WHAT CAUSES THE NAUSEA AND VOMITING OF PREGNANCY AND HYPEREMESIS GRAVIDARUM

SOME REASONS WHY PROSTAGLANDIN E₂ CAN BE ASSOCIATED WITH NAUSEA AND VOMITING OF EARLY PREGNANCY AND HYPEREMESIS GRAVIDARUM

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We have presented the case that nausea and vomiting of pregnancy (NVP) has an organic cause by applying information from our own work and the work of 143 other researchers working in the field of early pregnancy presented in 25 separate sections.

A temporal relationship between maternal serum human chorionic gonadotrophin (hCG) and NVP during weeks 7-14 from the first day of the last period (L.M.P.) for a group of pregnant women became a starting point. Other investigators found raised maternal serum hCG in women who had hyperemesis gravidarum, twin pregnancies or hydatidiform moles all three of these conditions are known to be associated with an increase incidence of severe NVP or hyperemesis gravidarum (HG). One outstanding fact is that very high serum hCG in women with choriocarcinoma is not associated with nausea and vomiting. However it is now known that the hCG in this condition is 100% ‘nicked’ in the β sub-unit of the hCG molecule, so becoming biologically inactive. Therefore maternal hCG in normal pregnancy can be related to NVP between weeks 6-14 from LMP. NVP is episodic in nature for the majority of women who have two or more episodes of nausea daily. This type of episodic pattern is also seen in the maternal serum concentration of hCG. However it has not been demonstrated that hCG is a substantially emetic hormone, it may be possible that some other factor either stimulating maternal hCG or which is stimulated by maternal hCG may be the cause of NVP.

Using Med-Line and associated medical papers we found there are at least 14 hormones, cytokines or eicosanoids including Prostaglandin E₂, which stimulate the synthesis and release of hCG in syncytiotrophoblast cells in the early trophoblast of the developing placenta. Only Prostaglandin E₂ (PGE₂) and Prostaglandin F₂ alpha (PGF₂α) of these substances are known to be emetic which was shown when the prostaglandins were given to procure legal terminations of pregnancy.

PGE₂ has been demonstrated to be synthesised in the syncytiotrophoblast cells of the human trophoblast at 7-11 weeks from LMP. These cells are in direct contact with maternal blood at the materno-fetal interface. The production of PGE₂ in these cells is stimulated by several hormones and cytokines, notably Interleukin-1 and hCG itself. In order for the synthesis of PGE₂ in early pregnancy to be properly controlled, the activity of the enzyme Prostaglandin dehydrogenase (PGDH) is present in early trophoblast cells. PGDH controls the first stage in the oxidisation of PGE₂ into its inactive metabolite 15 Ketoprostaglandin E₂. The syncytiotrophoblast contains little PGDH which therefore contains high levels of PGE₂. These syncytiotrophoblast cells of the chorionic villi are in direct contact with maternal blood. PGDH is under progesterone control. As progesterone levels in maternal blood fall during weeks 5-9 from LMP, so will PGDH activity in decidual and chorionic villi cells levels fall, while PGE₂ in trophoblast and maternal serum levels will rise. Weeks 6-10 from LMP are the weeks of pregnancy when NVP symptoms increase most sharply. A rise in PGE₂ has also be associated with the increased NVP when anti-progesterones are given to obtain a legal therapeutic abortion in modern times. Search of medline and associated scientific literature has shown that Prostaglandin E₂ is synthesised and released in early pregnancy into the maternal circulation from syncytiotrophoblast and decidual cells as well as in the extra cellular matrix of the decidua. Endovascular trophoblast alter the configuration of the decidual spiral arteries to become low resistance sinusoid sacs which maintain the utero-placental blood flow at very low pressures in early pregnancy. Later the placental blood flow and pressure rises to increase the exchange of substances, for example oxygen from the maternal to the fetal circulation. We have shown that maternal serum PGE₂ was higher when women had NVP than when they had no NVP on the same day. This remained true whether the sample taken at the time she had higher NVP was before or after mid-day. However it would be inadvisable to reduce maternal PGE₂ due to its functions of immune suppression and glycogenolytic effects in early pregnancy.
The formation of chorionic villi begins at the end of the 4th week of gestation that is from LMP. Histologically they are classified into primary, secondary and tertiary villi. The primary villi are composed of a central mass of cytotrophoblast cells surrounded by a thick layer of syncytiotrophoblast cells. During the following weeks of gestation they acquire a central mesenchymal core from the extra embryonic mesoderm and become branched forming secondary villi (Fig.1). The appearance of embryonic blood vessels within their mesenchymal cores transforms the secondary into tertiary villi (Fig.1). At the end of the 5th gestational week all three primitive types of placental villi can be found, but tertiary villi progressively predominate (1). By six weeks of gestation the villous tree is essentially composed morphologically of long immature trunks, branching mesenchymal villi and syncytial sprouts. The mesenchymal villi are characterised by a thick syncytiotrophoblast layer, a more or less continuous cytotrophoblast layer, a villous core and capillaries with lumina containing nucleated red blood cells (Fig.1). At 8 weeks of gestation the chorionic villi are well developed and surround the whole periphery of the gestational sac (Fig.2). Up to 10 weeks of gestation villi cover the entire surface of the gestational sac (1). Syncytium means a mass of cytoplasm with several nuclei not divided into separate cells (Oxford dictionary).

Ultrasound Features Of The Early Gestational Sac

A few days after the expected menstrual period it is possible to clearly distinguish the presence of an intra uterine gestational sac, distinct from the uterine cavity, it is buried in the uterine mucosa and appears as a round structure (Fig.2). This peripheral zone or ring is rich in echos and surrounding the embryo is the ultrasound expression of a structure containing a large number of interfaces. It represents a collection of tissues comprising the chorionic villi, the inter villous lakes, the extra villous trophoblast. This ring is the site of maternal/embryonic exchanges. From the moment the gestational sac is ultrasonographically evident the peripheral ring is complete (Fig.3). At this time, the liquid cavities surrounding the embryo are themselves surrounded by the chorion and trophoblastic structures. The latter progressively infiltrates the maternal decidua and remains uniformly thick all along the periphery of the gestational sac until the age of 7 weeks. At 5 weeks the ring is 3-4mm thick and triples within 15 days (Fig.2). After 10 weeks of gestation, two thirds of the trophoblastic ring ceases to grow, while the rest, which turns into the definitive placenta, continues to develop (Fig.3). There is no difference in echogenicity between these two structures, merely a disparity in size (2).

The syncytial sprouts are the most numerous villous off-shoots of the early placenta. The sprouting activity of the trophoblast is maximal at 10 weeks of pregnancy and decreases towards term. During villous development the sprouts are invaded first by cytotrophoblast and then by mesenchymal elements. Mesenchymal villi are continuously formed from trophoblastic sprouts throughout gestation and are the basis for the growth and development of all villous types (1). Trophoblast means a layer on the outside of mammalian blastula providing nourishment to an embryo (Oxford dictionary) for example, cytotrophoblast, syncytiotrophoblast cells and a thin sheet of connective tissue and the fetal vascular endothelium (Fig. 4).
**Mesenchymal Stroma of Chorionic Villi**

Mesenchymal cells gradually transform into small and large reticulum cells, fibroblasts and macrophages (hobauer cells). About 8 weeks gestation a basal lamina appears around the capillaries and is only complete at the beginning of the third trimester. Fetal capillaries rapidly establish contact with the basal trophoblastic surface. They are in intimate contact with the trophoblastic layer as early as the 6th week of gestation (1).

**The Early Villous Tree**

It is well known that in the early stage of gestation the villi are quite large in diameter and progressively decrease in diameter as term approaches. Initially the cytotrophoblast cells form a continuous layer beneath the syncytiotrophoblast but as pregnancy advances these cells become less prominent, which will have a major influence on the trophoblast thickness of the placenta. The volume fraction for the trophoblast increases until 11 weeks and then decreases, while the volume fraction of the villi occupied by the fetal capillaries increase progressively between 6 and 15 weeks (1).

**The Villous Trophoblast**

The primitive cytotrophoblast is derived from the cells of the wall of the blastocyst and gives rise to all populations of trophoblast cells. The syncytiotrophoblast is the most active component of the human placenta from embryonic period until the end of gestation (Fig.1). It has the highest concentration of organelles (1) which are key to the complexity of function that can be achieved by a single cell (3). The first trimester syncytiotrophoblast is rich in electron-dense granules, some of the granules are hormone-secretory granules. Human chorionic gonadotrophin (hCG) granules which predominate during the first trimester can be separated from human placental lactogen (hPL) granules which are usually found in second and third trimester placentas. The β hCG levels cannot be correlated with the growth of the placental mass. The trophoblast thickness decreases progressively with gestation, while hCG levels rise during the second month of gestation reaching a maximum between 8 and 10 weeks of gestation, at a time when most of the placental mass degenerates to form the smooth chorion. This suggests that the hCG synthesis depends on rate of differentiation of cytotrophoblast into syncytiotrophoblast and reflects the invasive role of the developing placenta (1).

The trophoblast surface is covered with microvilli. At 10 weeks the microvilli are long (measuring approximately 1.5µm) and become shorter as pregnancy progresses. Microvilli increase the surface area of the villi in contact with maternal blood and being richly endowed with enzymes and receptors facilitate placental transfer of water, gases and nutrients to the embryo or fetus (Fig.4).
The Villous Stroma

In early pregnancy the villous core is filled by small, spindle shaped undifferentiated cells with long thin cytoplasmic processes. Large reticulum cells and fibroblasts also develop and secrete the collagenous matrix that evidently fills most of the inter-cellular spaces of the tertiary villous core as well as providing structural support. Stromal cells form intimate connections with each other and also with the trophoblastic basement membrane. Hofbauer cells or villous macrophages are observed in chorionic villi of an 18-day embryo and are present in their highest concentration early in pregnancy. Immature or intermediate Hofbauer cells can be found in the core of secondary villi, even before angiogenesis begins and all through the first half of gestation they are the numerically dominant cell type in the villous mesenchyme (1).

Figure 1

![Diagram](image1)

ARLEY L.B. Developmental Anatomy in W.B. Saunders 1940 Philadelphia USA

Figure 2.

Ultrasound features of the early gestational sac

These chorionic villi surround the whole gestational sac as a complete circle at 5 weeks gestation.
Figure 3

Photograph of a gestational sac at 8 weeks of gestation. The chorionic villi are well developed and surround the whole periphery of sac. Reference (1).

Figure 4

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MATERNAL SERUM HUMAN CHORIONIC GONADOTROPHIN (hCG) βhCG AND PROGESTERONE LEVELS IN WEEKS 5-17 FROM LMP ASSOCIATED WITH SYMPTOMS OF PREGNANCY SICKNESS

Clinical relationship between maternal serum hCG and pregnancy sickness.

Several studies comparing emetic pregnancy to serum maternal hCG have been written spanning more than half a century. Schoeneck et al (1) presented evidence that there was an increased concentration of gonadotrophin in the urine of pregnant women who have the symptoms of nausea and vomiting, compared to pregnant women who are free of these symptoms. They also stated that the greatest concentration of maternal urinary hCG was present from the 6th to the 14th week of pregnancy when nausea and vomiting were mostly encountered. However, Soules et al (2) observed there was no obvious correlation between the concentration of maternal serum hCG and the severity of nausea and vomiting of pregnancy. These symptoms were graded 0-4 in severity but the mean levels of serum hCG did not differ significantly among any of the grades in individual women.

Masson et al (3) agreed with the findings of Schoeneck for a group of women rather than individual women, finding higher serum hCG levels in pregnant women with nausea and vomiting than in asymptomatic women. Jarnfelt Samsioe et al (4) formed a similar opinion, finding that serum levels of hCG in emetic women were significantly higher in early pregnancy, than the serum hCG levels of non-emetic women. Kaupilla et al (5) confirmed this view, observing that women suffering from hyperemesis gravidarum had serum hCG concentrations significantly higher than normal between weeks 7 and 14 of gestation.

Holder et al (unpublished work 1979) found that in a series of 13 women who gave weekly samples of blood between weeks 6 & 14 from LMP with a final sample at 17 weeks from which serum hCG, βhCG, progesterone and oestriol were estimated. These values were related to the women’s symptoms of nausea and vomiting in the previous week that each sample of blood was taken (6). The correlations between maternal serum hCG levels and pregnancy sickness symptoms in individual patients were so limited that they were clearly insignificant, agreeing with the findings of Soules. However, when serum hCG levels of those women with severe symptoms were compared with those with mild symptoms, raised hCG and βhCG levels were significantly associated with severe symptoms during weeks 7 and 9-12 from first day of last menstrual period (LMP), agreeing with the findings of Kaupilla, Masson and Jarnfelt Samsioe. In addition, when the overall maternal serum hCG levels were compared with overall sickness symptoms for several weeks throughout early pregnancy, there was a correlation for maternal serum hCG (P<0.02) and βhCG (P<0.01) levels with sickness symptoms from weeks 6-11 from LMP. This association became highly significant when βhCG and hCG serum levels and sickness symptoms were compared for weeks 6-17 from LMP (P<0.001). At the same stage of pregnancy the fall of serum progesterone during weeks 5-9 from LMP and its subsequent rise to week 17 showed an inverse association with the rise of pregnancy sickness symptoms in weeks 6-9 and fall of these symptoms from weeks 10-17 from LMP. Table 1 (6).

There are then some pointers to a association between rising maternal serum hCG and increasing symptoms of nausea and vomiting of pregnancy (NVP) at 5-10 weeks from LMP and declining symptoms of NVP in weeks 11-17 from LMP. However, when individual women’s symptoms of NVP are related to their maternal serum hCG, no significant association is found. This may be due
to the hCG/LH “spare receptor” syndrome, please see the section “Some Functions of hCG”. Added to that, we have been given the important information by Professor K D Bagshawe, Professor of Medical Oncology, Charing Cross Hospital School, London “very raised serum and cerebrospinal fluid levels of human chiorionic gonadotrophin occur with choriocarcinoma in the absence of nausea and vomiting provided there are no gastrointestinal or cerebral metastases”. We now know that the isoform of hCG formed in choriocarcinoma is 100% nicked at the beta-subunit residuals 44 and 45 or 47 and 48. Therefore the hCG’s bioactivity in choriocarcinoma is ablated (7). A certain association continues to be established between maternal serum hCG and pregnancy sickness symptoms. When seeking the aetiology of this condition, we should consider hormones, cytokines or prostaglandins which stimulate the synthesis of hCG in syncytiotrophoblast cells (8) or which hCG stimulates, during weeks 6-10 from LMP being particularly interested in any that cause nausea and vomiting.

Goodwin et al have shown in 39 women presenting to the emergency room of Los Angeles County University of Southern California Women’s Hospital with a complaint of severe vomiting, hyperemesis gravidarum, compared to 23 control subjects presenting for confirmation of pregnancy or routine prenatal clinics of the same age, weight and gestational age that the concentration of maternal hCG was greater in hyperemesis patients, as was the concentration of free β hCG. A percentage free β hCG greater than 0.6 percent was found in 33/39 hyperemesis patients (85%) compared to 5/23 controls 22% p=0.01. The difference between hyperemesis and control subjects with respect to free β did not vary with gestational age (9). This observation links hyperemesis gravidarum with conditions such as trophoblastic disease and Down’s syndrome in which the placenta is abnormal with respect to architecture and hormone production. This provides support for the view that hyperemesis gravidarum represents not an abnormal response of the mother to pregnancy itself, but is probably related as placental hormone metabolism. It would be important to know whether the increased free β sub-unit (and increased hCG itself) found in hyperemesis gravidarum is one end of a spectrum of abnormal placental production of hCG corresponding to the wide range in severity of nausea and vomiting seen in normal pregnancy (9). Holder demonstrated that raised β hCG also occurred with raised hCG in weeks 6-10 from LMP when symptoms of pregnancy sickness are increasing (6).
THE RELATIONSHIP BETWEEN HORMONE LEVELS AND EMESIS GRAVIDARUM IN EARLY PREGNANCY

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GRADED SYMPTOMS OF PREGNANCY SICKNESS
-Total grades for all patients added together for each week of pregnancy

○ = no. of patients in that week.

Reference 6
Table 1. Hormone levels measured in early pregnancy in patients with EG at individual weeks

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<td>91±32 (10)</td>
<td>22.1±6.10 (10)</td>
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*Median, range.
Numbers of observations in parenthesis.
EG = Emesis Gravidarum.
P = Progesterone.
E₂ = Estrodiol.
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THE SOURCE OF HUMAN CHORIONIC GONADOTROPHIN FROM WITHIN TROPHOBLAST CELLS

It is important to fully understand the differentiation of trophoblast cells which separate production of hyperglycosylated hCG and normal hCG (see figure). Stem cytotrophoblast cells differentiate to make extra villous invasive cytotrophoblasts which produce hyperglycosylated hCG. They also differentiate to make villous cytotrophoblast cells which do not produce hyperglycosylated hCG. These fuse to form multinucleated syncytiotrophoblast cells with 2 to 50 nuclei. The fusion of cytotrophoblast cells is controlled and promoted by regular hCG. Cytotrophoblast cells are the principal cells present at the time of pregnancy implantation. These cells rapidly differentiate to extra villous invasive cytotrophoblast and villous cytotrophoblast cells which fuse to become syncytiotrophoblast cells as pregnancy advances. Syncytiotrophoblast cells produce the hormone regular hCG. Hyperglycosylated hCG is an autocrine factor, it functions separate to regular hCG to promote invasion of implantation of pregnancy and malignancy in trophoblastic diseases. (1)

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THE ISOFORMS VARIANTS AND NICKING OF HUMAN CHORIONIC GONADOTROPHIN

Human Chorionic Gonadotrophin consists of two units, alpha hCG and beta hCG which combine to form the biologically active hormone hCG.

Several factors have to be considered in the relationship of hCG to pregnancy nausea and vomiting. hCG exhibit’s a considerable heterogeneity in maternal blood during pregnancy (1). Typical isoelectric focusing (IEF) pattern of immuno-reactive (IR) hCG is sera of normal pregnant women at 7 weeks of pregnancy (these components were designated for convenience). A PI (isoelectric point) 3.9, B PI 4.1, C PI 4.4, D PI 4.7, E PI 5.0, F PI 5.8, G approx PI 6.7. In general the reactive amounts of B and C were large while A, D, E, F and G were small (2).

The isoforms of hCG produced in early and late pregnancy are different (1). The isoforms of hCG in sera from early pregnancy were more acidic (have a lower isoelectric point) than those obtained from later pregnancy (1). The hCG molecules with the highest in vivo biological activity were produced during the first trimester (1). hCG from early pregnancy has a longer half-life (when tested in mice) than hCG from late pregnancy which explains the higher activity in the in vivo bioassay of hCG in early pregnancy (1).

In the alpha unit of hCG, carbohydrate constitutes 30% of the total weight of which 27.4% is sialic acid. Similarly, the beta subunit contains 36% carbohydrate of which 28% is sialic acid. The sialic acid residues as well as the entire carbohydrate moiety of hCG have been shown to be essential for the full expression of hCG’s in vitro and in vivo gonadotrophic activities (3).

The electrophoretic technique measures the overall charge of the isoforms of hCG and the change in the median charge of the isoforms of hCG reflects a change in the carbohydrate of the polypeptide structure (1). This change of iso-electric charge during pregnancy is not a continuous process but occurred at a limited period of time, namely 11-15 weeks of gestation. All the values of median mobility were higher at weeks 6-10 than at weeks 16-43 of gestation. The mean values of the degree of charge of heterogeneity of hCG was significantly (P<0.05 and P<0.01 respectively) higher at 11-15 weeks and at 16-43 weeks than at 6-10 weeks (1). The concentration of serum hCG decreased at the same period of gestation as the median charge of hCG changed. There was a significant (r= 0.394; P<0.01; n=3g) positive correlation between the median mobility and the concentration of hCG in serum during the weeks 6-10 or the weeks 16-43 of gestation (1).

In patients with hyperemesis gravidarum (HG), ten European and ten Samoan women the hCG profiles differed significantly from those without HG (p<0.001). The HG subjects had higher concentrations in the acidic third when compared with control subjects. Peak 6 (PH 3.3) was observed only in hCG profiles of women suffering from HG. Peak 5 (PH 3.6) occurred significantly more frequently in hCG profiles of HG women, than with control subjects (p<0.05). Therefore, the acidic forms might be responsible for pregnancy related nausea either by direct effect on the brain stem centres or in intestinal mobility or by indirect effects through secretion of hormones normally related to other glycoproteins, such as thyroid hormones (4).
Binding of hCG variants to Liver Receptors

The biological activities of desialyted forms of hCG are greatly reduced in vivo due to the high affinity of asiolo-glycoproteins for hepatic receptors and their consequent rapid clearance from the circulation. Intact purified hCG on the other hand has little affinity for hepatic receptors and has a relatively long life in vivo (5).

The β-subunit sialic acid seems to be more critical than the alpha-sialic acid in preventing hepatic binding and prolonging plasma half-life (7). Intact hCG exhibited the slowest metabolism as, very little, if any, of it was removed from the circulation during the thirty minute observation period (in mice). Asiolo (desialyted) hCG by contrast was rapidly downgraded and only 2% of the initial concentration was detectable after the first two minutes and less than 0.4% remaining after 30 minutes. Intact alpha asiolo-beta was also rapidly removed as about 1% of its residual concentration was detectable even after 30 minutes. Asiolo-alpha intact beta was cleared much more slowly as more than 30% of the initial concentration was present at two minutes, whereas, at 30 minutes nearly 10% was still detectable (5).

Yoshimura et al state other mutations of hCG cause it to be unable to dimerise i.e. for alpha and beta subunits to join which they must for normal activity, such mutations may manifest as elevated free β-hCG. This is seen in molar gestations. They found that compared to 23 gestational agematched controls, 39 patients with hyperemesis had elevated free β-hCG 101 ± 70ng/ml, v31 ± 31ng/ml p<0.001. There was also a significant difference between groups for total hCG 9,327 ± 3,613 ng/ml, 5,543 ± 2,290 ng/ml p<0.01 but not for free alpha hCG 399 ± 231, 377 ± ng/ml (6).

What was commonly referred to as immuno-reactive hCG is a mixture of free-beta hCG, free-alpha hCG, deglycosylated hCG, desialyted (asiolo) hCG and hCG completely lacking the four sites of glycosylation (BCTP). Molar tissue in which only 3% of the measured hCG is fully glycosylated and a majority of hCG is not intact. Large amounts of the basic fractions of hCG were much more potent than intact hCG in stimulating the release of cyclic ‘5’ adenosine monophosphate (c’AMP) when combined with human TSH or LH Receptors. These basic fractions correspond to the deglycosylated hCG lacking the β-CTP. Thus, part of the puzzle of the relationship between hCG and NVP may lie in the fact that measurements of intact hCG do not reflect various fractions of different potency (6).

Alpha and Beta hCG gene profiles

Whereas the human alpha-gene is present as a simple copy gene on chromosome 6 there are six genes which enclose hCG beta-like products on chromosome 19. Each of these hCG-beta genes are transcribed in vivo but with highly variable levels of expression. The genes hCG beta 5 and hCG beta 3 are expressed at about a 7:1 ratio. Furthermore, analysis of first trimester RNA expression using gene-specific oligonucleotide indicated a 20:1 ratio for the two hCG-beta genes. Could it be that the increased beta hCG unit found in molar gestations, or the change in charge of hCG isoforms around 13 weeks of gestation are due to a change in the relative expression between the genes encoding different hCG proteins? (1)

“Nicking of hCG”
A proportion of hCG molecules in pregnancy serum and urine samples have nicks or a missing peptide linkage between either beta subunit residues 44 and 45 or beta subunit residues 47 and 48. The nick causes a rapid dissociation of hCG into free alpha and free beta subunits, with consequent ablation of the steroidogenic activity of hCG (7).

Once nicked, hCG rapidly dissociates into free alpha and beta subunits. Standard hCG (batch CR 127, 20% nicked) and hCG preparation C5 (100% nicked) were incubated for varying times in whole blood. C5 hCG dissociated rapidly into free alpha and beta subunits (dissociation half-life 22 ± 5.2 hours) over 30 times faster than standard hCG (dissociation half-life 700 hours). It was inferred that nicked hCG rapidly dissociates and that the relative amount of nicked molecules produced by trophoblast may considering circulating (37-41h) and dissociation half-lives be 3.4-3.6 times higher than measured in serum samples (7).

Levels of total hCG (nicked and non-nicked) and intact hCG (non-nicked) were determined in 233 serum and 168 urine samples from 4-40 weeks of pregnancy. A linear relationship was indicated between advancing weeks of gestation and increasing extent of nicking. Minimum ‘nicking’ was observed in serum from the first two months of pregnancy (mean = 9% of hCG molecules) and increased ‘nicking’ in the months thereafter, with maximum ‘nicking’ in samples from the last months of pregnancy (mean = 21% of hCG molecules P<0.00005). It was concluded that nicking is more prevalent after hCG peak (after two months of pregnancy) (7).

The increased degree of ‘nicking’ of the hCG molecules was reported to be a gradual process throughout gestation, whereas, the median charge changed at a restricted period and then remained constant throughout the second and third trimesters. It, therefore, seems unlikely that the two processes are related (1).

**The Source of “Nicking”**

Human leukocyte elastase secreted by neutrophils can ‘nick’ hCG. Type IV collagenases secreted by macrophages are also elastases with the same specificity as the leukocyte enzyme. This enzyme may also ‘nick’ hCG. We postulate that an elastase or type IV collagenase like enzyme, associated with or present in trophoblast tissue, specifically ‘nicks’ and thus deactivates hCG. The progressive increase in proportions of ‘nicked’ hCG may simply reflect the increase of placental mass that occurs throughout pregnancy. We infer that ‘nicking’ occurs before or immediately upon secretion of hCG by trophoblast tissue (7).

**Summary**

hCG exhibits considerable heterogeneity in maternal blood during pregnancy. Isoforms of hCG in sera from early pregnancy were more acidic than those obtained from later pregnancy. This acidic hCG is also more glycosylated hCG and has a longer half-life than basic hCG. A significant change in the charge of hCG and its glycosylation takes place between 11-15 weeks gestation, the hCG becoming more basic with altered glycosylation.
Desialyted (asiolo) hCG has a high affinity for specific liver receptors and is, consequently, rapidly removed from the circulation, while intact hCG has little affinity for these liver receptors and, therefore, has a relatively long circulation half-life. hCG can also be ‘nicked’ when a peptide linkage becomes missing at the beta subunit residues 44 and 45 or 47 and 48. The nick causes a rapid dissociation of hCG into free alpha and free β-subunits with consequent ablation of hCG’s steroidogenic activity. This ‘nicking’ is minimal until 8 weeks of gestation and gradually increases throughout pregnancy.

If either of these processes (a) desialylation of hCG with increased binding to liver receptors or (b) ‘nicking’ of hCG does not act efficiently, the resultant fully glycosylated hCG may continue later into pregnancy, possibly causing nausea and vomiting of pregnancy to persist longer than usual until these changes occur.

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THE RELATIONSHIP OF THE START OF NAUSEA AND VOMITING OF PREGNANCY (NVP) TO THE ISOFORM OF hCG IN MATERNAL BLOOD.

Early pregnancy produces a type of hCG that resembles in terms of immunoreactivity, a major form of hCG excreted in Choriocarcinoma known as isoform β152. This isoform predominates in maternal blood for the first 5-6 weeks of gestation and is then replaced by a less glycosylated hCG known as β109 increasingly which predominates until 11 weeks of gestation (1). Carbohydrate moieties such as glycols and sialic acid appear to be related to bioactivity of hCG. Indeed N-linked oligosaccharides in the α chain (ASN) of hCG play an important role in signal transduction resulting in a stimulation of steroid genesis and Adenosine 3’ 5’ monophosphate (cyclic AMP) production (2). There was a higher proportion of β152 like hCG isoforms when hCG secretion was low in very early pregnancy (2).

There was a strong negative correlation between the isotopes of hCG β152/β109 and gestational age of women between 32 and 50 days from the first day of last menstrual period (LMP) (3). There is also a considerable increase of β109 hCG with reduction of β152 being produced from the early trophoblast at this early stage of pregnancy (3). This altered glycosylation of β109 hCG compared to β152 hCG is associated with altered bioactivity of hCG (2) and might be related to the development of nausea and vomiting of pregnancy NVP.

The week in which NVP most frequently started was week 6 or more specifically, day 39 from LMP (4). This is the week in which there is a significant fall in the ratio between hyperglycosylated hCG and regular hCG from 87% hyperglycosylated hCG in days 21-27 to 43% hyperglycosylated hCG in days 35-41 from LMP (3) and there is at the same gestational time an enormous rise in maternal serum hCG (3) suggesting a relationship between regular hCG and the start of nausea and vomiting of pregnancy.

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THE RELATIONSHIP OF PEAK SYMPTOMS AND CESSATION OF NVP TO THE ISOFORMS OF hCG IN MATERNAL BLOOD.

There are two main types of hCG secreted from the trophoblast into maternal blood: hyperglycosylated hCG or regular hCG (1). In fact each type is produced from different cells of the early trophoblast: hyperglycosylated is derived from cytotrophoblasts whereas the less glycosylated regular hCG is derived from syncytiotrophoblasts cells (1). In the first 3-6 weeks from LMP the ratio between the two hCGs moves away from being highly in favour of hyperglycosylated (β152) increasing toward a higher quantity of regular hCG (1). This change of ratio continues to become a marked increase of regular hCG between 6-10 weeks from LMP. At the same time the total synthesis of regular hCG is greatly increased (2). The regular hCG has been separated into 5 different isoforms moving from acidic to a more basic form of hCG (3). Regular hCG of early pregnancy has a greater ratio of the acidic form of the compound, as it has a higher carbohydrate content which has been shown to be essential for the bioactivity of hCG (4). The acidic form is also the more biologically active as it has a longer half life in maternal blood (5). It is more prominent in maternal blood in weeks 6-10 from LMP whereas from week 11-15 the more basic lower carbohydrate content less active type of regular hCG becomes more prominent in maternal blood. The major change occurs at week 13 from LMP (5). There is a decrease in the amount of regular hCG in maternal blood synthesised by week 11 until week 14 from LMP after which the level of hCG remains fairly constant throughout the pregnancy (5). Nausea and vomiting rapidly increases in incidence and severity from week 6 from LMP becoming markedly worse from weeks 6-9, then generally decreases at first in week 10 then gradually declines in weeks 11-12 and typically ceases between weeks 12-14 from LMP (6). There appears to be an association between the changes in the regular hCG to the acidic isoform secreted from syncytiotrophoblast cells of the early trophoblast causing the severity of nausea and vomiting symptoms at their peak weeks 6-10 from LMP, followed by a change to the basic form of hCG weeks 11-15 from LMP associated with the reduction and cessation of these symptoms of NVP.

In Jordan’s investigation of 20 women with hyperemesis gravidarum the hCG profile differed significantly from those without HG (p<0.001). The ten European and ten Samoan women with hyperemesis gravidarum had higher concentrations in the acidic third of hCG when compared to controls. Peak 6 (ph 3.3) was observed only in hCG profiles of women suffering from HG. Peak 5 (ph 3.6) occurs significantly more frequently in hCG profiles of HG women than with control subjects (p <0.05). Therefore the acidic forms of hCG might be responsible for pregnancy related nausea and vomiting either by direct action on the brain stem centres or in intestinal mobility or by indirect effects through secretion of hormones normally related to other glycoproteins such as thyroid hormones (7).

As there are LH/hCG receptors in at least three areas of human brains such as hippocampus, hypothalamus and brain stem (1) these receptors may explain the cause of hyperemesis gravidarum or the grades of severity of nausea and vomiting which occur during normal pregnancy.

It will also be necessary in considering the aetiology of various grades of pregnancy sickness which women may experience to remember that pregnancy sickness is an episodic condition, about 85% of women who have the condition have at least two episodes of nausea daily (6). There does appear to be a similar episodic daily pattern for maternal serum hCG (8).

Goodwin writes in his significant article, despite the evidence that hCG is very closely related to the symptoms of hyperemesis gravidarum there still lacks a good explanation of how hCG causes hyperemesis gravidarum. We ask could the association of maternal prostaglandin E₂ and the
symptoms of NVP and HG be a further link in the detection of the mystery, what causes this condition in otherwise normal pregnancies?

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SOME FUNCTIONS OF HUMAN CHORIONIC GONADOTROPHIN (hCG)


The acute in vitro effects of hCG and Prostaglandin E2 on human placental glycogen metabolism have been studied in immature 8-20 week placental villi in short-term culture. hCG elicited within 15 minutes of culture an acute glycogenolytic response in placental tissue which included a decrease in placental glycogen, an activation of the glycogen phosphorylase enzyme system, and a pronounced elevation of cyclic AMP concentration of the placenta. The presence of Prostaglandin E2 in the culture medium evoked a similar glycogenolytic effect in immature placental villi including the increase in tissue cyclic AMP levels (1).

The activity of glycogen phosphorylase was consistently and markedly affected by hCG in all the immature placentas studied. The activation of phosphorylase and subsequent glycogenolysis after exposure to hCG may be a function of cyclic AMP as observed in other tissues. Within 30 minutes of exposure to 50 iu/ml hCG the placental glycogen content was decreased. There was an increase in the active form of the phosphorylated enzyme and a pronounced elevation in the ratio of phosphorylase a (active form)/b. Accompanying this glycogenolytic response was an 82% increase in placental cyclic AMP concentration (1).

The effects of 10mg/ml Prostaglandin E2 were more marked than those of hCG on activation of phosphorylase. Prostaglandin E2 induced a larger decrease in placental glycogen and a 149% increase in cyclic AMP concentration in immature placental tissue (1). Reports on human placental adenyl cyclase and its sensitivity to be prostaglandins (2) and catecholamines (3) further suggests the possibility that glycogen turnover in the placenta may be controlled by gonadotrophins operating on an adenyl cyclase AMP phosphorylase system (1).


B. \( \beta \)-hCG subunit is thought to be hormone specific and responsible for receptor activation. The hCG formed by the human placenta stimulates the steroidogenesis of the corpus luteum in early pregnancy. Until 8-9 weeks of gestation the corpus luteum produces the progesterone necessary for maintaining pregnancy (4).


C. Trophoblast-derived human chorionic gonadotrophin acted as a growth factor because trophoblast proliferation (measured by uptake of thymidine labeled with tritium) was reduced by 60% in the presence of an anti-human chorionic gonadotrophin antibody (5).


D. (i) hCG showed a significantly positive correlation with T4 (0.05) lymphocyte count (<0.01)1GM (<0.05) C3 (<0.05) in the hyperemesis group compared to normal pregnant women (controls). There is a structural homology between hCG and TSH molecules and their receptors. It is conjectured that hCG is the thyroid stimulator of pregnancy. The thyroid gland of normal women may be stimulated by hCG secrete slightly excessive quantities of T4 and also induces slight suppression of TSH. It has also been suggested that clinical thyrotoxicosis is caused by circulating hCG with higher biological activity (6).

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D. (ii) If hCG is the thyroid stimulator of pregnancy how can there be a weak association between hCG and the degree of hyperthyroidism (in individual patients)? The answer to this question may lie in the action of variants of hCG of differing thyrotropic potential. The principal difference between hCG and other glycoprotein hormones (LH, FSH, TSH) is the large 31 amino acid tail, the carboxyterminal portion (B-CTP). Whereas LH is 10 times more potent than intact hCG at stimulating the TSH receptor, mutant hCG lacking the B-CTP is as potent as LH. This variant form of hCG having greater thyrotropic potential may also be found in otherwise normal pregnancies characterised by hyperemesis gravidarum. What is commonly referred to as immuno-reactive hCG is a mixture of Free \( \beta \)-hCG, Free \( \alpha \)-hCG, deglycosylated hCG, asiolo-hCG and hCG completely lacking the CTP (7).
**SOME FUNCTIONS OF HUMAN CHORIONIC GONADOTROPHIN (hCG) (Contd.)**

Basic fractions of hCG were much more potent than intact hCG in stimulating the release of c’AMP when they combined with TSH and LH receptors. These basic fractions correspond to deglycosylated hCG lacking the B-CTP. This type of hCG is seen in molar pregnancies. Goodwin found that compared to 23 gestational matched controls 39 patients with hyperemesis gravidarum had elevated free β- hCG. Part of the puzzle of the relationship between hCG and NVP may lie in the fact that measurements of intact hCG do not reflect various factions of differing potency (7).

Another important clue to the hormonal etiology of NVP may lie in variations within the hCG receptor. Mutations have been described in the glycoprotein hormone receptors, just as for the hormones themselves. A patient with hyperemesis gravidarum was found to have a familial gain of function mutation in the TSH receptor making her extremely sensitive to hCG in pregnancy (7). Such cases could be explained by a mutation in the glycoprotein hormone receptor in an area common to the TSH and LH/hCG receptor or by variants of hCG that have greater potency in stimulating not only the TSH receptor but also the LH/hCG receptor. How does hCG cause HG? Despite the evidence that hCG is very closely related to the cause of hyperemesis there still lacks a good explanation for how hCG can cause hyperemesis (7). These findings strongly suggest that whatever the cause of thyroid stimulation in pregnancy it is closely linked to the cause of hyperemesis gravidarum. It would not appear to be hyperthyroidism itself however, since hyperthyroidism itself rarely causes nausea and vomiting. (7)


E. In human term placentas hCG has a stimulating action on the armatisation process (testosterone to oestrogen) as on glycogenolysis. c’AMP is a mediator of the effects of HCG in the placenta. Young placentas obtained by therapeutic abortions during the first trimester were also stimulated by hCG. hCG probably acts initially by increasing the adenyl cyclase and the intra cellular concentration of cyclic AMP which in turn activates the phosphorylase of the gland. The increased glycogenolysis probably produces great amounts of NADPH via the pentose pathway. The addition of NADPH generating system or of only 500mg of glucose- 6-phosphate increased the estrogenic production from testosterone (aromatisation) as markedly as gonadotrophin addition. (ACTH is totally inactive) (8).

F. hCG produced by fused and differentiated villous syncytiotrophoblast cells has numerous functions, for example promotes progesterone production by corpus luteal cells, promotes angiogenesis in uterine vasculature, promotes the fusion of cytotrophoblast cells and differentiation to make syncytiotrophoblast cells, causes blockage of any immune or macrophage action by mother on foreign invading placental cells, suppresses any myometrial contractions during the course of pregnancy, acts on receptor in mother's brain causing hyperemesis gravidarum (1). Considering these critical biological functions of hCG there is a major paradox that exists. Individual hCG levels in serum of total hCG and hyperglycosylated hCG vary extremely widely. In serum at 4 weeks of gestation (weeks from the start of the last menstrual period) individual total hCG values vary by 824 fold amongst different women with singleton term outcome pregnancies. Hyperglycosylated hCG values vary even wider during this week of pregnancy 888 fold. In the 5th week of gestation total hCG values vary by 704 fold amongst different women with singleton term outcome pregnancies. Hyperglycosylated hCG values vary once again slightly wider 734 fold. How can all these pregnancies go to term and produce similar size babies?

The day of implantation is 3 to 16 days after the LH peak. The day of implantation is the day of starting viable pregnancy. Dating this important date to the time of the start of the last menses (week of gestation) is a source of great variability. Dating pregnancies to the time of implantation is preferable to dating to the start of the last menstrual period but is difficult in that it requires daily hCG measurements while attempting to achieve pregnancy to determine time of implantation. Dating pregnancies is a cause of variation in early pregnancy hCG values (1).

Examining 594 pregnancies from implantation to term only one other cause could be found for individual variations of hCG. That is hCG daily increase rate in the first 4 weeks following implantation. The increase rate per day ranged from 1.52 fold per day to 2.92 fold per day among 82 women. If this is considered over 7 days to be an increase of 18.7 fold v’s an increase of 1810 fold. Individual hCG daily amplification rate is also a major cause of variation in early pregnancy hCG values. It is a fact that pregnancy to pregnancy hCG levels vary greatly. One hCG-related biological activity was measurable, promotion of progesterone production by corpus luteal cells at 3-6 weeks of gestation. During the 4th week of pregnancy serum hCG ranged widely a variation of 824 fold. Serum progesterone during the same period in the same women did not vary widely 16 fold (1). Interestingly the case with extremely low serum hCG concentration the progesterone was slightly higher than the medium. In the case with extremely high hCG concentration the progesterone concentration was slightly lower than the medium. Why? This is apparently due to the high hCG/LH receptor “spare receptor” concept. Under the spare receptor concept, when only a tiny proportion of receptors is activated in a cell it may yield a similar cellular response to all receptors on the cell being activated. Conversely in case with extremely high serum hCG concentration lower than normal progesterone occurred, this is due to a receptor down regulation in the presence of high hCG concentration. As demonstrated high concentrations of hCG decrease the number of receptors on the cell by degrading the receptor transcript in cells, reducing their half-life (9). Therefore the severity of symptoms of nausea and vomiting of pregnancy is unlikely to be directly related to the maternal hCG serum levels in individual pregnant women.
From Cedared L, Alkat E, Urtasan M-Jose, Vaaoner J.
Studies on the mode of action of utilizing hormone and chorionic gonadotrophin on Estrogenic Biosynthesis and Glycogenolysis by Human Placenta perfused in vitro 1970, Oct 16: 361-375

Schema of the possible mechanism of action LH and hCG on the biosynthesis of estrogen in human placenta. All the enclosed products are able to increase the aromatization of testosterone during vitro placenta perfusion experiments.
HORMONES, CYTOKINES AND PROSTAGLANDINS WHICH INCREASE hCG SYNTHESIS OR RELEASE FROM SYNCYTIOTROPHOBLAST CELLS

Many substances, hormones, cytokines and prostaglandins are known to stimulate the production or release of hCG from maternal syncytiotrophoblast cells in the first trimester of pregnancy.

1. **Gonadotrophin Releasing Hormone (GNRH)**

1a. Pulsatile release of hCG into the medium. Pulse amplitude and frequency were increased in response to GNRH in higher dosage (1).

1b. GNRH stimulated hCG secretory response by 80%. IL1 beta stimulated a rapid and transient hCG stimulatory response increase approximately 150%, but lower concentrations were ineffective. Combined treatment stimulated response by 150%. GNRH and IL-1 beta produce an increased stimulation but by different pathways. 8-12 weeks placental trophoblast used (2).

2. **Epidermal Growth Factor (EGF)**

2a. EGF 7-10 week (gestational weeks placentae). In superficial explants, short (1-4 minutes) pulses of EGF increased both rate and amplitude of spontaneous pulsatility of hCG. The frequency increased from 3 to 5 hours. This effect was dose-dependent and the concentration of 50ng/ml was the lowest tested and the most effective. In explants cultured for 24 hours EGF caused a two-fold increase in hCG secretion compared to controls. EGF added daily for the first week caused 180% increase in hCG secretion.

2b. EGF stimulated proliferate potential of cytотrophoblast in early (4-5 week) placental explants. The EGF stimulation of trophoblast proliferation was apparent at a 12 hour EGF treated period. By contrast, 6-12 week placental explants did not respond to EGF increase in trophoblast proliferation. Instead, in early placental explant culture, EGF stimulated hCG and Human Placental Lactogen (HPL) secretion with a lag period of 72 hours, whereas, very early placental explants did not respond to EGF with increase in hCG and HPL secretion. Therefore, EGF stimulated trophoblast proliferation in 4-5 week placenta and stimulates differentiated trophoblast function in 6-12 week placentae (4).

2c. EGF binding sites and EGFR production increase in human placentae throughout the gestation period (5).
3. **Parathyroid Hormone (1-34PTH)**

Gestational age-dependent effects of parathyroid hormone (1-34 PTH) were noted. In static cultures 1-34 PTH stimulated hCG secretion in 7-9 week placentae in a biphasic fashion, the maximal effect being noted at 10-25ng/ml concentration (250-270%) while at 1 and 100ng/ml the effect was mild. Effects of 1-34 PTH at 11-14 weeks were inhibitory. In static cultures at 7-9 weeks the stimulatory effects of 25ng 1-34 PTH was increased by 70% when EGF 100 ng/ml was added (6).

4. **Oxytocin (OT) Arginine-Vasopressin (AVP) and Prolactin (PRL) Effects on hCG**

In static cultures OT and AVP significantly increase hCG secretion, whereas PRL had no effect. In superperfusion, one minute pulses of OT induce a significant 2 to 10 fold rise in hCG pulse amplitude. PRL pulses caused a progressive inhibition of spontaneous hCG pulsatility (7).

5. **Calcium**

Secretion of hCG by first trimester human placental minces. Depletion of calcium (Ca) in the medium by addition of EGTA resulted in a dramatic decrease in the levels of immunoreactive hCG in the medium with consequent accumulation of hCG in the tissue. Calcium is essential for normal secretion of hCG by human placentae (8).

6. **c’AMP**

Any factor which increases c’AMP in trophoblast tissue will increase maternal hCG secretion (9, 10).

Factors known to increase c’AMP in trophoblast cells:
(i) Catecholamines
(ii) GNRH
(iii) hCG itself, hCG variants,
(iv) Prostaglandin E<sub>2</sub>

7. **Catecholamines stimulate c’AMP in early Trophoblast Cells**

7a. The catecholamine Epinephrine stimulated adenylate cyclase activity from 10 weeks of gestation to term (11).

7b. High density of B adrenergic receptors in human placentae composed of B1 and B2 receptors. It is likely that cell size or membrane surface area changes contribute greatly to the decrease in beta adrenergic receptor densities observed with increasing gestational age (12). 3H-DNA binding to early human placentae, the binding capacity of early crude placental membrane is about three times higher than described in term placentae (13).
hCG and its Variants Stimulate c’AMP in early Trophoblast Cells

The acute in vitro effects of human chorionic gonadotrophin (hCG) on human placental glycogen metabolism have been studied in immature placental villi (8-20 weeks) in short term culture. hCG elicited within 15 minutes of culture an acute glycosynthetic response in placental tissue which included a decrease in placental glycogen, an activation of the glycogen phosphorylase enzyme system and a pronounced elevation in the cyclic AMP concentration of the placenta. (14)

hCG Variants

The principal difference between hCG and other glycoprotein hormones (leuteinizing hormone LH), follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) is the large 31 amino acid tail, the β-carboxyterminal portion (β-CTP). LH is ten times more potent than intact hCG at stimulating the TSH receptor; mutant hCG’s lacking the β-CTP were much more potent than intact hCG in stimulating the release of c’AMP when they combined with human TSH and LH receptors (15). Normal human placenta express hCG/LH receptor gene (16).

Prostaglandin E₂ Stimulates c’AMP in early Trophoblast Cells

The acute in vitro effects of Prostaglandin E₂ on human placental glycogen metabolism have been studied in immature placental villi in short term culture. 10 ng/ml Prostaglandin E₂ induced a similar glycosynthetic effect including a larger decrease in placental glycogen than 50 iu/ml of hCG and an increase in tissue cyclic AMP concentration (14).

hCG itself has also been shown to stimulate PGE₂ synthesis in 9-12 week placentas at physiological conditions. The rate of PGE₂ synthesis increased with a longer incubation period, particularly in placentas of younger gestation. Significant stimulation of PGE₂ synthesis occurred at 10⁴ iu/1 hCG and continued to increase in a dose-dependent manner up to 5 x 10⁶ iu/1 as seen in 9-10 week placental organ cultures. There was considerable variation of PG production between placentas of the same gestation (17).

Interleukin-1β Stimulates hCG Secretion

IL1β (10⁻⁹m) increased basal hCG secretion in placental trophoblast. The response peaked within 25 minutes after IL1β perfusion was initiated, and hCG secretion returned to basal concentration 10 minutes later. IL1β (10⁻⁹m) stimulated a rapid and transient hCG secretory response. hCG release increased by approximately 150% in response to the cytokine, but lower concentrations were ineffective. IL1β (10⁻⁹m) is within the physiological range. 8-12 week placental trophoblast (18).

Tumour Necrosis Factor Alpha (TNF ALPHA) Stimulates hCG Secretion

Trophoblast stimulated with rTNF alpha released hCG in a dose-dependent fashion. Simultaneous stimulation of trophoblasts (placentas at 7-9 weeks gestation) with rTNF alpha and IL-1 alpha resulted in synergistic enhancement of IL-6 release, subsequently leading to enhanced hCG release. Although TNF alpha and IL-1 share the intracellular...
signalling pathway, a comparative study of their potency to stimulate IL-6 production demonstrated that the level induced by rTNF alpha is much lower than the level induced by rIL-1. Similar results were obtained with regard to the capacity of these cytokines to induce hCG release. rTNF alpha induced IL-6 release at doses greater than 200 ng/ml while IL-1 alpha induced IL-6 releases at doses 2.0 ng/ml (19).

13. Human Macrophage Colony-Stimulating Factor (M-CSF)

When human cytotrophoblast cells in the early stage of pregnancy (6-11 week human villous tissue used) were cultured in a serum-free medium in the presence of M-CSF, the cytotrophoblast cells fused and formed a typical syncytiotrophoblast. On the other hand, cytotrophoblasts incubated with anti-M-CSF antibody showed hardly any syncytiotrophoblast formation.

When cytotrophoblasts were incubated in the presence of M-CSF the supernatant of the culture showed an increase in human chorionic gonadotrophin and human placental lactogen (HPL) levels in proportion to the concentration of M-CSF added. When cytotrophoblasts were incubated in the presence of anti-M-CSF antibody or anti-FMS antibody, hCG and HPL secretion were suppressed. Thus, M-CSF was morphologically and endocrinologically found to induce the differentiation of chorionic cells and hCG synthesis (20).

14. Transforming Growth Factor B1 (TGFB-1) Suppresses hCG Release

Trophoblast-derived TGFB-1 suppresses cytokine but not GNRH induced release of hCG by normal human 7-9 week trophoblasts. Trophoblast produced predominantly a latent rather than an active form of TGFB-1 (21). rTGFB-1 markedly suppressed rIL-1 alpha and rTNF alpha and IL-6 induced hCG release. In contrast to the TGFB-1 mediated regulatory activity on IL-6 and hCG release, TGFB-1 exerted no inhibitory or augmenting effect on IL-6 or hCG production. These findings, together with TGFB-1’s effect on GNRH-induced hCG release exclude the possibility that rTGFB-1 is toxic to trophoblasts and thereby reduces IL-6 and hCG release. This indicates that TGFB-1, produced by trophoblasts, platelets and monocytes in the placenta might act as a physiological regulator of cytokine-dependent hCG release mechanism in an autocrine or paracrine fashion (21).

These are some of the organic substances which increase hCG synthesis from syncytiotrophoblast cells in early pregnancy: gonadotrophin releasing hormone, epidermal growth factor, parathyroid hormone, oxytocin arginine-vasopressin hormones, cyclic AMP, catecholamines, hCG itself and variants of hCG, Prostaglandin E2, Interleukin-1β, tumour necrosis factor alpha and macrophage colony-stimulating factor. Prostaglandin E2 is the only substance in this group that is known to cause nausea and vomiting. For further information please see appendix B.
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Trophoblast-derived transforming growth factor B suppresses cytokine-induced but not gonadotrophin-releasing hormone induced release of Human Chorionic Gonadotrophin by normal human trophoblasts.
THE FORMATION OF PROSTAGLANDIN E₂ FROM STIMULATION BY HUMAN CHORIONIC GONADOTROPIN IN SYNCYTIOTROPHOBLAST CELL MEMBRANES

Molecules act as local chemical messengers binding to specific receptors in adjacent cells to give a concerted tissue response. This cell stimulation will result in a release of arachidonic acid from the cell wall which after further oxidative metabolism via the eicosanoid pathways can result in the production of prostaglandins (1). Cell activators stimulate PGE₂ from arachidonic acid released from the cell wall (see Fig.2). The ability of IL-1 to initiate prostaglandin synthesis is perhaps one of its most important biological properties accounting for many local systemic effects (2). As regular human chorionic gonadotrophin acts on receptors in early trophoblast cells, transforming cytotrophoblast cells to syncytiotrophoblasts, prostaglandin E₂ will be produced from all those membranes. The prostaglandin E₂ is known to be a very emetic eicosanoid.

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Figure 2. Steps in the transcription, translation and processing of IL-1. Cell activators stimulate PGE, leukotrienes (LT), and intracellular calcium levels. Transcription and translation are enhanced by calcium and LT. Transcription is short-lived because of the synthesis of a transcriptional repressor protein (or proteins), and translation is reduced by PGE-induced cAMP. The 31,000-Da IL-1 precursor (pro IL-1) undergoes limited cleavage into 220,000- and 17,000-Da peptides that can be found associated with the cell as well as in the extracellular compartment. Membrane-bound IL-1 can be 31,000-Da product. Lysosomal enzymes and serine proteases are responsible for cleavage of pro IL-1 into various peptides with molecular masses of 17,000 (the prominent extracellular form) and also of 11,000, 4000 and 2000 forms as determined by gel electrophoresis.

Diagram from Reference 2.
PROSTAGLANDIN E₂ (PGE₂) IS KNOWN TO CAUSE NAUSEA AND VOMITING IN EARLY PREGNANCY WHEN USED FOR TREATMENT TO OBTAIN LEGAL ABORTION IN EARLY PREGNANCY

Nausea and vomiting were the most troublesome side effects when PGE₂ was first used to procure a termination of pregnancy in the early 1970’s. These side effects of nausea and vomiting associated with giving Prostaglandin E₂ and F₂α (PG’s) have been clearly and persistently described when given by intravenous infusion (1) (2) by single intra-amniotic injection (3) by intrauterine infusion (4 and 5). The oral route of PG administration is quite unsuitable because of the severity of side effects (6).

Gillett (7) has shown raised plasma levels of prostaglandins were associated with an increased incidence of nausea and vomiting, these side effects regressed rapidly when the infusion was reduced (2) or stopped (8).

Wiqvist (2) found continuous intravenous infusion of PGF₂α caused nausea and vomiting, these symptoms disappeared on reducing the infusion rate, and the dose which produced side effects varied considerably from one woman to another. Some patients can tolerate a high dose before symptoms of nausea and vomiting appear while other patients develop these symptoms on a considerably lower dosage. Wiqvist et al also state that it is a well-known clinical experience that suitable dose levels of intravenous prostaglandins vary within a wide range and have to be adjusted to suit an individual patient (9) they showed that prostaglandins were metabolised at different rates by individual patients. It has been shown by Karim et al (10) and Beazley et al (11) that side effects of nausea and vomiting occur more readily when PGE₂ or PGF₂ are given in the first or second trimester of pregnancy. In the third trimester a much lower dose of prostaglandin is required to induce labour so that fewer side effects are associated with the dosage given at this stage of pregnancy. A more modern paper, Millar et al (12) describing the use of Prostaglandin E₂ pessaries 10milligrams given pre-operatively before termination of pregnancy states, the use of these pessaries was found to be associated with an unacceptably high incidence of nausea and vomiting. The incidence of nausea and vomiting is only too apparent to those providing an anaesthetic service to patients who have received prostaglandins.

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Nausea and Vomiting in Pregnancy: An Association between Symptoms and Maternal Prostaglandin E2

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Key Words
Pregnancy  Nausea  Prostaglandin E2  Interleukin 1-β  Tumour necrosis factor α

Abstract
This study investigated the aetiology of nausea and vomiting in pregnancy (NVP) in primary care using a new methodology. Eighteen women in early pregnancy had 2 blood samples taken in one 24-hour time period, one when they were symptom-free, and another when they were symptom-free. Maternal serum levels of the candidate agents PGE2, IL-1β, and TNFα were measured. The study shows a positive relationship between NVP and maternal serum PGE2 levels in early pregnancy.

Introduction
Pregnancy sickness is a condition which causes considerable morbidity [1]. Its aetiology remains uncertain. The symptoms of nausea and vomiting in pregnancy (NVP) increase significantly from week 6 (day 42) from the first day of the last menstrual period (LMP) to the end of week 9 (day 63) from LMP, and gradually improve during and after week 10 from LMP [1]. The aetiology of NVP should therefore relate to what is happening at the developing feto-maternal interface during this time period, and investigations into its cause need to be carried out then. Ultrasound and histological examination of the maternal-fetal interface at this time suggest that the secondary and tertiary chorionic villi could be the site of production of the aetiological factor(s) of NVP [2, 3].

Maternal serum levels of human chorionic gonadotrophin (HCG) have been related to symptoms of NVP by a number of researchers. Some have reported a negative correlation [4], no correlation [5] or a positive correlation [6–9]. Our hypothesis is that substances arising from the chorionic villi which are involved in the synthesis or effects of HCG are implicated in the aetiology of NVP. Trophoblast cells of the chorionic villi secrete HCG [10], interleukin 1β (IL-1β) [11], tumour necrosis factor-α (TNFα) [12], other hormones, cytokines, and prostaglandin E2 (PGE2) [13].

PGE2 is known to be a powerful emetic agent in early pregnancy [14–16]. NVP is episodic with 85% of women experiencing days of nausea with 2 episodes [1]. In this study, we have been able to take blood samples from pregnant women in the community between 7 and 9 weeks of...
Table 1. Material serum levels of IL-1β, TNFα and PGE2 in symptomatic (nascent present) and control (no nascent) samples for each individual woman.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Day from LMP</th>
<th>Time of day</th>
<th>IL-1β control (pg/ml)</th>
<th>IL-1β symptomatic (pg/ml)</th>
<th>TNFα control (pg/ml)</th>
<th>TNFα symptomatic (pg/ml)</th>
<th>PGE2 control (pg/ml)</th>
<th>PGE2 symptomatic (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62</td>
<td>18.40 h</td>
<td>09.40 h</td>
<td>0</td>
<td>0.53</td>
<td>1.04</td>
<td>0.4</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>16.50 h</td>
<td>08.50 h</td>
<td>0.12</td>
<td>0</td>
<td>1.2</td>
<td>0.54</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>10.10 h</td>
<td>19.00 h</td>
<td>0.1</td>
<td>0</td>
<td>0.42</td>
<td>0.56</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>16.45 h</td>
<td>11.20 h</td>
<td>0.09</td>
<td>0.07</td>
<td>0.71</td>
<td>0.57</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>18.50 h</td>
<td>10.20 h</td>
<td>0.76</td>
<td>0</td>
<td>0.47</td>
<td>0.67</td>
<td>17.8</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>17.50 h</td>
<td>09.05 h</td>
<td>0.4</td>
<td>0.26</td>
<td>1.01</td>
<td>0.91</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>16.45 h</td>
<td>10.15 h</td>
<td>0.12</td>
<td>0</td>
<td>0.8</td>
<td>3.18</td>
<td>16.2</td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>10.00 h</td>
<td>15.45 h</td>
<td>0.16</td>
<td>0.56</td>
<td>1.31</td>
<td>19.5</td>
<td>23</td>
</tr>
<tr>
<td>9</td>
<td>56</td>
<td>10.00 h</td>
<td>13.30 h</td>
<td>0.2</td>
<td>0.82</td>
<td>0.37</td>
<td>18.4</td>
<td>19.2</td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>10.45 h</td>
<td>15.00 h</td>
<td>0.1</td>
<td>0.2</td>
<td>1.06</td>
<td>0.45</td>
<td>17.8</td>
</tr>
<tr>
<td>11</td>
<td>84</td>
<td>19.25 h</td>
<td>10.10 h</td>
<td>0</td>
<td>0</td>
<td>0.56</td>
<td>0.71</td>
<td>18</td>
</tr>
<tr>
<td>12</td>
<td>52</td>
<td>14.15 h</td>
<td>10.45 h</td>
<td>0</td>
<td>0.75</td>
<td>0.8</td>
<td>13.4</td>
<td>21.3</td>
</tr>
<tr>
<td>13</td>
<td>66</td>
<td>09.45 h</td>
<td>14.00 h</td>
<td>0.14</td>
<td>0.9</td>
<td>0.24</td>
<td>16.6</td>
<td>20.7</td>
</tr>
<tr>
<td>14</td>
<td>49</td>
<td>10.30 h</td>
<td>18.30 h</td>
<td>0</td>
<td>0.07</td>
<td>0.75</td>
<td>0.35</td>
<td>12.5</td>
</tr>
<tr>
<td>15</td>
<td>53</td>
<td>16.45 h</td>
<td>10.30 h</td>
<td>0</td>
<td>0.59</td>
<td>0.47</td>
<td>14.3</td>
<td>18.6</td>
</tr>
<tr>
<td>16</td>
<td>74</td>
<td>10.30 h</td>
<td>16.35 h</td>
<td>0</td>
<td>0.6</td>
<td>0.62</td>
<td>17.2</td>
<td>18.5</td>
</tr>
<tr>
<td>17</td>
<td>62</td>
<td>14.00 h</td>
<td>10.00 h</td>
<td>0</td>
<td>0.18</td>
<td>0.53</td>
<td>0.51</td>
<td>12.1</td>
</tr>
<tr>
<td>18</td>
<td>64</td>
<td>09.35 h</td>
<td>16.10 h</td>
<td>0.08</td>
<td>0.89</td>
<td>0.44</td>
<td>15.7</td>
<td>23.3</td>
</tr>
</tbody>
</table>

pregnancy, whilst (1) women were nauseaed (symptomatic sample) and (2) within the same 24-hour period when they were not nauseaed (control sample). Women were thus able to be their own controls. Material serum IL-1β, TNFα and PGE2 were measured and related to the presence or absence of NVP at the time each sample was taken.

**Subjects and Methods**

This study was based in an eight-carte urban teaching practice of 14,450 patients of mixed socio-economic class. It received ethical approval from the Warwickshire Research Ethics Committee. Women first consulting about pregnancy over a 12-month period and who had pregnancy sickness symptoms by week 7 from LMP were informed about the study by their general practitioner.

Those who were willing to take part were referred to one of the authors (R.G.) who fully explained the study and ensured that a signed consent form was completed. The women were then visited at home by another author (P.G.) who explained the daily diary forms. Women completed these forms for 2 weeks so that their daily symptom pattern was documented. Arrangements were then made to take two blood samples within one 24-hour period, once when the woman had a symptom episode (symptomatic sample) and again when she was symptom free (control sample). None of the women received any medication for NVP, or any other conditions.

The samples for TNFα estimations were collected in a plain tube, which was left to clot and centrifuged. Plasma was then collected and frozen. The samples for IL-1β estimations were collected in heparin tubes, centrifuged and stored as above. The samples for PGE2 analysis were collected in tubes which had been pre-prepared by adding 0.55 ml of an EDTA anticoagulant mixture, and 0.05 ml of an indomethacin mixture (50 mg in 3.5 ml of absolute ethanol) to avoid rapid PG degradation. The samples were centrifuged and stored as above. They were transported frozen to the School of Biological Sciences at Warwick University and stored at −70°C until thawed for analysis.

TNFα and IL-1β were measured with a human cytokine ELISA system (Amersham International, Aylesbury, UK). The sensitivity for both assays was 0.1 pg/ml. PGE2 was measured using a radiommunoassay (RUA) system (Amersham International). The sensitivity of the assay was 16 pg/ml. All assays had a within and between assay CV of <16%. Samples were analysed in duplicate. Data were first tested for normality using the Kolmogorov-Smirnov test. Statistical differences in the mean concentrations of maternal serum cytokines were determined with Student’s t test, when the experimental and control groups were compared.

**Results**

Eighteen Caucasian Women with symptoms of NVP whose mean age was 27.4 years (range 17–33) gave two blood samples in one 24-hour period, 16 of them between weeks 7 and 9 from LMP. The maternal serum levels for the control and symptomatic samples of IL-1β, TNFα and PGE2, along with the time of day and the day from LMP.
the samples were taken, are given in table 1. All 18 went on to deliver a normal, single live baby.

IL-1β maternal serum levels were mostly below the levels of sensitivity of the assay. There were no significant differences between the control and symptomatic samples for the cytokines IL-1β or TNFα (table 1). However, there was a statistically significant difference (p < 0.001) between the mean symptomatic and control results of PGE2 (table 2).

Moreover, for each woman the symptomatic PGE2 level was always higher than the control (table 1). This was independent of the time of day that the symptomatic samples were taken. Eight symptomatic samples were taken in the mornings whereas 10 were taken after midday (table 1).

**Discussion**

It would seem logical that investigations of the aetiology of NVP should be done at the time when the symptoms are at their worst, which is at 7–9 weeks from LMP [1]. This is usually soon after women first realise that they are pregnant and present to their general practitioner. Therefore, this kind of research needs to be undertaken in primary care.

We now know that 85% of women with NVP experience days with two episodes of nausea, and that NVP symptoms occur for 71% of women in 2- to 4-hour episodes throughout the day [1]. In this study, we have for the first time been able to exploit the episodic symptomatology of NVP by taking maternal blood samples twice within the same 24-hour period, once when the woman was nauseated, and again when she was symptom-free, a strategy that minimises their own and external variables.

Detailed ultrasound scanning at an early stage of pregnancy shows that from the moment when the gestational sac is evident, the peripheral ring, the site of maternal/embryonic exchange, is complete. The echogenicity of this region is the consequence of a number of interfaces including the chorionic villi, the intervillosus space, and the extravillous trophoblast. At 5 weeks from LMP, the ring is 3–4 mm thick and triples over the next 15 days (day 50). After 10 weeks from LMP (day 70), two thirds of the trophoblastic ring ceases to grow while the rest, which becomes the definitive placent, continues to develop [2].

The formation of chorionic villi begins at the end of the fourth week after LMP. They are classified into primary, secondary and tertiary villi. At the end of the fifth week,

**Table 2. Mean levels of IL-1β, TNFα and prostaglandin E2 in control and symptomatic samples of maternal serum (± SE)**

<table>
<thead>
<tr>
<th></th>
<th>IL-1β pg/ml</th>
<th>TNFα pg/ml</th>
<th>PGE2 pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.21 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>16.8 ± 2.4*</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>0.18 ± 0.13</td>
<td>0.62 ± 0.3</td>
<td>22.3 ± 4.6*</td>
</tr>
</tbody>
</table>

*p < 0.001.

(day 35 from LMP), all three primitive types of placental villi can be found. By 6 weeks from LMP, the villi are characterised by a thick syncytiotrophoblastic layer, a more or less continuous cytotrophoblastic layer, and a villous core [3]. These syncytiotrophoblast and cytotrophoblast cells secrete a number of hormones, cytokines and prostaglandins, including HCG [10], IL-1β [11], TNFα [12] and PGE2 [13].

HCG has been related to the symptoms of NVP [6–9], but HCG itself cannot be the cause of NVP, as very raised serum and cerebrospinal fluid levels of HCG occur in chorangioma in the absence of nausea and vomiting, provided there are no gastrointestinal or cerebral secondaries [Bagshaw KD, private communication]. HCG can therefore be regarded as a marker for NVP, although not its cause.

Our hypothesis is that substances arising from the chorionic villi which are involved in the synthesis or effects of HCG are implicated in the aetiology of NVP.

Both IL-1β [10] and TNFα [17] have been shown to induce HCG release from human trophoblastic cells: IL-1β was effective at a minimum concentration of 10⁻⁹ M, which is within the physiological range [10]. r-TNFα induced a sharp increase (2.6-fold) in HCG release at 120 min [17], and synergistic effects on HCG release were observed with r-TNFα and r-IL-1β [17].

HCG stimulated PGE2 from human placental tissue in a dose-dependent manner. The synthesis of PGE2 was maximal in the younger (9–12 week) placentas, and at 9–10 weeks gestation the maximal response of PGE2 to HCG was 4.5-fold [18]. In order to test our hypothesis, we have measured maternal serum levels of IL-1β, TNFα and PGE2 between 7 and 9 weeks of pregnancy and related them to the presence or absence of NVP at the time the samples were taken. As far as we are aware, maternal serum PGE2 has not previously been related to symptoms of NVP. Our results show no relationship between maternal serum IL-1β or TNFα and symptoms of NVP, al-
though serum levels of IL-1β were mostly below the level of sensitivity for the assay.

We have demonstrated a statistically significant relationship between maternal PGE2 levels and NVP symptoms (p < 0.001). The result in each woman showed a raised PGE2 serum level in the symptomatic sample versus the control, as we predicted in our descriptive study [1]. The results could not be due to any diurnal variation of serum PGE2 because eight experimental samples were taken in the mornings and 10 were taken after midnight. PGE2 is known to cause nausea and vomiting when given by intravenous infusion for therapeutic abortion [14, 15]. Nausea and vomiting also occurred when the PGE analog gameprost was given by vaginal pessary [16]. Therefore, the increased maternal serum levels of PGE2 measured when a woman was having an episode of nausea could indicate a cause and effect relationship. There may be theoretical concerns about reducing maternal PGE2 at such an early stage of pregnancy. For example, PGE2 with cytokines, notably TGFβ [19], play a significant role in reducing the immunological reaction, which would otherwise take place between the maternal decidua and the developing fetus [20, 21]. Therefore, these preliminary findings should not be used as a basis for the treatment of NVP without careful consideration and further study. Whilst interesting, these results connecting maternal serum PGE2 levels and NVP need confirmation in larger studies. Our results support the hypothesis of an organic astiology for nausea and vomiting of pregnancy and demonstrate PGE2 as a possible causative agent.

Acknowledgments

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tocytes in the decidua with potential anti-tro
THE PRESENCE OF PROSTAGLANDIN E₂ IN THE EARLY SYNCYTIOTROPHOBLAST CELLS

As we have found a relationship between hCG and pregnancy sickness between weeks 7 and 17 from LMP and a relationship between hCG and PGE₂ synthesis in early trophoblast cells, it seems reasonable to make further investigation of the possible association between Prostaglandin E₂ levels and pregnancy sickness.

Kelly et al wrote, a mechanism by which the trophoblast could control its immediate environment in the decidua and maternal blood may be through the release of prostaglandins (PG’s) and cytokines. This possibility has been investigated by measuring PG and several cytokines released from first trimester trophoblastic villi cultured for 24 hours. PGE is the main primary prostaglandin with a release of 240 ± 95 pg/mg/24 hours (1). Because PGE can influence the function of many leukocytes (particularly macrophages) by raising intracellular c’AMP concentrations, the release of hormonal factors from the villous trophoblast might be important in pregnancy maintenance. Trophoblastic villi were obtained from women undergoing a first trimester 7-11 week termination of pregnancy (1). Johnson et al found the PGE₂ was produced by a syncytial (differentiated) trophoblast. Cells grown beyond 24 hours in fibrin showed sustained expression of cyclo-oxygenase and this enzyme protein expression correlated with increased PGE₂ production in differentiated (syncytial) trophoblast. Term placertas were used (2). Cheng et al found there was clear distinction in the distribution of immunoreactive PGE₂ among the different cell types of trophoblastic villi at 56 days of amenorrhoea or less. The cytoplasm of syncytiotrophoblast cells stained positively for PGE₂, whereas, there was very little, if any, staining in the cytotrophoblastic layer (3). At this stage of pregnancy prostaglandin dehydrogenase (PGDH) was present in abundance in cytotrophoblast cells of chorionic villi but is virtually absent from syncytiotrophoblast. This PGDH in cytotrophoblast cells would prevent access to prostaglandin E₂ (PGE₂) generated in the syncytiotrophoblast to the fetal blood vessels (4).

The syncytiotrophoblast is devoid of Prostaglandin dehydrogenase (PGDH) and, therefore, contains high levels of PGE₂. This distribution of catabolising enzyme leaving some unmetabolised PGE₂ only on the maternal aspect of syncytiotrophoblast cells might be important where these villi, and particularly the syncytiotrophoblast cells are in direct contact with maternal blood (3).

Infusion of PGE₂ into the maternal circulation of an in vitro dual perfused human placental cotyledon preparation did not result in increased PGE₂ efflux but PGEM (metabolite of PGE₂) was increased demonstrating a rapid efficient metabolism by the placenta. There was no significant transfer of PGE₂ across to the fetal circulation, although there was some transfer but in the form of inactivated PGEM. There was no significant interconversion of PGE₂ to PGF₂α by the 9-keto-reducase pathway (5).

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HORMONES AND CYTOKINES WHICH INCREASE THE SYNTHESIS OF PROSTAGLANDIN E\textsubscript{2} IN SYNCYTIOTROPHOBLAST CELLS

Activators which stimulate cells by means of cell surface receptors also cause release of prostaglandins from these cell membranes. This mechanism of prostaglandin stimulation is illustrated in Figure 2 from Dinarello’s paper Biology of Interleukin-1 (1). Examples of this mechanism include Interleukin-1 which induces a five-fold increase in Prostaglandin E\textsubscript{2} production, a response that was density, time and dose dependent. 8-10 week human placentas used (2). hCG itself has also been shown to stimulate PGE\textsubscript{2} synthesis in 9-12 week placentas at physiological conditions. The rate of PGE\textsubscript{2} synthesis increased with a longer incubation period, particularly in placentas of younger gestation. Significant stimulation of PGE\textsubscript{2} synthesis occurred at $10^4$ iu/1 hCG and continued to increase in a dose-dependent manner up to $5 \times 10^6$ iu/1 as seen in 9-10 week placental organ cultures. There was considerable variation of PG production between placentas of the same gestation (3).

Prostaglandin E\textsubscript{2} is also synthesised in early pregnancy by macrophages and monocytes present in the decidua (4) and at the materno-fetal interface (5) and by decidual stroma cells (6). There is therefore, clear evidence of the presence of prostaglandin synthesis and stimulation of Prostaglandin E\textsubscript{2} production in the various cells of the materno-fetal interface in early pregnancy.

This study investigated the regulation of cyclo-oxygenase-2 (COX-2) gene by human CG (hCG) in mucosal cells from fallopian tubes. Culturing mucosal cells with increasing concentrations of hCG also resulted in a dose-dependent increase in media PGE\textsubscript{2} levels suggesting that the COX-2 protein increased by hCG is catalytically active. Although hCG treatment had no effect on the transcription rate of COX-2 gene, it significantly increased the stability of COX-2 transcripts from 3.7h in the control to 7.3h after treatment. (7)

GSH (reduced glutathione) stimulates prostaglandin synthesis but the presence of both L-Epinephrine (catecholamine) and GSH are needed to achieve maximum conversion of arachidonic acid into prostaglandins E2 in human term placentae. (8)

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DIFFERENTIAL LOCALISATION OF PROSTAGLANDIN E₂ SYNTHASE ISOFORMS IN HUMAN PLACENTAL CELL TYPES

Increased prostaglandin E₂ (PGE₂) synthesis involves multiple enzymes and two isoforms of the terminal enzyme of this biosynthetic pathway PGE synthase (PGES) were recently identified. Cytosolic PGES (cPGES) is identical to the Hsp90 chaperone 23 and is reportedly functionally coupled to constitutive PG endoperoxide H synthase –1 (PGHS-1). Microsomal (mPGES) on the other hand is inducible by pro-inflammatory cytokines such as IL-1β. The authors studied the cellular localisation of both enzyme isoforms of the human placenta in early gestation (6-10 weeks from LMP). Cytosolic PGES was immunolocalised to fibroblasts and macrophages in villous stroma at 6 weeks gestation whereas PGES was localised in extravillous trophoblasts (EVT’s) as well as macrophages in early gestation (6 weeks) tissues. Microsomal PGES was observed in cytotrophoblast (CT’s) but not in syncytiotrophoblast (ST’s) in early gestation (6 weeks). Apoptotic early gestational ST’s were heavily stained with cPGES, previous studies have shown that mPGES-1 is co-localised with PGHS-2. PGHS-2 has immunolocalised to both extravillous and syncytiotrophoblasts in early gestation. Microsomal PGES-1 appears to be associated with the invasive phenotype EVT in basal plate and all columns suggesting PGE₂ may play a part in the invasive process of cytotrophoblast cells, whereas cytosolic PGES would be involved in apoptosis or repair mechanisms (1).

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PROSTAGLANDIN E₂ RECEPTORS

Prostaglandin E₂ acts via 4 G protein coupled receptors EP₁-EP₄. PGE₂ receptor analogue gamepost has been demonstrated to possess EP₃ receptor agonist activities. Additionally PGE₂ can enhance ingraft of neutrophils into tissues by synergetic action with chemotactic agents such as IL-8, such effects likely to be demonstrated through the EP₂/EP₄ pathway which is implicated in vasoactive rather than chemotactive actions of PGE₂ (1).

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Prostaglandin dehydrogenase is an enzyme required for the first step of prostaglandin metabolism. In human uterine and intrauterine tissues, oxidation of the 15-hydroxyl group of prostaglandins is catalysed almost entirely by a NAD-linked 15-hydroxyprostaglandin dehydrogenase (PGDH) (1). PGDH activity was measured in homogenates of 25 human placentae between 7 and 10 weeks of gestation. Between 7 and 8 weeks of gestation and 15 and 16 weeks, mean values for PGDH increased 10 fold. The data indicate that the development of terminal villi and the migration of trophoblast into the maternal spinal arteries is associated with a substantial increase in the placental capacity for prostaglandin metabolism (1). There was a large variation in PGDH activity between individual placentae of each of the early gestational stages (1).

Prostaglandin E\textsubscript{2} is a better substrate for this enzyme than Prostaglandin E\textsubscript{1}. The first step in the metabolism of Prostaglandin E\textsubscript{2} involves oxidation of the 15-hydroxyl group to its inactive metabolite 15 Ketoprostaglandin E\textsubscript{2} (2). There is a wide variation in the PGDH content in individual placentas (2).

In early human placentae 15-OH PGDH activity decreases between 5 and 9 weeks and then gradually increases in week 9. The activity was significantly lower than at other times during gestation. The progesterone content of the placenta changes in a similar way to PGDH activity and is lowest in week 9. On regression analysis there is a fairly close positive correlation ($r^2 = 0.69$) between PGDH activity and the concentration of progesterone in the placenta during the period studied (3). Prostaglandin dehydrogenase is under progesterone control in reproductive tissues. That is, if progesterone is raised, PGDH will be raised and PGE\textsubscript{2} will be lowered. After treatment with anti-progesterone RU 486, in vivo levels of PGDH fall in uterine tissues and PGE\textsubscript{2} will be raised (4).

It is relevant to relate anti-progesterone treatment for induction of early termination of pregnancy to nausea and vomiting of pregnancy. Epostane, the competitive inhibitor of the enzyme 3B-Hydroxysteroid dehydrogenase, therefore a specific anti-progesterone, when given in a dose of 200mg orally every 6 hours for 7 days in 50 women in the 5\textsuperscript{th} through the 8\textsuperscript{th} week of pregnancy, nausea occurred in 86% of women (5). Thirty-four early pregnant women (duration of amenorrhoea for up to 49 days) admitted to hospital for termination of their pregnancy received 25mg RU 486 (anti-progesterone, a progesterone receptor antagonist) twice or four times daily for 4 days. Seven patients (20%) had increased nausea during RU 486 treatment (6). It is reasonable to suggest that the cause of increased nausea in both instances is due to lowered progesterone activity in decidual and chorionic villus tissues, with lower prostaglandin dehydrogenase and raised PGE\textsubscript{2} tissue and maternal serum levels.
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WHY IS RAISED MATERNAL SERUM PROSTAGLANDIN E\(_2\) NOT ASSOCIATED WITH AN INCREASED INCIDENCE OF MISCARRIAGE?

Both anti-progesterones such as Mifepristone (RU 486), and Prostaglandins have achieved considerable success in their ability to induce abortion. The most effective treatment has been the administration of a primary dose of anti-progesterone and the subsequent treatment with a small dose of synthetic prostaglandin, eg. Misoprostol (1). Only a small dose of PGE\(_2\) needs to be used, a considerable advantage due to the side effects of NV (nausea and vomiting) it causes.

If a raised maternal serum PGE\(_2\) is associated with increased NVP (nausea and vomiting of pregnancy) as we suggest (2), why is this raised maternal serum PGE\(_2\) not associated with an increased incidence of miscarriage?

Prostaglandin dehydrogenase (PGDH) is the primary inactivating enzyme for prostaglandins and, therefore, controls local levels of prostaglandins. PGDH is under the control of progesterone, as we have described in this paper. As progesterone synthesis increases in pregnancy, so PGDH will also increase and inactivate PGE\(_2\). Cheng et al (3) wrote, in early pregnancy at 55 days of gestation PGDH staining in the cells of the decidual mucosa taken from a patient who had undergone surgical termination of pregnancy with no prior treatment, showed intense staining for PGDH in decidual stroma cells, in decidual glands and in the endothelial lining of cells of small blood vessels, both veins and arteries, of the decidua. In the small arteries the cells of the muscle layer were also PGDH positive, and intensely positive decidual stroma cells were arranged around the circumference of the vessel. In contrast in RU 486 treated tissue, the endothelial cell lining of small blood vessels was commonly immunonegative for PGDH or with only weak and focal reactivity, and with reduced immunoassaying for PGDH of the surrounding muscle and decidualised stroma cells. Thus, PGDH in decidual tissues would normally protect the uterine myometrium from the side effects of maternal PGE\(_2\) (3). Indeed, Bygdeman et al (4) have found that within 24 hours of the administration of Mifepristone (RU 486 anti-progesterone which lowers PGDH activity in cells) to women in early pregnancy, there is an increase in the contractility of the uterus and the uterus becomes very sensitive to exogenous prostaglandins.

In contrast, the chorionic villi from a patient after surgical termination of pregnancy stained intensely for PGDH in the cytotrophoblast cells, but with much less staining for PGDH in the syncytiotrophoblast cells (3) suggesting the Prostaglandin E\(_2\) would still be synthesised by these syncytiotrophoblast cells which bathe in maternal blood at the materno-fetal interface. This would enable the maternal serum PGE\(_2\) to be raised at this stage of pregnancy. In the natural state it is PGDH in decidual cells and small blood vessels which prevents the PGE\(_2\) from causing an abortion, although the maternal PGE\(_2\) produced by the syncytiotrophoblast cells of the chorionic villi can still be the cause of NVP.
Cheng et al (5) investigating the effect of Mifepristone (RU 486) on the immunohistochemical distribution of Prostaglandin E and its metabolite in decidual and chorionic tissue in early pregnancy wrote, there was a clear distinction in the distribution of immunoreactive PGE₂ among the different cell types. The cytoplasm of syncytiotrophoblast cells stained positively for PGE₂, whereas, there was very little, if any, staining in the cytotrophoblast layer. There was no obvious difference in staining between control villi from women who had a surgical termination of pregnancy and villi from women treated with RU 486. The intensity of PGE₂ immunostaining was greater in decidual tissue after RU 486 treatment compared to that in controls. These changes were most obvious in blood vessels (P<0.001) after only 12 hours of RU 486 treatment. Overall, PGE₂ staining of decidual stroma cells increased compared to that in controls, although changes were less marked than the changes in corresponding blood vessels. Cheng continues, the early appearance after RU 486 administration of a change in metabolism as indicated by the striking increase in PGE₂ in blood vessels, suggests that one of the main mechanisms by which progesterone ensures a continuing successful pregnancy is the maintenance of low PG levels in the vasculature (5).

In summary, the reason that raised maternal serum PGE₂ which is associated with increased NVP is not associated with an increased incidence of miscarriage, may be that the presence of PGDH in decidual cells and decidual small arteries and veins results in a low level of PGE₂ in the decidual cells and small decidual blood vessels, thus protecting the uterine myometrium from PGE₂. On the other hand, PGE₂ continues to be synthesized in syncytiotrophoblast cells of the chorionic villi, where there is very little PGDH, and which bathe in maternal blood. Released from these syncytiotrophoblast cells of the chorionic villi, PGE₂ can pass from the materno-fetal interface into the uterine veins, thence to the cerebral chemoreceptor trigger zone causing nausea and vomiting of pregnancy.
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HUMAN DECIDUA AND DECIDUALISATION

1. The formation of a specialised decidua from endometrium the normal lining of the non-pregnant uterus is called decidualisation (1).

Decidualisation includes the process of differentiation of spindle-shaped stromal cells of the endometrium into the plump secretory decidual cells, which create a pericellular and entracellular matrix rich in fibrorectin and laminin (1).

Vascularity, as well as vascular permeability, is enhanced in the decidualising endometrium (1).

It’s leukocyte population is distinct with the pressure of large endometrial granular leukocytes and B-cells are scant. These large granular lymphocytes (CD56\textsuperscript{bright}) are called “uterine NK cells” or LGL cells (1).

Role of the Human Decidua

At the maternal interface to the embryo the decidua participates in the exchanges of nutrition, gas and waste with embryo. It also protects the pregnancy from the maternal immune system. Further the decidua has to allow a very controlled invasion by the trophoblast (1).

2. Changes in human endometrium are essential to allow the establishment of pregnancy. These changes are induced in vivo by progesterone and include appearance within the tissue of a specific uterine natural killer cell characterised by an abundant expression of CD56. Changes also occur in the stroma cells, which undergo a characteristic decidualisation reaction. Decidualised stroma cells are derived from the fibroblast like cells within the endometrium which maintain their progesterone receptors in the presence of progesterone. In vitro elevated intracellular cAMP as well as progesterone is necessary for decidualisation. In vivo these changes may be provided by progesterone from the corpus luteum and by Prostoglandin E\textsubscript{2}, a stimulator of adenyl cyclase and relaxin which has been shown to be a phosphodiasterase inhibitor (2).

3. Decidual layer identified constantly at 5-6 weeks of gestation. Thickness peaked at 6-7 weeks. It was seen inconsistently at 8-9 weeks and was not identified at 10 weeks. There is a window of opportunity in the first trimester for sonographic examination of the decidua. This will allow screening at an early stage for conditions that effect the decidua during pregnancy. 105 women with uncomplicated pregnancies who later delivered at term (3).

WONG H S, CHEUNG Y K, TAIT J.
Sonographic study of decidua basalis in the first trimester pregnancy.
4. Human trophoblast cells are sensitive to lysis by IL-2 stimulated decidual NK cells. The majority of decidual white cells are potential killer cells. The major population of leucocytes present during the time that the extra villous trophoblast infiltrates the decidua are uterine large granulated lymphocytes (LGL) which constitute 70% of the decidual white cells CD+56 LGL/NK cells, together with 20% macrophages and a few CD3 + T cells 10%. The relative surface density of CD+56 is greatly increased in decidual LGL to 22 times that found in peripheral classic NK cells. Peripheral LGL’s also respond to interleukin2 (IL-2) by proliferating and becoming potent lymphokine activated killer cells capable of greatly increased killing of K562 cells. They will also kill cytotrophoblast cells (4).

5. Decidual CD-16 CD56 Bright NK cells possess a high affinity receptor for IL-2. These NK cells have both interleukin-2 receptors, alpha and beta (5). Their NK activity is markedly elevated even by treatment with small amounts of IL-2. These cells have high affinity for IL-2 (5). Therefore there needs to be suppression if IL-2 cytokine at the feto-maternal interface (5).

6. Decidual endometrium includes endometrial granulocytes was not apparent in choriocarcinoma. Fetal trophoblast antigen expression in both molar pregnancy and choriocarcinoma has been shown to obey the same general principles according to morphological and anatomical classification as previously described for normal pregnancy. Choriocarcinoma contained syncytio and cytotrophoblastic elements but no villous structure (6).
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mole and choriocarcinoma.
Fig 4: Uterine insertion of the human placenta: AV = anchoring villi, CCC = cytotrophoblast cell column, CL = villous cytotrophoblast, Langhans layer, CTS = cytotrophoblast shell, DC = cells of the maternal decidua basalis, IAC = intra-arterial trophoblast cells in the walls and lumen of the maternal spiral artery, ICT = interstitial cytotrophoblast at the insertion of the basal plate, IVS = intervillous space, Sb = syncytiotrophoblast bud, SEM = syncytiotrophoblast embolus carried by the venous blood returning from the placenta to the maternal circulation, SpA = maternal uteroplacental spiral artery, TGC = trophoblast multinucleate giant cell, UVL = uterine vein lumen, VST = syncytiotrophoblast of the chorionic villous tissue.

We have recently reviewed (Panigel et al., 1985; Panigel, 1986) the distribution in the human placenta of the cellular constituents of the maternal-fetal junctional zone in the ‘placenta bed’. These constituents are described in Fig. 4. Metabolically active trophoblast is not only found in the cellular and syncytial covering of the chorionic villi, but is also observed in the amniochorion, in the cell columns at the tip of the anchoring villi and in the ‘basal plate’ of the placenta. Giant multinucleate trophoblast cells migrate into the decidua basalis and the myometrium. In addition, endovascular or ‘intra-arterial’ cytotrophoblasts migrate against the blood stream, along the lumen and the wall of the uteroplacental spiral arteries carrying maternal blood into the intervillous space of the placenta. Finally, ‘deported’ trophoblasts disseminate through the uteroplacental veins into the maternal circulation, and embolize in the lungs.
Figure 1. Organization of the fetal–maternal interface in the 10-wk human placenta. (A) Diagram of the fetal–maternal interface showing floating villi (FV), an anchoring villus (AV) with an associated cell column, and the uterine wall. The spatial organization of this tissue (as indicated by demarcation into zones) recapitulates the differentiation of cytotrophoblasts (CTB) along the invasive pathway. Most of zone I has the structure of a floating chorionic villus: mononuclear CTB stem cells, separated from the stromal core (S) by a basement membrane (BM), fuse to form the overlying syncytiotrophoblast (STB) layer. However, at discrete sites in anchoring villi, CTB form a cell column that connects the fetal and maternal compartments of the placenta (zones II and III). The column spreads laterally in regions of contact with the uterine wall, and there is shallow penetration (this begins zone IV). The column then breaks up into clusters of CTB which penetrate the decidua layer and superficial myometrium (MY). The expression of adhesion and matrix components in these four zones is described in Results and summarized in Table III. MBV, maternal blood vessel; ENDO, endometrium. (B) and (C) Sections of a 10-wk human placental bed biopsy, embedded in JB-4 resin and stained with hematoxylin-eosin. These sections show all stages of CTB differentiation along the invasive pathway. (B) Both floating and an-

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**THE PRESENCE OF PROSTAGLANDIN E\(_2\) IN DECIDUA**

1. The mechanism by which prostaglandin E\(_2\) (PGE\(_2\)) inhibits human T-lymphocyte activation was studied. Analysis of physiological concentrations of PGE\(_2\) on interleukin-2 (IL-2) production, expression of IL-2 receptor (TAC antigen) and expression of transferrin receptor after in vitro activation with phytohaemagglutinin. PGE\(_2\) inhibited T lymphocyte proliferation by 80-90% of control values. This was associated with a similar degree of inhibition of IL-2 production while the expression of IL-2 receptor was not affected. In marked contrast to the expression of transferrin receptor which was inhibited 65% after 72 hours if in vitro activation (1).

   These studies demonstrated that PGE\(_2\) exerts its inhibitory effect on T cell activation and proliferation via two distinct pathways: inhibition of IL-2 production and inhibition of transferrin receptor expression. The transferrin receptor expression is mediated via the cAMP pathway and is IL-2 independent (1).

2. Since pregnancy impairs neither the primary or the secondary response of the mother against a heterotopic allograft the paternal or fetal allotype, the protective mechanism must be sought locally at the feto maternal interface. Early decidual stroma cells seem to represent an important suppressor cell class in the early gestation decidua. The major cell class of mediator molecules was identified as prostaglandin E\(_2\) on the basis of the following results (a) the presence of indomethacin (10\(^{-5}\)M) or varying dilutions of an anti-PGE\(_2\) antibody abrogated this suppression substantially or completely. (b) the addition of pure PGE\(_2\) (3 x 10\(^{-7}\) to 1.1 x 10\(^{-5}\)M) but not PGF\(_2\alpha\) reproduced a dose-dependent suppressor effect. (c) PGE\(_2\) levels measured in the day 4 of the mixed lymphocyte cultured cells containing decidual cells were positively correlated with the decidual cell dose or the degree of suppression.

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<tr>
<th>Menstrual Age (from LMP in weeks)</th>
<th>PGE(_2) concn in the culture ng/ml decidual tissue</th>
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<td>7.5</td>
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<td>8.5</td>
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<td>12.0</td>
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2. A strong dose dependent suppressor activity was exhibited by Ficoll-Paque separated nucleated cell preparations having a high incidence (70-94%) of typical decidual stroma cells at 6.5-9.5 weeks from LMP (2).

3. At the fetal maternal interface the physiological level of PGE₂ primarily secreted by decidual cells might be higher than 10⁸ nanograms/ml. Macrophages secrete large amounts of IL-1 and PGE₂ (3).

4. The authors have previously shown that prostaglandin (PGE₂) produced in abundance by decidual stroma cells, now show that prostaglandin E₂ promotes the migration of first trimester human extra villous trophoblast (EVT) by increasing the cellular concentration of calcium and activating calpain, furthermore CD42 silencing using siRNA inhibited PGE₂ migration of HTR-8/SV neo cells (EVT cell line) (4).

5. Conclusion. In this study we demonstrate that the inflammatory response induced by antiprogesterone in combination with prostaglandin E₂ analogue in first trimester decidua is accomplished by both increases in macrophages and neutrophils numbers and decreases in progesterone receptors (PR) and estrogen receptor A (ERA) (5).

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EXTRA CELLULAR MATRIX OF THE DECIDUA AND PROSTAGLANDIN E₂ SYNTHESIS

Tissue remodelling occurs in trophoblast population (villous, columns interstitial) is associated with changes in the constituents of the extracellular matrix and in the expression of appropriate matrix receptors by cells. In most biological systems cells require a substrate of cellular matrix proteins for anchorage and to derive traction for migration. During the first trimester of pregnancy the cellular matrix proteins laminin and fibronectin are particularly abundant in the uterus. These proteins are distributed pericellularly around each individual decidual stroma cell (1).

Trophoblast cells bind the matrix of laminin and fibronectin secreted by decidual stroma cells and this interaction is mediated by a combination of functionally active intigrin molecules (1).

It has now become clear that integrins can function as true receptors which are capable of transducing signals to the cell interior. A possible downstream effect of integrin mediated signal transduction in trophoblast cells by matrix proteins is the production of enzymes which facilitate trophoblast invasion such as a family of metalloproteinases (1). The synthesis of metalloproteinases (MMPS) is dependent on PGE₂ production (1).

Ref 1. LOKE Y W, KING A, BURROWS T D
Decidua in human implantation

There was a positive correlation between MMP and PGE₂ production. Suspended monocytes treated with soluble SIKVAV (100 nanogram/ml) peptide had a 5 fold increase in PGE₂ levels (8.5 ± 0.1ng/ml) as compared with untreated cells (1.6 ± 0.9ng/ml) (2).

Prostaglandin E₂ plays an essential role in the metabolism of metalloproteinases which are involved with laminin in the basic structure of the human decidua extracellular matrix (2).

Ref 2. CORCORAN M L, KIBBEY M C, KLEINMAN H K, WAHL L M
Laminin SIKVAV peptide induction of monocyte/macrophage prostaglandin E₂ and matrix metalloproteinases.
IMMUNOLOGY OF EARLY HUMAN PLACENTA INCLUDING FUNCTION OF DECIDUAL NK CELLS

It was generally assumed that implantation of the human trophoblast would be governed by the laws of classical transplantation immunology and that there would be a maternal T-cell response to the non-self-histocompatibility antigens of the fetal placenta. Surprisingly the situation is more complex in that implantation appears to be predominantly influenced by an unusual immune system. The mechanism of this system probably involves natural killer cells (NK cell) rather than T cells.

Trophoblast expression of MHC antigens.

The genes responsible for recognition of non-self in graft rejection are those encoding the major histocompatibility complex (MHC) antigens. HMC Class I antigens are expressed on the surface of most nucleated cells and in humans are known as human leukocyte antigens (HLA’s). Six HLA class I loci that have expressed protein products are recognised: three classical loci (HLA-A –B – C) and three non-classical loci (HLA-E –F –G). The three classic antigens are those normally expressed by nucleated cells. The population of trophoblast cells invading the uterus (collectively known as extra villous trophoblast) express at least one classical molecule (HLA-C) and one non-classical molecule (HLA-G).

The balance of evidence seem to warrant the conclusion that abundant expression of HLA-G is probably restricted to extra villous trophoblast. HLA-C was detected in extra villous trophoblast by a specific antibody. In the formulation of any hypothesis regarding immune recognition of extra villous trophoblast by the mother it is necessary to consider HLA-G and HLA-C. The overall impression is that HLA-G and HLA-C might be relatively unimportant as ligands for T cells compared to HLA-A and HLA-B. NK cells appear to be increasingly strong candidates for binding HLA-C and HLA-G.

Leukocyte population in the uterus

Analysis of leukocytes in the uterus has shown NK cells are the predominant populations. They are referred to as large granular lymphocytes (LGL’s) because of the prominent granules in their cytoplasm. The total number of these cells in the uterine mucosa varies throughout the menstrual cycle. They are sparse during the proliferative phase, increase significantly throughout the secretory phase and remain high in the decidua during the early stages of gestation. Their numbers are particularly high in the decidua basalis at the site where trophoblast cells invade the uterus. Phenotypically decidual NK cells (CD56bright CD16-) differ from NK cells in the peripheral blood.

T cells are sparse in the decidua. In humans in vitro experiments analogous to the mixed lymphocyte reaction (MLR) have shown that decidual lymphocytes (including T cells) do not proliferate in response to trophoblast. Decidual T cells appear to be relatively anergic as are lymphocytes in intestinal mucosa. It is proposed that interaction between the invading trophoblast and decidual NK cells could provide the basic mechanism for allogenic recognition of the placenta by the mother.

NK cells express receptors capable of recognising HLA class I molecules. The first family of NK receptors to be found belong to the immunological super family and is known as the killer inhibitory receptor (KIR) family because interaction of these receptors with class I HLA molecules
leads to the transmission of signals that inhibit cytolysis and cytokine production. Subsequently other members of the same receptor family killer activatory receptors (KAR’s) were observed to transmit positive signals that trigger effector functions such as cell killing or production of cytokines. The class I locus that might be most pertinent in influencing NK function is HLA-C. Two KIR’s that are specific for HLA-C alleles who recognise HLA-G lending support to the notion that HLA-G might be as universal inhibitor of cytolysis by NK cells. The repertoire of KIR’s and KAR’s has been shown to vary among individuals. Investigation accords to the idea of HLA-G triggering inhibitory signals to decidual NK effectors.

A potential in vivo scenario could be that decidual NK cells recognise HLA-C or HLA-G expressed by invading trophoblast via KIR’s or KAR’s resulting in a combination of positive and negative signals that regulate cytokine production and cytolysis. Decidual NK cells are known to secrete a variety of cytokines and trophoblast cells express receptors for many of these cytokines. This would suggest that this recognition system between maternal uterus and placenta is capable of variable outcomes in different pregnancies.

Every paragraph in this account of immunology in early human pregnancy are taken from the following reference.

LOKE Y W, KING A.
Immunology of Human Placental Implantation: Clinical implications of our current understanding.
Molecular Medicine Today. Review April 1997;3(4):153-159

As decidual NK cells are known to secrete cytokines and have receptors for these cytokines and invading trophoblast cells secrete cytokines and express receptors for many of these cytokines, these interactions at cell wall linings will have the effect that PGE$_2$ is synthesised from these cell wall linings in the decidua.

Dinarello C A
Biology of Interleukia 1

**Progesterone and Glucocorticoid Immunology Functions**

Progesterone blocks the response of T cells to allogeneic cells in the mixed lymphocyte reaction (1). Contrasting progesterone and glucocorticoids immunological reaction. Glucocorticoids besides blocking T cells and macrophage functions, inhibit prostaglandin synthesis by interfering with membrane phospholipases and decreasing arachadonic acid release. They were not able to find a similar effect of progesterone on lymphocytes (1). This may be one reason that glucocorticoids reduce symptoms of nausea and vomiting of pregnancy.

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ENDOVASCULAR TROPHOBLAST AND PGE$_2$ SYNTHESIS

Endovascular trophoblast presumably derived from the cytotrophoblast shell appears within the lumina of the decidual spiral arteries. This type of invasion seems to begin during the first month of pregnancy. The relations of endovascular trophoblast with the spiral vessel walls shows degenerative changes in the spiral vessel walls that is endothelial hypertrophy, muscular retrogression and the appearance of swollen cells that are the consequence of the invasion of the wall by the endovascular trophoblast cells (1).

A conspicuous feature of the human implantation site is the presence of trophoblast cells (endovascular trophoblast) within the lumen of decidual spiral arteries. These trophoblast plugs are continuous with the trophoblast shell occluding the orifice of the spiral arteries at the point of entry to the inter villous space (Boyd and Hamilton) (2). The plugs consist of loosely cohesive cells which move down the inner wall of the vessel like wax dripping down the side of a candle. These loose plugs are thought to act as valves allowing plasma to seep into the inter villous space at low pressures. In addition the trophoblast cells can be seen embedded in the walls of these spiral arteries together with fibroid chains of musculo-elastic tissues to form spiral vessels into low resistance sinusoid sacs which can maintain the utero-placental blood flow under all physiological conditions (3).
Clinical implications of defective implantation
An important function of the invading trophoblast is to destroy the muscular walls of the uterine spiral arteries converting them into large, flaccid vessels that are no longer capable of responding to vasoactive stimuli and are capable of high conductance (Fig. 1). This vascular transformation is essential to provide an adequate blood supply to the rapidly growing fetus and placenta. The blood flow will be compromised if there is inadequate trophoblast invasion. This can lead to a variety of clinical pathological conditions such as miscarriage, unexplained intrauterine growth retardation (IUGR), stillbirth or pre-eclampsia.

At the other end of the spectrum, trophoblast over-invasion deep into the myometrium, and even through to the peritoneum, can occur. In this condition, placenta percreta, implantation usually occurs on areas of the uterus where the decidua is deficient, such as on a scar from a previous caesarian section. This illustrates that the inherently invasive nature of trophoblast is normally modulated by the maternal decidua. Another condition in which trophoblast is abnormally invasive is hydatidiform mole, which typically arises when the blastocyst is formed by two paternal sets of chromosomes. This behaviour can be explained by the concept of genomic imprinting, which proposes that the paternal genome provides the necessary information for trophoblast growth while the maternal genome acts in an inhibitory way. Without the restraining influence of maternal genes, these pregnancies become highly invasive. Thus, implantation can be viewed as a parental “tug-of-war” where the aggressive behaviour of the fetal trophoblast is constantly kept in check by the mother.

In in vitro fertilization (IVF) programmes, it is well recognized that only 10-15% of successfully fertilized eggs placed in the uterus will produce a viable offspring. In many of these cases, there is biochemical evidence of pregnancy, indicating that the initial stages of implantation have been achieved. This suggests that the

YOKE Y W, King A
Figure 1. Immunology of human placental implantation: clinical implications of our current understanding.
Spiral arteries are functionally occluded by trophoblastic plugs. These are integral parts of the trophoblastic shell. The cohesivity between trophoblastic cells within the plugs is rather loose. Fluid and very small calibre particles can percolate through the plugs. The intra vascular trophoblast plugs do not incorporate with the vessel wall, nor do they acquire tight junctions with endothelial cells. This implies that they are loose and must act like valves. Direct observation with the chorionscope confirms this fact. Spiral arteries run a very tortuous course which is subject to continuous changes. Physiologically there are always necrotic foci within the decidua. Arterial segments also undergo necrosis. There are however new channels which are open. In serial reconstructions a given artery may be opened several times and occluded by several trophoblastic plugs. The length of a spiral artery is the same at 8 weeks as it is at term (4).

In the first few months of pregnancy endovascular trophoblast is encountered in the decidual spiral arteries but not beyond the decidual myometrial junction. At 16-18 weeks endovascular trophoblast was noticed in the myometrial segments of many spiral arteries which means a second wave of endovascular migration is triggered off quickly after a resting phase of several weeks (1).

Endovascular trophoblast cells stained positive of IL-1β(5) recombinant IL-1 stimulates PGE₂ on cell surfaces (6).

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POSSIBLE REASONS WHY NAUSEA AND VOMITING DOES NOT OCCUR IN CHORIOCARCINOMA

Human Chorionic Gonadotrophin (hCG) in Choriocarcinoma

Very raised serum and cerebrospinal fluid levels of hCG occur in choriocarcinoma in the absence of nausea and vomiting provided there are no gastrointestinal or cerebral matestases (personal communication Bagshaw KD 1997). As in choriocarcinoma high maternal serum levels of hCG did not cause nausea and vomiting it seemed unlikely that the maternal serum hCG did cause nausea and vomiting in pregnancy. However it is now known that there are major changes in the hCG beta sub unit secreted in choriocarcinoma particularly significant to this is that it is 100% nicked at the B47-48 level (1). A macrophage or circulating leukocyte elastase proteus cleaves hCG beta subunit at Val residue 44 (residue 44-45 cleavage) or glyresidue 47 (residue 47-48 cleavage) generating nicked hCG. Both nicking and dissociation eliminates hCG hormone activity (2). The combination of nicking of beta subunit and dissociation of alpha and beta units in either order leads to a rapid deactivation and clearance of hyperglycosylated hCG (2). Therefore the fact that hCG produced by choriocarcinoma cells lacks hormone activity due to being 100% nicked at residue 47-48 will explain why high levels of hCG which occur with choriocarcinoma are not associated with nausea and vomiting. It is therefore possible that regular hCG can be associated with nausea and vomiting in pregnancy.

PGE\textsubscript{2} is not or is only minimally synthesised by choriocarcinoma cells. The authors were unable to demonstrate the presence or synthesis of prostaglandins in human malignant choriocarcinoma cells (four lines BEWO, JAR, 2 and Omega 2) growing in continuous culture (3).

To determine the production of PGE\textsubscript{2} and PGF\textsubscript{2}\alpha by JEG-3 choriocarcinoma cells, the cells were incubated in either (a) control media or (b) media containing TNF-\alpha (1-20ng/ml). After 15 mins - 23 hours media were collected and analysed directly for PGF\textsubscript{2}\alpha and derivisited for the determination of PGE\textsubscript{2} as the methyloxinate derivative. TNF\alpha (1-20ng/ml) appeared to have no effect on the viability of JEG-3 cells. The effect of TNF\alpha on the formation of PGE\textsubscript{2} and PGF\textsubscript{2}\alpha was studied at 15 mins and 24 hours. Levels of PGE\textsubscript{2} and PGF\textsubscript{2}\alpha were below the limit of assay sensitivity in all cultures (1.25 and 3.1pg respectively). We were unable to demonstrate any effect of TNF-\alpha on the production of either PGE\textsubscript{2} or PGF\textsubscript{2}\alpha (4).

Decrease in Nicotinamide Adenine Dinucleotide-dependent 15-Hydroxyprostaglandin Dehydrogenase Activity (PGDH) may be important in the accumulation of PGs in neoplastic tissues. The activity of PGDH in neoplastic trophoblast tissue, namely hydatidiform mole tissues and in choriocarcinoma cells grown in monolayer culture and the activity of PGDH in term placenta which is known to be high, was compared at the same time. The specific activity of PGDH in hydatidiform mole tissue (0 to 1.2nmol 15 ketoprostaglandin E\textsubscript{2} formed x min^{-1} x mg^{-1} cytosolic protein) and in choriocarcinoma cells (BEWOline) (1.0nmol 15-ketoprostaglandin E\textsubscript{2} x min^{-1} x mg^{-1} protein) was strikingly less than that found in normal term placental tissue (11.4 ± 2.3 (SE) nmol ketoprostaglandin x min^{-1} x mg^{-1} protein). PGDH activity in neoplastic tissues was found to be one tenth or less of that in normal term human placenta (5). If choriocarcinoma cells were able to synthesise prostaglandin E\textsubscript{2} or PGF\textsubscript{2}\alpha then the very limited amounts of PGDH in these cells would allow considerable quantities of these prostaglandins to be produced. The lack of PGDH in these cells suggests that very little or no PGE\textsubscript{2} is synthesised in choriocarcinoma cells (5).
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CLINICAL FEATURES OF NAUSEA AND VOMITING OF PREGNANCY (NVP) WHICH CAN BE RELATED TO MATERNAL SERUM PROSTAGLANDIN E$_2$

There are some features present in the symptom complex of nausea and vomiting of pregnancy (NVP) which can be associated with maternal serum PGE$_2$.

Firstly, the variation in NVP from pregnancy to pregnancy, indeed no two pregnancies have exactly similar symptoms, and the variation in NVP from one pregnancy to the next in the same mother, which occurs in between 33-50% of pregnant women. These variations can be explained by the many different maternal serum concentrations of placental specific hormones and cytokines in each individual pregnancy. They stimulate the synthesis of hCG which, consequently, varies in each pregnancy. hCG itself is one of the factors which will stimulate PGE$_2$ production, which consequently differs in each pregnancy. There is also a large variation in the amount of Prostaglandin dehydrogenase (PGDH) activity between individual placentae at the same stage of gestation.

Secondly, the finding that whether NVP begins early or late, severely or mildly, it ceases on average at approximately day 84 from LMP (1) suggests that another substance, possibly PGDH activity, is required to reduce NVP.

Thirdly, the median week of peak NVP is week 9 from LMP (ration 8-10 weeks) (1). This week corresponds with the week of peak maternal hCG serum levels, and the nadir of maternal serum progesterone. High serum hCG gives maximum stimulation of maternal PGE$_2$ synthesis. Low maternal progesterone leads to reduced placental PGDH. Both raised PGE$_2$ and low PGDH will be related to increased NVP at that time of gestation.

Fourthly, the positive correlation with NVP and non-smoking status (2), agreeing with the finding of 10 other authors, can be due to the damage cigarette smoking in pregnancy causes to the placental cells, with resultant marked decrease in maternal hCG (3) and PGE$_2$.

Fifthly, we have published the paper Nausea and Vomiting of Pregnancy: An Association between Symptoms of NVP and Maternal Prostaglandin E$_2$ (4), which demonstrates a positive relationship between NVP and maternal serum PGE$_2$ levels. For each of 18 women the maternal serum PGE$_2$ was higher when she had NVP than when she had no NVP on the same day. This remained true whether the sample taken at the time she had NVP was before or after midday. However, we would not recommend any attempt to reduce maternal PGE$_2$ during early pregnancy, because of the vital functions PGE$_2$ has at that time of gestation. We are, however, of the opinion that treatment of NVP can and should be given safely and effectively as it is now in Canada and in America (2013).
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SOME FUNCTIONS OF PROSTAGLANDIN E₂ IN EARLY PREGNANCY

Although we attempt to make the case for maternal serum Prostaglandin E₂ being a cause of pregnancy sickness, we realise that no attempt should be made to reduce the synthesis of Prostaglandin E₂ in early pregnancy because of the vital functions of Prostaglandin E₂ at that time. These functions include:

A. Immunosuppression of Decidual CD-16 CD56 Bright NK Cells (LGL White Cells)

Uterine large granulated lymphocytes (LGL), constitute 70% of the decidual white cells, together with macrophages 20% and CD₃ T Cells 10%. Peripheral LGLs respond to Interleukin-2 (IL-2) by proliferating and becoming potent lymphokine activated killer cells capable of greatly increased killing of K 562 cells (1) and human trophoblast cells (2). These decidual CD-16 CD56 Bright NK cells possess a high affinity receptor for IL-2. These NK cells have both Interleukin-2 receptors alpha and beta (3). Their NK (killer) activity is markedly elevated even by treatment with small amounts of IL-2 (3). Therefore, there needs to be suppression of IL-2 at the feto-maternal interface.

Prostaglandin E₂ inhibited T lymphocyte proliferation by 80-90% of control values. This was associated with a similar degree of inhibition of IL-2 production while the expression of IL-2 receptors was not affected. This was in marked contrast to the expression of the transferrin receptor, which was inhibited 65% after 72 hours of in vitro action (4). These studies demonstrate that Prostaglandin E₂ exerts its inhibitory effect on T cell activation and proliferation via two distinct pathways; inhibition of IL-2 production and inhibition of transferrin receptor expression. The transferrin receptor expression is mediated via the c’AMP pathway and is IL-2 dependent. (4)

For additional information see section Presence of Prostaglandins E₂ in Decidua.

B. Stimulation of Cyclic AMP Levels in Immature Placental Villi

The presence of Prostaglandin E₂ in the culture medium evoked a glycogenolytic effect in immature human placental villi, 8-20 weeks placentae used, including the increase in tissue cyclic AMP levels. The effects of 10mg/ml Prostaglandin E₂ were more marked than those of hCG on activation of phosphorylase. Prostaglandin E₂ induced a larger decrease in placental glycogen and a 149% increase in cyclic AMP concentration in immature placental tissue. (5)

The fact that Prostaglandin E₂ stimulates c’AMP and glycogenolysis has significant effects on trophoblast cells as c’AMP mediates the action of a wide variety of hormones on target tissues. (6)

Prostaglandin E₂ has other functions in early pregnancy, for example, the synthesis of matrix metalloproteinases which assist cytotrophoblast invasion of the decidua is partly dependent upon PGE₂ production (7). However, the two significant functions mentioned above alone serve to show the importance of Prostaglandin E₂ in early pregnancy. Therefore, any attempt to improve pregnancy sickness by reducing Prostaglandin E₂ synthesis in early pregnancy could adversely effect that pregnancy.
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FLOW CHART OF THE POSSIBLE ASSOCIATION BETWEEN MATERNAL PROSTAGLANDIN E₂ AND NAUSEA AND VOMITING

HORMONES AND CYTOKINES WHICH STIMULATE AND RELEASE HUMAN CHORIONIC GONADOTROPHIN (HCG) FROM SYNCYTIOTROPHOBLAST CELLS

GNRH, EGF, 1-34 PTH, OT, AVP, Ca, CAMP, factors which stimulate CAMP, ie, catecholamines, HCG itself, HCG variants, PGE₂ and separately IL-1β, TNFα and M-CSF

HCG

Stimulates a dose-dependent increase of PGE₂ in 9 – 10 week placental organ cultures.

PGE₂

Vital functions of PGE₂ in early pregnancy:
1. Immunosuppression of decidual large granulated lymphocytes (LGL cells).
2. Stimulation of cyclic AMP levels in immature placental villi.

Raised maternal HCG associated with increased NVP in twins, hydatidiform moles, hyperemesis gravidarum and groups of women at 6 – 14 weeks from LMP.

PGE₂ is known to cause nausea and vomiting (NV) when given to procure a legal abortion. Raised maternal plasma levels of PGs associated with IV infusion of PGs caused increased NV. These side effects regressed rapidly when infusion was terminated.

PGE₂ is the main primary prostaglandin released from first trimester trophoblast villi 240 – 295 pg/mg/24 hours. Cytoplasm of syncytiotrophoblast cells stained positively for PGE₂ whereas there was very little if any staining in cytotrophoblast cells.

Activators which stimulate cell surface receptors also cause the release of prostaglandins from these cells membranes, eg. Interleukin-1β in 8 – 10 week placentas and HCG in 9 – 12 week placentas.

PGE₂ is only minimally synthesised by JEG-3 choriocarcinoma cells. RFWO choriocarcinoma cells PGE₂ activity is found to be one tenth or less than in term placentas.

Prostaglandin dehydrogenase (PGDH) oxidises the 15-hydroxyl group of prostaglandins to its inactive metabolite 15 keto prostaglandins. PGDH is under progesterone control. Progesterone  

in reproductive tissues. After treatment with anti-progesterone (RU486) in vivo levels of PGDH fall in uterine tissues and PGE₂ will be raised. Anti-progesterone treatment is associated with increased NVP.

Full names for all abbreviations can be found in the text. References for each statement can be found in the text.
CONCLUSION

We have attempted to review from a study of various literature articles in twenty-five different subjects written by 143 separate investigations and our own work an assessment of factors involved in the cause of nausea and vomiting in pregnancy (NVP) including the most severe symptoms of the condition hyperemesis gravidarum (HG).

We and other researchers found that maternal serum human chronic gonadotrophin, beta human chorionic gonadotrophin and progesterone levels can each be associated with the symptoms of nausea and vomiting of pregnancy, the first two showing a positive association and the latter a negative association. A study of the early development of the trophoblast demonstrates that the primary, secondary and tertiary chorionic villi are present at the end of the 5th week of pregnancy at which time the symptoms of nausea and vomiting will typically start. These chorionic villi increase to surround the whole gestational sac by 6 weeks from the first day of the last menstrual period (LMP), that is called weeks of gestation, tripling in size from 6-8 weeks of gestation. During the weeks 6-10 of gestation the symptoms of nausea and vomiting are typically at their worst. After 10 weeks of gestation two thirds of the ring of chorionic villi ceases to grow while the rest which becomes the definitive placenta continues to develop. At this stage of gestation the nausea and vomiting typically begin to reduce in severity. Therefore we need to look at these chorionic villi for the source of the symptoms.

Villos cytotrophoblast cells of the chorionic villi produce little hormone but they unite to form syncytiotrophoblast cells which synthesize and secrete large quantities of regular hormones including chorionic gonadotrophin. At the weeks 6-10 of gestation the time of increasing severe symptoms of NVP to reach their peak typically at 9-10 weeks there is an enormous increase in the total synthesis of human chorionic gonadotrophin (hCG) again demonstrating an association between hCG and NVP. There are at least 5 isoforms of regular hCG from which the more acidic long acting isoform is produced in week 6-10 of gestation. This isoform of hCG changes to the more basic less active isoform of hCG at weeks 11-15 of gestation, the most significant change occurring at 13 weeks. Weeks 12-14 of gestation are the weeks in which symptoms of NVP most often cease. The relationship between maternal serum hCG and NVP are again associated at this stage of gestation but is hCG a significantly emetic hormone? One that can cause severe nausea and vomiting in about 1.5% of all pregnant women, even in some of these pregnant women throughout their pregnancy?

In order to dig a little further into the cause of nausea and vomiting of pregnancy we need to consider hormones cytokines and eicosanoids produced by trophoblast cells which will stimulate increased synthesis of human chorionic/gonadotrophin or which human chorionic gonadotrophin will stimulate from these cells. One of the eicosanoids is very emetic namely prostaglandin E₂ (PGE₂). PGE₂ is synthesised and released when a cell wall receptor is stimulated by its activator for example “the ability of IL-1 to initiate prostaglandin synthesis is perhaps one of its most important biological properties”. Prostaglandin E₂ has been shown to be a very emetic eicosanoid when given to obtain a legal abortion in early pregnancy. Prostaglandin E₂ has been shown to be secreted from syncytiotrophoblast villous cells which bathe in maternal blood in the inter villous space and from decidual stroma cells and macrophages as well as in the decidual extracellular matrix. Professor Sincha Yagel has told us in 1998 that there is plenty of prostaglandin E₂ in the trophoblast cells during the early weeks of pregnancy. However the first stage of the breakdown of prostaglandin E₂ to its inactive metabolite involves oxidation of the 15-hydroxyl group to 15 ketoprostandin E₂ by a NAD-linked 15 hydroxyprostaglandin dehydrogenese (PGDH). PGDH is present in syncytiotrophoblast cells of the chorionic villi, in the decidua and in the extracellular matrix in
early human pregnancy. In normal pregnancy PGDH, which is under progesterone control, falls in mean placental values in gestational weeks 5-9 then gradually rises to weeks 15-16. There is a ten fold increase in placental values between gestational weeks 7-15. There is also a large variation in PGDH activity between individual placentae for each early gestational age.

We have presented an investigation demonstrating a statistically significant association between maternal PGE2 levels and nausea and vomiting of pregnancy symptoms between weeks 7-9 of pregnancy (P <0.001). The result for each woman (number 18) showed a raised maternal PGE2 serum level in the symptomatic sample versus the control maternal serum PGE2 level on the same day. The results could not have been due to any diurnal variation of serum PGE2 because eight experimental samples were taken in the mornings and ten taken after mid-day. These results showing an association between prostaglandin E2 maternal serum levels and nausea and vomiting of pregnancy need confirmation in larger studies. However prostaglandin E2 plays such a vital role in early pregnancy that suppressing that eicosanoid at this stage of pregnancy might have adverse effects for the welfare of the pregnancy.

There is general agreement that maternal human chorionic gonadotrophin serum levels and βeta human chorionic gonadotrophin maternal serum levels can be associated with the cause of nausea and vomiting of pregnancy but are these hormones significantly emetic in nature to cause the variation of degree in pregnancy nausea and vomiting which occur possibly throughout all three trimesters of pregnancy? However prostaglandin E2 is an eicosanoid which is known to be a strong emetic substance when given to obtain legal abortion in early pregnancy. Could the finding that gonadotrophin stimulates the secretion of prostaglandin E2 from syncytiotrophoblast villous cells and from decidual cells and the metabolism of prostaglandin E2 by prostaglandin dehydrogenase, be a link to the cause of the various degrees of severity of episodic nausea and vomiting of pregnancy and the cessation of these symptoms in pregnancy. Certainly these symptoms are awful for nearly 30% of pregnancy women who develop nausea and vomiting and for about 1.5% of all pregnant women who suffer from the extreme form of this nausea and vomiting called hyperemesis gravidarum. We know from our experience working with the charity Pregnancy Sickness Support that those pregnant women would be most grateful if more sympathetic heed and investigation were given to the cause and treatment of this severe nausea and vomiting of pregnancy. In fact these symptoms are for them a frightening problem which may abolish their natural joy of being pregnant and even cause some women to have no further pregnancies, or worst of all a termination of their pregnancy.
APPENDIX A:

FOUR SHORT ARTICLES CONCERNING ASPECTS OF NAUSEA AND VOMITING.

An outline account of the anatomy of vomiting

Borison and Wang wrote an article entitled Physiology and Pharmacology of vomiting in 1953 in which they explained the anatomy of the vomiting centres.

They elicited vomiting in the cat by electrical stimulation of the lateral reticular formation in the immediate vicinity of the fasciculus solitaries. No other portion of the lower brainstem yielded such responses. At that time most physiologists had agreed only to the existence of the vomiting centre and not to its precise location in the medulla oblongata. This vomiting was elicited by electrical stimulation without prior retching following the short latency required for maximal inspiration and it was continuous for a period of five to fifteen seconds of stimulation. In a study of chronic dogs Wang and Borison found that superficial medullary lesions abolished the emetic response to intravenous apomorphine, certain cardiac glycosides (including digitalis) without disturbing the response to oral copper sulphate, whereas deeper lesions which also involved the lateral reticular formation impaired the responsiveness of oral copper sulphate as well as to the intravenous apomorphine. These results were interpreted to mean that the vomiting centre is situated in the dorsal portion of the lateral reticular formation and that there exists in the medullary surface a specialised chemoreceptor trigger zone which serves as a receptor site for certain central emetic agents(1).

The importance of this finding is that digitalis the most significant drug for treating heart conditions such as auricular fibrillation or congestive cardiac failure, because it increases the force of the myocardial contractions, had the unfortunate side effect of causing nausea and vomiting. It is an interesting story that other researchers thought that the nausea and vomiting must have originated from the heart because it was so effective at treating conditions of the heart, or another possibility because the digitalis was concentrated in liver cells, these symptoms of vomiting arose from the liver. Borison and Wang’s interpretation of a chemoreceptor trigger zone which serves as the receptor site for certain emetic agents has proved correct (1).

The Human chemoreceptive trigger zone has been localised in the area of postrema (AP) in the floor of the fourth ventricle and has both a blood supply and is in contact with the cerebrospinal fluid. The vomiting centre itself is situated in the medulla oblongata can be stimulated directly by certain chemicals such as copper sulphate, but hormones or chemicals which stimulate the chemoreceptor trigger zone also effect the vomiting centre via nervous elements and connections in the mid-brain. The final act of vomiting or retching being controlled by the vomiting centre(2).

As the chemoreceptor trigger zone is outside the blood brain barrier. Drugs which activate this centre have fewer side effects such as sedation and extrapyramidal effects which can induce incoordinated muscle spasms. All the substances that excite the AP neurones are also emetic in the dog (3). The response to all excitatory substances (except glutamate) were similar long latency relatively low mixed discharge frequency, and very long duration (3). The ultimate experiment was done in humans, Lindstrom treated five humans who suffered from intractable nausea and vomiting with local AP ablation and all patients experienced total relief of symptoms(4).

AP neurones are excited by serotonin, (the urinary excretion of serotonin is not associated with the intensity of nausea and pregnancy(5)) Histamine, Epinephrine and Norepinephrine about half the time and at least for histamine and Epinephrine these responses are complicated in that some neurones show inhibitory responses to these substances (3).

To test the hypothesis that circulating emetic substances activate neurones in the AP, the effects
of prostaglandins on electrical activity of neurones in canine area postrema were studied by Briggs and Carpenter using the techniques of extra cellular recording with iontophoresis. Excitatory responses were obtained upon application of prostaglandin A1, B1, B2, E1, F1 Alpha, F2 Alpha in between 24 and 50% of the cells studied. The percentage excitation was greatest in prostaglandin E1 47% and prostaglandin F2 Alpha 50%. The excitation was very similar in pattern to that observed for apomorphine, biogenic amines and several neuropeptides in that it had a relatively long latency, low frequency and prolonged duration. Since the area postrema is known to play a central receptive role in initiating emesis to circulating toxins these results suggest that prostaglandins may play a role in the initiation of some forms of emesis(6).

Prostaglandins arise in tissue after irradiation and the time course of increase is very similar to that of radiation induced emesis in that there is an initial peak of one-four hours often followed by a subsequent fall. The neurones of the AP could be excited by circulating prostaglandins evoking emesis (7).

The results are consistent with a possibility that c’amp is the common second messenger for common excitatory substances (3). A common messenger is probably the reason for all these substances having the same effect. The majority of cells were excited by eight-bromo-c’amp as well as forskolin an activator of adenyl cyclase. All twenty one substances effect cells mediated by a common second messenger c’amp (3).

A prolonged action from the brief application of a transmitter onto a single neurone is usual. These observations suggest the possibility that responses to all of these substances have a common step or are mediated through a common second messenger. The responses are indeed mediated through c’amp. Pretreatment of an animal with phosphodiasterase inhibitors should retard the breakdown of c’amp and if involved in mediation of the response should reduce the threshold dose necessary to induce emesis. We tested theophylline. Thresholds of all substances were reduced (8).

Multiple applications of several substances would cause the neurone to become spontaneously active at a low frequency; this spontaneous discharge would be maintained for many minutes (8).

One of the major difficulties of the study of the emetic reflex is its great variability among animal species (9). Rodents do not vomit at all and the sensitivity of other species various considerably. Man and dog are clearly among the most sensitive species to emesis from humoral agents, while both show emesis in response to motion, ionising radiation and drugs for cancer chemotherapy. Monkey and cat are considerably less sensitive to most of these stimuli (9).
References For An outline account of the anatomy of vomiting


APPENDIX A:

Vomiting Centre Excitation

1. In animals with ablation of the area postrema (AP), vomiting seen in control animals upon IV administration of a variety of drugs is abolished, as in the vomiting that follows motion, ionising irradiation and chemotherapeutic drugs, at least in the species like the dog, which show emetic responses similar to that of humans (1).

2. The AP, which lies outside the blood brain barrier, has the role of surveying the blood for noxious substances (1).

3. All the substances that excite the AP neurones are also emetic in the dog (1).

4. The ultimate experiment was done in humans (2). Lindstrom treated 5 humans who suffered from intractable nausea and vomiting, with local AP ablation and all patients experience total relief of these symptoms.

5. The response to all excitatory substances (except glutamate) were similar, long latency, relatively low mixed discharge frequency and a very long duration (1)

6. A prolonged action from the brief application of a transmitter on to a single neurone is usual. These observations suggest the possibility that responses to all of these substances have a common step or are mediated through a common second messenger (3). The responses are indeed mediated through C’AMP. Pre-treatment of an animal with a phosphodiasterase inhibitor should retard the breakdown of cyclic AMP and if involved in mediation of the response, should reduce the threshold dose necessary to induce emesis. We tested theophylline. Thresholds for all substances tested were reduced (3).

7. Our results are consistent with the possibility that C’AMP is the common second messenger for these common excitatory substances. (1)

8. AP neurones were excited by serotonin (the urinary excretion of serotonin is not associated with the intensity of NVP (2)) Histamine, Epinephrine and Norepinephrine about half the time and at least for Histamine and Epinephrine, these responses are complicated in that some neurones show inhibitory responses to these substances (1).

9. The percentage of responses from AP cells is so high, an average 50% of neurones studied responded to the excitatory substances, the rate of responsiveness is so high as to suggest that all of the cells have all of the receptors (1).
10. Dogs with bilateral subphrenic vagotomy show no change in their sensitivity of IV Apomorphin and vomited after irradiation with a pattern almost identical to that of the controls. Therefore, radiation induced emesis is humorally mediated through some agent released into the circulation subsequent to irradiation (4).

11. The effects of PH on electrical activity of neurones in the canine AP were studied. Excitatory responses were obtained upon application of PGs A1, B1, B2, F1a and F2a in between 25-50% of cells studied. The excitation was very similar in pattern to that observed to apomorphine, in that it had a relatively long latency, low maximal frequency and prolonged duration (60 seconds). Since the AP is known to play a central receptive role in initiating emesis to circulation toxins, these results suggest that prostaglandins may play a role in the initiation of some forms of emesis (5).

12. Glucose application to 16 cells and zinc to 14 cells never had any effect (6).

13. Frequently multiple applications of several substances would cause the neurone to become spontaneously active at a low frequency; this spontaneous discharge would be maintained for many minutes (3).

14. The area postrema of the human brain does not have a blood brain barrier and is known to be involved in nausea and vomiting reflexes. It contains LH/hCG receptors which suggests they are involved in nausea and vomiting of pregnancy (7).

References for vomiting centre excitation


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APPENDIX A:

Clinical and treatment similarities for nausea and vomiting associated with palliative care and the nausea and vomiting associated with pregnancy (NVP).

In one study related to palliative care 62% have both nausea and vomiting 34% had nausea alone and 4% had isolated vomiting (1). In one study of nausea and vomiting of pregnancy 73% had nausea with vomiting, 32% had nausea alone and 0.6% had vomiting alone.

In the palliative care study, nausea was aggravated by movements and alleviated by resting (1) a most significant way to improve nausea and vomiting of pregnancy is getting more rest (2 section 39).

Nausea and vomiting have been reported to be frequently undertreated in palliative care and surprisingly it is patients with more severe symptoms who often miss out on anti-emetic treatment (1). If you heard that a women might be left to suffer from nausea and vomiting for at least a couple of months without any nursing or medical attention you would perturbed. This is exactly what happens when women suffer from severe pregnancy sickness (2 section 42).

Nausea and vomiting rarely occur in isolation but tend to occur with other symptoms for example fatigue, drowsiness, lack of energy or lack of appetite (1). This is similar to nausea and vomiting of pregnancy when in the most severe symptoms patients are unable to get out of bed due to their severe vomiting plus fatigue, lack of energy and become depressed due to the constant awful nausea they suffer (2 section 42).

The history of nausea should include questions which aid determination of the cause of the nausea in palliative care. Intermittent nausea associated with early satiety and postprandial fullness or bloating. Nausea is relived by vomiting which is usually small volume, occasionally forceful and may contain food. This clinical picture suggests impaired gastric emptying in both palliative care and pregnancy nausea and vomiting(1),(2). In either group of patients nausea is aggravated by sight and smell of food or relived by vomiting again similar in both conditions(1),(2). This suggests chemical causes activating chemoreceptor trigger zone(1),(2).

The picture of the treatments of nausea and vomiting associated with palliative care is very similar to these same treatments of pregnancy (NVP). These substances and their affects are well expressed by the table 4 in the article nausea and vomiting palliative care (1). Incidentally their definition of nausea is very appropriate, the sensation that immediately precedes vomiting. One or two additions in their script which goes with that table. Prokinetic agents stimulates the upper gastrointestinal tract, block aid 5HT3 receptors releasing a break on gastric emptying. These agents include metaclopromide (maxolon) which is a dopamine antagonist(1). Glare and his colleagues make two points, first the dose of metaclopromide is 10mgs three of four times daily. The dopamine antagonist affect is only achieved with high doses 10mgs every four-six hours orally. Secondly, in America the food and drug administration state administration of metaclopromide beyond a twelve week period is not recommended in elderly patients due to side effects of restlessness, drowsiness and fatigue. Domperidone is a similar drug to metaclopromide but has the advantage that it does not cross the blood brain barrier so only acts on peripheral dopemine receptors releasing the dopeminergic brake. As a result side effects particularly uncoordinated movements are much less likely to occur. However cardiac toxicity associated with QT prolongation may occur.

Phenothiazines are antipsychotic agents which block D2 receptors in the chemoreceptor trigger zone, also blocking histamine serotonergic and alpha-adrenergic receptors. They may cause side effects notably sedation, hypotension and extrapyramidal symptoms such as in coordination with muscle spasms. When given to elderly patients who have dementia with aggression they were found to increase the incidence of deep vein thrombosis and fatal pulmonary embolism. This has to
be borne in mind when treating nausea and vomiting of pregnancy but these clinical conditions have not been recorded as complications when treating NVP. The dosage of prochlorperazine (stemetil) is 5-10mgs tds orally.

**Antihistamines.** First generation H1 receptor antagonist block H1 receptors in the medullary vomiting centre and in the Chemoreceptor trigger zone. Cyclizine 50mgs 1tds (valoid) or Avomine 25mgs are the most frequently used H1 receptor antagonist antihistamines in treating NVP in this country as Diclectin is not at present available here. Avomine (Promethazine Theoclate) is available in a dose of 25mgs two at night plus one twice during the day. The side effect is sedation, but tolerance to sedation usually develops in a few days. Phenergan another Promethazine Maleate usually causes more sedation than Avomine.

**Pyridoxine= Vitamin B6** has been shown to be effective in treating the nausea of NVP shown in two randomised controlled trials (3,4). The safety of pyridoxine was confirmed in a cohort study which found no association with major malformations (5). Pyridoxine has been shown to be non-teratogenic when combined with Doxylamine in the tablet called diclectin 10mgs of each up to four times a day. Pyridoxine may be used to treat NVP on its own up to 10mgs one four times daily. In that dosage it causes no significant side effects and no increased risk of a foetal abnormality(6).

**Selective 5HT3 receptor antagonists.** Ondansetron is the oldest selective 5HT3 receptor antagonist. It exerts its antiemetic effects via blockaid of peripheral and central 5HT3 receptors. These receptors are found in the vagus nerve which feeds into the emetic centre, in the nucleus tractus solitaries and the chemoreceptor trigger zone(1). The 5HT3 receptor antagonists block the amplifying effect of serotonin on the vagus nerve(1). They are primary used for chemotherapy induced emesis but are increasingly used to treat nausea and vomiting of pregnancy particularly in America(7). A recent paper published in the New England Journal Of Medicine by Pasternak B et al found that in 1233 women exposed to ondansetron after 6 weeks from LMP caused no increase risk of any major birth defect(7).

**Corticosteroids** have chiefly been studied as anti-emetics in chemotherapy induced emesis but are also used in treatment of severe nausea and vomiting of pregnancy (NVP) and hyperemesis gravidarum (HG). Jarvis S and colleague in their article management of nausea and vomiting published in the BMJ(8) state one small RCT showed that compared with placebo corticosteroids improved symptoms with reduced dependence on intravenous fluids. This treatment is best undertaken in secondary care so that the dosage regime can be carefully monitored with maternal blood electrolytes controlled. There is some evidence steroids are best used only after the end of the tenth week from LMP to avoid a small increased risk of oral clefting. They should only be used for intractable cases of severe hyperemesis gravidarum cases after other treatments have been unsuccessful.
Glare P, Miller J, Nikolova T, Tickoo R.
Treating nausea and vomiting in palliative care: A review.

### Table 4 Receptor site affinities of commonly used antiemetics

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dopamine antagonist</th>
<th>Histamine antagonist</th>
<th>Acetylcholine (muscarinic) antagonist</th>
<th>Serotonin type 2 antagonist</th>
<th>Serotonin type 3 antagonist</th>
<th>Serotonin type 4 agonist</th>
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<tbody>
<tr>
<td>Chlorpromazine</td>
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<td>Cisapride</td>
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<td>Cyclizine</td>
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<td>Domperidone</td>
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<td>Haloperidol</td>
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<td>Hyoscine</td>
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<td>Levomepromazine</td>
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<tr>
<td>Metoclopramide</td>
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<tr>
<td>Ondansetron</td>
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<td>Prochlorperazine</td>
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<tr>
<td>Promethazine</td>
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</table>

**Notes:** Black, high affinity for receptor; dark gray, moderate affinity; light gray, low affinity; white, no known affinity
References for Clinical and Treatment Similarities of Nausea And Vomiting associated with palliative care and the nausea and vomiting associated with pregnancy (NVP).


APPENDIX A:

**Nausea and vomiting related to age, sex, postoperative recovery and chemotherapy**

Recent evidence shows that postoperative recovery may differ between men and women. The authors planned a prospective cohort study to examine the impact of gender or postoperative outcome. Secondary endpoints included the incidence of complications one of which was postoperative nausea and vomiting. The men (N=200) and women (N=222) in the authors study were similar in terms of age, American society anaesthesiologists physical status and likely to have a history of postoperative nausea and vomiting, 42 (19%) women versus 18(7.4%) men (P<0.001) and to have received prophylactic antiemetic agents 102 (46%) women versus 70 (29%) men (P<0.001) (1). The higher incidence of some complications among women, maybe attributable to greater willingness to report them, however participants in the study were directly questioned about nausea rather than being obliged to mention them without promoting. This makes it more likely that the differences in nausea and vomiting between the sexes are genuine and important (1).

There is little available data on the incidence and causes of vomiting and nausea in an otherwise healthy population. Therefore the authors of this article decided to undertake a survey to examine the influence of age and sex on the emetic response. 596 participants completed questionnaires. There were 65% female and 35% male participants ages ranging from 18-91 years. The overall incidence of vomiting and nausea at least once in the twelve months prior to completing the questionnaire were 39%-54% respectively. There was no significant association between the incidence of symptoms of gender although a higher frequency of nausea but not vomiting was reported amongst women as compared to men (P<0.005). In contrast when the population was grouped according to age 18-30 years N=215, 31-60 years N=197, >60 years N=185. There was a highly significant decrease in the incidence of both vomiting (P<0.0001) and nausea (P<0.0001) with increasing age (2). Postmenopausal women (N=175) were less likely to vomit (P<0.0001) or feel nauseous (P<0.0001) compared with those women who were still menstruating N=217(2). The results show an age dependency in the incidence of emesis and support the evidence that many autonomic functions become altered with age (2).

Age, sex, previous exposure to chemotherapy, type of chemotherapy, history of alcohol intake and susceptibility to motion sickness have all been proposed as predictors of chemotherapy induced emesis (3). Recently the authors analyzed the role of the new prognostic factor in chemotherapy induced emesis in female patients receiving a first cause of FAC adjuvant chemotherapy; the intensity of emesis during past pregnancy (EDPP)(3). 113 breast cancer patients receiving a first course of FAC adjuvant chemotherapy in an out patients setting entered the study. The antiemetic treatment was identical in all patients. The patients were classified in one of the following groups according to the intensity of emesis during past pregnancy (EDPP).
Intensity of emesis in the first course of FAC chemotherapy according to EDPP grade.

<table>
<thead>
<tr>
<th>EDPP Grade</th>
<th>FAC induced emesis</th>
<th>Number of patients with</th>
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<tbody>
<tr>
<td>0</td>
<td>0 vomiting 25(81%)</td>
<td>1-5 vomiting 5(16%)</td>
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<tr>
<td>1</td>
<td>16(57%)</td>
<td>10(36%)</td>
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<tr>
<td>2</td>
<td>10(36%)</td>
<td>5(18%)</td>
</tr>
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</table>

EDPP= Intensity of emesis during past pregnancy.

A. Grade 0 EDPP no nausea and vomiting during any pregnancy.
B. Grade 1 EDPP nausea and vomiting limited to the early morning only in the first trimester of pregnancy (normal emesis gravidarum).
C. Grade 2 EDPP nausea and or vomiting present during the entire day and/or extended to the second or third trimester in at least one pregnancy (pathological emesis gravidarum)(3).

The mean age of the 87 evaluable patients was similar in the three groups (mean age 54, mean age 55, mean age 50 groups ABC respectively.

The intensity of emesis in the first course of FAC chemotherapy in these three groups shows a statistical analysis a significant positive correlation between the intensity of EDPP and the intensity of FAC induced emesis (P<0.001). Eighty one percent of patients with grade 0 EDPP obtained complete protection from FAC induced emesis while respective figures for grades 1 and 2 were 57% and 36% (3). This study suggests that EDPP may be an important prognostic factor in FAC induced emesis and could be useful as a clinical marker for those patients who require a more intensive antiemetic treatment(3).
Nausea and vomiting related to age, sex, postoperative recovery and chemotherapy
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APPENDIX B: FLOWCHART - HCG STIMULATION AND RELEASE

HCG is released by the placenta during pregnancy, stimulating the synthesis and release of PGE2. PGE2, in turn, regulates the release of LH and FSH, which are essential for ovulation and implantation.

PGE2 synthesis is regulated by a complex interplay of factors, including prostaglandin synthase (PGS) activity, which is increased during pregnancy. The release of PGE2 is also influenced by the levels of estrogen and progesterone in the maternal circulation.

The flowchart illustrates the key steps in the HCG stimulation and release process, highlighting the roles of PGE2 and other signaling molecules in the regulation of fertility.

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