Studies on the Early Development and Implantation in the Cat:

1. Cleavage and Blastocyst Formation

By H.-W. Denker[1], L. A. Eng[2], U. Mootz[1] and C. E. Hamner[2]

[1]Abteilung Anatomie der Medizinischen Fakultät an der Rheinisch-Westfälischen Technischen Hochschule Aachen, F. R. G., and [2]Department of Obstetrics and Gynecology, University of Virginia School of Medicine, Charlottesville, Virginia, U.S.A.

With 2 Figures and 2 Tables

Abstract

This investigation persues 2 goals: 1. to give data on the time course of cleavage and blastocyst development in the cat, and 2. to study the differentiation of trophoblast and embryonic knot cells during cleavage and blastocyst formation in this species.

Timed matings under standardized laboratory conditions are used in order to obtain exactly dated embryonic stages which are described in detail. It is found that all preimplantation stages take comparatively long in the cat, i.e. roughly twice as long as in the rabbit; e.g. the embryos enter the uterus at 6 (—7) days post coitum when they are in the morula or pre-blastocyst stage.

After fixation in formol-alcohol-acetic acid which extracts certain fractions of proteins, it is found possible to distinguish, in cleavage stages, 2 categories of blastomeres which differ in density of remaining cytoplasmatic protein as well as in cell polarity. Using serial sections and comparing successive stages of development it is concluded that this technique allows to identify progenitors of trophoblast and embryonic knot cells in early cleavage stages of the cat in basically the same manner as in the rabbit, although morphological details tend to be obscured by copious lipid vacuoles in the cat and differential staining of blastomeres is very obvious only in a proportion of the embryos. Of particular interest is the observation that the presumptive trophoblast cells are grouped together as a polar cap. If we assume that the divergent properties of the described 2 groups of blastomeres have some physiological significance, the polar arrangement is incompatible with the "*inside-outside theory*" of determination of embryonic knot and trophoblast cells favored recently. This rather points to a preexisting polarity of the early embryo. In order to take into account conflicting results of, on one hand, histochemical findings of this type as well as the results of deletion experiments, and on the other hand transplantation experiments which favor the "*inside-outside model*", a unifying hypothesis is discussed which postulates the concerted action of both environmental/positional (epigenetic) factors and a preexisting pattern of intrinsic morphogenetic factors of the egg.

Introduction

The mechanism of determination of trophoblast and embryonic knot cells of the mammalian blastocyst has recently received much attention, in particular because blastomere transplantation and embryo fusion experiments have revealed vast regulative capabilities of mammalian cleavage stage embryos (for review see Denker 1976). Detailed analysis of chimeric embryos produced by appropriate fusion proce-

30*

dures has impressively shown that, in rodents, up to at least the 8-cell stage all blasto-
meres can be forced to differentiate into either trophoblast or embryonic knot cells,
their fate depending on their position in the resulting compound embryo: outside cells
become trophoblast while enveloped cells will form the embryonic knot. It was as-
sumed, therefore, that also in regular development, the inside or outside position of
blastomeres provides the determinative stimulus (*"inside-outside model"*, Mintz,
Tarkowski; for review see Herbert, Graham 1974; Denker 1976). The validity of
the theory has been questioned, however, on the basis of conflicting results obtained
by cell isolation experiments and by histochemical studies (for review see Mulnard
1966; Denker 1976). A histochemical investigation of cleavage stages and early
blastocysts of the rabbit gave evidence for an early segregation of presumptive tropho-
blast and embryonic knot cells. The most significant result was that the presumptive
trophoblast cells are arranged in a cap or cup-like manner on *one pole of the morula*
rather than completely surrounding the other blastomeres (which will later from the
embryonic knot) (Denker 1970b, 1971, 1976). This finding is inconsistent with the
„*inside-outside model*" which implies that all outer cells become determined to form
trophoblast.

In the rabbit, the 2 groups of blastomeres were found to differ, after fixation with
formol-alcohol-acetic acid (FAA), in their density of cytoplasmic protein stainable
with Hg-bromophenol blue (HgBPB). It seemed to be of great interest to investigate
whether this type of cytochemical differences can also be demonstrated in other
species. While the FAA-HgBPB technique failed to reveal clear-cut differences be-
tween blastomeres in mouse and hamster cleavage stages (Denker 1972), cat em-
bryos again do give evidence for an early segregation of presumptive trophoblast
and embryonic knot cells as described in the present communication.

Material and Methods

Sexually mature female and male cats were kept in individual cages under standardized con-
ditions (light phase 15 hours, dark phase 9 hours) and were mated as described before (Hamner,
Jennings, Sojka 1970; Sojka, Jennings, Hamner 1970). The estrous cycle of the females was
observed by taking daily vaginal smears. At estrus, they were mated twice to a tame trained
male, followed by intravenous injection of 100 IE of HCG (Follutein, E. R. Squibb & Sons) to
ensure ovulation.

Cats No. 6 and 9 were superovulated by giving daily intramuscular injections of PMS (Gestyl,
Organon) for 6 days (150 IE, 100 IE, 4 times 50 IE) followed by mating and HCG injections as
described above.

At the desired stage (time counted from the first coitus and HCG injection, and given as days
or hours post coitum = d p.c. or h p.c.), the animals were sacrificed by an overdose of sodium
pentobarbital, the genital tract was quickly removed and flushed with 38 °C warm 0.9 % saline
solution to recover embryos. Morulae and blastocysts were immediately pipetted into freshly pre-
pared formol-alcohol-acetic acid solution (FAA) (36 % formaldehyde solution 3 ml, 60 % ethanol
6 ml, glacial acetic acid 1 ml) kept at room temperature. Further processing, paraffin embedding,
serial sectioning and protein staining with the Hg-bromophenol blue method (HgBPB) were per-
formed as described before (Denker 1970b). Photographs taken from all serial sections were
used for evaluating spatial relationships and numbers of blastomeres.

Table 1. Time course of cleavage and blastocyst formation, and position of embryos in the genital tract

Cat No.	Stage of pregnancy (d and h p.c.)	Location of embryos				Developmental stage[1]			Unfertilized eggs (No. of eggs)
		Tube (No. of embryos)	Uterus (No. of embryos)	n. d. (No. of embryos)	Total (No. of embryos)	cleavage stages (No. of embryos)	preblasto-cysts[2] (No. of embryos)	blasto-cysts (No. of embryos)	
1	~3 d (2 d 21 h)	5			5	3			2
2	~3 d (3 d 1 h)	5			5	5			
3	~4 d (3 d 22 h)	4			4	1			
4	~4 d (4 d 4 h)	4			4	2			2
5	~5 d (5 d 3 h)	4			4	4			
6[3]	~5 d (5 d 3 h)	14			14	10		4	
7	5 d 12 h			1	n.d.	1			
8	~6 d (6 d 2 h)	5			5	5			
9[3]	6 d	10	7	15	32	17	5	1	
10	10 d		4		4			4	
11	12 d		2		n. d.			2	

1) Indicated only for sectioned embryos.

2) Small intercellular vacuoles as precursors of the blastocyst cavity are just appearing.

3) Superovulated.

Results

Embryo transport

Up to 5 d 3 h p.c., all embryos were recovered from the tubes (Table 1). At 6 d 2 h p.c., the embryos were still found in the tubes in one animal (non-superovulated), while some had already entered the uterus in another female sacrificed at 6 d p.c.; the latter had been superovulated.

Time course of cleavage and blastocyst formation

Cleavage was found to proceed very slowly in the cat (Table 2). Around 3 d p.c., most of the embryos were in the 8-cell stage. Around 5 d p.c., embryos were in the morula stage, and in most cases between 24 and 40 blastomeres were counted. The first signs of beginning formation of a blastocyst cavity were observed in some embryos at 6 d p.c. (approximately 60—100 cells) when fluid began to accumulate

Table 2. Observations on histochemical differences between presumptive trophoblast and embryonic knot cells

Cat No.	Stage of pregnancy (d p.c.)	Developmental stage of embryos	Histochemical findings (HgBPB reaction)	
			Differences not detectable or moderate (No. of embryos)	Differences obvious (No. of embryos)
1 + 2	∼ 3 d	(4—)8 cells	5[1])	2[1])
3 + 4	∼ 4 d	10—16 cells	2	1
5 + 6	∼ 5 d	24—48 cells	6	8
7	5 d 12 h	60 cells	1	
8 + 9	∼ 6 d	60—100 cells; morulae or pre-blastocysts	13	14
10	10 d	blastocysts	1	3
11	12 d	blastocysts		2

1) Even though differential staining is recognizable, a clear distinction of 2 categories of blastomeres is problematical.

between some of the blastomeres. At 10 d p.c., the expansion of the blastocyst had caused transformation of the embryonic knot into an embryonic disc, the morphological details of which are described below.

Morphological and histochemical aspects of differentiation of trophoblast and embryonic knot cells

Around *3 d p.c.*, the cytoplasm of the mostly 8-celled embryos contained copious *lipid vacuoles* as typical for the feline egg. After paraffin embedding, these vacuoles appeared empty and gave the cytoplasm a mesh-like appearance (Figs. 1, 2). Cytoplasmic HgBPB stain uptake was, in all eggs studied, not uniform in different blastomeres, i.e. lighter and more intensely stained cells could be recognized. However, differences were only slight and no regular pattern of cell positioning was apparent. In 2 of the embryos, the blastomeres differed more obviously in stain uptake, but morphological details were not sufficiently well preserved to allow more definite statements on the relative positions of the cells.

Basically the same difficulties with the cytological analysis were encountered in case of the 3 *4-day* embryos studied. One of them (14 blastomeres), however, showed very obvious differences between lightly stained and intensely stained cells. 2 light cells were in the center of the embryo, while one reached to the perivitelline space.

Fig. 1. Diagrammatic representation of basic morphological features and differential staining of cells in cat cleavage stages and blastocysts. FAA fixation, paraffin sections, HgBPB staining.
a: 5 d p.c. A group of outer layer blastomeres is more intensely stained than the others, with maximal stain uptake in the basal cytoplasm directed towards the center of the embryo. They do not completely surround the other blastomeres but rather form a kind of a "cup". The lightly
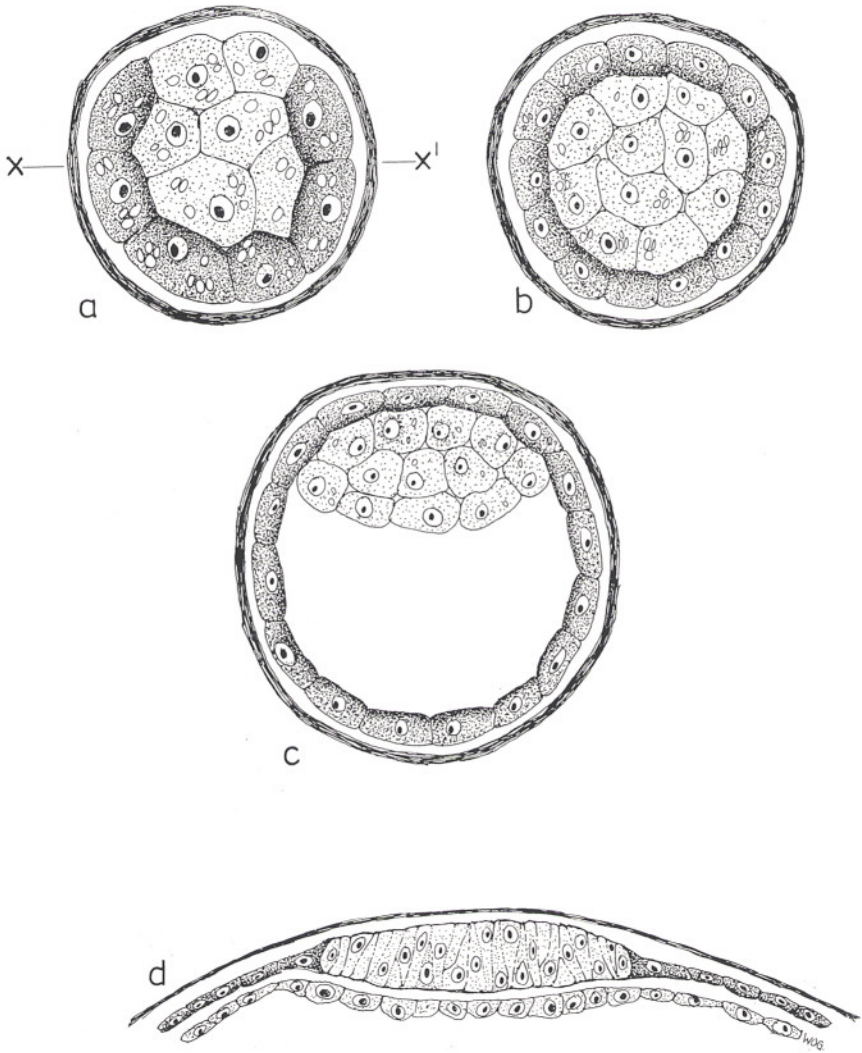
stained cells do not show cytoplasmatic polarity. Numerous fat vacuoles are present in all blasto-
meres.

b: At 5 $^1/_2$ — 6 d p.c., the basic cytological details have not changed, but most of the sections
show the pattern indicated here: the more heavily stained blastomeres surround the others com-
pletely. The same pattern would also result if in an embryo as shown in Fig. 1a, the plane of
sectioning would correspond to x — x′.

c: Early blastocyst. The embryonic knot is composed exclusively of lightly stained cells while
the dark cells form the trophoblast, i.e. abembryonic pole and lateral trophoblast as well as
embryonic pole trophoblast (Rauber's layer, above).

d: 10 d p.c., embryonic pole. The embryonic disc ectoderm is composed of highly columnar,
lightly stained cells. The entoderm also shows only little HgBPB stain uptake. The trophoblast,
however, still stains heavily. Rauber's layer has disappeared, and the embryonic ectoderm is
intercalated into the trophoblast layer the ends of which can be recognized easily due to different
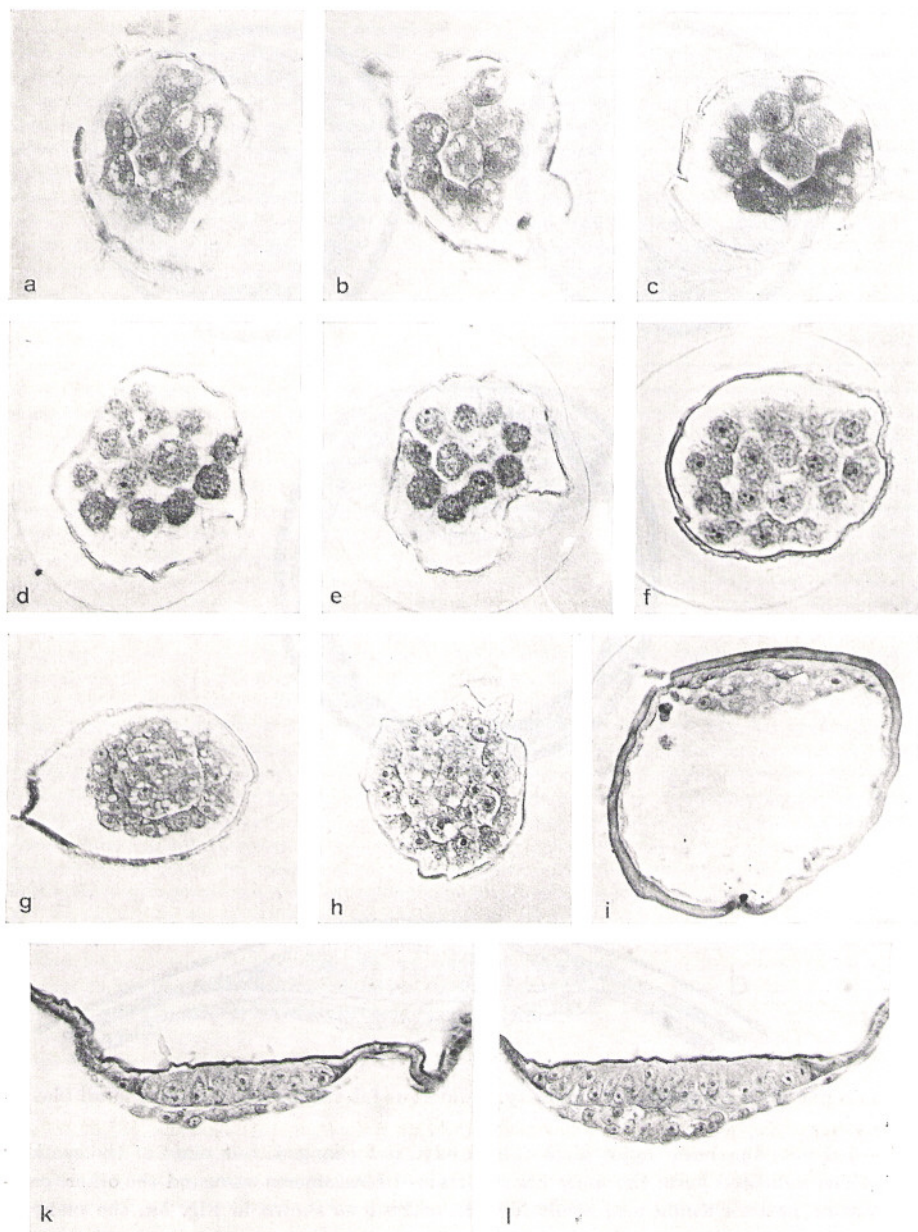stainability. The blastocyst is still surrounded by the zona pellucida.

Fig. 2. Photographs of paraffin sections of FAA-fixed cat embryos stained with the HgBPB reaction for proteins. 230×.

a—f: Cleavage stages, 5 d 3 h p.c.; a, b and d, e represent pairs of adjacent sections from one embryo each, c and f are taken from different embryos. A group of more intensely stained blastomeres can be recognized in a—e but is more indistinct in f.

g, h: Morula, 6 d p.c. More intensely stained outer blastomeres seem to surround the centrally located lighter cells completely.

i: Early blastocyst. The trophoblast (note Rauber's layer, above) is stained more intensely.

This group was incompletely surrounded by at least 6 dark blastomeres. The remaining blastomeres could not be classified unequivocally as belonging to any of these 2 groups because they came either to lie in the first or last sections of the series, or their morphology was not sufficiently preserved.

The most relevant observations were made with *5-day* embryos (24—48 cell stage). In 8 out of 14 embryos, the HgBPB staining showed marked differences between 2 groups of blastomeres: 1. a knot-like group of lightly stained cells, and 2. a cap of more intensely stained blastomeres which formed always a single layer at the periphery of the first group. This typical arrangement is diagrammatically shown in Fig. 1 a. Interestingly, the peripheral layer of *darkly stained cells did not completely surround* the group of light blastomeres, but rather formed a cap or cup-like structure so that at one pole some of the light cells faced the perivitelline space. As the serial sections revealed there did not seem to be any exception from this rule. Cytological details did not differ between the 2 categories of blastomeres, except that the dark cells mostly showed maximum stain uptake in their basal parts directed towards the center of the morula.

A segregation into 2 groups of blastomeres was equally obvious in 14 out of 27 embryos at the *6-day* stage when a cell number of approximately 60—100 was reached. However, in the majority of those sections a pattern was seen which corresponded to Fig. 1 b, i.e. the more intensely stained blastomeres surrounded the light ones *completely.* Although in a number of cases blastomeres were seen which appeared to belong to the group of "light" cells but which were nevertheless positioned in the outer layer, it was felt that a heterogeneity of outer layer cells could not be shown conclusively. On the other hand it was also not possible to exclude this completely, either, because the *plane of sectioning* lies certainly often, by chance, as indicated by x—x' in Fig. 1 a. In such cases a pattern is seen which does indeed correspond to Fig. 1 b, i.e. a complete belt of dark cells appears on the outside. The "gap" in the outer layer is then met in the first or the last sections of the series which are difficult to evaluate. If the "gap" in the dark cell covering is only narrow, we must expect that most sections of such embryos will show the pattern seen in Fig. 1 b.

In 13 other embryos of this stage, the blastomeres differed only minimally in their protein staining characteristics, and it was not possible to distinguish 2 populations of cells unequivocally on the basis of this criterium. No morphological differences could be recognized between embryos which had been flushed out of the tubes as compared to those which had already entered the uterus. In all embryos was the number of *lipid vacuoles* per cell markedly reduced as compared to earlier stages, and this trend continued until 10 d p.c. when most of them had disappeared. At 6 d p.c.

k, l: Embryonic disc region of a blastocyst, 10 d p.c. The lightly stained embryonic ectoderm is intercalated into the trophoblast layer (dark); no Rauber's layer present anymore. The dark line on top of the embryonic disc is the zona pellucida. Entoderm below embryonic ectoderm and trophoblast.

first signs of the beginning of *blastocyst formation* could be recognized because fluid began to accumulate in the intercellular spaces in a number of embryos (Table 1).

At *10 d p.c.* the blastocysts were expanded but still encased in the zona pellucida. The trophoblast layer of the embryonic disc (Rauber's layer) had disappeared, and the ectoderm of the embryonic disc was intercalated in the extraembryonic tropho-blast (Fig. 1d). The ectoderm cells were columnar and tall and showed only little HgBPB stain uptake while the trophoblast cells were flat to cuboid and exhibited very intense staining. There was a well-developed entoderm which consisted of cuboid cells in the embryonic disc area and of flat cells in the extraembryonic parts.

At *12 d p.c.*, the embryonic disc ectoderm had considerably expanded both in area and in thickness. The trophoblast cells were more cuboid in shape. Differences in HgBPB stain uptake between trophoblast and embryonic disc ectoderm were still as obvious as at the 10-day stage. No zona pellucida was found at the surface of these blastocysts which had been flushed out from the uterus.

Discussion

The light microscopical morphology of cleavage and blastocyst formation in the cat has been described in much detail by HILL and TRIBE (1924). However, the mate-rial studied by those authors was "obtained from cats of unknown origin, which came into (their) hands shortly after death" (HILL, TRIBE 1924, p. 516) so that the age of the embryos could not be determined. Furthermore, no differences in staining charac-teristics were observed between blastomeres of early cleavage stages so that the cell lineage leading to the formation of trophoblast and embryonic knot remained very speculative (see below).

Timed mating of cats under laboratory conditions as used in the investigations described here makes it possible now to give exact data on the time course of early developmental stages in this species, particularly because the cat is a reflex ovulator like the rabbit, i.e. ovulation is induced by the coitus (although it may occasionally also occur spontaneously if no mating takes place, see GREULICH 1934).

Early embryonic development proceeds at a much slower rate in the cat than in those other mammalian species commonly used in embryology and reproduction re-search, i.e. in rodents and the rabbit. All observed developmental steps take roughly twice the time as in the rabbit (for the latter see SEIDEL 1960; DENKER 1970a, b): ovulation occurs at an average of $26-28$ h p.c. (25 h p.c. as a minimum) (cf. COUR-RIER, GROS 1933; GREULICH 1934) (rabbit: around $10\,^1/_2$ h p.c.); passage from the tubes into the uterus and appearance of the blastocyst cavity at 6 (-7) d p.c. (rabbit: 3 d p.c.); unattached large blastocysts with an expanded embryonic disc are found at 12 d p.c. (rabbit: $6-6\,^1/_2$ d p.c.); the dissolution of the blastocyst coverings (zona pellucida) takes place at 12 d p.c. and attachment of the trophoblast starts between 13 and 14 d p.c. (DENKER, ENG, HAMNER 1978 (rabbit: 7 d p.c.). The transport of the embryos from the tube into the uterus is possibly accelerated in case of superovulation: in the superovulated animal (No. 9), a proportion of embryos

had already entered the uterus at 6 d 0 h p.c. (Table 1) while in the non-super-
ovulated one (No. 8) all embryos were still in the tubes at 6 d 2 h p.c. This is con-
sistent with the observation of MANWELL and WICKENS (1928) who recovered a morula
from the tube at 150 h (6 d 6 h) p.c.

From the embryological point of view, the most interesting result obtained in
the course of this study seems to be the observation of *2 categories of blastomeres*
which differ, in relatively early cleavage stages, in their staining characteristics if
the FAA-HgBPB technique is applied. These staining characteristics remind very
much of those seen in the rabbit (DENKER 1970b, 1971, 1972, 1976). However, the dif-
ferences between both categories of blastomeres are not as obvious in the cat as in
the latter species, and in the cat they can be clearly recognized only in a proportion
of the embryos (Table 2) while in the rabibt they are seen in nearly all embryos (after
the 16-cell stage). The morphological and histochemical analysis is made much more
difficult in the cat because cytological details are obscured by the large number of
lipid vacuoles present, and the cells are usually less well preserved in the paraffin
sections than in case of rabbit embryos. Nevertheless, the study of a sufficient number
of embryos and the comparison of subsequent stages of development allows us to
conclude that the peripherally located, intensely stained blastomeres represent pro-
genitors of *trophoblast* cells while the lightly stained cells will form the *embryonic knot*.
The presumptive trophoblast cells show some polarity: stain uptake is maximal in
those parts of the cytoplasm which are directed towards the center of the morula.
The light blastomeres, on contrast, do not show any obvious polarity. This corres-
ponds perfectly to observations made in the rabbit (DENKER l.c.).

The mechanism and specificity of the HgBPB reaction has been discussed else-
where (DENKER 1970b, 1972). The HgBPB reaction is used as a general protein stain-
ing and is usually assumed to lack marked selectively for any individual classes of
proteins. It was found to stain all blastomeres equally, in the rabbit, when neutral
formaldehyde, glutaraldehyde or Bouin *fixatives* were employed. Only after fixation
in the formaldehyde-alcohol-acetic acid (FAA) mixture was a differential staining of
the 2 categories of blastomeres observed. After a detailed analysis it seemed improb-
able that any diffusion and displacement artifacts were responsible for the results
although this possibility should still be kept in mind (DENKER 1970b). Since it is
well established that the FAA mixture extracts certain fractions of proteins (and nu-
cleoproteins) from cells (MERRIAM 1958; DICK, JOHNS 1968) it appears that *FAA-non
extractable cytoplasmic protein fractions* are the ones which are present in different
proportions in presumptive trophoblast and embryonic knot cells.

The importance of using the right kind of fixative is also underlined by a compari-
son with the observations of HILL and TRIBE (1924) who did not observe any differen-
tial stain uptake or other differences between inside and outside blastomeres in the
cat up to the 63-cell stage. The fixatives which they used included: Flemming's fluid
(chromic acid, osmium tetroxyde, acetic acid), Hermann's fluid (platinous chloride,
osmium tetroxyde, acetic acid) and picro-nitro-osmic acid. These authors observed
differential stain uptake only in late morulae from the uterus which they described

as exhibiting a "more deeply staining layer of trophoblast, more or less clearly marked off from the somewhat lighter-staining mass of central or embryonal cells" (Hill, Tribe 1924 p. 555). In the analysis of earlier stages where they did not observe differences in stain uptake, those authors had to rely on purely morphological criteria like cell size, shape and position. Basing themselves, in addition, on comparative aspects derived from classical embryological work of the end of last century (for references see Denker 1976), Hill and Tribe (1924) felt that there was „conclusive evidence in favour of the occurrence of epiboly, i.e. of a process of overgrowth whereby the central cells become secondarily enclosed by the peripheral" (Hill, Tribe 1924, p. 543 f.). However, they were puzzled by the observation that, in their material, it seemed that even in the 59—63 cell stage "the central cells ... do not appear to differ in any essential respect from the trophoblastic cells" (Hill, Tribe 1924, p. 550). Obviously the FAA fixation followed by HgBPB staining as used in the present investigation offers the advantage of showing differential staining of both categories of blastomeres in earlier stages.

Most relevant for the discussion of the physiological mechanisms of the determination of trophoblast and embryonic knot cells is the spatial arrangement of the 2 groups of blastomeres, which was found to correspond to Fig. 1a. In the cat this was seen particularly well at the 24—48 cell stages. The *"inside-outside theory"* (Tarkowski, Mintz) mentioned in the introduction implies that all blastomeres of early cleavage stages are at first equal in their developmental potentials. Those cells which (by chance) happen to come to lie in the center of the morula become determined to form the embryonic knot, because they are in a micro-environment which differs from that of the outer cells because the latter face the perivitelline space. The specific micro-environment is thought to provide the determinative stimulus. This assumption of course implies that all of the outside cells will form trophoblast because the positional/environmental situation is identical for all of them. In our investigations, the spatial arrangement of the 2 groups of blastomeres was found, however, to be as shown in Fig. 1a, in both the cat and the rabbit. The observation that only part of the peripheral cells show the staining characteristics of presumptive trophoblast is inconsistent with the named assumptions of the *"inside-outside model"*. Rather than completely surrounding the other blastomeres, the presumptive trophoblast cells form a *cap on one pole* of the morula. In the rabbit it was found to be located at the future abembryonic pole of the embryo, i.e. opposite to the future embryonic knot. The blastocyst cavity develops at the interface between this cap of presumptive trophoblast cells and the rest of the blastomeres which become the embryonic knot. On the basis of these observations it was not possible, so far, to decide whether the embryonic pole trophoblast cells (Rauber's layer) gain their position by an epiboly-like overgrowth by the abembryonic pole cap cells or whether they might derive from the outer layer cells of the embryonic pole which possibly might undergo determination in a somewhat later phase and maybe controlled by positional effects.

In the cat we are not completely sure, with the material at hand, whether the polar organization indicated by the "gap" in the covering layer of presumptive tropho-

blast cells persists until the late morula stage, i.e. until 6 d p.c. The hypothesis had been presented before that in some species (possibly including e.g. the rodents) a polar arrangement of the described type may disappear again in later cleavage stages because the presumptive trophoblast cells grow around the future embryonic knot in an epiboly-like manner, and they may finally come to surround it completely already before the blastocyst cavity begins to appear (DENKER 1972). This could perhaps also be the case in the cat. In any case, the polarity seen in the 5-day stage in the cat, if it has any physiological meaning, contradicts the *"inside-outside theory"* in its strict form. We tend to imagine that *both the distribution of morphogenetic factors inside the uncleaved egg and epigenetic factors like the microenvironment provided by the inside or outside position of the blastomeres act together* in bringing about the final determination of the blastomeres.

An epiboly-like overgrowth is only one possible explantation for the developmental steps which may lead from a polary organized cleavage stage as shown in Fig. 1a to a blastocyst which is completely surrounded by trophoblast (Fig. 1c). An alternative hypothesis would be that the *differentiation* of outside cells of a morula into trophoblast, which may be triggered by micro-environmental (positional) factors, always *starts at one specific pole* of the morula: the pole which possesses the highest (or lowest) concentration of morphogenetic factors. These factors could well be derived from a cytoplasmatic field of factors (cf. Seidel's "Bildungszentrum", see SEIDEL 1960; cf. also DALCQ 1954) of the uncleaved egg. A hypothesis of this type which takes into account the cooperation of both a polar organization of the uncleaved egg and the action of epigenetic factors would allow to unify divergent views derived from seemingly conflicting results obtained by morphological and histochemical investigations and deletion experiments on one hand, and by transplantation (fusion) experiments on the other hand (see DENKER 1976).

References

COURRIER, R., and G. GROS: Données complémentaires sur le cycle génital de la Chatte. C. R. hebdom. Séances Soc. Biol. Filiales, Paris **114** (1933) 275—277.

DALCQ, A.-M.: Nouvelles données structurales et cytochimiques sur l'oeuf des mammifères. Rev. Gén. Sciences Pures Appl., Paris **61** (1954) 19—41.

DENKER, H.-W.: Topochemie hochmolekularer Kohlenhydratsubstanzen in Frühentwicklung und Implantation des Kaninchens. I. Allgemeine Lokalisierung und Charakterisierung hochmolekularer Kohlenhydratsubstanzen in frühen Embryonalstadien. Zool. Jb. Physiol., Jena **75** (1970a) 141—245.

— Topochemie hochmolekularer Kohlenhydratsubstanzen in Frühentwicklung und Implantation des Kaninchens. II. Beiträge zu entwicklungsphysiologischen Fragestellungen. Zool. Jb. Physiol., Jena **75** (1970b) 246—308.

Denker, H.-W.: In discussion to J. G. Mulnard: Manipulation of cleaving mammalian embryo with special reference to a time-lapse cinematographic analysis of centrifuged and fused mouse eggs. In: Schering Symposium on Intrinsic and Extrinsic Factors in Early Mammalian Development, Venice 1970. Advances in the Biosciences (G. Raspé, ed.) 6 (1971) 275—277.
— Furchung beim Säugetier: Differenzierung von Trophoblast- und Embryonalknotenzellen. Verh. Anat. Ges. 66; Anat. Anz. Suppl., Jena 130 (1972) 267—272.
— Formation of the blastocyst: determination of trophoblast and embryonic knot. In: Current Topics in Pathology (E. Grundmann and W. H. Kirsten, eds.) Vol. 62: Developmental Biology and Pathology (A. Gropp and K. Benirschke, Vol.-eds.). Springer, Berlin-Heidelberg-New York 1976, 59—79.
— L. A. Eng and C. E. Hamner: Studies on the early development and implantation in the cat. II. Implantation: proteinases. Anat. Embryol. 154 (1978) 139—154.
Dick, C., and E. W. Johns: The effect of two acetic acid containing fixatives on the histone content of calf thymus deoxyribonucleoprotein and calf thymus tissue. Exp. Cell Res., New York 51 (1968) 626—632.
Greulich, W. W.: Artificially induced ovulation in the cat (Felis domestica). Anat. Rec., Philadelphia 58 (1934) 217—224.
Hamner, C. E., L. L. Jennings and N. J. Sojka: Cat (Felis catus L.) spermatozoa require capacitation. J. Reprod. Fertil., Oxford 23 (1970) 477—480.
Herbert, M. C., and C. F. Graham: Cell determination and biochemical differentiation of the early mammalian embryo. Curr. Topics Developm. Biol., New York-London 8 (1974) 151—178.
Herron, M. A., and R. F. Sis: Ovum transport in the cat and the effect of estrogen administration. Amer. J. Vet. Res., Chicago 35 (1974) 1277—1279.
Hill, J. P., and M. Tribe: The early development of the cat (Felis domestica). Quart. J. microsc. Sci., London 68 (1924) 513—602.
Manwell, E. J., and P. G. Wickens: The mechanism of ovulation and implantation in the domestic cat (Abstr.). Anat. Rec., Philadelphia 38 Suppl. (1928) 54.
Merriam, R. W.: Standard chemical fixations as a basis for quantitative investigations of substances other than deoxyribonucleic acid. J. Histochem. & Cytochem., Baltimore 6 (1958) 43—51.
Mulnard, J. G.: Les mécanismes de la régulation aux premiers stades du développement des mammifères. Bull. Soc. Zool. France, Paris 91 (1966) 253—277.
Seidel, F.: Die Entwicklungsfähigkeiten isolierter Furchungszellen aus dem Ei des Kaninchens. Wilhelm Roux' Arch. Entwickl.-Mech. Organismen, Berlin (West) 152 (1960) 43—130.
Sojka, N. J., L. L. Jennings and C. E. Hamner: Artificial insemination in the cat (Felis catus L.). Labor. Animal Care, Baltimore 20 (1970) 198—204.
Tarkowski, A. K., and J. Wróblewska: Development of blastomeres of mouse eggs isolated at the 4- and 8-cell stage. J. Embryol. exper. Morphol., Oxford 18 (1967) 155—180.

Prof. Dr. Dr. H.-W. Denker, Abteilung Anatomie der RWTH, Melatener Straße 211, D - 5100 Aachen.