attach on maternal tissues which have not been prepared by decidual changes is not always sucessful and if sucessful in the initial phase the chances of it forming a functional placenta are poor. Decidual tissue is not necessary for invasion of trophoblast as demonstrated by eye implants into castrate mice. However, neither did these trophoblast go on to develop functioning placental tissue.

What is the cause of the decidual reaction? Partially it is the result of interaction of progesterone and estrogen on the endometrial stroma. Chorionic gonadotrophin plays a indirect role by serving as a lutenizing hormone to perpetuate the activity span of the corpus luteum.

Fana (33) by employing the method of Dustin, controlling the formation of decidua in female rats, 9, 15, 27, 39, 51 and 91 hours post coitum believes he has proven the connective tissue origin of the decidual cells in rats. Undifferentiated elements primarily histocytes occurring in the connective tissue and more especially in the tunica of the vessels, and even elements of the connective tissue of the myometrium differentiate and form decidua.

Whether chorionic gonadotrophin has an direct effect on decidual reaction is not known at this time. If any other hormones are involved is not known. It is interesting to speculate what part ectopic decidua plays in normal trophoblastic metastasis. According to Novak (48) the one main difference between physiological trophoblastic metastasis and malignant metastasis is lack of control by the maternal host. Therefore does not ectopic decidua suggest inability of maternal host to limit this reaction to endometrium; or perhaps

it could also mean inability to control the amount of proliferating trophoblast and therefore a higher serum concentration of chorionic gonadotrophin which in turn cause more areas of decidual reaction all over the body. It may also represent abnormal reaction of maternal host to normal hormonal pattern of pregnancy. It seems only logical to assume that the many trophoblastic emboli which occur would find this most fertile soil to establish new metastasis and thereby complete the viscious circle. Hertig (87) has categorized the metastasis occurring in chorioepithelioma. He found that there were metastasis in the lung 80% of the time, Vagina 50%, kidney, spleen and ovary 13%, pelvis 10%, and brain 10% of the time. The areas of ectopic decidual reaction were previously given, however, no percentage figures are known. Because the spread is primarily hematogenous perhaps the high percentage of metastasis to brain, lung, kidney and spleen where decidual reaction is not known to occur is not so much against ectopic decidual metastasis as would appear at first glance.

Novak (83) states that he encountered only two cases of ovarian metastasis in approximately eighty cases of chorioepithelioma and neither were accompanied by decidual reaction.

A.T. Hertig (84) also believes that ectopic decidua has nothing to do with chorioepitheliomas. He has never seen ectopic decidua associated with physiologic pulmonary emboli nor with metastasis in chorioepithelioma.

Klebanow (20) claims to have isolated in extra uterine pregnancy a hormone from the decidua which is almost exclusively follicle stimulating and which is higher early in pregnancy. If this is true

it cast little light on the final answer as to trophoblastic and decidual relationships.

It would be interesting to note how a patient with chorio-epithelioma responded to aminopterin. Since Velardo (9) was able to completely inhibit deciduomata reaction from trauma in pseudo pregnant rats by subcutaneous injection of 30 micrograms of aminopterin. Deciduomata reaction which occurred in castrated rats after 1.5 mgm. of progesterone could also be suppressed by aminopterin. Both suppressions could be reversed by doses of leucovorin in 1:1 ratio. This indicates that this antifolic acid agent has a direct effect on the formation of decidua. The authors in attempting to prove that the aminopterin effect was not just one of toxcity administered strychnine and dibenamine both of which failed to inhibit decidua.

SUMMARY

G. Schmorl in 1893 was the first to call attention to the medical profession that trophoblastic fragments are frequently if not physiologically carried to the lungs during pregnancy. He found embolic displacement of placental fragments to the lungs in 80 of 158 females who died from eclampsia but only active cell growth had occurred in 3.

Since so little is known of trophoblastic growth in the ectopic sites let us see what is known about it in the uterus.

By 8 to 8½ days cytotrophoblast and syncytiotrophoblast are identifiable. By the 10th day erosion of maternal vessels occurs. On the 12th day primordial villi are being formed. Decidual reaction is beginning to occur by the 14th day.

Most authorities agree that gonadotrophin is produced by the cytotrophoblast, whereas the syncytiotrophoblast produce estrogen and progesterone. The cytotrophoblast and syncytiotrophoblast are very rich in ribose nucleoprotein. This is thought to be indicative of active protein synthesis. Glycogen is abundant in cytotrophoblastic columns, but little occurs in cytotrophoblast or syncytiotrophoblast. It is generally more plentiful over the avascular or anaerobic areas. There are many mitochondria in syncytiotrophoblast, but few in cytotrophoblasts.

The multiplicity of substances dissolved by the growing trophoblasts suggests that the invasive agent or agents must be powerful proteolytic and cytolytic enzymes.

In rabbit tissue cultures trophoblast show no selectivity as to what they destroy as they will invade and destroy the original embryo's. At this time they resemble cells seen in chorioepithelioma very markedly. The giant cells present in these culture showed marked amoeboid motion. Since mouse trophoblasts can be grown in male, hypophysectomized mice, mouse trophoblastic growth is independent of any hormonal pattern which occurs in pregnancy. Foreign objects and ova in rat uteri implant at similar rates thus negating the rat endometriums passive role in implantation.

Human trophoblast fulfill criterion for malignancy since they are heterologously transplantable into rabbit's eye.

At this time, as far as it is possible to tell, the histological elements that make up chorioepitheliomas are identical with normal trophoblasts.

In multiple ova tranplants as soon as the most mature one begins to implant the rest of the ova are destroyed apparently from enzymes emanating from the implanting ova. Since hemorrhage occurs before the trophoblast are in contact with maternal tissue some cytolytic substances must be elaborated by the trophoblast.

At present all that is known of chorionic gonadotrophin is its role in prolonging the function of the corpus luteum. It is found only in the equidae and primates and apparently other mammalian trophoblast are able to implant without it. The glycogen content of the endometrium early may be very important in normal trophoblastic growth.

Some workers believe that chorionic gonadotrophin is detectable

in many extra genital cancers.

Chorionic gonadotrophin was found to have a constant renal clearance throughout pregnancy of .38cc/min. Also better than 90% of the hormone is destroyed in the body with less than 10% excreted in the urine. Chorionic gonadotrophin urinary excretion peaks between the 50th and 70th day at levels from 40,000 to 200,000 R.U. from then on it declines to levels of about 5,000 to 10,000 R.U./24 hours. Progesterone urinary levels peak between the 220 - 240 day levels of 50-100 mgm/24 hour urine volume. Estrogen peaks at 230 days at levels of 40,000 to 50,000 micrograms/24 hour urine volume.

Experimental work seems to indicate that progesterone and estrogen in small dosages cause decidual reaction, whereas estrogen in larger doses is an inhibitor of decidua and lowers chorionic gonadotrophin levels. In rabbits and rats progesterone adapts the uterus to enlarging fetal contents.

Frankel at about 1900 demonstrated a lytic substance capable of destroying placental tissue in the sera of pregnant females. He was unable to demonstrate this in females suffering from chorioepithelioma. The opinion of the function of the Layer of Nitabuch is divided as to whether it is a defense against trophoblastic invasion or not. The theory that the trophoblast may have a predetermined growth potential and cease growing of their own accord must be considered in explaining trophoblastic behavior. Most authorities, however, feel that there must be some antitrophoblastic element in pregnant females, the nature of which has not been identified as yet. In support of this concept there are approximately four bona-

fide cases of chorioepithelioma in the literature which have regressed. This supposedly never occurs in a truly malignant tumor.

A rise in antichymotripsin levels in pregnant females occurs reaching maximum levels of 10 to 20 units, normal is 5 units. Anti-rennin levels rose from normals of 7 to 12 units to 90 units. Trypsin levels also are increased to 360 to 630 inhibitor units. The normal was not given.

Decidua plays an important role in normal trophoblastic development. Decidual cells may originally be connective tissue cells. Apparently ectopic decidua plays no part in systemic trophoblastic metastasis.

Amminopterin has been shown to inhibit decidual reaction. It would be interesting to know how a patient with chorioepithelioma would respond to its usage.

CONCLUSION

It must be noted that no work has been done on a similar scale to Schmorl's in the last 50 years to confirm or refute his observations that trophoblastic emboli and metastasis are physiologic in pregnancy.

Later Schmorl recommended caution in interpreting "syncytial structures" in the lungs having seen similar structures, if not identical in case of aspiration preumonia. According to William, Park and Lees (68) some of Schmorl's illustrations are certainly suggestive of placental fragments and villi within the lung while others are unconvincing. It is also amazing that if trophoblastic emboli are such a constant feature in eclampsia why in the last 50 years they haven't become established as one of the features of the disease.

If we are to ever decide how the maternal organism resist trophoblastic invasion we must first understand how the trophoblast accomplishes invasion. There is much that we do not know but some of what we know of trophoblastic invasion is listed below:

1. In mice at least trophoblastic invasion is entirely independent of any hormonal pattern during pregnancy and also it is entirely independent of hormones from the pituitary.

2. The trophoblast elaborates powerful cytologic enzymes before trophoblastic elements are even in contact with maternal elements.

3. The multiplicity of substances which are destroyed by the trophoblast suggest that the enzymes must be of many types and very

powerful. That these agents are indeed enyzmes is partially substantiated by a rise in anti enzymes in the mother's serum.

4. The trophoblast apparently does not make use of a spreading factor in its invasion since structures stain in undiminished intensity up to the maternal-fetal junction. In addition no anti-hyaluronidase is detectable in maternal sera.

That there definitely is an antitrophoblastic element in pregnancy is demonstrated by (1) proven cases of chorioepithelioma which have recovered and later have gave birth to children, (2) regression of lung metastasis in hydatiform mole. (3) Frankel demonstrated that there was a lytic substance in pregnant mother sera which would destroy placental tissue. He also demonstrated that this substance was not present in patients suffering from chorioepithelioma.

I was unable to find in the literature any work to support or refute these observations. Work is definitely needed to establish the validity of the observations and if indeed such a substance in present isolation and identification of it would be a major step forward.

Trophoblast resemble cancer cells in that:

1. Histiologically normal trophoblast and chorioepithelioma trophoblasts are indistinquishable.

2. Trophoblast fulfill the criterion of malignant cells in that they are heterologously transplantable.

3. Though some tumors approach the growth rate of trophoblast none grow faster.

4. Trophoblast in tissue culture invade and destroy their own

embryo resembling at this time chorioepithelioma histologically.

5. All types of cells known to be malignant are represented in trophoblastic growth.

6. Many extra genital cancer have been demonstrated by some to produce chorionic gonadotrophin. Thereby suggesting that trophoblast are the invasive element in all carcinoma.

Decidua:

1. In rats the decidual plays an active role in implantation of the embryo.

2. The nutritive state of the decidua is thought early to play an important part in normal trophoblastic growth.

3. It is suggested in this paper that ectopic decidua may represent inability to limit trophoblastic growth to the uterus and thereby provide fertile ground for systemic metastasis. However, this is not substantiated by pathological evidence.

Hormones:

Chorionic gonadotrophins is present in only two mammals, the primates and the horse. Other than prolonging the corpus luteum activity nothing else is known about its function. Since it is not necessary for other mammalian trophoblasts to implant and invade its necessity in man is debatable. But its constant presence and high concentration in chorioepithelioma and its sbrupt fall when regression commences is suggestive that it plays a more active part in maintaining trophoblastic growth.

Estrogen rise shortly after chorionic gonadotrophin peaks and its sudden drop while estrogen urinary excretion keeps climbing is

very suggestive that estrogen tends to regulate chorionic gonadrtrophin production. In support of this is experimental evidence that stilbesterol lowers chorionic gonadotrophin levels in chorioepithelioma's.

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