STAGE 4

Probably approximately 0.1-0.2 mm in diameter
Approximately 5-6 postovulatory days
Characteristic feature: attaching blastocyst

Stage 4, the onset of implantation, is reserved for the attaching blastocyst, which is probably 5-6 days old.

Although the criteria for the first three stages are those of the first three horizons, it has not proved practicable in stages 4-10 to retain the criteria for horizons IV-X. This abandonment had already been begun by Hertig, Rock, and Adams (1956) and by Heuser and Corner (1957).

It should be noted that certain specimens that are now listed in stages 5a and 5b (Hertig, Rock, and Adams, 1956) were formerly included in horizon IV. In other words, because such specimens represent "significantly different stages of development" (ibid.), they have been transferred, and, as a result, stage 4 has been made more restricted. Healing of the uterine epithelium over the conceptus, for example, is too variable and has been eliminated as a criterion for stage 5; it usually occurs after horizon VI has begun (Böving, 1965).

Implantation is the specific process that leads to the formation of a specialized, intimate cellular contact between the trophoblast and the endometrium, or other tissue in the case of ectopic implantation (Denker, 1983).

Implantation is a highly complicated and ill-understood phenomenon "by which the conceptus is transported to its site of attachment, held there, oriented properly, and then attached by adhesion, trophoblastic penetration, spread, proliferation, envelopment of vessels, and other developments of the placenta, both conceptal and maternal parts" (Boving, 1963). In this broad sense, implantation includes at least stages 4 and 5.

Implantation, then, includes (1) dissolution of the zona pellucida, and contact and attachment (adhesion) between the blastocyst and the endometrium, (2) penetration, and (3) migration of the blastocyst through the endometrium. On the basis of comparative studies, it has been suggested (Boving, 1965) that stage 4 might be subdivided into these three phases. Human (but not macaque) implantation is interstitial in type: i.e., the blastocyst comes to lie entirely within the substance of the endometrium. In the human (as also in the macaque), implantation occurs into an edematous, non-deciduous endometrium. In other words, decidualization takes place at the end of implantation.

In his important study of the early development of the primates, Hill (1932) concluded as follows:

> The outstanding feature of the early human blastocyst is its extraordinary precocity as exemplified in the relations it very early acquires to the uterine lining and in the remarkably early differentiation of its trophoblast and its extra-embryonal mesoderm. It is no longer content to undergo its development in the uterine lumen as does that of all the lower Primates, but, whilst still quite minute, burrows its way through the uterine epithelium and implants itself in the very vascular subepithelial decidual tissue of the uterus. Therein it forms for itself a decidual cavity and undergoes its subsequent development, completely embedded in the maternal tissue. In this way the Primate germ reaches the acme of its endeavour to maintain itself in the uterus and to obtain an adequate supply of nutriment at the earliest possible moment.

The mammalian stage 2 organism and the early blastocyst are surrounded by an intact zona pellucida, which disappears at the beginning of implantation. Hence, implantation "is taken as beginning when the zona pellucida is lost and the trophoblast is in contact with the uterine epithelium throughout its circumference" (Young, Whicher, and Potts, 1968). Although claims have been made that the blastocyst emerges from its zona pellucida by "shedding" or by "hatching," it has also been maintained that, at least in the mouse, the zona undergoes rapid dissolution all around the blastocyst *in situ*, that is, at the actual site of implantation.

After the blastocyst becomes attached at random to the uterine epithelium in the mouse, it is believed that the inner cell mass can travel around the inside of the trophoblastic shell (presumably somewhat like a satellite gear). Although the nature of the stimulus responsible for the final orientation of the inner cell mass is unknown, it is postulated that the final position is determined either by a morphogenetic gradient across the vertical axis of the uterus or by changes in the trophoblast associated with its attachment to the underlying tissues, or by both.

The implantation site has been studied by electron microscopy in several mammals, such as the mouse. The cell membranes of the trophoblast and uterine epithelium become intimately related, and large cytoplasmic inclusions are found in the trophoblastic cells. The ultrastructural changes taking place at implantation suggest that there may be a high degree of permeability between maternal and embryonic cells. In addition, there may be an exchange of cellular material between uterus and embryo.

After the zona pellucida has become dissolved, the surface membranes of the trophoblast and uterine epithelium are separated by a very narrow interval (in the mouse). This first morphological sign of implantation can be detected only by electron microscopy.

In at least some macaque specimens a distinction between cytotrophoblast and syncytiotrophoblast can be made (Heuser and Streeter, 1941, e.g., No. C-520, their fig. 38). Moreover, amniogenic cells have been claimed to be "separating from the trophoblast above and distinct from the germ disk" (ibid., No. C-610, their fig. 53). Finally, epiblastic cells and hypoblast can be distinguished ***(ibid.,*** No. C-520, their fig. 40).

Of the several macaque specimens of stage 4 that have been described, in one instance (No. C-560), the uterine epithelium at the site of attachment showed a disturbed arrangement of its nuclei. Moreover, the cytoplasm had become paler, which was taken to indicate beginning cytolysis. In the conceptus, the site of attachment was formed by syncytiotrophoblast, which is initially formed by the coalescence of polar trophoblast (Hertig, 1968). Some of the increased number of nuclei appeared to have been released from the uterine epithelium and then engulfed by the rapidly expanding trophoblast. Within the cavity of the blastocyst, disintegrating embryonic cells were interpreted as a mechanical accident caused by displacement of the cells.

At the site of attachment, fused multinucleated cells of the uterine epithelium constitute a "symplasma," which fuses with the syncytiotrophoblast (in macaque No. C-610, and also in the rabbit; in the latter it has been studied by electron microscopy by Larsen, 1970).

A specimen of stage 4 in the baboon has been illustrated (Hendrickx, 1971). The single layer of abembryonic trophoblast was continuous with the cytotrophoblast dorsally. The syncytiotrophoblast was in contact with the uterine epithelium, which had lost its columnar appearance. Moreover, as much as one-half of the surface portion of the uterine cells had disappeared at the site of attachment. The inner cell mass showed occasional "endoblastic" cells bordering the blastocystic cavity. The age of the specimen was estimated as 9 days.

Specimen of Stage 4 Already Described

In the human, the only report of stage 4 seems to be two not altogether satisfactory photographs in a Supplement to ***Ovum humanum*** (Shettles, 1960, figs. 65 and 66). One illustration is captioned "attachment of the blastocyst to the uterine epithelium during the sixth day after ovulation. The encapsulating zona pellucida has disappeared." The second figure is a high-power view to show that "pseudopodia-like protoplasmic projections from blastocyst traverse the adjacent zona pellucida at area of contact with endometrium."

No specimen of stage 4 in the human has been recorded that would be comparable to those in the macaque illustrated by Heuser and Streeter (1941, plate 3, fig. 31, and plate 5, fig. ***48).***