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Experimental Studies on Regulation in the Development of Isolated Blastomeres of Mouse Eggs*)

Badania eksperymentalne nad rozwojem izolowanych blastomerów jaj myszy

I. INTRODUCTION

Embryological research of an experimental character is still limited to a few selected groups of invertebrate and vertebrate animals. Of the verte-brates it is perhaps mammals which form the least-known group. This is partiqularly true of the early stages of development, analysed from the

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standpoint of the prospective potency of the individual blastomeres, and of their regulative capacity.

Up to the present only two attempts have been made at determining by means of experiment the regulative capacity of the blastomeres of the 2-cell egg of mammals. N i c h o l a s and H a l l (1942) isolated the blastomeres of the 2-cell egg of a rat, and after transplanting them to the uterus of the recipient, established that normal development of „¹/₂" embryos took place during the first few days after implantation. Of special significance was the observation that embryos of identical structure could develop from both blastomeres of one 2-cell egg. Their development did not, however, continue beyond the egg cylinder stage and all the embryos died before the 10th day.

S e i d e l's (1952) experiments on the ova of rabbits were carried out slightly differently. In the first place — one of the blastomeres was destroyed by piercing it with a glass needle through the zona pellucida, which was retained, and in the second — absolutely no check was made of the embryonic development during pregnancy, since the sole aim was to achieve the final effect — the birth of the young. Two rabbits were born as the result of these experiments. On account of the very way in which they were planned, these experiments did not supply. however, any new data as to the potency of both primary blastomeres.

A third attempt at this type of experiment was recorded by P i n c u s (1936), who in his book „Eggs of Mammals" (p. 97) refers to the transplantation of single blastomeres of the 2-cell egg of a rabbit and the obtaining of blastocysts which differed from the normal forms only as regards smaller dimensions. The author does not, however, give any fuller information on this subject.

The results obtained by N i c h o l a s and H a l l (1942) and S e i d e l (1952), although very interesting, are somewhat fragmentary and do not give a full picture of the whole phenomenon. The basic problem as to whether at the 2-cell stage both blastomeres are as a rule totipotent, or whether regulation is possible only in certain definite cases, was not solved, neither is there exact information available as to the course of development of „¹/₂" embryos as regards the rate of their morphogenesis and growth. In this connection it is not clear in which period of pregnancy these embryos attain the degree of development and the size characteristic of normal development. Neither of the works cited above supplies any observations on the development of „¹/₂" blastomeres before implantation. It would seem, on the other hand, that a knowledge of the structure of blastocysts creates the first possibility of preliminary determination of the potency of the blastomeres examined, and that without a knowledge of these facts it is difficult to arrive at a correct interpretation of development after implantation.

In view of the above it was considered useful to undertake experiments wider in scope, which might provide as full a picture as possible of development from the moment of the first cleavage of a „¹/₂" blastomere until the birth of the young individual. The present work, based on mouse ova, deals with two problems simultaneously.

1) Development before implantation with particular regard to the blasto-cyst stage. Special emphasis was laid on the determination of the degree of formation of the inner cell mass and trophoblast in the blastocysts.

2) Analysis of the whole embryonic development after implantation from the point of view of rate of morphogenesis and growth.

These researches concern in the main the developmental potency of bla-stomeres in the 2-cell stage. They also include, however, experiments in which the development of 3 or 1 blastomeres was analysed, using 4-cell eggs as the starting point of the experiment. The results of these experiments, although as yet somewhat fragmentary, throw some light on the problem of differentiation in blastomeres after the second cleavage division, and the possibilities of their further development.

The value of experimental research on the potency of blastomeres and their capacity for regulation is fundamentally dependent on a knowledge of the internal organisation of the undivided egg. It may confidently be asserted that it was not until Dalcq and his co-workers discovered the existence in the eggs of mammals of bilateral symmetry and two cytoplas-matic territories, differing from each other as regards the cytochemical aspect, and defined the course of segregation of the cytoplasm of both zones into the cells of the inner cell mass and the trophoblast, that the possibility of causal explanation of the results of experimental research arose.

The results obtained in this work reveal that blastomeres of the 2-cell stage (and also 4-cell) possess regulating capacities, which are however li-mited to a large extent and determined by the character of the cytoplasmic material forming the blastomeres. Totipotency of both primary blastomeres is only one (and not the only) of the possible types of relations characteri-stic of the 2-cell stage.

II. MATERIAL AND METHODS

1. Donors and recipients

Mice of several different colours, which were not defined exactly from the genetic standpoint, were used for the experiments. Eggs intended for trans-plantation were obtained from mice of dark colouring (black or brown), or from albino mice crossed with „grey" or „beige" individuals. The black and brown mice were homozygous as regards colouring. Cross-breeding between the albino mice and the „grey" and „beige" mice resulted as a rule in the F_1 generation in progeny of dark colouring. Recipients were albino, „beige" or „grey" mice mated with vasectomized males of the same colour. The use of sterile males and the suitable selection of donor and recipient from the point of view of colouring guaranteed a double check on the experiments.

2. Method of transplantation

The method of egg transplantation used in the work described below was worked out previously (Tarkowski, 1959) and checked by experiments on the transplantation of normal mouse ova. This technique consists in introducing the eggs by means of a thin pipette (after an incision had pre-

viously been made in the ovarian capsule) directly into the lumen of the oviduct through the infundibulum.

Regardless of the character of the material transplanted (2-cell eggs with 1 blastomere destroyed, or 4-cell eggs with either 1, 2 or 3 blastomeres destroyed) the recipients were as a rule females mated the previous night. In the first case the difference between the mating of the donor and of the recipient was 1 day, and in the second 2 days.

3. Preparation of material for transplantation

2-cell eggs were washed out from the oviduct of the donors on the second day after mating. Serum diluted with an equal quantity of normal saline solution was used for carrying out all manipulations with the eggs. This medium was of room temperature. The eggs were next transferred to a fresh drop of the medium on the slide. The destruction of the blastomeres was carried out by piercing them with a thin glass needle fixed to a simple ,,micromanipulator", as constructed by G o l d a c r e (1954). At the actual moment of piercing the blastomere the egg was drawn to and held against the mouth of a thin micropipette (external diameter about 40 microns) held at its thick end in a clip and connected by a rubber tube to a rubber bulb. This bulb was compressed before the operation, which caused the creation of negative pressure in the pipette and increased the suction power of the micropipette. The suction power could easily be regulated by holding the bulb in the hand throughout the operation, and made it possible both to avoid the possible suction of the egg into the pipette, and to disengage the egg from the pipette at any time. The micropipette and needle were placed low on the surface of the slide and the eggs moved under them by moving the slide. Endeavour was made to destroy the blastomere by one piercing movement only, so as to interfere with the zona pellucida as little as possible. Immediately after piercing the blastomere, or possibly within the next few minutes, the cytoplasm became cloudy, and partial or total destruction of the pierced blastomere took place. It should be emphasised that this process takes place so rapidly that there is absolutely no doubt that the operation has been properly carried out (Fig. 1).

4-blastomere eggs were obtained in two ways: 1) from mature females, on which autopsy was carried out in the late evening of the following day, or early in the morning (up to 8 a. m.) on the third day after mating, and 2) from immature females in which

ovulation was produced by the injection of gonadotropic hormones (P.M.S. and human chorionic gonadotrophin), given in doses of 2 I.U., at intervals of 35 hours. The hours of injection were so planned that ovulation took place about noon (acc. to R u n n e r & P a l m, 1953, ovulation takes place after $13^{\pm}2$ hours after the injection). The females were kept with the males from the early hours of morning, and in the majority of cases mated. In order to obtain 4-cell eggs, autopsy was carried out on the females about 48 hours after the presumed moment of ovulation. The results of the works by R u n n e r & G a t e s (1954) and G a t e s (1956) were complete authority for making use in these experiments of eggs obtained from immature females and also for treating them as fully equivalent to eggs produced by spontaneous ovulation.

Destruction of blastomeres in a 4-cell egg was carried out similarly to the operation on 2-cell eggs. In all cases the choice of the blastomere (or blastomeres) for destruction was completely fortuitous. Figs. 3 and 4 show 4-blastomere eggs in which 1 or 3 blastomeres were destroyed.

In addition, as incidental to these experiments, several attempts were made at transplanting naked blastomeres obtained from 2-cell eggs. The zona pellucida was removed by mechanical means. This operation consisted in the sudden introduction of the egg to the micropipette (of external diameter of about 70 microns) and immediate expulsion of the egg from it. As a result of this manipulation the zona pellucida was as a rule ruptured. The naked blastomeres fall from the micropipette either already separated or still connected with each other. In this latter case they must be separated by a thin capillary glass needle or by directing a stream of liquid on to them from the pipette. The disadvantage of this method is that very often, simultaneously with the rupture of the zona pellucida, one of the blastomeres is also destroyed.

The period the eggs are in vitro varied from 16 to 28 minutes.

4. Investigation of development before implantation

In order to follow the development of the blastomeres in the pre-implantation period, a check was made on the 1, 2 and 3rd days after transplantation had been carried out. The „eggs" were washed out from the oviduct or from the uterine horn and observed in the living state, measured and photographed. In the begin-

ning stage of the work, in order to determine the number of cells, .the „eggs" were fixed under a cover-slip with 5% acetic acid in absolute alcohol and stained with aqueous solution of toluidine blue. In the overwhelming majority of cases, however, after observations of the living state had been made, the „eggs" were fixed with H e i d e n h a i n fixative and mounted in toto in canadian balsam (technique similar to that described by S e m b r a t 1951). E h r- li c h's haematoxyline was used for staining.

The majority of the experiments were examined at a time which permitted of completely formed blastocysts being obtained from the transplanted blastomeres. Measurements were made of the size of the zona pellucida and of the blastocyst itself (length of both axes), and measurement of the height of the inner cell mass. On the basis of these measurements the volume of the blastocyst was calculated, taking it as an ellipsoid, and of the volume of the inner cell mass, treated as the elliptical cap.

For purposes of comparison with experimental material several completely formed normal $3^1/_2$ day blastocysts (autopsy made on the 4th day post-coitum) were collected from several females. Identical measurements and calculations were made in respect of these as for the blastocysts obtained from the experiments.

5. Investigation of development after implantation

As a result of the experiments embryos were obtained developed from one blastomere of the 2-cell stage, from all the successive days of pregnancy, beginning at the 5th and continuing to the 15th. The stages of development reached between the 5th and 10th day of pregnancy were fixed in situ in the uterine horn (in B o u i n's fluid), and then cut into sections 10 microns thick and stained with E h r l i c h's haematoxyline and eosine.

Embryos from the 10th day (apart from those fixated in situ in the uterus) and from the 11, 12, 13, 14 and 15th day of pregnancy were taken from the uterus and after removal of the foetal membranes, were fixed in 70% alcohol.

Control material was also taken from all the days of pregnancy to give a picture of normal development. Endeavours were made so to select this material that from the genetic point of view it was similar to that used in the experiments. Autopsy on the „experimental" and „normal" (control) pregnant females was made at the same time of day.

When analysing the development of embryos coming solely from certain blastomeres of the cleaving egg, it is essential to compare it with normal development, not only from the point of view of the course and rate of developmental processes, but also from the point of view of size relations of the corresponding stages. The important point here is to distinguish between development and growth, which on account of the reduced initial embryonic mass must take a different course from that in normal development.

With this aim in view measurements were made of the volume of the egg cylinders in embryos from the 6th to 10th day of pregnancy, and the weight of embryos from the later days of development determined. The technique of calculating the volume of the egg cylinder consisted in exposing all the histological sections by means of a projector and mapping their contours. The area of the figures was then calculated by means of a planimeter. The total of areas obtained from all figures was then converted into the true area of the sections and multiplied by their thickness, which was taken as equalling 10 microns.

Analysis of the early stages of development was based chiefly on S n e l l's (1941) work. The degree of development of embryos from the later days of pregnancy (10—15) were related to the stages of normal development, using my own control material and the data given by G r ü n e b e r g (1943).

6. Birth and development after birth

Among females in which it was established halfway through pregnancy that the development of embryos from transferred blastomeres was following a normal course (occurrence of vaginal bleeding and the characteristic mucous smear), several were left to give birth normally. Beginning with the afternoon hours on the 19th day of pregnancy, check on the cages was made at intervals of every few hours to determine more accurately the time of birth.

The young were weighed daily at the same time of day, beginning with the first and continuing to the 21st day after birth.

Similar measurements were made of the young which had developed from normal eggs and came from litters of the same size.

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In order to avoid constant descriptive definition of material the following symbols were used in this work. Single blastomeres of

the 2-cell stage, and the blastocysts and embryos which developed from them are marked „$^1/_2$". If the initial material consists of the 4-cell eggs the corresponding symbols are as follows: „$^1/_4$", „$^2/_4$", „$^3/_4$". This whole material is often defined as „experimental". In cases in which the number of the experiment is given, the letter, A indicates the transplantation of „$^1/_2$" blastomeres, the letters Aa — „$^3/_4$" and the letters Ab — „$^1/_4$" blastomeres.

Control material concerning the development of normal eggs and embryos is marked as normal (N).

III. DEVELOPMENT BEFORE IMPLANTATION

The observations discussed below concern the possibilities and course of development in the pre-implantation period of 1 blastomere from the 2-cell stage, and 1, 2 or 3 blastomeres from the 4-cell stage. Cleavage has been given superficial treatment only. Attention has basically been concentrated in this part of the work on the stage of the formed blastocyst. The justification for this arrangement of experiments is two-fold.

1. At the blastocyst stage we reach a definite and clear formation of the trophoblast and inner cell mass. This makes it possible to determine precisely the participation of the cells of the blastocyst in the formation of both these elements.

2. Information is obtained in this way on the starting form of the embryo at the moment of implantation, which is essential in order to understand and correctly to interpret development after implantation.

In the case of transfer of „$^1/_2$" blastomeres, completely formed blastocysts are present in the uterus of recipients during the 4th day post-coitum (5th day of their development). When using 4-cell eggs for experiments, completely formed blastocysts, irrespective of the number of destroyed blastomeres (1, 2 or 3), are present in the oviduct of the recipient on the 3rd day post-coitum (also the 5th day of their development). In both cases, similarly to what was established in the case of normal mouse eggs (T a r k o w s k i, 1959) the blastocyst stage is achieved from one-half to 1 day later than would be the case with development uninterrupted by transplantation. It should here be pointed out that when destruction of 2 blastomeres of one pair is carried out in the 4-cell stage, initial material „$^2/_4$" can be obtained, which in essentials is identical with the „$^1/_2$" material obtained from 2-cell eggs.

The general numerical comparison of the experiments, a check on which was made before implantation, is given in Table 1. In connection with the basic subject of this work, the main part of the research work is constituted by the exeperiments using starting material „$^1/_2$". In the series of experiments in which 4-cell eggs were used, fundamental emphasis is laid on the analysis of the development of „$^3/_4$" and „$^1/_4$" blastomeres. In several cases „$^3/_4$" and „$^2/_4$" blastomeres were jointly transferred. As the origin of the blastocysts obtained from the above „mixed" experiments cannot be

accurately defined, this material was used only conditionally in the various comparisons (with an appropriate note) or was completely omitted.

When carrying out autopsies on female-recipients, about 50% of the transferred „eggs" were found in their genital tract. Certain of these eggs must have died directly after transplantation was carried out (complete absence of cleavage) or underwent complete destruction into loose detritus, leaving behind them only empty zonae pellucidae. In the case of the remainder, which constituted the majority of the material (see Table 1), cleavage took place followed by further more or less normal development.

1. Cleavage

a. „$^1/_2$" b l a s t o m e r e s

Out of a total number of 25 experiments, only in one case was a check made 1 day after transplantation and in 3 cases after 2 days. After the blastomeres had been 24 hours in the recipient, one division was found to occur. After 2 days in the oviduct of the recipient the „$^1/_2$" blastomeres can be, depending on the time of day at which a check was made, at a stage from 8 (Figs. 7 and 8) to 16 cells. The most advanced stage, found in a recipient on the 3rd day post coitum, shows a 16-cell morula with a small cavity forming (Fig. 9). The group of cells which will form the inner cell mass can already be distinguished in the interior of the morula.

During this period the cleaving „$^1/_2$" blastomeres, even at a mere glance, can be seen to differ clearly from normal eggs at a similar stage of development (cf. Figs. 7, 8, 9 and Figs. 5 and 6), since the cells occupy only part of the interior of the zona pellucida. The remaining space is completely empty or partly filled with loose detritus from the destroyed blastomere.

b. „$^3/_4$" b l a s t o m e r e s

A check made on one experiment carried out one day after transplantation revealed that „$^3/_4$" blastomeres may at this time reach the morula stage consisting respectively of 12 (Fig. 10), 18 (Fig. 11) and 24 cells, or at the stage of the early blastocysts (Fig. 12), composed of about 26 cells. As in the case of the developing „$^1/_2$" blastomeres the cells do not occupy the entire interior of the zona pellucida

2. Blastocyst stage

a. „$^1/_2$" b l a s t o c y s t s

In normal (N) completely formed blastocysts their wall adheres closely to the internal surface of the zona pellucida. As a result

of the great internal pressure the zona undergoes extension and
its volume increases considerably in relation to its primary size
(unfertilized eggs or eggs in the cleavage period). Most often the
blastocyst is the shape of an ellipsoid and the inner cell mass is
at the pole of the longer axis. For purposes of comparison with
experimental material a normal $3^{1/2}$ day blastocysts is shown in
Fig. 13.

As regards their general appearance the majority of the comple-
tely formed „$^{1/2}$" and „$^{3/4}$" blastocysts were similar to normal bla-
stocysts. Certain differences occur, however, in their structure,
which can already be established during the observations made
of the live material.

They consist in essentials of two features. 1. The size of the
blastocyst itself expressed in the degree to which it occupies the
space within the zona pellucida. 2. The size and shape of the inner
cell mass.

Table 1.

Results of experiments checked before implantation.

Material	No. of Exp.	Successful Exp.		No. of transferred blastomeres	Blastomeres recovered		Blastomeres developing	
		No	%		No.	%	No.	%
1/2	25	21	84 0	113	59	52.2	53	46.9
3/4	7	6	85.7	38	26	68.4	25	65.7
3/4 + 2/4	3	3		23	14	60.8	14	60 8
Total	35	30	85.7	174	99	56.8	92	52.8

The general shape and size of „$^{1/2}$" blastocysts makes it possible
to distinguish several groups within them. This classification, of
course completely arbitrary and schematic, is intended solely to
provide some sort of system for somewhat heterogeneous material.

1. Blastocysts of follicle-like shape occupying the entire interior
of the zona pellucida (Figs. 15, 16, 17, 18).

2. Follicle-like blastocysts, with large cavity, lying loosely wit-
hin the zona pellucida (Figs. 19, 20, 21, 22, 23).

3. Shrunken blastocysts with very small or almost entirely con-
tracted cavity (Figs. 24, 25). This group includes both blastocysts
which possessed this shape directly after they had been washed
out from the genital tract of the recipient, and also those blasto-
cysts which had at first the shape of a follicle, but which after

a very short period in vitro underwent partial or complete shrinkage.

The behaviour of blastocysts in vitro observed while alive may be of two kinds. Some of them, even during a long period in normal saline solution, do not change at all, or only change very slightly, their appearance, and preserve the shape which they possessed immediately after being washed out. Others very quickly lose the appearance of a follicle and undergo greater or lesser shrinkage, and become similar to morulae. The ' differentiation into cells of the inner cell mass and the trophoblast can however always be established, during the observations made either of the live material or after fixation and staining. The question then arises as to whether it is not the degree of destruction of the zona pellucida, from among the various factors which might influence the variations found in the size and turgor of the blastocysts, and its behaviour in vitro, that plays the fundamental part. With experimental eggs the zona was damaged during the piercing of the blastomeres. This damage usually consists of a small opening (Fig. 16) but in extreme cases may take on the shape of a large split (Fig. 24). Analysis of the entire material obtained revealed, however, that the degree of damage to the zona pellucida is not the only factor on which the turgor of the blastocysts depends. Blastocysts have several times been observed, which despite the lack of the zona, have preserved even during a long period in vitro, their primary follicle-like shape. Blastocysts are often characterised by so powerful a turgor that part of the wall of the trophoblast, or even of the inner cell mass, herniates through the hole in the zona (Fig. 18) [1].

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[1] W h i t t e n (1957) observed similar herniation of the trophoblast through the zona pellucida in normal mouse blastocysts under culture in vitro. This phenomenon is more often found in experiments in which the period of incubation of the eggs was prolonged; it is presumably caused by the increasing internal pressure. W h i t t e n considers it probable that herniation occurs at the site of the small cleft made by the passage of the sperm.

Herniation of the trophoblast or the inner cell mass in certain „$1/2$" blastocysts can be explained as due to two causes. 1. As a result of prolongation of the period of development, the internal pressure in the cavity of the blastocoele is greater than with normal $3^{1}/2$ day blastocysts. 2. A comparatively large opening is present in the zona pellucida, caused by piercing the zona pellucida with glass needle.

On the other hand it must be pointed out that the overwhel-
ming majority of naked blastocystes had very small cavities (e. g.
the blastocysts shown on Fig. 27). An exception to this is the
naked, follicle-like blastocysts with a very large cavity (Fig. 26
a and b), which throughout the observations made of the live state
did not change its primary appearance.

The observations discussed above would seem to indicate that
maintenance of turgor by the blastocysts depends not only on the
presence of the zona pellucida, but is also dependent on the phy-
siological state of the cells themselves. During observations made
of normal mouse blastocysts I several times found that in a group
of blastocysts washed out from one female, some of them which
at first appeared completely normal very rapidly underwent a lar-
ge degree of shrinking. At the same time others preserved the
follicle-like shape for a very long period of time. In such cases
it was impossible to connect this phenomenon with the mechani-
cal damage of the zona pellucida. It is difficult to form an opinion
on the basis of these observations and decide whether the diffe-
rences perceived in appearance and in the behaviour of the bla-
stocysts in vitro have any real significance, on which the possi-
bilities of their further normal development are dependent.

On the basis of measurements made of living material, calcu-
lations were made acc. to the formula for the volume of the ellip-
soid:

$$V = \frac{4}{3}\pi \, abc,$$

(a, b, c — semi-axis; b = c)

of the volume of the zonae pellucidae [2]) and of the blastocysts
themselves. The volume of the inner cell masses was calculated
by the use of formula for the volume of the cap of the ellipsoid:

$$V = \frac{\pi \, b^2 \, z^2}{a^2} \left(a - \frac{z}{3} \right),$$

(a, b — semi-axis, z — height of inner cell mass).

[2]) Volume of the zona pellucida — space limited by the external surface
of the zona.

Only those blastocysts were used for measurements and calculations which were characterised by a regular follicle-like appearance and which at the same time possessed inner cell masses shaped in the form of a cap.

The volume of the zonae pellucidae of „$^1/_2$" and „$^3/_4$" blastocysts is similar to the volume of the zonae pellucidae of normal blastocysts. At the same time, however, the size of the blastocysts themselves is markedly less (Table 2 and 3). This might seem to indicate that in the genital tracts of recipients „$^1/_2$" (and „$^3/_4$") blastocysts have a similar volume to normal blastocysts and do not undergo any shrinkage until after being washed out. This matter is not however completely clear.

Table 2.

Volume of zonae pellucidae, blastocysts and inner cell masses (in $10^3 \, \mu^3$).

Material	Zona pellucida			Blastocyst			Inner cell mass			Participation of inner cell mass in volume of whole blastocust (in %)
	No.	Av.	Range	No.	Av.	Range	No.	Av.	Range	
N	6	565	670-421	20	431	690-263	20	92	132-70	21.3
3/4	8	555	711-450	8	361	579 227	7	73	126-38	19.9
1/2	9	546	581-477	16	314	465-158	10	41	65-19	13.0
1/4	1	505		1	119		1	22		18.5

It is indeed difficult to overlook the observations discussed previously, which show that in the very first moment immediately after the blastocysts have been washed out from the recipient, great differences are found in their size and the degree to which they occupy the space within the zona pellucida.

Data concerning the volume of the blastocysts do not define the mass itself of the cell material from which they are constructed, but only reflect the degree of formation of the blastocoele. Since the mass of the whole cystoplasmatic material of „$^1/_2$" blastocysts must be less by one-half than in the case of normal blastocysts, only the determination of the ratio between the mass of trophoblastic cells and inner cell mass cells becomes significant. In practice it is only possible to estimate the volume of the inner cell mass, since its compact and relatively regular structure makes it possible to apply geometrical formulas for purposes of calcu-

lation. A knowledge of the mass of the inner cell mass is, howe-
ver, particularly important since it represents that part of the
cell material which, as the result of later transformations, creates
the egg cylinder, consituting an essential part of the embryonic
tissue. At the same time it contains material intended for strictly
embryonic purposes — the embryo develops in fact from a defi-
nite area of the egg cylinder. This justifies the size of the inner
cell mass being considered as one of the fundamental features
which determine the further developmental possibilities of the
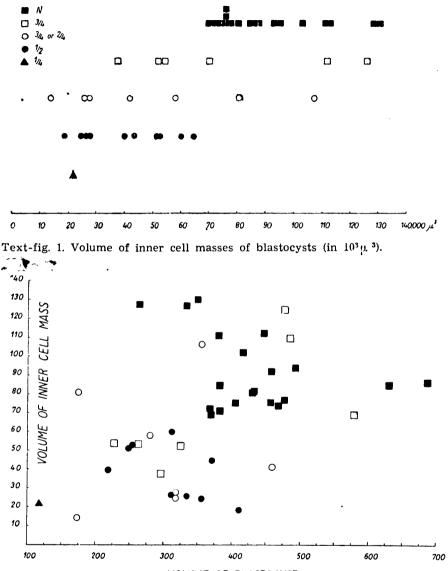individual blastocysts.

Table 3.

Volume of inner cell masses, zonae pellucidae and of the „$^3/_4$", „$^1/_2$", and „$^1/_4$"
blastocysts themselves expressed in percentages of corresponding values
of normal blastocysts.

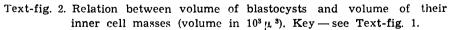|	„$^3/_4$"	„$^1/_2$"	„$^1/_4$"
zona pellucida	98.2	96.6	89.3
blastocyst	83.7	72.8	27.6
inner cell mass	79.3	44.5	23.9

It should moreover be pointed out, that the size of the inner
cell mass depends on the degree of formation ,of the blastocysts.
In developing blastocysts, or blastocysts which have developed
and then shrunk again, the inner cell mass occupies the greater
part of their volume. The increase in pressure in the cavity of
the blastocele exhibits itself by the increase in the volume of
the blastocyst itself, and undoubtedly at the same time influen-
ces the compression of the cells in the inner cell mass and the
reduction of its dimensions. The connection between the size of
the inner cell mass and the size of the blastocyst is visible on
Text-fig. 2, especially in relation to the experimental blastocysts.
It is expressed by an apparent increase in the volume of the inner
cell masses together with a decrease in the volume of the blasto-
cysts.

The data obtained (Table 2, 3 and Text-figs. 1, 2) despite their
great variations, undoubtedly connected with the lack of pre-
cision in the method used, are however very convincing. The vo-
lume of the inner cell masses of „$^1/_2$" blastocysts does not in any

Text-fig. 1. Volume of inner cell masses of blastocysts (in $10^3 \mu^3$).

Text-fig. 2. Relation between volume of blastocysts and volume of their
inner cell masses (volume in $10^3 \mu^3$). Key — see Text-fig. 1.

case attain the size found in normal blastocysts, and in general
is slightly below one half of their average size. The size of the
inner cell mass is, as can be seen from this, the most striking fea-

ture distinguishing the „$\frac{1}{2}$" blastocysts from normal ones. The large variation in the size of the inner cell mass observed in „$\frac{1}{2}$" blastocysts represents to a certain degree the relations undoubtedly existing (see analysis of their cell composition). Conclusions based solely on these data would have to be drawn with great care, since large variations in this size are also observed in the case of normal blastocysts. It should, however, be once again emphasised, that despite the wide range of variation, these values are grouped in separate aggregations, the scope of one not encroaching on the other.

Table 4.
Cell composition of experimental and normal blastocysts.

Material	Total no. of cells			No. of inner cell mass cells			Participation of inner cell mass cells in total no. of cells (in %)		
	No. of speci-mens	Av.	Range	No. of speci-mens	Av.	Range	No. of speci-mens	Av.	Range
N	10	59.1	50 — 65	10	37.3	30 — 46	10	63.1	52.3 -- 70.7
3/4	13	40.4	22 — 59	5	21.8	15 — 27	5	49.8	33.9 — 68.4
1/2	26	40 5	17 — 55	24	12.3	0 — 24	24	29.9	0 — 52.1
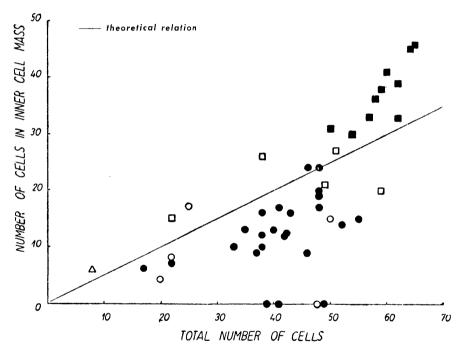
Table 5.
Cell composition of „$\frac{3}{4}$" and „$\frac{1}{2}$" blastocysts in relation to normal blastocysts (in %).

	„$\frac{3}{4}$"	„$\frac{1}{2}$"
Total number of cells	68.3	68.5
Number of cells of the inner cell mass	58.4	33.0

The above remarks concerned only those forms in which both component elements of the blastocyst were formed — the inner cell mass and the trophoblast. Extreme forms were not taken into consideration, that is, „blastocysts" composed solely of trophoblast (Figs. 33a and b), and morulae, in which despite the accumulation of a large number of cells, differentiation of trophoblastic cells did not take place (Fig. 32).
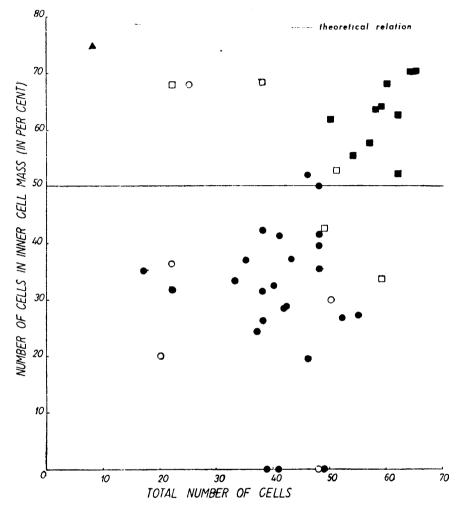
A total of 4 blastocysts was obtained, in which there was complete absence of any aggregation of inner cell mass cells, and

which were shaped in the form of a follicle consisting of one
layer of cells. Three of these are undoubtedly from „$^1/_2$" blasto-
meres, while the fourth was obtained as a result of the experi-
ment in which „$^3/_4$" and „$^2/_4$" blastomeres were transferred simul-
taneously. Most probably this blastocyst developed from a „$^2/_4$" bla-
stomere. Histological sections were obtained from one of these
blastocysts, and the remaining three were mounted in toto. Anal-
lysis of the fixed material confirmed the observations made of

Text-fig. 3. Participation of inner cell mass cells in total number of cells of
        blastocysts. Key — see Text-fig. 1.

live material as to the complete lack of formation of the inner
cell mass. The developmental forms shaped like morulae without
differentiated trophoblastic cells will be discussed in greater de-
tail in a later part of this work.

Observations made of living material are capable of determi-
ning only the external effects of differentiation of the cells of the
blastocysts into the inner cell mass and trophoblast, the only re-
ally perceptible feature being the absolute size of the inner cell

Text-fig. 4. Percentage participation of inner cell mass cells in total num-
ber of cells of blastocysts. Key — see Text-fig. 1.

mass itself. An exact numerical determination of which cells of
the blastocysts belong respectively to these two elements is, ho-
wever, of great importance.

The data given in Table 4 and 5, and Text-figs. 3 and 4 are
based in the great majority on calculations made from total pre-
parations. With ordinary haematoxyline preparations there is,
however, a fundamental difficulty in distinguishing against the
background of the inner cell mass the trophoblastic cells (nuclei)

which cover it. In this connection it was decided to treat as cells of the inner cell mass, all cells within its boundary. On this assumption the numerical ratio between cells of the trophoblast, and the inner cell mass is artificially allocated to the advantage of the latter. The data obtained, although not exactly correspon- ding to the true state of things, are however capable of compa- rison with each other.

The first fundamental observation concerns the total number of cells in the blastocysts. Although subject to relatively great variation, in the vast majority of cases however they group them- selves around 40. In relation to the corresponding values found in normal blastocysts, the total average number of cells of „$\frac{1}{2}$" blastocysts is about 70%.

This statement is especially interesting in comparison with the numbers of cells of the inner cell mass. From this it would seem that the number of cells of the inner cell mass of „$\frac{1}{2}$" blastocysts is exceptionally low. What is more important is that the parti- cipation of cells of the inner cell mass in the total number of cells forming the whole blastocysts is far smaller than that found in the case of normal blastocysts. While cells of the inner cell mass ($+$ the trophoblastic cells covering them) in normal blastocysts form 63% of the total number of cells, in „$\frac{1}{2}$" blastocysts the figure is only 30% (Table 4). An example in point of this different numerical ratio between inner cell mass cells and trophoblastic cells is given by the normal blastocysts and „$\frac{1}{2}$" blastocyst shown in Figs. 14 and 36. The first of them (N) consists of 54 cells, of which 30 are included in the composition of the inner cell mass. In the second („$\frac{1}{2}$") — of the total of 52 cells only 14 form the inner cell mass.

The average of the total number of cells of the blastocysts and the average of the number of cells of the inner cell mass are gi- ven in Table 5 as percentages of the corresponding values found in normal blastocysts. These data show that in the case of „$\frac{1}{2}$" blastocysts, the numerical ratio between cells of the inner cell mass and the trophoblast are transferred to the advantage of the trophoblastic cells. This means that their structure is based on slightly different „proportions" than is the case with normal bla- stocysts. The points on Text-figures 3 and 4 defining the partici- pation of cells of the inner cell mass in the total number of cells of the „$\frac{1}{2}$" and „N" blastocysts form separate aggregations above

and below the line corresponding to a theoretical ratio. In normal blastocysts the preponderance of participation of the so-called cells of the inner cell mass can be explained as the result of including, together with the cells proper of the inner cell mass, the trophoblastic cells covering them. In „$\frac{1}{2}$" blstocysts this ratio is below the theoretical value despite the fact that in these cases also, certain of the cells defined as „inner cell mass cells" undoubtedly belong to the trophoblast.

The extent of the error made in determining whether the cells belong to the inner cell mass or the trophoblast differs according to the degree of formation of the blastocyst. The observation is therefore of particular significance, that in the different blastocysts composed of a similar or nearly similar number of cells, the degree of formation of the inner cell mass and the trophoblast differs widely (Text-fig. 4).

All the data obtained as a result of analysis of fixed material confirm with greater exactitude the wide variation in the size of the inner cell mass found while making observations of living material. They also indicate that the „$\frac{1}{2}$" blastocysts obtained as the results of experiments, from the point of view of formation of the two separate elements — the inner cell mass and the throphoblast — do not constitute a homogeneous group here. This means that the „$\frac{1}{2}$" initial material obtained as the result of fortuitous selection of one of two blastomeres at the 2-cell stage, is also non-homogeneous. At the same time the suggestion must be made that achievement of the stage of a blastocyst does not necessarily imply full capacity for further normal development.

In 5 experiments transplantations were made of 17 naked, isolated blastomeres from the 2-cell stage. The absence of the zona pellucida caused a considerable degree of difficulty in finding them among the contents of the oviduct or the uterus of the recipients. Only 3 „progeny forms" were found — 1 blastocyst in process of formation, with a very small cavity, and 2 morulae. The possibilities of implantation by such blastocysts was not investigated.

b. „$\frac{3}{4}$" and „$\frac{2}{4}$" blastocysts

All identical measurements and calculations were carried out on these blastocysts as had been made for „$\frac{1}{2}$" blastocysts, and the data obtained are given in the tables and Text-figures already cited.

From the point of view of average values of various features, the „³/₄" blastocysts occupy a middle position between the „¹/₂" and normal blastocysts. This also applies to all measurements made on living material, and to calculations of the cell composition. Only as regards one feature — the average of the total number of cells — are they similar to the „¹/₂" blastocysts. Attention is however drawn to the fact that all features concerning the „³/₄" blastocysts fluctuate within very wide limits of variability, and do not in the least tend to group themselves around the average values. This peculiarity is especially clearly expressed in the size of the inner cell mass (Text-fig. 1 and 2). From this point of view the „³/₄" blastocysts are similar either to the „¹/₂" blastocysts or to normal ones.

The wide limits of variability of the various features are undoubtedly connected in this case with the heterogeneity of the initial material. This heterogeneity is of a qualitative character and results from the fact that during the operation of destruction, the selection of one of the 4 blastomeres, which are not of identical prospective significance is completely fortuitous [3]).

Several of the „³/₄" blastocysts obtained are shown in Figs. 37—39.

At the begining of this section reference was made to the fact that the fifth day of development of „³/₄" blastomeres, on which they take on the form of completely formed blastocysts, took place within the recipient on the third day post-coitum. On this day the blastocysts were still in the oviduct. In one case autopsy was made of the recipient on the 4th day post-coitum (6th of development of the blastomeres). The blastocysts found no longer possessed the zona pellucida and had lost their follicle-like shape. Their general state indicated that the process of implantation had begun. In cases of normal development, in the midday hours of the 4th day post-coitum the blastocysts are still lying loosely in the lumen of the uterus, and are characterised by the appearance shown by the blastocysts on Fig. 13. It is, however,, probable that under the conditions of the experiment, in connection with the

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³) A similar dispersion of the values of features is shown by the blastocysts obtained as the result of „mixed" experiments (transplatation of „³/₄" and „²/₄" blastomeres). This clearly confirms their originating from different initial material, differing in this case also as to quantity.

complete formation of the blastocysts in the oviduct, the moment of implantation may become hastened.

c. „$^1/_4$" blastocysts

Observations of the development of „$^1/_4$" blastomeres are very fragmentary at present. The fundamental cause which makes it difficult to obtain more material were obstacles of a technical nature. These are the difficulties experienced in destroying 3 blastomeres without considerable damage to the zona pellucida. On the other hand, although from the technical point of view it is relatively easy to obtain naked „$^1/_4$" blastomeres, it is extremly difficult to find their „progeny forms".

All these difficulties contribute to the very small degree of effectiveness of the experiments. As the result of 11 transplantation operations, during which a total of 28 „$^1/_4$" blastomeres (in zona pellucida) and 14 naked blastomeres were transplanted, only 2 blastocysts in the zona pellucida and 3 naked developmental forms were obtained. The finding of only 2 blastocysts in the zona pellucida would indicate that in the majority of cases the damage to the zona pellucida must be so considerable that the blastomeres fall from it during the period spent in the oviduct of the recipient.

The blastocyst shown on Fig. 41 possesses a regular folliclelike shape and is lying loosely in the interior of the zona pellucida. It is formed of 20—24 cells, of which 4—6 form the inner cell mass.

The second blastocyst (Figs. 40a and b) is composed of 8 cells only, of which 6 form the inner cell mass and 2 the trophoblast. As a result of the powerful deformation and compression of the zona, the blastocyst had to form in a very limited space. This blastocyst presents a very interesting case, proving that this developmental stage can be formed on the basis of 8 cells.

The above observations indicate that at least in certain cases, the single blastomere from the 4-cell stage is still capable of organised development and contains (although in a different ratio) structural material intended both for trophoblast and inner cell mass.

3. Incidental observations

A completely separate but interesting case is that of the blasto-cyst (Fig. 44) obtained as the result of transplantation of the group of „³/₄" and „²/₄" eggs. The „inner cell mass" is formed of only one large cell, which as regards measurements and calculated volume corresponds closely to one of the 4 blastomeres of the 4-cell stage [4]). The trophoblast composed of 24 cells does not cover the blastomere — inner cell mass — at the top, and joins only its side walls. Despite the cessation of division of one of the blasto-meres, the regular form of the blastocyst was created. At the same time, which is especially interesting, the blastomere which was not developing became installed in the general structure in a manner which precisely defined its primary predestination.

In several cases when carrying out checks on experiments in which „³/₄" or „¹/₄" blastomeres were transplanted naked groups of cells were found clustered together without any visible inter-nal organisation (Figs. 42 and 43). These forms consisted, accor-ding to size, of from 20 to over 30 cells. In certain cells metaphase plates were found. In the first type of experiments these groups must originate from blastomeres which fell from the zona pellu-cida during the period spent in the oviduct of the recipient. The differences in their size indicate that they developed from differ-ing preliminary numbers of „¹/₁" blastomeres, which in indivi-dual cases might separate from each other. In the second type of experiments they originate either from the primarily naked „¹/₄" blastomeres, or from blastomeres which fell from their zonae pellucidae after transplantation. At the present time no definite opinion can be formed from these observations.

4. Some general remarks

The observations discussed above make it possible to draw some general conclusions as to the development of isolated blastome-res during the cleavage and blastulation period.

During the cleavage period of normal eggs, fairly characte-

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[4]) To give a picture of the size of this blastomere, a normal 6-cell egg is shown on Fig. 45, in which in addition to „¹/₄" blastomeres, „¹/₈" blastomeres are also visible.

ristic differences are found in the appearance of the 8-cell and 16-cell stage (Figs. 5 and 6). In the 8-cell egg the blastomeres, irrespective of their mutual positions in relation to each other, are in fairly loose contact and preserve their spherical form. The 16-cell stage is characterised by a more compact arrangement of the blastomeres, which adhere closely to each other and lose their regular spherical form.

It is typical of ,,$^{1}/_{2}$'' 8-cell forms (Figs. 7 and 8) that they possess a general form like that represented in normal development by the 16-cell stage. This would mean that with the development of ,,$^{1}/_{2}$'' blastomeres, this form is attained with the number of cells half as small as is the case in normal development.

The possibility of more precise connection of the form or the stage of development with the amount of aggregated cell material becomes apparent only when the process of blastulation starts. The formation of the blastocyst constitutes the first clear expression of organised transformation extending beyond the mere quantitative increase in the number of cells. The question therefore arises as to what minimum number of cells is essential for the distinct perceptibility of this process and for the formation of the blastocyst stage [5]).

Material on the development of ,,$^{1}/_{2}$'' blastomeres was collected with the aim of obtaining the most fully formed blastocysts. For this reason the 16—32 cell stages of development are only infrequently represented. In spite of this it proved possible to obtain several fairly advanced or even completely formed blastocysts composed of about 20 cells (Figs. 26a and b, 34, 35). The 16-cell morula described previously (Fig. 9) possesses, it is true, a cavity only in process of formation, but the inner cell mass material already distinctly differs from the trophoblast elements surrounding it. At the same time 16-cell eggs are also encountered in which neither the distinct differentiation of the cells has yet taken place, nor the beginning of the formation of the blastocoele.

The occurrence of the blastulation processes was observed in developmental form originating from ,,$^{3}/_{4}$'' blastomeres on a basis of about 24 cells, (although this is not an invariable rule).

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[5]) Only those forms were considered in which the degree of development (formation of the blastocoele, differentiation into inner cell mass and trophoblast) correspond to the stage attained by $\pm$ 32-cell normal blastocysts.

If a blastocyst develops from 1 blastomere originating from the 4-cell stage, there is the possibility of attaining this form on the basis of a bare 8 cells.

Generally speaking it may be stated that the start of the blastulation process in the development of „$^3/_4$", „$^1/_2$" and „$^1/_4$" blastomeres does not require the aggregation of a number of cells similar to that at which this process becomes visible in the case of normal development. To put it precisely the process begins, irrespective of the mass of initial material, after a definite (and identical in all the eggs discussed) number of divisions undergone by the blastomeres from the beginning of development. The question then arises as to why the average of the total number of cells of „$^1/_2$" blastocysts is higher than half of the analogical average of normal blastocysts. The reason for this state of things is undoubtedly the prolongation of the period of development of „$^1/_2$" blastomeres before implantation. The check to the process of cleavage, which takes place directly after transplantation, does not last long enough completely to liquidate the difference in the age of „$^1/_2$" blastomeres and normal eggs i. e. one day. In connection with this the number of divisions of cells which take place is greater than in the case of normal blastocysts.

The above observations make it possible to form an opinion to a certain extent on the several developmental forms in which, despite the aggregation of a large number of cells (from over 30 to about 50), the differentiation of the trophoblast and formation of the cavity of the blastocoele cannot be observed (Fig. 32). The question then arises as to whether they are not composed exclusively of material destined for the formation of the inner cell mass, and whether they do not represent forms in extreme opposition to „blastocysts" in which only the trophoblast was formed. Since the blastocyst stage (of „$^1/_2$" origin) may be formed on the basis of about 20 cells, it may be stated with a great degree of probability that morulae composed of over 32 blastomeres and not exhibiting the slightest sign of differentiation of the trophoblast, are not in fact capable of forming this stage, even when the cells are further multiplied. It is, however, a cause for reflection that such forms are encountered in material originating not only from „$^1/_2$" blastomeres, but also from „$^3/_4$" blastomeres. In this last case, for theoretical reasons which will be discussed later, the occurrence of this type of form should not however be expected.

Finally, to end this section, the question of regularity of structure of the blastocyst, which developed from an artificially reduced number of blastomeres, should be mentioned. Such blastocysts do not always attain a completely regular form identical with that of normal blastocysts. Forms are sometimes encountered in which the inner cell masses do not possess a regular shape, or else the group of inner cell mass cells does not specially differ from the trophoblastic elements surrounding it (Figs. 29, 30 and 31). In such cases it is quite often only after fixation and staining of the object that this differentiation becomes plain. It sometimes happens that the inner cell mass does not occupy the pole of the long axis of the blastocyst but is shifted to its side wall (Figs. 16 and 29). Irregularity of structure may be expressed in the earlier period of development. A sporadic case is represented by the 16-cell morula with an asymmetrically placed very small cavity (Fig. 28). It is difficult to determine to what degree all these „deviations" are an expression of the immanent properties of the blastomeres examined, and to what degree the result of the interference caused by the experiment itself (and its brutality), and to establish the capacity (or incapacity) of such blastocysts for further normal development. The „non-typical" cases cited above of structure of developing eggs and blastocysts are, however, only an inconsiderable part of the material collected.

IV. DEVELOPMENT AFTER IMPLANTATION

The post-implantation development of embryos originating from „¹/₂" blastomeres was traced over a period lasting from the 5th to the 15th day of pregnancy. Each day of development is represented by at least one embryo. The early stages of development, both experimental and control, were compared with the description of normal development given by S n e l l (1941). The value of the control material, on account of the small quantity, is only for purposes of guidance, and the material itself does not in the least pretend to represent typical normal development.

In this work the age of the embryos has been determined according to the day of pregnancy from which they originate (taking as the first day that on which the presence of the vaginal plug was confirmed), and not according to the age itself of the embryos. This must be taken into account in any possible comparison of the

material presented here with S n e l l's (1941) results, or in relation
to later stages of development, with G r ü n e b e r g's data (1943)
and that of other authors.

1. Description of the structure of embryos

5th d a y o f p r e g n a n c y. Experiment no. 52A. The presence of
4 blastocysts was confirmed in the lumen of the uterine horn (Figs. 46—49).
In all 4 cases the trophoblast is in contact with the wall of the uterine crypt,
but the degree of contact varies greatly — from completely loose (Fig. 49)
to complete adhesion of the trophectoderm with the mucosa (Fig. 47). The
uterine epithelium is destroyed at the places of contact with the trophoblast.
On the edges of the inner cell masses of the blastocysts, shown in figures 46
and 47, flattened trophoblastic cells are visible which also occur in the form
of distinctly differentiated groups on the upper (mesometrial) pole of the
inner cell masses. These cells differ from the cells proper of the inner cell
mass by their small and flattened nuclei. An especially distinct line of de-
marcation between the trophoblast and the cells proper of the inner cell
mass can be observed in the blastocyst shown on Fig. 48. The loosely arran-
ged cells of the inner cell mass do not occupy the actual pole of the blasto-
cyst, but are placed slightly to the side. The trophoblast round the cells of
the inner cell mass forms a single-layer wall clearly separated from them.
In the blastocysts shown on Figures 46 and 47, large entodermal cells occur,
with deeply stained nuclei, lying under the inner cell mass and beginning
to spread beneath the trophectoderm. In the remaining 2 blastocysts the
presence of entodermal cells is problematical. Entodermal cells lying under
the inner cell mass (Fig. 46) already possess vacuolised cytoplasm. Material
used for control purposes consisted of 10 blastocysts from 3 females. Blasto-
cysts from the various litters exhibit fairly wide differences in the state of
development and size.

6th d a y. Experiment no. 15A. When autopsy was made on the recipient,
2 implantations of equal size were found in the uterine horn. After sectio-
ning the uterus it was found that in one foetal chamber there was complete
absence of embryo, the tissues of which must have previously undergone
total destruction. The decidual changes in the uterine mucosa are however
identical with those in the normal foetal chamber.

A developing embryo (Fig. 50) has a distinctly shaped egg cylinder, sur-
rounded by the proximal entoderm. The ectoderm of the egg cylinder is
divided into the embryonic and extra-embryonic parts. The lumen begins
to appear in the embryonic ectoderm. The cells of the proximal entoderm
are vacuolised. The cells of the ectoplacental cone begin to extend in the
mesometrial direction. The lumen of the uterus still reaches directly to the
embryo. The cavity of the yolk sac is very small and its wall is situated
fairly close to the egg cylinder.

Experiment no. 13Aa. 3 embryos obtained from one transplantation opera-
tion are shown on Figs. 52, 53 and 54. Two embryos (Figs. 52 and 53) have

an egg cylinder of regular shape and clearly marked division of the ecto-
derm into the embryonic and extra-embryonic parts. The egg cylinder of
the third embryo (Fig. 54) is not of very regular shape, but on the other hand
it is difficult to assert that it is abnormal. Its tissue exhibits no sign of
degeneration. As yet no lumen has appeared in the embryonic ectoderm in
any of the embryos. In two cases the cavity of the yolk sac is large, in the
third small. In all embryos the differentiated grouping of the cells of the
ectoplacental cone is visible to a greater or lesser degree. Vacuolisation
of the cells of the proximal entoderm is clearly marked. In all foetal cham-
bers the lumen of the uterus above the embryos is completely open.

Control material consisted of 4 embryos from 1 female. One of the em-
bryos is shown on Fig. 51. A lumen has appeared in the embryonic ecto-
derm of all the egg cylinders (in Fig. 51 it is not visible, as it is situated on
a different focal plane). The separation of the embryonic ectoderm from the
extra-embryonic is very distinct. All the embryos are completely isolated
by the decidua from the lumen of the uterus.

7th day. Experiment no. 17A (Fig. 55). A cleft forms in the egg cylinder
running through the area of the embryonic and extra-embryonic ectoderm.
The ectoplacental cone is well developed. Isolation of the embryo from the
lumen of the uterus is not yet complete. The transverse bridges of the de-
cidua tissue occur only in places in the cleft running from the lumen of the
uterus (covered by epithelium) to the ectoplacental cone. This embryo, as
regards stage of development, is only slightly inferior to the normal embryo
(Fig. 56) and only the egg cylinder is considerably smaller than in the nor-
mal embryo. The normal embryo shown here is less advanced in develop-
ment than the embryos of similar age described by S n e l l (1941).

8th day. Experiment no. 19A (Fig. 57). The embryo represents the stage
of development slightly later than that of the previously described embryo
from the 7th day of pregnancy (17A). A distinct cleft (primary cavity of
amnion) runs through the entire area of the ectoderm of the egg cylinder.
The embryonic ectoderm is clearly divided from the extra-embryonic ecto-
derm, and exhibits a certain internal organisation which is expressed in the
arrangement of the nuclei perpendicularly to the edge of the cylinder. This
is undoubtedly the beginning stage of formation of the primitive streak,
despite the fact that the marks of the proliferation of the mesodermal cells
cannot as yet be seen. The ectoplacental cone is well developed. For the
first time amongst the cases so far discussed, the embryo is completely iso-
lated by decidua from the lumen of the uterus. The appearance of a new
lumen of the uterus (on the antimesometrial side) is characteristic, although
does not occur as yet in the normal foetal chambers of a similar age.

The normal embryo is considerably more advanced in development (Fig.
58). The amnion and chorion are completely formed, separating 3 cavities: the
ectoplacental cavity, the extra-embryonic coelom, and the amniotic cavity.
In the area occupied by the embryo proper, a well-developed primitive
streak and head process occur. R e i c h e r t's membrane is present.

9th day. The ninth day of development is represented by 2 embryos ob-
tained from two separate experiments.

Experiment no.. 24A (Fig. 60). The plane of the section of the embryo runs obliquely in relation to its anterior-posterior axis, and is more similar to the transverse section. This plane of section was obtained by making a transverse section of the uterine horn. S n e l l (1941) states that although up to the moment of attainment by the embryos of the age of 8—8¹/₂ days, their anterior-posterior axis most often lies in the plane of the transverse section of the uterus, deviations can take place reaching up to 45°. In this case the deviation is even greater and fluctuates between 45 and 90°.

The notochord and large mesodermal wings are already distinctly formed in the embryo. The ectoplacental cavity is almost entirely closed and the chorion adheres very closely in places to the ectoplacental cone. The allantois is large and reaches aproximately to half of the exocoelom.

Experiment no. 48A (Figs. 59a and b). The plane of section of the embryo is slightly oblique but closer to the sagittal plane. The ectoplacental cavity is strongly constricted and the chorion, in the central part, contacts with the ectoplacental cone over a large area. The allantois reaches as far as half the height of the extra-embryonic coelom. As regards the fundamental characteristics of structure the embryo represents a stage of development similar to the embryo discussed previously. A different plane of section makes it possible, in addition, to establish the presence of the first signs of formation of the head fold and of the archenteron. In both embryos a well-developed R e i c h e r t's membrane is visible.

A normal embryo (Fig. 61) possesses 5 somites and is less advanced in development than the embryos of similar age described by S n e l l (1941). Far more advanced embryos, possessing about 12 somites, were encountered in other control litters from the 9th day of pregnancy.

10th d a y. Experiment no. 22A (Figs. 63—66). Of four embryos obtained from one experiment, 2 were removed from the foetal membranes, and histological sections were made from the other two after their fixation in situ in the uterus. All the embryos are at a similar stage of development — the number of somites varies from 7 to 10. The neural groove is closed only in the central part of the body, while in the caudal and head parts the neural folds are still open. The degree of closing of the folds in the head section is slightly different (cf. Fig. 65, embryo with 7 somites, and Fig. 66, an embryo of probably 10 somites). In the more advanced embryo the outlines of the mandibular and hyoid arch (Fig. 66b), and the formation of the hind gut, are visible. In all embryos the allantois has come into contact with and joined the chorion. The stage of development of the two remaining embryos (transverse section of one is shown on Fig. 64) is similar to the stage represented by the embryos discussed above.

Experiment no. 16Aa (Fig. 70). The embryo is characterised by external morphological features which correspond to the stage between the 11th and 12th day of normal development (with a preponderance of features observed in embryos from the 12th day of pregnancy). The ring of pigment surrounding the pupil is as yet invisible, and the differentiation of the foot plate in the fore limb is only lightly marked.

11th d a y. Experiment no. 36A (Figs. 67a, b, c). 3 embryos. The largest

embryo (a) is distinguished by the full group of features characteristic of the 11th day of development. Embryo (b), which is the medium one as regards size, is slightly less advanced in development than (a). This is expressed in the inferior formation of the nasal processes and the limbs. The differences in size between these two embryos is considerably greater than the differences in degree of advancement. The smallest embryo (Figs. 67c and 68) is abnormal — the neural folds on the head have not undergone closing, and in this connection the whole head is abnormally developed. Apart from the head, the neural groove is closed. The ear vesicle is formed. The gill arches and heart are normally developed. The fore limb is in the form of an elongated swelling, the hind one not yet formed. The general degree of advancement (not including the abnormal development of the head) corresponds to that of the 10th day of development.

Experiment no. 45A (Figs. 69a and b). 2 embryos. The larger of the two is slightly less advanced in development than normal embryos of the 11th day of pregnancy. This is expressed in its most obvious form, in the very slight formation of the nasal processes. The smaller embryo represents the stage of development typical of the 10th day of normal development.

A normal embryo at the 11th day of pregnancy is shown on Fig. 71.

12th d a y. Experiment no. 32A (Fig. 72). 1 embryo. The group of external features corresponds to the 12th day of development. The ring of pigment surrounding the pupil is scarcely visible.

13th d a y. Experiment no. 38A (Fig. 74). 1 embryo. The embryo is characterised by features typical of between the 13th and 14th day of development. In relation to the stage of development characteristic of the 13th day of pregnancy, the differences are as follows: presence of rudiments of 4th and 5th row of sinus hairs (whiskers), presence of sinus hair follicle over the eye and on the cheek, oval shape of the external auditory meatus and rudiment of the pinna. The rudiments of the fingers can be distinguished in the footplate of the fore limb. The rudiments of toes and indentations on the edge are only lightly marked on the footplate of the hind limb. Although the various features are of a character midway between the 13th and 14th day of development, the general habitus of the embryo is however closer to the stage of the 13th day of pregnancy.

14th d a y. Experiment no. 35A (Fig. 75). 1 embryo. Group of features characteristic of embryos of the 14th day of pregnancy.

15th d a y. Experiment no. 34A (Figs. 76 and 77). 2 embryos. Both embryos exhibit the features characteristic of the 15th day of development, but as regards size differ slightly from each other.

Experiment no. 1Aa (Fig. 78). 1 embryo. State of advancement characteristic of the 15th day of development.

As it was found that beginning with the 13th day of development, complete regulation to the „normal" level takes place in the state of development and size of all „experimental" embryos, the collection of material was ended on the 15th day of pregnancy.

2. Rate of morphogenesis and rate of growth

The discussion following in this section on the rate of morpho-
genesis and growth after implantation constitutes the final sum-
ming up of the results dealing with development of „$\frac{1}{2}$" and„$\frac{3}{4}$"
embryos during this period of pregnancy. This question will not
therefore be dealt with again in the final discussion of results.

The morphological description given above of „$\frac{1}{2}$" and „$\frac{3}{4}$"
embryos from the period from the 5th to 15th day of pregnancy
shows that the course of development is completely normal, and
the structure of the various stages of development and morpho-
logical transformations do not deviate in any way from that of
the normally developing embryos [6]). This state of things justifies
the limitation of discussion of development after implantation to
the analysis of the rate of morphogenesis and growth.

When discussing the structure of the embryos, their size was
omitted entirely from consideration. Regulation of the develop-
ment of experimental embryos to „normal" level, however, inclu-
des both regulation of the rate of morphogenesis and of the rate
of growth. If track is kept of these phenomena it is possible, on
the one hand, to define the moment in which the experimental
embryos become similar, as regards state of advancement and size,
to normal embryos, and one the other to form a basis for determi-
ning the connection between development and growth.

The size of the embryos is expressed by the use of linear mea-
surements and of volume or weight. Linear measurements do, it
is true, show the differences in size of experimental and normal
embryos, but it is difficult on this basis to imagine the actual
differences between the masses of their tissue. Data as to the
volume or weight of the embryos are far more decisive and formed
the basic foundation for determination of the rate of growth.

„$\frac{1}{2}$" blastocyst from the 5th day of development, as regards the
state of development attained, are not only not inferior in any
way to the normal blastocysts examined, but are even more advan-

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*) This statement was not refuted by the presence in the material of one
abnormally formed embryo (Exp. no. 36A; p. 220 Figs. 67c and 68)   The
monster type it represents cannot in any way be connected with the fact of
its origin from a „$\frac{1}{2}$" blastomere, and it merely constitutes an example of
a certain type of disturbance in morphogenesis which can occur sporadically
in the development of normal embryos.

ced than the majority of them. It is presumably for this reason
that the size of their inner cells masses and total number of cells
comes within the limits of variability of normal blastocysts (with
the exception of the blastocyst shown on Fig. 49) — Table 6.
Within the various litters the variability of normal blastocysts
is slight, considerably less than between the 4 „¹/₂" blastocysts
obtained. The wide limits of variability of both features examined
is the result of the large differences between the blastocysts from
different litters.

Table 6.

Volume of inner cell masses and total number of cells of „¹/₂" and N blasto-
cysts from 5th day of pregnancy (Volume — V — in $10^3 \mu^3$).

Exp. No.	„¹/₂"		N				
	V of inner cell mass	No. of cells	No. of blastocysts	V of inner cell mass		No. of cells	
				Av.	Range	Av.	Range
52 A	77.6 58.5 28.7 53.5	182 131 — * 175	10 **	78.9	43.3 — 129.9	162.8	125 — 221

* The blastocyst underwent destruction before calculation of the number of cells.
** Blastocysts obtained from 3 females.

On account of the great variability of the control material the
sole significant observation seems to be the fact that „¹/₂" blasto-
cysts from one litter are characterised in the same way as during
the period of their „free" development, by distinct differences
in the size of the inner cell mass and the degree of formation of
the inner cell mass and the trophoblast.

As the degree of contact of the „¹/₂" blastocyst with the uterine
tissue is as yet inconsiderable, it may be assumed that the size
of their inner cell masses is proportional to the size which they
possessed in the final period of „free" development. Evidence of
this is the fact that in the blastocyst shown on Fig. 47 the inner
cell mass is smaller than in the blastocyst shown in the adjacent
figure 46, despite the fact that the contact of the trophoblast with
the mucosa of the uterus is far closer.

This heterogeneity of form in the implanted blastocysts indicates
the possibility of a non-uniform rate of development and growth

of the various embryos in the successive days of pregnancy. This assumption finds confirmation in the material obtained.

„¹/₂" embryos from the 6th (Fig. 50) and 7th (Fig. 55) days of pregnancy are, as regards the stage of development attained, very similar to the normal embryos of corresponding age. The embryo representing the 8th day of development (Fig 57) has not yet reached the normal stage and is only slightly more advanced than the normal embryos from the 7th day of pregnancy. Both embryos from the 9th day of pregnancy (Figs. 59a, b and 60) (obtained from 2 different experiments) represent a stage of development halfway between the 8th and 9th day of normal development. Embryos from the 10th day of pregnancy (Figs. 63—66) exhibit a greater delay in rate of development, since an identical state of advancement is found in normal embryos 1 day younger.

The above indicates that up to the 10th day of pregnancy the development of „¹/₂" embryos is subject to great individual variation, extending far beyond the limits of variability found in normal development. In certain cases the rate of morphogenesis may be completely normal and in others it is considerably slower and causes delay in attaining the various stages of development, sometimes of as much as 24 hours.

The great heterogeneity of „¹/₂" developmental material from this period of pregnancy is also confirmed by data on the size of the individual embryos and the calculated indices of rate of growth. When calculating the volume of embryos only the egg cylinder was taken into consideration. The ectoplacental cone was not considered at all, on account of the difficulty in defining its limits·with accuracy. The line connecting points of transition of the proximal entoderm into the distal entoderm was accepted as the upper (mesometrial) limit of the egg cylinder. Egg cylinders of embryos from the 6th and 7th day of pregnancy are of compact structure and the size of the free space is inconsiderable. Beginning with the 8th day, at the moment the foetal membranes and cavities form, the tissue itself constitutes only an inconsiderable part of the whole cylinder. In this connection calculation was made for embryos from the 9th day of pregnancy both of the volume of the whole cylinder and of the embryo itself (sensu stricto), the borders of which are defined by the amnion. In material from the 10th day of development only the volume of the embryo itself was defined. On the basis of data obtained in this

Table 7.

Volume of egg cylinders of „$^1/_2$" and N embryos (in $10^4\,\mu^3$).

Day of preg-nancy	„$^1/_2$"					N			
	Exp. No.	No. of embryos	Volume	Daily increase of volume	Vol as %,of V of normal embryos	No. of embryos	Volume Measure-ments	Av.	Daily increase of volume
6	15 A	1	17.8	—	62.0	4	23.4 26.5 28.7 36 3	28.7	—
7	17 A	1	96	5.4 ×	32.2	2	287 310	298.5	10.4 ×
8	19 A	1	227	2.4 ×	11.1	2	2050 2050	2050	6.9 ×
9	24 A	1	7770	34.2 ×	32.8	1	23650		11.5 ×
	48 A	1	8370	36.9 ×	35.4				

Table 8.

Volume of „$^1/_2$" and N embryos (sensu stricto) from 9th and 10th day of pregnancy (in $10^4\,\mu^3$).

Day of preg-nancy	„$^1/_2$"					N		
	Exp. No.	No. of embryos	Volume	Daily increase of V	Volume as % of V of N embryos	No. of embryos	Volume	Daily increase of V
9	24 A	1	1100		33.3	1	3298	.
	48 A	1	1210		36.7			
10	22 A	1	13745	11.9 ×*	16.0	1	85950	26.1 ×

* Calculated in relation to average volume of embryos from Exp. 24A and 48A.

way, the ratio was calculated of the volume of „$^1/_2$" embryos in relation to normal for each day of development, and also the relative increase in volume (by comparison with embryos 1 day younger). All these data are set out in Tables 7 and 8.

In the period from the 6th to the 10th day of pregnancy the size of the „$^1/_2$" embryos is less than half the size of normal embryos. The only exception to this is the embryo from the 6th day

of pregnancy, the volume of which exceeds 50 % of the average volume of normal embryos. It is particularly worthy of emphasis that this ratio is not maintained on the following days at the same level, but varies within very wide limits. Both comparison of the absolute size of „$\frac{1}{2}$" embryos and normal ones, and of the relative increases in size indicate that the embryos from successive days do not represent successive stages of one line of development.

Table 9.
Dimensions of egg cylinders of „$\frac{1}{2}$", „$\frac{3}{4}$" and N embryos (in $\mu\mu$).

Day of preg- nancy	Material	Length	Width	No. of sections
6	„$\frac{1}{2}$" Exp. 15 A	102.0	50.9	6
	„$\frac{3}{4}$" Exp. 13 A a	91.6 96.7 122.1	48.3 40.7 56.0	7 5 7
	N	76 3 137.4 122.1 132 3 } 117.0	45 8 45.8 45.8 50.9 } 47.1	12 9 8 9
7	„$\frac{1}{2}$" Exp. 17 A	168.0	102.0	9
	N	303.3 324.0	122.1 108.8	17 15
8	„$\frac{1}{2}$" Exp. 19 A	254.5	112.0	11
	N	518.4 453.8	259.0 284.4	28 31

The most striking example of this state of affairs is the embryo from the 8th day of pregnancy, which both as regards the state of advancement and size does not constitute a halfway stage between the 7th and 9th day of development. It is even smaller than the normal embryos from the preceding day of pregnancy and only slightly more advanced than they are in development. There is no „$\frac{1}{2}$" embryo in the material obtained, the developmental stage of which corresponds to the 8th day of normal development. Both embryos from the 9th day of pregnancy are of uniform size.

The increase in their volume in relation to the embryo from the 8th day is three times greater than in normal development. This is additional proof that the embryo from the 8th day of pregnancy is exceptionally delayed in development.

Table 8 shows the volumes of embryos (sensu stricto) from the 9th and 10th day of development. It is a characteristic thing that the ratio of volume between „½" and normal embryos is identical with the ratio of volume of the whole egg cylinders. This

Table 10.

Weight of „¹/₂", „³/₄" and N embryos (10—15th day pregnancy) in mg.

Day of pregnancy	Exp. No.	Experiment		Control — own observations				Average weight acc. Mac Dowell et al. (1927)
		No. of embryos	Weight	No. of litters	No. of embryos	Average weight	Range	
10	22 A	2	0.2 0.18	3	8	1.61	1.1— 2.2	1.47
	16Aa	1	11.6					
11	45 A	2	3.6 9.75	2	14	8.56	4.0— 13.8	8.60
	36 A	3	7.7 4.3 1.2					
12	32 A	1	12.0	2	14	28.0	15.7— 39.3	32.9
13	38 A	1	73.8	3	17	76.6	54.9—107.8	76 2
14	35 A	1	160.7	2	10	119 2	98.5—138.2	129.8
15	34 A	2	245.1 183 5	3	15	223.2	187 5—279.4	228.8
	1Aa	1	232.5					

means that the proportions in the structure of the whole cylinder of the „¹/₂" embryo are completely normal.

„¹/₂" embryos from the 10th day of development possess a body mass of about 15% of the mass of normal embryos. The ratio of their size was determined by comparing the volumes (Table 8) and weights (Table 10). The coincidence of the data obtained by these two methods (16,0% and 11,8%) is satisfactory. Although the experimental embryos are considerably smaller than normal 10-day ones, as regard volume they surpass the normal 9-day

embryo by 4 times as much. In this case the situation is not, therefore, completely similar to that found on the 8th day of pregnancy.

Data on the size of „$\frac{1}{2}$" embryos from the period between 6—10th day of pregnancy make it necessary to form an opinion as to the results of similar investigations carried out on rats by N i c h o l a s & H a l l (1942). These authors unfortunately give only very general remarks on the size of the egg cylinders, based in addition only on linear measurements. On page 449 of their work they write in relation to the 5th day stage: „... the blasto-mere embryos were typical and although of smaller linear size (ca 20%) than the normal at this time, were definitely larger than the half embryo which might have been expected". A second re-mark on this subject concerns the 7th day egg cylinders (page 451) „The egg cylinders were small but well within the normal range of variability".

These results are at complete variance with those obtained in this work. Even on the basis of linear measurements only of the egg cylinders from the 6th, 7th and 8th day of pregnancy (Ta-ble 9) there cannot be the slightest doubt that the experimental embryos are considerably smaller during this period than normal ones, and in no case do their dimensions fall within the range of normal variability. An additional indicator is the number of sections of each egg cylinder given in this table. They supply in-formation as to the thickness of the cylinder and indicate to a cer-tain extent the connection between the measurement obtained of the length of the cylinder and the plane of the section itself.

Leaving the question of the value of linear measurement, it is difficult on the basis of these fragmentary observations in the work by N i c h o l a s & H a l l, (l. c.) to form an opinion as to the growth of „$\frac{1}{2}$" embryos of the rat. It can, however, be most definitely stated that in the case of mice, the „$\frac{1}{2}$" embryos dur-ing the period between the 5th and 10th day of pregnancy are as a rule considerably smaller than the normal embryos correspon-ding to them in age, and it is doubtful whether regulation of size could in any case occur before the 10th day. It is worth while emphasising once again, that with the exception of one case (em-bryo from 6th day of pregnancy) the size of the egg cylinders and of the embryos themselves did not attain even 50% of the corre-sponding values found in normal development. The ratio of size

which the inner cell mass of „½" blastocysts and normal blastocysts from the 4th day of development represent, was not exceeded between the „½" embryos and the normal ones throughout this whole period. It is of course impossible to establish the exact link between the size of the embryos after implantation and the size of the inner cell masses which they possessed at the blastocyst stage. Treating the whole problem generally it would, however, seem that the absence of uniformity in rate of development and growth of the individual embryos after implantation is the

Table 11.

Body length of „½", „³/₄" and N embryos (10—15th day of pregnancy).

| Day of pregnancy | „½" and „³/₄" | | | | N | | | | | |
| | | | | | Own observations | | | | Grüneberg, 1943 | |
	Material	No. of implantations	No. of live embryos	Length	No. of litters	No. of embryos	Average length	Range	Average length	Range
10	„³/₄"17Aa	1	1	5.2	3	8	3.1	2.9- 3.4	1.9	1.5- 2.5
11	„½" 45 A	5	2	2.9; 3.9	2	14	4.6	3.3- 5.5	3.7	3.0- 4.0
	„½" 36 A	3	3	2.7; 3.8; 4.3						
12	„½" 32 A	5	1	5.3	2	12	6.6	5.8- 7.6	5.9	5.0- 7.0
13	„½" 38 A	2	1	8.7	3	18	8.2	7.0- 9.3	7.5	7.0- 8.5
14	„½" 35 A	4	1	11.0	2	10	9.7	9.4-10.4	8.8	8.0- 9.5
15	„½" 34 A	2	2	11.1; 12.0	4	18	11.2	10.6-11.4	10.5	10,0-11.5
	„³/₄" 1 Aa	2	1	11.6						

simple consequence of the wide variability in structure (size of inner cell mass, ratio of inner cell mass: trophoblast) of the blastocysts before implantation.

A fundamental change in the rate of development and growth of the „½" embryos does not take place until after the 10th day of pregnancy.

Embryos from the 11th day of pregnancy, obtained as the result of two experiments, exhibit enormous differences between each other, both as regards the degree of advancement in development and as regards size (Figs. 67 and 69). In fact only 2 embryos (Figs.

67c, 68 and 69b) of which one, nota bene, is not formed normally, can be considered as the succesive stage of development of „$\frac{1}{2}$" embryos from the 10th day of pregnancy. Both these embryos, both as regards the stage of development and their size, correspond to normal embryos from the 10th day of development. Of the 3 remaining, two (Figs. 67a and 67b) show the stage typical of the 11th day of development, and one (Fig. 69a) is slightly less advanced. Their size (length and weight) falls within the range of variability of normal embryos (Tables 10 and 11). The material from the 11th day of pregnancy is especially interesting for two reasons. In the first case — on no other day of pregnancy were such wide differences found between „$\frac{1}{2}$" embryos, and in the second — for the first time certain of them attained the size and state of advancement completely typical of normal development. There is not, however, the slightest doubt that in certain cases (e. g. the embryo shown on Fig. 67a) complete regulation of rate of development and growth must have taken place earlier.

The embryo from the 12th day of pregnancy (Fig. 72) is characterised almost completely by features typical of this day of development, but as regards size is still slightly inferior to the smallest normal embryos (Tables 10 and 11).

Embryos from the 13th, 14th and 15th day of pregnancy (Figs. 74—77) have completely attained the size and state of advancement characteristic of the normal embryos of corresponding age (Tables 10 and 11). In certain cases, even, a certain acceleration was found in the rate of development (embryo from the 13th day) or in the rate of growth (embryo from 14th day).

The question then arises as to whether the period of the 10th—11th day is in fact the turning-point in the development of „$\frac{1}{2}$" embryos, and what are the causes of the lack of continuity found between embryos obtained up to the 10th day of pregnancy, and the older ones. In order to settle this question it would appear of importance to consider two phenomena which manifest themselves during this period — formation of the chorio-allantoic placenta and the intensified resorption of „$\frac{1}{2}$" embryos.

In the development of normal embryos the joining of the allantois with the chorion, leading as a result to formation of the allantoic placenta, takes place at the moment when the embryos attain the stage of 7 or slightly more somites (S n e l l, 1941). With a normal embryo from the 9th day of pregnancy (Fig. 61) which

represents normal development in all the previously given cal-
culations, the joining of the allantois with the chorion has not
taken place. The existence of this contact is found, on the other
hand, in embryos, possessing about 12 somites, from a different
litter. The start of the functioning of the allantoic placenta pre-
sumably takes place between the 9th and 10th day of pregnancy.
With „$^{1}/_{2}$" embryos from the 10th day of pregnancy, in which the
number of somites varies from 7—10, this joining has also taken
place.

The start of the functioning of the allantoic placenta is undoub-
tedly an extremely important moment in embryonic develop-
ment, considered only from the point of view of the fundamental
increase in the source of nutriment for the foetus. A comparison
of normal embryos from the 9th and 10th day of pregnancy shows
a 26-fold increase in their volume (Table 8). From a comparison
of the body weight of embryos from these two days of develop-
ment (data from work by M a c D o w e l l et al., 1927) it appears
that the increase in weight is 17.6 times as great in this period
(average weight of embryos — 0.08 mg. and 1.41 mg.). On none
of the previous nor the successive days of pregnancy was so great
a relative increase in body weight found. On account of the sligh-
tly slower rate of development of „$^{1}/_{2}$" embryos this sudden
acceleration in rate of growth must take place slightly later. Re-
gulation of size, at a quicker or slower rate, undoubtedly occurs
at the moment of formation of the allantoic placenta. It would
indeed be difficult to presume that all the previously described
embryos from the period between the 5th and 10th day of pre-
gnancy were exceptionally delayed in development, and were not
the developmental „forerunners" of embryos from later days.

The developmental material obtained from the period 11th—
15th day of pregnancy, consists only of a certain selected group
of embryos (of all those implanted) which had survived up to the
day that autopsy was made of the foster-mothers. Beginning with
the 11th day of pregnancy, however, a considerable intensification
was found in the resorption of the embryos. In the period from
the 6th to the 10th day of pregnancy also some of the embryos
were undergoing resorption, but this was limited to the period
following immediately after implantation. In addition, the inten-
sity of mortality in this period is considerably less than after the
10th day of pregnancy (Table 12). All the embryos from the 6th—

10th day of development (discussed previously), regardless of the state of advancement, appeared completely normal and did not exhibit any symptoms of degeneration. The increased mortality after the 10th day of pregnancy suggests, however, that certain of them, which when checked had appeared to be developing normally would have undergone resorption in the following days. It could therefore be assumed that the embryos which are smallest and most delayed in development die at this time.

Table 12.

Mortality among „$^{1}/_{2}$" embryos after implantation.

	D a y o f p r e g n a n c y											Placental scars found after birth
	6	7	8	9	10	11	12	13	14	15	16 — 20	21	
No. of females	1	1	1	2	3	2	2	1	1	1		1	3
No. of implantations	2	2	1	2	8	8	6	2	4	2	No data available	3	9
Dead embryos	1	1	—	—	4*	3	5	1	5	—		3	3

* Death of embryos must have taken place directly after implantation.

The increasing degree of regressive degeneration of foetal chambers, encountered on the 12th, 13th or 14th day of pregnancy shows that they all come from the same period, which is limited to the 11th day. The resorption of the embryos in this period is a fundamental factor reducing the number of normally developing „$^{1}/_{2}$" embryos after implantation. Evidence of this is the fact that of 24 dead embryos, only 8 died soon after implantation, and 16 about the 11th day of pregnancy.

The 11th day of pregnancy is the last day on which wide differences are found in the state of advancement of the different embryos. Beginning with the 12th day only those embryos which have attained the normal „level" of development, or resorbed embryos, are encountered.

The occurrence of mortality of „$^{1}/_{2}$" embryos of mice is completely different from that in similar experiments carried out by N i c h o l a s & H a l l (1942) on the rat. These authors found intensive resorption to take place of „$^{1}/_{2}$" embryos, increasing gradually from the first day after implantation. The main intensification of this mortality fell on the 8th and 9th day, and then included all the embryos still alive. The differences in results

are quite significant and require special consideration on account
of the relatively close relationship of the two species and the very
similar rate of embryonic development. It would, however, seem
that the causes of this state of affairs are not so much the result
of differences in the developmental capacities of the blastomeres
themselves of mice and rats, but rather are caused by the diffe-
rences in the method used. I am even inclined to assert that in
the experiments made by N i c h o l s & H a l l (l. c.) the resorption
of all the „$^1/_2$" embryos before the 10th day of pregnancy is the
result of the harmful effect of the operations carried out in vitro
(the long period during which eggs were kept in acidified R i n -
g e r's solution), and does not in any way indicate the impossibi-
lity of full development taking place from the 1 blastomere at
the 2-cell stage. The author (1959) found when transferring nor-
mal mouse blastocysts through uterine cervix, that intensive resor-
ption of the embryos takes place, beginning in the overwhelming
majority of cases before the 10th day of pregnancy. It was then
assumed that the cause of this phenomenon is the harmful effect
of the medium itself in which the blastocysts were kept in vitro
(L o c k e's solution used). Since, however, the work of other
authors on the transplantation of mouse eggs were not carried
out with the aim of determining the actual moment of occurrence
during pregnancy of embryonic mortality, there are no direct
proof available which argue in favour of the view put forward [7]).
It is however difficult to provide a different explanation of the
fact that despite the transfer by these authors of a large number
of blastomeres (about 250), and the very effective transplantation
technique (high percentage of implantation), not one of the em-
bryos survived to the later days of pregnancy. On the other hand
my own observations revealed that the probability of normal de-
velopment of „$^1/_2$" embryos is very high (Table 18, p.242).

A second factor, which may to a certain extent be connected
with the intensive mortality among embryos observed by N i c h o-
l a s & Ha l l (l. c.) is the lack of synchronisation between the age
of „$^1/_2$" and normal embryos of the recipient female, the difference
being as much as 24 or even 48 hours [8]).

The method of carrying out experiments described in the pre-

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[7]), [8]) These questions are treated in greater detail in the discussion of
methods (p.p. 243—249).

sent work does not provide the possibility of determining the influence of the manipulations themselves, carried out in vitro (not of course, including the destruction itself of the blastomeres), on the further development of the embryos. In all experiments the conditions were identical, in essentials, and the only varying factor which could be accurately determined was the time spent

Table 13.
Development of „$^1/_2$" blastomeres depending on time spent in vitro.

Period of time	No. of Exp.	No of trans-ferred blasto-meres	Implantations		Normally developing embryos	
			No.	%	No.	%
16 — 22	22	89	29	32.5	17	19.1
22 — 28	17	86	24	27.8	12	13.7

Table 14.
Mortality of „$^1/_2$" embryos in litters with varying numbers of implantations (from 6th day of pregnancy).

No. of Implantations	No. of females	Total number of implanted embryos	Dead embryos
1	6	6	2
2	5	10	3
3	4	12	8
4	3	12	4
5	2	10	7
Total	20	50	24 (48 0%)

by the eggs in vitro. The time the eggs spent outside the organism varied from 16 to 28 minutes. Within the limits of this period two time groups were distinguished (16—22 and 22—28 mins.) and the results obtained were compared on this principle (Table 13). Prolongation of the period spent by the eggs outside the organism affects, but to a very inconsiderable extent, the capacity for survival of the embryos before and after implantation. The results are not, however, and cannot be, convincing, on account of the small scale of the range of this factor.

It might be assumed that on account of the „handicapped" start of „$\frac{1}{2}$" embryos and their great food requirements that the foster-mother is not capable of breeding a large number of them. In such case the existence might be expected of correlation between intensity of mortality and the number of implanted embryos. This treatment of the question a priori already very problematical, is not confirmed by the arrangement of the data in Table 14. The small numbers do not permit of making an estimate of their significance, but it would seem that variations in the intensity of resorption are purely fortuitous in character. Mortality after implantation is rather merely the result of the limited possibilities of development of the individual „$\frac{1}{2}$" embryos, which is expressed by their slow rate of development and growth presumably determined from the actual moment of implantation.

All the above observations on the size and state of advancement of „$\frac{1}{2}$" embryos before and after the 10th day, and the occurrence during this period of intensified resorption of embryos indicate that the lack of continuity in the developmental material obtained is the result of two factors. 1. Real and sudden hastening of the rate of morphogenesis and growth after formation of the allantoic placenta, and 2. Elimination from among the developing embryos of the smallest and least developed, by resorption, giving in effect an increase of the differences between the embryos from these two periods.

The discussion given in this section of the state of advancement and size of „$\frac{1}{2}$" embryos proves that the mutual connection between development (morphogenesis) and growth takes a different form from that in the case of the development of normal embryos.

The structure of embryos from the 5th, 6th and 7th day of pregnancy indicates that the rate of morphogenesis can be completely normal from the very moment of implantation. It is not, on the other hand, completely clear if it is maintained at the normal level to the end of embryonic development in such cases.

Embryos from the 8th, 9th and 10th day of pregnancy are examples of retarded development. Regulation of the rate of morphogenesis presumably takes place later on (10—11th day), but would appear to embrace only relatively slightly retarded embryos. On the other hand in cases of extreme retardation of morphogenesis during the period 5th—10th days, further normal

development is probably impossible. Such forms are the precursors of the embryos which die about the 11th day.

In the first half of pregnancy, regardless of the rate of development (normal or retarded) ,the successive stages of morphogenesis come into being, as a rule, on the foundation of a smaller mass of the embryo itself than is usually the case. Regulation of the size of ,,$^1/_2$'' embryos to the normal level does not take place until the beginning of the second half of pregnancy.

The origin of the embryo from the ,,$^1/_2$'' blastomere affects, to a different extent, the rate of morphogenesis and growth. Both these phenomena take place to a certain degree independently of each other, and completely non-parallel, lead to the occurrence of complete regulation in the development of ,,$^1/_2$'' embryos.

The embryos which developed from 3 blastomeres of the 4-cell stage (,,$^3/_4$'') require separate discussion.

Of 3 ,,$^3/_4$'' embryos from the 6th day of pregnancy (Figs. 52—54) 2 are similar as regards size to the ,,$^1/_2$'' embryos of corresponding age, and the third is of a size coming within the range of variability for normal egg cylinders (their volume is 15.9 ; 16.2 and 24.2 × 10^{4}μ^3). The state of advancement is similar to that of normal 6-day embryos.

The embryo from the 10th day of pregnancy (Fig. 70, Tables 10 and 11) occcupies an exceptional position, since both from the point of view of state of advancement and of its size, it corresponds to normal embryos of mid-way between the 11th and 12th day of development. Two causes may lie at the root of such exceptional hastening of rate of development: 1. The embryo developed from an egg, in which the ,,$^1/_4$'' blastomere had been destroyed, containing chiefly cytoplasm from which the trophoblast would have been formed (the blastocyst must have possessed an inner cell mass approximating as regards size to the inner cell masses of normal blastocysts), 2. It is possible that in this case the moment of implantation was hastened (see page 211).

The embryo from the 15th day of pregnancy (Fig. 78, Tables 10 and 11) completely corresponds as regards state of advancement and size both to normal embryos and to ,,$^1/_2$'' embryos of similar age.

This material is too scanty to permit of establishing connection with the observations made before implantation. Treated theoretically, the exceptionally wide variability in structure of ,,$^3/_4$''

blastocysts (expressed by the different degree of formation of the inner cell mass and trophoblast) should be expressed by a very varying rate of development and growth between the individual embryos in the first half of pregnancy.

V. BIRTH AND DEVELOPMENT AFTER BIRTH

Three females gave birth to young originating from transplanted „$^1/_2$" blastomeres, and 1 female to young which developed from „$^3/_4$" blastomeres. The body weight of the newly-born mice (experimental and normal) is given in Table 15, and the body weight of the young ones on the 21st day of life in Table 16.

Table 15.

Weight of „$^1/_2$", „$^3/_4$" and N young on the first day after birth (in grm.).

Size of litter	Material	No. of litters	Measu-rements	Range	Average	Gates, 1925	Parkes, 1926	Bluhm, 1929*	Crozier, Enzmann, 1935**
						Data from literature			
1	„$^1/_2$" 25 A	1	1.92			—	1.80	1.29	1.83
2	„$^1/_2$" 39 A	1	2.00 2.10			1.87	1.60	1.38	1.53
	„$^3/_4$" 17 Aa	1	1.92 1.98						
	N	4		1.55—2.10	1.83				
3	„$^1/_2$" 44 A	1	1.88 1.90 2.02			1.78	1.54	1.33	1.49
	N	3		1.45—1 80	1.65				

*, ** Unfed young.

Experiment no. 25A. Birth took place on the 21st day post coitum between 3.30 and 8.30 a.m. The retardation of birth in relation to the average time of normal births was about 24 hours. The young mouse (♀) was completely normal (Fig. 79) and was nursed by the mother until weaned (Fig. 80). Autopsy was made on the foster-mother after it had nursed the young one. The presence of three placental scars (1 large, 2 small) was found in the uterine horn on the operated side. In the 9th week of its life the young female was put with a male. After being 37 days together, the young female gave birth to a litter, but bit the young to death immediately after birth. The next birth took place 23 days after. This litter consisted of 9 young, of which 7 survived (4 ♂♂, 3 ♀♀).

E x p e r i m e n t no. 39A. Birth took place between 10 p.m. on the 19th
day and 6 a.m. on the 20th day post-coitum. Two female mice were born.
One of them died on the 17th day of life. Observation of the external appea-
rance during development, and posthumous autopsy did not reveal any ab-
normalities in structure. The second female survived and was put with
a male (also born from „¹/₃" blastomere — Exp. no. 44A) in the 7th week of
life. After 4 days the female mated and after 19 days gave birth to 5 young
ones, which it bit to death 3 days later. The next litter consisted of 12 young
ones; only 4 of them (2 ♂ ♂ and 2 ♀ ♀) survived to the 21st day of life. Two
placental scars were found in the uterine horn when autopsy was made on
the foster-mother.

Table 16.

Weight of „¹/₂", „³/₄" and N young on 21st day of life (in grm.).

Size of litter	Material	No. of litters	Measu-rements	Range	Average	Data from literature	
						Gates, 1925	Parkes, 1926
1	„¹/₂" 25 A	1	11.10			—	16.00
2	„¹/₂" 39 A	1	9.50*			6.85	11.75
	„³/₄" 17 Aa	1	8.42 8.67				
	N	3		6 70—8.30	7.60		
3	„¹/₂" 44 A	1	8.50 8.80 8.90			8.55	8.82
	N	1		7.50—8.60	8.03		

* Second young one from this litter died on 17th day, weighing on that day 7.90 g.

E x p e r i m e n t no. 44A. Birth took place between 10 p.m. on the 20th
day and 8.30 a.m. on the 21st day after mating. Similarly to the first of the
cases described, birth was delayed and pregnancy prolonged to ± 24 hours.
Sex of the young — 2 ♂ ♂ and 1 ♀. All 3 young ones survived. Four
placental scars were discovered in the foster-mother during autopsy. The
young female was mated with one of the brother males. After 80 days of
life the female gave birth to 9 young ones, of which 7 (1 ♂ and 6 ♀ ♀) survi-
ved the period of development in the nest.

E x p e r i m e n t no. 17Aa. 2 young ones (♀ and ♂) were born between
10 p.m. on the 19th and 8 a.m. on the 20th day post-coitum. Four placental
scars (3 large, 1 small) were found in the foster-mother. Development of the
youngs proceeded normally. The young female was mated with its brother
and after 2 months of life gave birth to 5 young ones (2 ♂ ♂ and 3 ♀ ♀). One

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month later this female gave birth to a second litter, also consisting of 5 young ones.

In the 3 cases described above, in which the birth took place of young ones developing from „$\frac{1}{2}$" blastomeres, the length of pregnancy of the recipients varied, and lasted 19 or 20 days.

It is generally accepted that pregnancy in mice most often lasts 19 days, but that variations are possible up to the extent of 1 day (A s d e l l, 1946). F e k e t e (data not published, cited by S n e l l, 1941) found variations in females belonging to the C 57 Black and dba strains of up to 4 days. Of course extreme cases are rarely represented. It is, however, characteristic that in the C 57 strain pregnancies of 19 and 20 days occurred in almost equal numbers of cases (41 and 51 cases of a total of 99 cases examined).

The animal material used in these experiments was fairly heterogeneous, but in all the control observations, when the date of mating was known, pregnancies lasted as a rule 19 days. A similar length of pregnancy was found in the case of the 4 females to which normal 2-blastomere eggs were transferred (T a r k o - w s k i, 1959).

The question then arises as to whether the length of pregnancy equal to 20 days found in 2 cases (Exp. no. 25A and 44A) was, in fact, governed by the slower rate of development of the „$\frac{1}{2}$" embryos, or whether this is merely the expression of the normal variability of this phenomenon in mice. The observations discussed previously on development after implantation revealed great differences in size and degree of advancement of „$\frac{1}{2}$" embryos from the same days of pregnancy. Theoretically speaking, it might be assumed that the length of pregnancy may, within certain limits, be dependent on the rapidity of occurrence of full regulation of the size and state of advancement of the developing „$\frac{1}{2}$" embryos. It should, however, be noted that a considerable degree of variation in rate of development and growth of „$\frac{1}{2}$" embryos is in principle found only up to the 11th day. All normally developing embryos from the later days of pregnancy, which presumably correspond to the largest embryos surviving from the previous period, represent the stages typical of normal development. In such cases there are no grounds for expecting prolongation of pregnancy

The number of developing embryos does not seem to play a significant part in the problem discussed. A length of pregnancy

equal to 19 days was found in the case of a female which gave birth to 2 young ones, and 20 days, in cases in which 1 and 3 young ones were born. It should be added that with mice, no distinct connection between the number of embryos and length of pregnancy has as yet been established.

In the experiments described by S e i d e l (1952), as a result of which he obtained 2 young rabbits developed from one blasto-mere of the 2-cell stage, pregnancy in the recipients was 2 and 4 days longer than the generally accepted average. An estimation of the significance of these facts is however even more difficult than in analogical cases found in mice, since in normal pregnancies of the rabbit there is a connection between the number of embryos and the duration of pregnancy (W i s h a r t & H a m m o n d, 1933; H a m m o n d, 1934; V e n g e, 1952), (S e i d e l also draws attention to this).

On the first day after birth the young „experimental" mice do not differ as regards weight (Table 15) and body dimensions (and proportions) from normal newly-born mice. All data on young „experimental" mice are grouped nearer the upper limit of varia-bility found in normal young ones, or even exceed this. The grea-ter weight of young ones (both „experimental" and normal) in comparison with data from literature (Table 15) is presumably caused by the fact that weighing was not done until a few hours after birth.

Development of the young ones during the period of nest life followed a similar course to that in normal control litters, and in accordance with P a r k e s' observations (1926). The body weight during the period from 2 to 20 days after birth, although noted, has not for this reason been included in this work. In Table 16 only the last measurements, made on the 21st day of life of the animals, are given. The body weight of the young „experimental" mice was greater in this period than the average weight of young ones from „normal" litters of similar size.

No abnormality of structure was found in any of the young ones born. The death of one animal (Exp. no. 39A) on the 17th day of life is rather an example of the increased mortality normally observed in the young during this period, connected with the end of nursing by the mother, and transition to independent feeding. As has been mentioned already, the posthumous autopsy of this female revealed completely normal formation of all organs. In no

case, either among the young ones or among the embryos in a more advanced stage of development, was the abnormal formation of organs on one side of the body, described by S e i d e l (1952), found.

Attainment of sexual maturity by all the animals (males and females) and normal course of reproductive activities is yet another proof indicating the full biological value of individuals developed from „$^1/_2$" and „$^3/_4$" blastomeres.

On the basis of occurrence of vaginal bleeding, and, on its termination, of the typical pregnancy smear (abundant mucus) birth was expected to take place in two further females. One of them aborted 1 young one on the 18th day of pregnancy. Unfortunately it was not possible to recreate the appearance of the embryo at the moment of abortion, as the mother bit it to death and only small fragments of the body were found in the cage.

Autopsy was made on the second female 21 days post-coitum, as birth had not taken place by this time. A large placenta still firmly attached to the wall of the uterus was found in the uterine horn on the operated side[9]). Judging by its external appearance this placenta must be regarded as completely normal and composed of healthy tissue. Not even the slightest fragments of foetal membranes or embryo were, however, found on its surface. The possibility of abortion should therefore rather be excluded in this case (absence of any traces in the cage, retention of placenta in uterus). I myself am inclined to consider that the death of the embryo itself took place in the early part of pregnancy, without involving the gradual necrosis of the placenta. In cases of spontaneous resorption of the embryo, such a phenomenon does not usually occur (cf. discussion of this question — T a r k o w s k i, 1956). N e w t o n (1935) with mice, and H u g g e t t & P r i t c h a r d (1945) with rats, showed, however, that if the embryos themselves are destroyed in the second half of pregnancy, the placentae can remain in the uterus and develop normally. The possibility cannot therefore be excluded, that in the case discussed above, although there was no interference by experimentation, the situation was in essentials similar to that described by these authors.

VI. NUMERICAL ASPECT OF EXPERIMENTS CHECKED AFTER IMPLANTATION

The embryos discussed previously, from the successive days of pregnancy, were obtained as the result of carrying out a large number of experiments. It is obvious, of course that they represent a certain percentage of the transplanted blastomeres. It is,

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[9]) Traces of two other foetal chambers, which must have undergone resorption in the early period of pregnancy, were found in this same horn.

however, very essential to present the summarised figures embracing all the controlled experiments after implantation. Such data may constitute a certain basis for consideration of the developmental potency of both blastomeres at the 2-cell stage. Since the actual method of carrying out the experiments (destruction of one blastomere) does not permit of obtaining direct proofs of the above, it is only possible to draw indirect conclusions based on an extensive amount of numerical material. Arriving at conclusions in this way is, however, rendered considerably more difficult by the fact that the results obtained are not only the simple expression of developmental possibilities of transferred blastomeres, but are also dependent to a considerable degree on the effectiveness of the actual transplantation technique. A certain indicator of the effectiveness of the technique used is that provided by the controlled experiments before implantation. From the summarised comparisons given in Table 1 it will be seen that only about 50% of the transferred blastomeres are in the genital tract of the recipients at this time. This figure defines the actual

Table 17.

Results of experiments checked after implantation.

Material	Total no. of exp. ($\female\female$)	Females in which implantation took place		Total no. of transferred blastomeres	Implantations		Normally developing embryos		
							No.	%	
		No.	%		No.	%		in relation to no. of blastomeres	in relation to no. of implantations
1/2	39	25	64.1	175	54	30.8	30	17.1	55.5
3/4	5	4	80.0	21	10	47.6	7	33.3	70.0

number of eggs at the moment of implantation, and thus the effectiveness of the transplantation technique in the experiments discussed. It would seem that acceptance of such a correction eliminates to a considerable degree the results of deficiencies in methods, and makes it possible to connect the phenomena observed exclusively with the actual developmental capacities only of the ,,1/2" (or ,,3/4") developmental material.

The general numerical comparison of experiments checked after implantation is given in Table 17. Of a total of 39 females to which ,,1/2" blastomeres were transferred, implantation occurred

in 25 (64.1%). It is worth while adding that in only 84% of the females checked before implantation was the presence of transferred blastomeres established. From a total number of 175 „½" blastomeres transferred, 54 implanted themselves (30.8%), and 30 (17.1%) were developing normally at the moment autopsy was carried out. If the correction suggested previously is taken into consideration, these figures increase by twice as much. This would then mean that at the moment of implantation more than half of the „½"

Table 18.

Results of experiments in relation to number of transferred blastomeres.

No. of transferred blastomeres	No. of exp. (♀♀)	Total no. of transferred blastomeres	Implantations		Normally developing embryos		Females in which implantation took place	Females in which embryos developed normally
			No.	%	No.	%		
1	2	2	1		—		1	—
2	2	4	—		—		—	—
3	8	24	6	25.0	4	16.7	4	4
4	8	32	9	28.1	5	15.6	4	3
5	10	50	13	26.0	10	20.0	9	6
6	4	24	9	37.5	3	12.5	3	2
7	3	21	11	52.4	7	33.3	3	2
8	—	—						
9	2	18	5	27.8	1	5.5	1	1
Total	39	175	54	30.8	30	17.1	25	18

blastocysts are capable of evoking a decidual reaction in the uterus and of continued development. As there is no possibility of discovering all the dead embryos directly after implantation (on account of the rapid course of the process of resorption in this period), the number of such blastocysts is in fact undoubtedly much greater.

Determination of mortality of embryos occurring in the later stages of pregnancy is much easier on account of the slower rate

of involution of the foetal chambers and the formation of placen-
tal scars. As C o n o w a y (1955) states in the case of rats, the
placental scars remain in the uterus in the case of resorbed em-
bryos as well, if death took place after the 7—8th day of pre-
gnancy (only certain embryos in the litter), or after the 11th day
(the entire litter). K e r r (1947) also, as additional notes to his
work, drew attention to the formation of such scars in mice after
the embryos had been killed on the 10th day of pregnancy. In
the case of several females which gave birth to ,,$^{1}/_{2}$" and ,,$^{3}/_{4}$"
young ones (see page 236), a preponderance was found of placental
scars over the number of young born. Although C o n o w a y
(1955) demonstrated the impossibility of completely accurate de-
termination of the origin of scars (either from normal embryos
or from resorbed ones), the differences in size and appearance
of placental scars discovered in these females were so distinct,
that certain of them had to be acknowledged as originating from
embryos which had died during pregnancy.

Comparison of the results of several experiments aimed
at obtaining ,,$^{3}/_{4}$" developmental material (Table 17) shows that
both the percentage of implantation and the percentage of nor-
mally developing embryos is markedly higher than in experiments
with ,,$^{1}/_{2}$" blastomeres. Despite the small number of these experi-
ments it would seem that the divergences between results are not of
a fortuitous character, but define the actual differences in the
developmental capacities of ,,$^{1}/_{2}$" and ,,$^{3}/_{4}$" blastomeres.

VII. DISCUSSION

1. Discussion of methods

In view of the fact that experimental research on the early sta-
ges of development of mammals has not so far proceeded beyond
the preliminary period, it would appear desirable to put forward
certain remarks on this problem from the point of view of methods
used.

The initial material can be obtained in two ways — either by
isolating the blastomeres (after previous removal of the zona
pellucida from the egg), or by destroying defined blastomeres
within the zona pellucida which is allowed to remain in place.
Only the first procedure can make it possible to determine directly
the potency of all the blastomeres of the cleaving egg. Theoreti-

cally speaking, this is the ideal aim — in practice, however, it has
certain disadvantages. When using the second method, investi-
gation of the potency of the blastomeres can be carried out solely
indirectly, by an analysis of the frequency of occurrence of deve-
lopmental forms of varying structure. In order to ensure that the
material so collected is suitable for such analysis, it must be obtai-
ned by completely fortuitous selection of blastomeres for destru-
ction.

The method of isolation of the blastomeres necessitates remo-
val of the zona pellucida in a way which guarantees that none
of the cells are damaged. N i c h o l a s & H a l l (1942) achieved
this by placing the eggs in an acidified R i n g e r's solution. If this
method is used, there are, however, fundamental difficulties in
selecting a pH of fluid at which the zona pellucida will dissolve
without involving any harmful effect to the vitality of the blasto-
meres themselves. This forced the above cited authors to apply
a very slightly acidified medium, and in consequence prolonged
the period the eggs were in vitro. This is a most inconvenient
necessity, since if the eggs spend a period of several hours in an
artificial physiological solution, in pH conditions far from opti-
mum, this may effect to a marked degree the later development
of the blastomeres. This in particular is true of the eggs of *Muridae,*
which are exeptionally intolerant even of a short storage in vitro.
W h i t t e n (1956) found that the development of 8-cell mouse
eggs up to the blastocyst stage can take place in vitro only within
the limits of pH 6.9—7.7, i. e. with a far greater pH than is essen-
tial for dissolving the zona pellucida. G a t e s & R u n n e r (1952)
obtained proof of the fact that during the transplantation of
normal mouse eggs, when the period spent in vitro is limited to
that absolutely essential for washing the eggs out from the donor
and for transfer, the use of L o c k e's solution as medium (by
comparison with the parallel use of semen-diluter) affects the
future capacity for survival of the eggs to a certain extent.

The „mechanical" technique described in this work of breaking
up the zona pellucida undoubtedly makes it possible to avoid seve-
ral of the disadvantages of the „chemical" method. In the first
place it is very simple and permits of removal of the zona pellu-
cida from several eggs literally in a few minutes. In the second
place it makes it possible to keep the eggs and carry out the whole
operation in serum, which is undoubtedly the best of all the media

so far used. In the third place, the possible harmful influence of manipulation is expressed in the immediate and distinct degeneration of the blastomeres. Blastomeres which do not undergo degeneration within a very few minutes preserve their normal form for a very long period in vitro. Generally speaking there is basically no danger here such as must always be taken into account when employing any of the methods of the „chemical" type, of the possible harmful effect of the operation not manifesting itself immediately in distinct morphological changes, but occurring later when the eggs are already inside the recipient. At the present time the disadvantages of this method consist in the fact that it is not possible to obtain 2 naked blastomeres from all the eggs in this way, as often one of them (exceedingly infrequently both of them) undergoes destruction. It would seem, however, that in cases in which isolation and preservation of both blastomeres is of importance, the perfected „mechanical" method has more promising prospects.

An experiment aimed at determining the fate of all isolated blastomeres necessitates, if it is to be carried out lege artis, further treatment of the group of blastomeres of each egg completely individually. This involves the necessity for transplanting in each operation only 1 pair of blastomeres (or „fours" in the cases of 4-cell eggs). This in turn would necessitate an enormous expansion in the scale of the experiments. At the same time, on account of the fact that the results depend to a great extent on the precision with which transplantation was done, and on several other factors, which cannot be exactly controlled, only certain experiments can be defined as „positive" [10]).

All the above remarks would seem to show that at the present time the most efficient method is the mass transplantation of eggs in which defined blastomeres have been destroyed. In this case, however, it is necessary to be completely sure that the pierced blastomere has in fact been destroyed, and that it will not parti-

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[10]) The significance of the zona pellucida in the development of the eggs themselves has been completely omitted here. Its presence, as Nicholas & Hall (1942) showed, and as is shown by several observations in this work, is not essential to the development of the blastomeres of mouse and rat eggs. This does not, however, indicate its complete lack of significance, especially in the case of the development of eggs of other mammals.

cipate in further development. When using the method of destruction applied in this work there was not the slightest doubt on this point, since the chosen blastomere underwent immediate partial or complete destruction into loose detritus. The degree of destruction of the blastomere, expressing itself either in its complete breakdown, or in its preservation in the form of a dead but compact element, may be of essential significance to the further development of the live blastomeres. The solution of this problem would, however, necessitate the use of a less brutal technique than that of piercing the blastomere with a glass needle.

When one of the blastomeres of the 2-cell stage is destroyed, the remaining blastomere, if damaged in a manner invisible to the experimenter, but of significance to the possibilities of its further development, degenerates and thus automatically excludes the „egg" from the experiment. On the other hand, in the case of destruction of one blastomere in the 4-cell stage, the danger exists that one or more of the remaining 3 may also degenerate in the course of further development, as the result of invisible preliminary damage. Such an „egg" can continue to develop, although it does not represents a developmental stage of „$^3/_4$" origin. These remarks should undoubtedly be taken into consideration during work, although this would not appear to be a phenomenon of a very general character. Observations made of the behaviour of eggs with a definite number of destroyed blastomeres, left for a comparatively long period in vitro, indicate that the remaining blastomeres preserve their normal form and appearance. Appropriately exact check made of the eggs when selecting them for the actual transplatation ensures as far as possible the accuracy of the experiment.

Investigation of the course of development of blastomeres (even only before implantation) necessitates, in view of the still unsatisfactory state of the technique of culture in vitro (especially in respect of mouse eggs and those of related species), their introduction into the reproductive organs of the recipient. The choice of the most suitable transplantation technique for this type of experiment remains open. It would seem that this question, as far as mice are concerned, has encountered somewhat fundamental difficulties. The transplantation techniques most often applied for cleaving eggs are based on transfer to the uterine horn. It has been found, however, that it is impossible to transfer the eggs

to the uterus during the second day post coitum, since when introduced during this time they are presumably soon afterwards discharged from the genital tract [11]).

Transplantation of eggs in this way gives satisfactory results only when the operation is conducted on the third or fourth day post coitum. When normal 2-cell eggs or „$1/2$" blastomeres are used for transplantation, however, this technique is unsuitable, as it does not ensure a sufficiently long period for the eggs to attain the blastocyst stage (up to the time at which implantation should begin). These difficulties are eliminated by the technique of transplantation to the oviduct (T a r k o w s k i, 1959). Use of this technique makes it possible, in the first place, to introduce the eggs to the part of the genital tract appropriate to them at this time, and in the second, maked it possible for the eggs to achieve full preimplantation development (even prolonging the time allotted for this development by one day), and in the third place, guarantees the transit of the eggs to the uterus in a natural manner, and at the moment most suitable for the beginning of implantation. Prolongation of the time allotted for pre-implantation development by one day proved to be of great importance, since in the case of „$1/2$" blastomeres a certain retardation of cleavage occurs directly after transplantation, delaying the attainment of the stage of the formed blastocyst by $1/2$ to 1 day. This phenomenon is not, however, connected with the destruction of the blastomeres, but is the normal reaction of the eggs to the actual operation of transplantation (T a r k o w s k i, 1959). Prolongation of this period where blastomeres originating from the 4-cell stage are concerned, by as much as 2 days, did not create any obstacles to continued normal development of the eggs. It has been found that when transplanting „$3/4$" blastomeres and normal 8-cell eggs and blastocysts (work by this author cited above), the blastocysts already formed in the oviduct pass in this stage to the uterus, where implantation takes place at the normal time. Although all works on the transplantation of mouse eggs are in agreement as to the fact that the best results are obtained in cases in which the transplanted eggs are more advanced in development than the eggs of the recipient, or are of the same age (this may also be expressed

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[11]) This problem is discussed in detail in the work by the author (T a r- k o w s k i, 1959), which also includes a list of relevant literature.

as advancement of the pregnancy of the donor and pregnancy or
pseudo-pregnancy of the recipient at the moment of operation),
N i c h o l a s (1933), on the basis of his experiments on the trans-
plantation of normal 2- and 4-cell rat eggs, arrives at contrary
conclusions. Basing their work on these observations, N i c h o l a s
& H a l l (1942) transplanted naked isolated „$^{1}/_{2}$" blastomeres to
recipients, the eggs of which were at that time 24 or even 48 hours
older. It is not clear whether such considerable delay in the time
of implantation, in relation to the proper time at which it ought
to take place, does not affect the normal development of the em-
bryos. The possibility cannot be excluded that this factor also
exerted some influence, in the experiments by these authors, on
the mortality of all „$^{1}/_{2}$" embryos.

It is an interesting fact that in both the previously cited works,
these authors carried out transplantation to the uterus on the
second day post coitum. On these grounds it must be concluded
that in the uterus of the rat, contrary to that of mice, conditions
prevail at this time which make it possible for the transplanted
eggs to remain there. As, however, experiments on the transplan-
tation of rat eggs were not continued, there is no comprehensive
comparative material available.

In the present work, the use of the technique of transplantation
to the oviduct ensured the implantation of 30% of the transplanted
„$^{1}/_{2}$ "blastomeres of which 17% were developing normally when
a check was made. Although transplantation, using this technique,
of normal 2-cell eggs gives markedly better results — 70% (T a r-
k o w s k i, 1959) — on account of the specific type of transplan-
ted material in this case the results obtained may be regarded
as satisfactory. It would appear that an increase in the „producti-
vity" of the experiments is possible only by means of perfecting
the precision with which the blastomeres are destroyed, and the
precision of the actual transplantation operation. An increase in
the number of transplanted eggs in each operation has no distinct
influence on the final effect of the experiments (Table 18). Appli-
cation of transplantation technique is sufficiently accurate to en-
sure good results even when the number of transplanted blasto-
meres is small.

It should be noted that only in one case was the presence of
implantation found in the uterine horn on the unoperated side.
As however this embryo was in a stage of very advanced resorp-

tion, it was not possible to determine its origin. It is possible that it developed from a transplanted „$^1/_2$" blastomere, which migrated to the opposite side in the blastocyst stage .The phenomenon of trans-uterine migration of ova is fairly uncommon in mice, but may occur sporadically. R u n n e r (1951) noted one such case after transferring eggs (oocytes) under the ovarian capsule, and M a c L a r e n & M i c h i e (1954 and 1956) found that several of the eggs transplanted migrated to the opposite uterine horn.

In all the remaining cases the embryos from transplanted „$^1/_2$" and „$^3/_4$" blastomeres became implanted in the uterine horn on the operated side, while the opposite horn was completely empty and devoid of any trace of any previous implantation.

2. Discussion of results

The problem of regulating capacities of „$^1/_2$" and „$^1/_4$" blastomeres, which forms the subject of this discussion, is considered solely in relation to the observations of the blastocyst stage. The value of the observations on development after implantation (summed up in the section „Rate of morphogenesis and rate of growth") is very limited as regards this problem, since they cannot explain the basis and causes of the non-uniform rate of development of the individual embryos, nor the fact that only certain of them are capable of beginning, and later continuing, development after implantation. A knowledge of the preliminary structure of the embryos (blastocysts) at the moment of implantation is of fundamental significance in providing a more exact explanation of these phenomena, and in determining the general principles which govern the development of isolated blastomeres.

The final form of development before implantation — the blastocyst — is, it is true, too early a stage to make it possible to speak categorically, on the basis of its structure, of the occurrence of regulation. The basic morphogenetic processes which would supply full authority for such conclusions do not take place until after implantation. The degree of formation in the blastocysts of the inner cell mass and trophoblast make it possible, however, to arrive at an approximate determination of their further developmental capacities, and in addition is the first perceptible expression of the regulating capacities of the isolated blastomeres. This makes it possible to explain several of the phenomena which

manifest themselves after implantation, on the basis of the stru-
cture of the embryos in the earlier stage of development. On the
other hand, only during this period of development is it possible,
on the basis of the structure of the „embryo", to establish the
probable factors which define the potency of the blastomeres.

Explanation of the principles governing the development of
normal eggs or the individual isolated blastomeres is only possible
when based on a knowledge of the internal organisation of the
undivided egg. The eggs of mammals are exceptionally difficult
material for this type of research, since both with living material,
and after application of the usual histological methods, it is diffi-
cult to discover the existence of clearly differentiated cytoplasma-
tic teritories and elements defining symmetry. Previous research
work on undivided eggs and the early stages of development of
many species of placental mammals, based on traditional methods
and aimed at determining the prospective significance of the indi-
vidual blastomeres, has not succeeded in solving this problem,
and the results obtained and the interpretations given are very
conflicting. Literature on this problem, although abundant, does
not in essentials provide any data which could be treated as the
basis for an interpretation of the results of the experimental re-
search work. It has not, therefore, been considered to be of any
practical use referring to these works when conducting further
examination of this problem. A review of investigations of this
type was recently made by J o n e s - S e a t o n (1950), B o y d &
H a m i l t o n (1952) and D e G e e t e r (1954).

Recently, thanks to the cytochemical works by D a l c q and
his co-workers, our knowledge of the internal structure of the
egg, and the process of differentiation of the cells of the inner
cell mass and trophoblast, has been greatly increased as regards
rodents. The observation of greatest significance to the problem
discussed here are those included in the works of J o n e s - S e a-
t o n (1954) and M u l n a r d (1955) (in the section on the distribu-
tion of acid phosphatase). A general summing up of results has
recently been made by D a l c q (1957). Among the results obtained
by these authors four basic points should be emphasised:

1. The oocyte and undivided fertilised egg are characterised by
polarity, bilateral symmetry and presence of 2 cytoplasmatic zones
with different cytochemical properties.

2. The plane of the first cleavage division has no fixed relation to the plane of symmetry.

3. The cytoplasm of the dorsal zone of the egg passes, as a result of cleavage, to the cells of the inner cell mass, the cytoplasm of the ventral zone to the cells of the trophoblast.

4. At the 8-cell stage the final differentation occurs of the cells destined respectively for the inner cell mass and the trophoblast. This means that the cytoplasmatic material of both zones is not completely segregated until the third division of cleavage takes place.

Since these facts consitute a fundamental basis for further deductions, it is worth while quoting, in the original, part of the work of one of these authors (M u l n a r d, 1955, p. 557), which includes a short summing up of the results so far obtained. Although the description principally concerns the eggs of the rat, the basic points are of equal importance in regard to mouse eggs also.

„La symétrie bilatérale est déjà indiquée dans l'oocyte par la répartition des mitochondries et de la basophilie ribonucléique du cytoplasme. Celle-ci charactérise une sphère d'endoplasme excentrique n'atteignant la surface de l'oocyte qu'au pôle apical et d'un seul côté de celui-ci. Après examen attentif des phases de segmentation, d'enveloppment et de creusement du léchitocoèle, cette zone de la „cape basophile" a été reconnue comme occupant la côté dorsal de l'oeuf; elle contient d'assez nombreuses mitochondries de grosse taille. A l'opposé, le cytoplasme ventral est moins dense et beaucoup moins basophile: il présente un aspect spumeux caractéristique et contient des nombreuses mitochondries dont la plupart de petite taille (microchondries).

Il n'existe pas de relation déterminée entre le premier sillon de segmentation et le plan de symétrie bilatérale, de telle sorte qu'au cours des deux premiers cycles mitotiques les matériaux des zone dorsale et ventrale se distribuent de manière variable entre les blastomères. Ce n'est qu'au stade VIII régulier (étalé en placula) qu'une distinction peut être constamment faite entre des blastomères de type dorsal on ventral".

It should be clearly emphasised that when examining the experimental material, no special cytological methods were applied, and interpretation of results was carried out solely indirectly on the basis of the examination cf normal development cited above.

The overwhelming majority of „$^{1}/_{2}$" blastomeres developed into blastocysts. The fundamental characteristic of this stage is the undetermined ratio, varying between wide limits, between the number of cells of the inner cell mass and the trophoblast. Re-

gardless of the degree of formation of the inner cell mass and tro-
phoblast, the predominant majority of blastocysts contain both ele-
ments. In certain, but infrequent, cases however, formation of one
of these elements does not occur. An example of such forms are the
„blastocysts" completely devoid of the inner cell mass,, and morulae
in which the trophoblastic cells are not differentiated. In view
of the fact that cytochemical methods were not applied, it was not
however possible to find whether such morulae are composed ex-
clusively of cytoplasmatic material intended to form the inner
cell mass. A certain indirect proof of the uniform character of all
(or at least the great majority) of the cells may, however, be the
lack of occurrence of the blastulation process.

In the light of the previously cited investigations of D a l c q's
school, the great variability in structure of „$\frac{1}{2}$" blastocyst would
appear to be the simple consequence of the variable relation be-
tween the plane of the first division and the plane of symmetry
of the egg. Since the position of the plane dividing the cytoplasm
from the basophile and vacuolar (dorsal and ventral) zones to the
two first blastomeres is not determined (J o n e s - S e a t o n, 1950;
De G e t t e r, 1954), in individual cases the blastomeres may con-
tain cytoplasmatic material from both zones in completely diffe-
ring proportions. This fact, which is of no significance in normal
development, becomes a decisive factor defining the developmental
capacities of isolated blastomeres.

The differentiation of blastomeres at the 4-cell stage is consi-
derably greater than at the 2 cell stage, since it is also dependent
on the situation of the planes of the 2nd division in both „mater-
nal" blastomeres. The consequence of both divisions, regardless
even of the mutual relation of their planes, must most often be
the formation of blastomeres with a preponderance of material
coming either from the dorsal or from the ventral zone. Only in
this way is it possible to explain the exceptionally wide variation
in structure of „$\frac{3}{4}$" blastocysts. Since the selection of the blasto-
mere intended for destruction was completely fortuitous, there
was a uniform probability of removing from further development
a blastomere with either greater inner cell mass tendencies or
greater trophoblast tendencies.

Some confirmation of the above may be found in the structure
of the 2 „$\frac{1}{4}$" blstocysts described in the text. The participation
of the inner cell mass and of the trophoblast in the structure of

these blastocyst is almost completey different. The „$\frac{1}{4}$" blasto-
mere may therefore change, to a very significant degree, the pro-
portions in which both these elements are formed in „$\frac{3}{4}$" blasto-
cysts.

Single „$\frac{1}{4}$" blastomeres, judging by their capacities for forma-
tion of the blastocyst, are capable of organised development. The
proportions of participation of the cells of the inner cell mass and
trophoblast must be even more variable, however, than in the
case of „$\frac{1}{2}$" blastocysts. The occurrence of forms composed solely
of cells of one of these elements should also be expected to occur
more frequently. These observations confirm the conclusion of the
above authors, that complete segregation of the cytoplasm of both
territories of the egg does not as yet occur in the 4-cell stage.
Since this process takes place as the result of the 3rd division, the
4-cell stage is therefore the last stage of development at which
certain blastomeres are still capable of forming a blastocyst com-
posed of the inner cell mass and trophoblast. Such blastocysts
cannot, however, possess both these elements formed in the pro-
portions characteristic of blastocysts developed from a whole egg,
or from such „$\frac{1}{2}$" blastomeres as formed as the result of symme-
trical division. The aim of future experiments will be the deter-
mination of the capacities for further development of „$\frac{1}{4}$" blasto-
cysts. It is, however, very likely that on account of the very small
initial mass of the embryo, development will not extend beyond
the early post-implantation period. The possibilities of occurrence
of disturbances in the actual process of implantation will also have
to be taken into account.

The interpretation of the development of isolated „$\frac{1}{2}$" and „$\frac{1}{4}$"
blastomeres, based on the principles governing the process of
segregation of the cytoplasm of the egg in normal development,
is undoubtedly of a somewhat schematic character. Since the cyto-
plasm in both zones is not, either in the egg (and also in the bla-
stomeres) clearly defined in its limits, the very reasonable obje-
ction may be made that the use of the term „zone" or „territory"
is to a certain extent a simplification. The acceptance of such sim-
plification is, however, justified, since the destiny of the cytoplasm
of both zones is exactly defined in normal development, and diffe-
rentiation in it can be discovered throughout the whole process
of cleavage and blastulation. The results of experimental investi-
gations also indicate that two cytoplasmatic areas with differing

creative properties occur in the egg. It may even be stated that the character of both territories is very strongly determined in the egg before the occurrence of the first division or cleavage. In fact no complete regulation is found in the development of isolated „$\frac{1}{2}$" or „$\frac{1}{4}$" blastomeres which would as a rule lead to formation of proportionately built blastocysts. It would only be possible to speak of complete regulation if each „$\frac{1}{2}$" and „$\frac{1}{4}$" blastomere (regardless of the character of its cytoplasm) were capable in the early period of development of formation of an embryo (blastocyst) which, as regards the proportions of participation of component elements, would be a faithful (and merely a smaller) copy of forms developed from a whole egg.

Determination of the extent of regulation occurring can be expressed at the blastocyst stage only by numerical categories, i. e. by the degree of formation of the inner cell mass and trophoblast. Numerical relations between these two elements define to a considerable extent the developmental potency of the maternal blastomere, but do not as yet give sufficient authority for accepting them as the only and basic criterion determining the possibilities of further development of the embryo. Indeed three essential questions remain unsolved: — 1. what proportions of participation of the inner cell mass and trophoblast in the blastocyst are the most „favourable" for further development, 2. what is the minimum size of the inner cell mass which ensures normal development of the embryo after implantation, and 3. is normal development dependent only on the proper quantitative proportions of structure.

Analysis of the structure of the blastocyst from the point of view of quantitative participation of the inner cell mass and trophoblast therefore makes it possible only to state that their further developmental capacities vary considerably, and are probably to a great extent dependent on the initial mass of the inner cell mass. Since, however, a suitable degree of formation of the trophoblast is also essential to the proper course of development, presumably the blastocysts closest as regards proportions to the structure of normal blastocysts are those with the greatest possibilities of further development.

Regulation in the development of „$\frac{1}{2}$" and „$\frac{1}{4}$" blastomeres therefore leads to the formation of a new organised system but is limited to a great degree by the determined character of the cyto-

plasm, intended for trophoblast and inner cell mass, present in the maternal blastomere.

It would be dangerous to state that the fate of the cytoplasm from both zones of the egg is completely and irrevocably determined from the very beginning of development. The possibility cannot be excluded that the degree of determination of the formative capacities of the cytoplasm is not uniform troughout the area of the whole egg, and that in the case of the development of an isolated blastomere, in which the participation of both territories is different from the proportions in which they enter into the composition of the egg, a secondary re-arrangement of the fate of the forming cells may not take place. One somewhat interesting observation inclines the author to refer to this question. In the majority of „$^1/_2$" blastocysts, a distinct preponderance of cells of the trophoblast is found over those of the inner cell mass. An average of only 30% of the total number of cells enters into the composition of the inner cell mass. Control calculations carried out on normal blastocysts gave a completely different result — 60%. The difference between the results is striking. If the plane of the first division has no established position, and the degree of determination of the cytoplasm in both zones of the egg is so strong that it does not permit of re-formation of the normal proportions of structure, then the average number for the participation of inner cell mass and trophoblast cells in „$^1/_2$" blastocysts should be similar to normal blastocysts. The above remarks would suggest that a certain tendency occurs in the development of isolated „$^1/_2$" blastomeres to formation of a preponderance of trophoblast. This is, of course, at present only a supposition, which requires confirmation and more exact documentation.

It is generally accepted that the 2 first blastomeres in placental mammals differ from each other in size (B o y d & H a m i l t o n, 1952). This fact is sometimes treated as one of the expressions of creative inequality of the two blastomeres. With the rat, an unequal division does not, however, constitute a general rule (J o n e s - S e a t o n ,1950; S e m b r a t, 1955). While carrying out my own experiments, several hundreds of mouse eggs were examined from this angle, and I found that both possibilities (equal and unequal division) are encountered equally frequently. The most significant is the fact recorded by J o n e s - S e a t o n (1950) that in cases of unequal division, no tendency is observed in the

cytoplasm of any territory to pass to the greater or smaller bla-
stomere. This authoress writes on page 338 of her work: „L'iné-
galité éventuelle des deux premieres blastomeres est une particular-
ité contingente, sans relation fixe avec le symétrisation". The only
significant factor determining the potency of „$^1/_2$" blastomeres is
therefore solely the arrangement of the plane of the 1st division.

The lack of an established connection between the plane of the
1st division and plane of symmetry does not mean, however, that
a certain kind of arrangement is not more frequently represented.
The only observations available at this time on this problem are
to be found in the work of J o n e s - S e a t o n (1950). This autho-
ress found that of 30 2-cell rat eggs examined, the arrangement
of the plane of division was converging in 14 cases, oblique in 10
and perpendicular to the plane of symmetry in 6 cases. There
is therefore an undoubted tendency to symmetrical or almost
symmetrical distribution of both territories.

In making conclusions indirectly on the basis of the experi-
mental material obtained, it might be thought that this pheno-
menon occurs in similar form in the case of mouse eggs. Evidence
of this would be the observation, that the great majority of „$^1/_2$"
blastocysts obtained are formed both from the inner cell mass
and from the trophoblast, and that extreme disproportions in the
formation of both elements are relatively seldom represented.

Since the arrangement of the plane of the 1st division is an essen-
tial factor determining the potency of both blastomeres, the exi-
stence of a tendency to symmetrical or almost symmetrical divi-
sion means that in the majority of cases both blastomeres have
similar (or almost similar) capacities for further development.
Together with the increasing deviation of the plane of division
from the plane of symmetry, the difference in the potency of both
blastomeres must increase. Simultaneously the probability decre-
ases of occurrence of normal development from both blastomeres.
In an extreme case — perpendicular arrangement of the plane
of division — both blastomeres are most probably incapable of
continued development beyond the period of cleavage and possibly
blastulation.

The mortality among embryos occurring directly after implan-
tation is a manifestation, at least to a certain extent, of the com-
plete incapability of such „extreme" forms of further develop-
ment. „Blastocysts" completely deprived of the inner cell mass

can perhaps evoke a decidual reaction in the uterine tissue, but must undoubtedly die directly after this.

Embryonic mortality occurring halfway through pregnancy embraces those embryos which on account of the state of advancement in development and normal structure should be considered as completely fulfilling the conditions of totipotency. It is for this reason that the phenomenon of resorption of „$\frac{1}{2}$" embryos in this period has no longer any significance for theoretical questions of potency and regulation.

For correct interpretation of this phenomenon, it is essential to take into consideration the specific features of embryonic development in mammals, which is formed both by the influence of immanent factors inherent in the embryo itself, and by external factors brought to bear on it by the organism of the mother. Disturbance of the normal relations between mother and foetus, resulting even from considerable retardation of the rate of development, may be a sufficient cause to start the conflict which in its consequences leads to the death of the embryo. Embryonic mortality in this period would therefore constitute only a certain external „sieve" eliminating the embryos most retarded in development. On account of its intensity it is, however, a basic factor decreasing the probability of the „$\frac{1}{2}$" embryos being capable of continuing full development up to the moment of birth.

The observations, as a whole, discussed in this work indicate that regulation in the development of isolated „$\frac{1}{2}$" mouse blastomeres (and presumably of mammals in general) is limited and defined in each actual case by the character of the cytoplasm forming the blastomere. Both factors — capacity for regulation and pre-formed creative character of cytoplasm of both zones of the egg — possess an equal significance.

In view of the fact that the character of the cytoplasm contained in the blastomere is of fundamental significance to its further development, and the arrangement of the first planes of cleavage is not determined, the blastomeres may be characterised in each case by a non-uniform developmental potency. This means that in relation to mammals, a static definition of the potency of „$\frac{1}{2}$" (and „$\frac{1}{4}$") blastomeres is not possible, and that this problem can only be considered from the statistical aspect. The totipotency of both „sister" „$\frac{1}{2}$" blastomeres does not represent a general rule, but only one of possible types of relations in 2-cell eggs.

VIII. SUMMARY

1. The aim of the investigations was to define the regulating capatities of the isolated blastomeres of 2- and 4-cell mouse eggs. The development of embryos originating from „$\frac{1}{2}$" blastomeres was traced from the point of view of the course and rate of morphogenesis and rate of growth.

2. The blastomeres were destroyed by piercing them with a glass needle. The remaining blastomere (or blastomeres) with the zona pellucida intact, were then transferred to the oviduct of recipient mated with a vasectomised male. The donors and recipients as a rule differed as to pigmentation.

3. About 100 cleaving eggs and blastocysts originating from 1 blastomere of the 2-cell stage, and 3 blastomeres from the 4-cell stage, were obtained. Measurements were made of the volume of live „$\frac{1}{2}$" and „$\frac{3}{4}$" blastocysts, and of the volume of their inner cell masses, and calculation made (on fixed material) of the numbers of cells entering into the composition of the inner cell mass and the trophoblast.

4. The size of the inner cell mass and the numerical ratio between cells of the inner cell mass and trophoblast is subject to wide wariation in „$\frac{1}{2}$" and „$\frac{3}{4}$" blastocyst. The average total number of cells (expressed in % of the appropriate values found in normal blastocysts) is 68.5 and 68.3, and the average number of cells of the inner cell mass — 33.0 and 58.4.

5. Among the developmental forms from the „$\frac{1}{2}$" blastomeres occur follicle-like „blastocysts" devoid of the inner cell mass, and morulae in which, despite the aggregation of a large number of cells, the process of blastulation does not take place.

6. Single blastomeres of the 4-cell stage are also capable (at least in certain cases) of forming a blastocyst composed of trophoblast and inner cell mass.

7. The beginning of the process of blastulation depends only on the occurrence of a defined number of divisions of the blastomeres, and is independent of their initial number and character, ($\frac{1}{2}$, $\frac{3}{4}$, $\frac{1}{4}$). A „$\frac{1}{2}$" blastocyst can form on the basis of 8 cells.

8. Among 175 transferred „$\frac{1}{2}$" blastomeres, 54 (30,8%) became implanted and 30 (17.1%) were developing normally at the mo-

ment autopsy was made. After transfer of 21 „³/₄" blastomeres, 10 implanted themselves and 7 developed normally. „¹/₂" embryos were obtained from all the successive days of pregnancy, from the 5th to the 15th.

9. No developmental abnormalities were found in the structure of the embryos.

10. In the first half of pregnancy the rate of morphogenesis of „¹/₂" embryos is either completely normal or slightly retarded — but retardation never exceeds 24 hours. Their size in this period is not greater than half the size of normal embryos of similar age.

11. About the 10—11th day intensive resorption of the embryos takes place. At the same time a sudden hastening of the rate of development of the surviving embryos is observed. This would seem to have a two-fold basis: a) A real hastening of the rate of development, connected with the beginning of functioning of the chorio-allantoic placenta, and b) Apparent hastening of the rate of development connected with the elimination (resorption) of the embryos most retarded in development. Beginning with the 12th day of pregnancy the embryos attain the size and state of advancement characteristic of normal ones.

12. Since in the first half of pregnancy all „¹/₂" embryos develop normally, and about the 10th day attain the stage of development which justifies the statement that complete regulation has taken place, resorption would seem to be connected with the disturbance of the normal relations between mother and foetus, resulting from the considerable retardation of the rate of development.

13. 3 females gave birth after 19 or 20 days to young developed from „¹/₂" blastomeres, and 1 female to young developed from „³/₄" blastomeres. All the young were fertile and of normal structure.

14. In the discussion of results, an analysis was made of the data obtained in relation to the cytochemical results of the investigations made by D a l c q and his co-workers on the structure of the eggs of rodents, and the normal course of cleavage and blastulation. The results of the experiments prove that although blastomeres at the 2-cell stage are capable of regulation, the extent of this regulation is, however, limited to a considerable degree by the character of the cytoplasm, the developmental capacity of which must be established from the beginning of development. Since in many „¹/₂" blastocyst the quantitative ratio between cells of the inner cell mass and of the trophoblast is different from the

normal ratio, and in certain cases one of these elements does not form at all, it cannot be considered that regulation is of an complete character. In fact observations do not show that it as a rule leads to the formation of proportionately built forms. In 2-cell mouse eggs the totipotency of both blastomeres is only one of several possible alternatives, although presumably it is the one most frequently represented.

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EXPLANATION OF PLATES

Plate XII.

Fig. 1. 2-cell egg with 1 blastomere destroyed („$^1/_2$"), ×400.

Fig. 2. Naked blastomere of the 2-cell stage, obtained by means of mechanical removal of the zona pellucida, ×400.

Fig. 3. 4-cell egg with 1 blastomere destroyed („$^3/_4$"), ×400.

Fig. 4. 4-cell egg with 3 blastomere destroyed („$^1/_4$"), ×400.

Fig. 5. Normal 8-cel egg, ×400.

Fig. 6. Normal 16-cell morula, ×400.

Plate XIII.

Fig. 7 and 8. 8-cell „$^1/_2$" eggs. The blastomeres occupy only part of the space within the zona pellucida. Traces are visible of cytoplasmatic detritus from the destroyed blastomere, ×400.

Fig. 9. 16-cell „$^1/_2$" morula. The small cavity of the blastocoele and the differentiated group of cells of the inner cell mass are visible. ×400.

Fig. 10. 12-cell „$^3/_4$" morula ×400.

Fig. 11. 18-cell „$^3/_4$" morula, ×400.

Fig. 12. Early „$^3/_4$" blastocyst composed of 26 cells, ×400.

Plate XIV.

Fig. 13. Normal $3^1/_2$-day blastocyst, ×400.

Fig. 14. Normal $3^1/_2$-day blastocyst, stained and mounted in toto, ×500.

Fig. 15. „$^1/_2$" blastocyst. Structure completely regular, only the inner cell mass is very small, ×400.

Fig. 16. „$^1/_2$" blastocyst. Small inner cell mass situated slightly sideways from the pole of the long axis of blastocyst, ×400.

Fig. 17. „$^1/_2$" blastocyst, ×400.

Fig. 18. „¹/₂" blastocyst. Inner cell mass herniates partly outwards through the opening in the zona pellucida, ×400.

Plate XV.

Figs. 19—23. „¹/₂" blastocysts characterised by follicle-like structure and clearly formed inner cell mass, but not occupying the entire interior of the zona pellucida, ×400.

Plate XVI.

Fig. 24. Shrunken „¹/₂" blastocyst with constricted blastocoele. Very strong rupture of zona pellucida, ×400.

Fig. 25. Shrunken „¹/₂" blastocyst. No distinct damage to zona pellucida perceptible, ×400.

Figs. 25a and b. Naked „¹/₂" blastocyst — (a) while living, ×400; and (b) after fixation, ×500. Total number of cells 22. One of the unflattened cells protruding from the trophoblast to the exterior presumably represents the preserved second polar body.

Fig. 27. Naked „¹/₂" blastocyst with small blastocoele situated laterally. ×400.

Fig. 28. Irregular 16-cell „¹/₂" morula with small cavity situated laterally, ×400.

Plate XVII.

Figs. 29—31. „¹/₂" blastocysts characterised by small and not very regularly, formed inner cell mass, ×400.

Fig. 32. „¹/₂" morula composed of about 45-cells not differentiated into inner cell mass and trophoblast. Morula slightly compressed, stained with haematoxylin and mounted in toto, ×500.

Figs. 33a and b. „¹/₂" blastocyst composed solely of trophoblast. Photograph taken from 2 different focal planes, ×400.

Plate XVIII.

Fig. 34. „¹/₂" blastocyst with regularly formed inner cell mass composed of 7 cells. 15 cells in trophoblast.

Fig. 35. „¹/₂" blastocyst composed of 20 cells. Inner cell mass, lying slightly below focus, composed of 4 cells.

Fig. 36. „¹/₂" blastocyst composed of 52 cells: only 14 cells in inner cell mass, Figs. 34, 35 and 36 show the eggs stained with haematoxylin and mounted in toto, ×500.

Figs. 37a and b. „³/₄" blastocyst — (a) when living, and (b), after fixation and staining with toluidine blue, ×400.

Fig. 38. „³/₄" blastocyst, ×400.

Fig. 39. „³/₄" blastocyst. Strongly ruptured zona pellucida. Inner cell mass small and not very regular.

Plate XIX.

Figs. 40a and b. „$^1/_4$" blastocyst composed of 8 cells. Figs. a and b taken from
2 focal planes to give a better idea of the structure. 2 cells in tropho-
blast, 6 in inner cell mass (one of them is not visible). Zona pellucida
slightly deformed, blastocyst developing in the space limited by the
compressed wall of the zona pellucida. Stained with haematoxylin,
mounted in toto, ×1000.

Fig. 41. „$^1/_4$" blastocyst with regular follicle-like shape. Inner cell mass
clearly formed, but majority of cells of blastocyst are included in
the composition of the trophoblast, ×400.

Figs. 42 and 43. Naked developmental form occasionally found after trans-
plantation of „$^3/_4$" and „$^1/_4$" blastomeres. Absence of distinct organi-
sation in arrangement of cells, ×400.

Fig. 44. Blastocyst formed from one large blastomere — „inner cell mass",
and 24 small trophoblastic cells. The large blastomere corresponds
to „$^1/_4$" blastomere as regards size (cf. Fig. 45). The blastocyst was
initially of follicle-like shape, and underwent shrinkage during the
observations, ×400.

Fig 45. Normal 6-cell egg, stained with haematoxylin, mounted in toto,
×500.

> Note: An exhaustive description of the structure of embryos, the
> figures of which are shown on Plates XX—XXVII, was given in the
> text of this work (page 217—220).

Plate XX.

Figs. 46—49. „$^1/_2$" blastocysts from the 5th day of development. All originate
from one experiment (52A). Wide differences in size of the inner
cell masses and different degree of advancement of the implantation
process are observed. The uterine epithelium was destroyed in the
places of contact with the trophoblast, ×400.

Plate XXI.

„$^1/_2$", „$^3/_4$" and N embryos from the 6th day of development, ×300.

Fig. 50. „$^1/_2$" embryo (Exp. 15A).

Fig. 51. Normal embryo.

Figs. 52—54. 3 „$^3/_4$" embryos originating from Experiment 13Aa. Separation
of the embryonic ectoderm from the extra-embryonic ectoderm has
taken place in all embryos, but is only clearly visibly in Fig. 53.

Plate XXII.

Fig. 55. „$^1/_2$" embryo from the 7th day of pregnancy (Exp. 17A), ×200.

Fig. 56. Normal embryo from 7th day of pregnancy, ×200.

Fig. 57. „$^1/_2$" embryo from 8th day of pregnancy, extremely retarded in de-
velopment (Exp. 19A), ×150.

Fig. 58. Normal embryo from 8th day of pregnancy, ×150.

Plate XXIII.

„$^1/_2$” and N embryos from 9th day of pregnancy, $\times$75.
Figs. 59a and b. „$^1/_2$” embryo from Exp. 48A. 2 sections taken from different levels. Plane of section close to sagittal plane of embryo.
Fig. 60. „$^1/_2$” embryo from Exp. 24A. Plane of section close to transverse plane of embryo.
Fig. 61. Normal embryo with 5 somites.

Plate XXIV.

10th day of pregnancy:
Fig. 62. Normal embryo, $\times$8.
Fig. 63. 2 „$^1/_2$” embryos removed from foetal membranes, $\times$8.
Fig. 64. Transverse section trough anterior part of body of „$^1/_2$” embryo fixed in situ in uterus, $\times$75.
Figs. 65a and b. „$^1/_2$” emrbyo (lower on Fig. 63) seen from (a) ventral side, and (b) dorsal side, $\times$30.
Figs. 66a—c. „$^1/_2$” embryo (upper on Fig. 63) seen from (a) ventral side, (b) side, and (c) dorsal side, $\times$30.

Plate XXV.

Figs. 67a—c. 3 „$^1/_2$” embryos from 11th day of pregnancy (Exp. 36A), $\times$8.
Fig. 68. Abnormal embryo, shown on Fig. 67c, $\times$20.
Figs. 69a and b. 2 „$^1/_2$” embryos from 11th day of pregnancy (Exp. 45A), $\times$8.
Fig. 70. „$^3/_4$” embryo from 10th day of pregnancy, $\times$8.
Fig. 71. Normal embryo from 11th day of pregnancy, $\times$8.

Plate XXVI.

Fig. 72. „$^1/_2$” embryo from 12th day of pregnancy (Exp. 32A), $\times$8.
Fig. 73. Normal embryo from 12th of pregnancy, $\times$8.
Fig. 74. „$^1/_2$” embryo from 13th day of pregnancy (Exp. 38A), $\times$5.
Fig. 75. „$^1/_2$” embryo from 14th day of pregnancy (Exp. 35A), $\times$5.
Figs. 76 and 77. 2 „$^1/_2$” embryos from 15th day of pregnancy (Exp. 34A), $\times$5.

Plate XXVII.

Fig. 78. „$^3/_4$” embryo from 15th day of pregnancy (Exp. 1A), $\times$5.
Fig. 79. Newly-born „$^1/_2$” mouse (Exp. 25A) on first day after birth, $\times$1.5.
Fig. 80. 21-day old mouse developed from „$^1/_2$” blastomere (Exp. 25A), together with foster-mother.

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STRESZCZENIE

1. Celem badań było określenie zdolności regulacyjnych blastomerów 2- i 4-komórkowego jaja myszy. Prześledzono rozwój zarodków pochodzących z „$^1/_2$” blastomerów pod względem przebiegu i tempa morfogenezy oraz tempa wzrostu.

2. Blastomery niszczono nakłuwając je igłą szklaną. Pozostały blastomer (blastomery) w zachowanej zona pellucida przeszczepiano następnie do jajowodu biorczyni pokrytej samcem sterylnym. Dawcy i biorcy różnili się z reguły pod względem pigmentacji.

3. Uzyskano około 100 bruzdkujących jaj i blastocyst pochodzących z 1 blastomeru stadium 2-komórkowego i 3 blastomerów stadium 4-komórkowego. Przeprowadzono pomiary objętości żywych blastocyst „$^1/_2$" i „$^3/_4$" i objętości ich węzłów zarodkowych oraz obliczono (na materiale utrwalonym) liczebność komórek wchodzących w skład węzła zarodkowego i trofoblastu.

4. Wielkość węzła zarodkowego i stosunek liczbowy między komórkami węzła zarodkowego i trofoblastu podlega w blastocystach „$^1/_2$" i „$^3/_4$" dużej zmienności. Średnia ogólna liczba komórek (wyrażona w % odpowiednich wartości stwierdzonych w blastocystach normalnych) wynosi 68,5 i 68,3, a średnia liczba komórek węzła zarodkowego — 33,0 i 58,4.

5. Wśród postaci rozwojowych pochodzących z „$^1/_2$" blastomerów występują pęcherzykowate „blastocysty" pozbawione węzła zarodkowego i morule, w których pomimo nagromadzenia dużej liczby komórek nie następuje proces blastulacji.

6. Pojedyńcze blastomery stadium 4-komórkowego są również zdolne (przynajmniej w niektórych przypadkach) do wykształcenia blastocysty, złożonej z trofoblastu i węzła zarodkowego.

7. Rozpoczęcie procesu blastulacji uzależnione jest jedynie od nastąpienia określonej liczby podziałów blastomerów i jest niezależne od ich wyjściowej liczby i charakteru ($^1/_2$, $^3/_4$, $^1/_4$). Blastocysta „$^1/_4$" może się wykształcić już na podłożu 8 komórek.

8. Spośród 175 przeszczepionych blastomerów „$^1/_2$" — 54 (30,8%) implantowało się a 30 (17,1%) rozwijało się normalnie w momencie przeprowadzania sekcji biorcy. Po przeszczepieniu 21 blastomerów „$^3/_4$" — 10 implantowało się a 7 rozwijało normalnie. Uzyskano zarodki „$^1/_2$" ze wszystkich kolejnych dni ciąży od 5 do 15-go.

9. Nie stwierdzono żadnych nienormalności rozwojowych w budowie zarodków.

10. W pierwszej połowie ciąży tempo morfogenezy jest całkowicie normalne albo nieco zwolnione — opóźnienie nie przekracza jednak nigdy 24 godzin. Wielkość zarodków „$^1/_2$" w tym okresie nie przekracza połowy wielkości zarodków normalnych o podobnym wieku.

11. Około 10—11 dnia ma miejsce intensywna resorpcja płodów. Jednocześnie obserwuje się gwałtowne przyśpieszenie tempa rozwoju pozostałych przy życiu zarodków. Wydaje się ono mieć dwojakie podłoże: 1. Rzeczywiste przyśpieszenie tempa rozwoju związane z rozpoczęciem funkcjonowania łożyska kosmówkowo-omoczniowego i 2. Pozorne przyśpieszenie tempa rozwoju związane z wyeliminowaniem (resorpcja) najbardziej opóźnionych w rozwoju zarodków. Począwszy od 12-go dnia ciąży zarodki osiągają już wielkość i stan zaawansowania charakterystyczne dla normalnych płodów.

12. Ponieważ w pierwszej połowie ciąży wszystkie zarodki „$^1/_2$" rozwijają się normalnie i około 10-go dnia osiągają już stadium rozwoju upoważniające do stwierdzenia, że nastąpiła całkowita regulacja, resorpcja musi być związana z naruszeniem normalnego układu stosunków między matką i płodem, wynikającym ze znacznego opóźnienia tempa rozwoju.
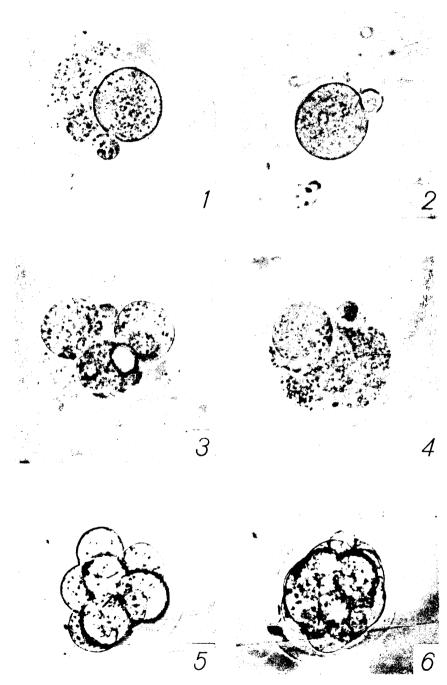
13. 3 samice urodziły po 19 lub 20 dniach młode rozwinięte z „$^1/_2$" blastomerów a 1 samica młode pochodzące z blastomerów „$^3/_4$". Wszystkie młode były normalnie zbudowane i płodne.
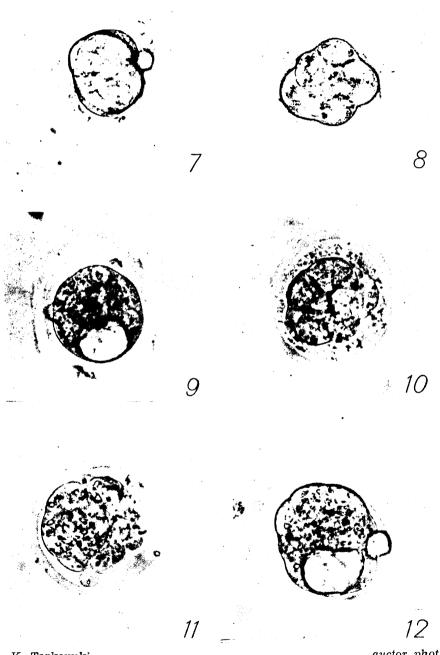
14. W dyskusji wyników przeprowadzono analizę uzyskanych danych w oparciu o wyniki cytochemicznych badań Dalq'a i współpracowników nad budową jaj gryzoni i normalnym przebiegiem bruzdkowania i blastulacji. Wyniki eksperymentów dowodzą, że jakkolwiek blastomery stadium 2-komórkowego są zdolne do regulacji, to jednak zasięg tej regulacji jest w znacznym stopniu ograniczony przez charakter cytoplazmy, której możliwości rozwojowe muszą być zdeterminowane już od początku rozwoju. Ponieważ w wielu blastocystach „$^1/_2$" stosunek liczbowy między komórkami węzła zarodkowego i trofoblastu jest daleki od stosunku normalnego, a w niektórych przypadkach jeden z tych elementów w ogóle się nie wykształca, nie można uważać, że regulacja ma charakter absolutny. Nie obserwuje się bowiem aby prowadziła ona z reguły do wykształcenia proporcjonalnie zbudowanych postaci. W 2-komórkowych jajach myszy totipotencja obu blastomerów jest tylko jednym, chociaż przypuszczalnie najczęściej reprezentowanym, z możliwych układów stosunków.
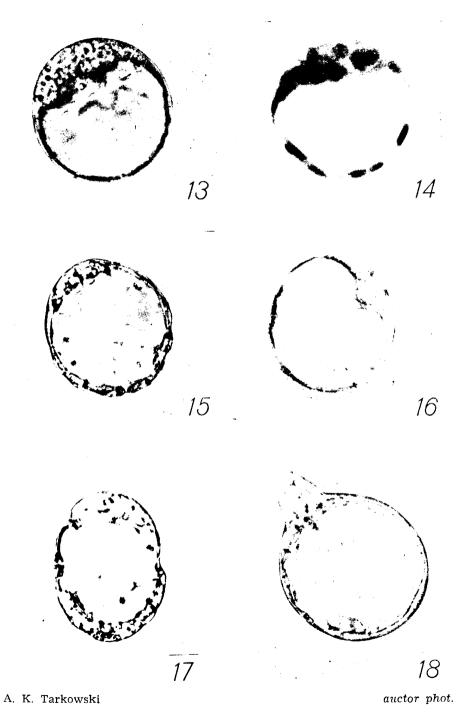
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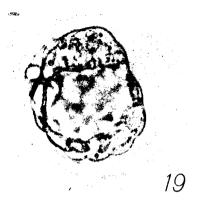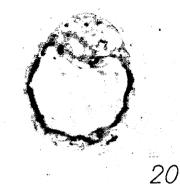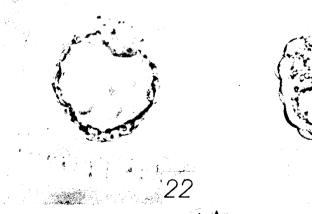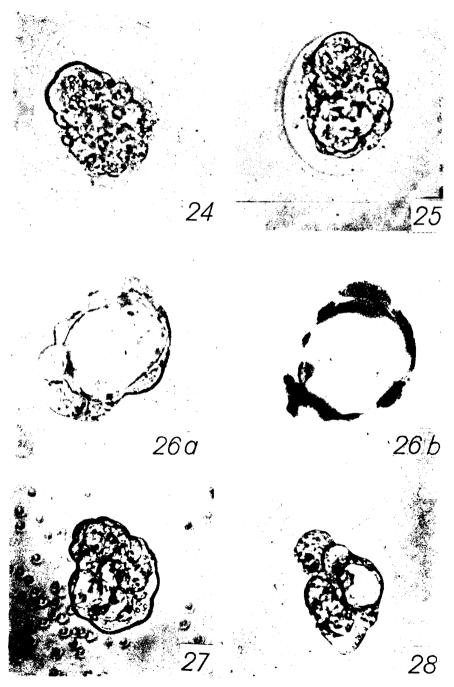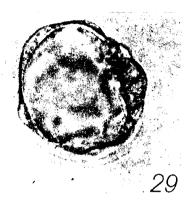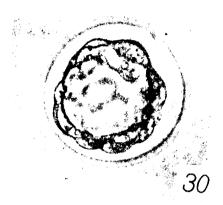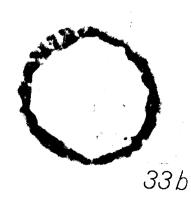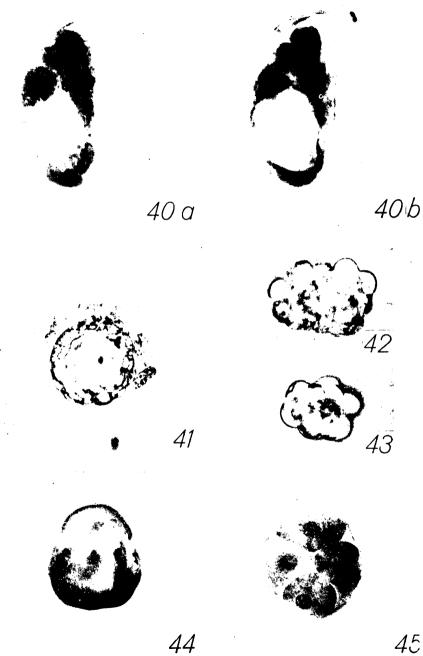
A. K. Tarkowski   *auctor phot.*

Plate XIII.

A. K. Tarkowski

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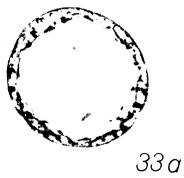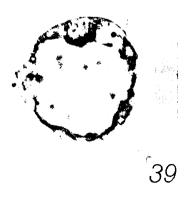
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